PROSTAGLANDIN PRODUCTION BY THE PSEUDOPREGNANT AND PREGNANT RABBIT UTERUS

by

FIONA D.C. LYTTON

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ABSTRACT OF THESIS (Regulation 6.9)

Name of Candidate FIONA D.C. LYTTON

Address ..

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Title of Thesis PROSTAGLANDIN PRODUCTION BY THE PSEUDOPREGNANT AND PREGNANT RABBIT UTERUS

There is now strong evidence to suggest that prostaglandin (PG)-$F_{2\alpha}$ acts as a uterine luteolysin in a number of sub-primate mammalian species. The purpose of the experiments reported here was to investigate whether $PGF_{2\alpha}$ acts as a uterine luteolysin in the pseudopregnant rabbit and to examine the effects of pregnancy and hormone treatment on $PGF_{2\alpha}$ secretion by the uterus.

Measurements of plasma progesterone and measurements of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ concentrations in plasma and uterine and placental tissue were made by radio-immunoassay following solvent extraction. The presence of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ was confirmed by combined gas chromatography - mass spectrometry wherever possible.

Peripheral plasma progesterone concentrations in intact and hysterectomised rabbits decreased from Day 14. The rate of decrease in intact, but not in hysterectomised rabbits was accelerated on Days 17 and 18 and was associated with an increase in $PGF_{2\alpha}$ levels in the uterine venous plasma. There was no change in uterine PGE_2 output during pseudopregnancy. The endogenous uterine tissue concentration of $PGF_{2\alpha}$ was significantly higher on Day 17 than on earlier days of pseudopregnancy but neither this nor the increase in uterine $PGF_{2\alpha}$ secretion on Day 17 could be related to an increase in the ability of uterine tissue homogenates to synthesise $PGF_{2\alpha}$ *in vitro*, or to an increase in substrate availability or a decrease in the ability of uterine tissue to metabolise $PGF_{2\alpha}$.

In the pregnant rabbit the peripheral plasma progesterone concentrations remained elevated up to Day 31 and there was no increase in uterine venous plasma levels of $PGF_{2\alpha}$ on Days 17 and 18. $PGF_{2\alpha}$ levels in the uterine venous plasma were elevated on Days 24 to 26 of pregnancy but this increase was not associated with a decrease in plasma progesterone concentrations. There was no significant difference in the ability of uterine tissue from Day 17 pregnant or Day 17 pseudopregnant rabbits to synthesise or metabolise $PGF_{2\alpha}$ *in vitro*. The uterine venous plasma levels of PGE_2 increased markedly after Day 11 of pregnancy and was positively correlated with the number of foetuses present in the uterus. The Day 17 pregnant uterus synthesised significantly more PGE_2 than the Day 17 pseudopregnant uterus and synthesised significantly less PGE_2 than the Day 17 foetal-placenta.

In ovariectomised rabbits the *in vivo* administration of progesterone significantly increased the ability of uterine tissue homogenates to synthesise $PGF_{2\alpha}$ when compared with oestrogen administration. This effect is due to a lack of substrate availability. The synthetic potential of the uterus is greatest following the sequential exposure of uterine tissue to progesterone followed by oestrogen.

It is concluded that $PGF_{2\alpha}$ does act as a uterine luteolysin in the pseudopregnant rabbit and that prostaglandin production by the pregnant uterus and its contents is important in maintaining placental function and is also involved in the process of parturition.

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In accordance with the requirements of Regulation 2.4.15. I declare that this thesis has been composed by Fiona D.C. Lytton and that the work presented herein is my own.

Signed:

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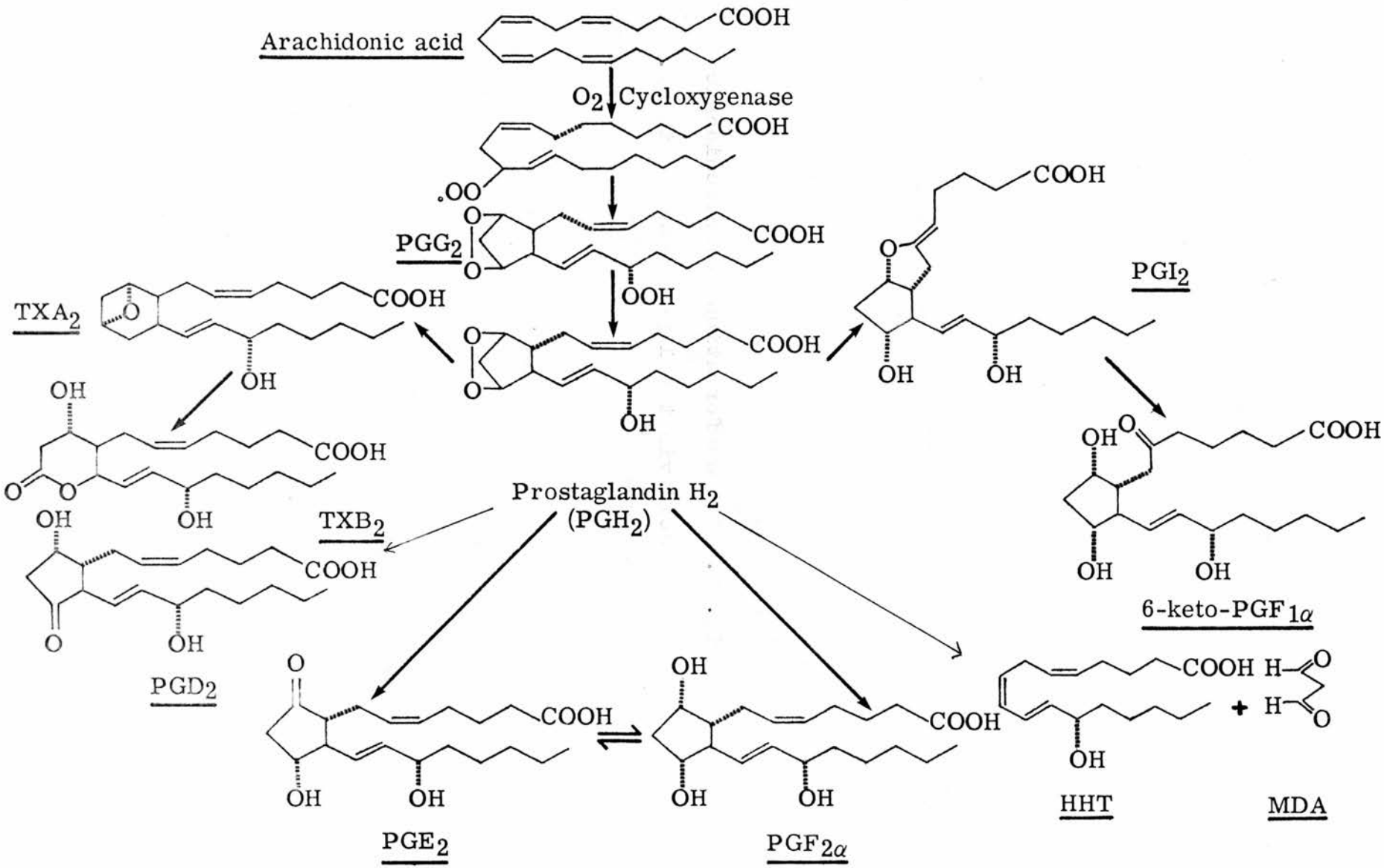
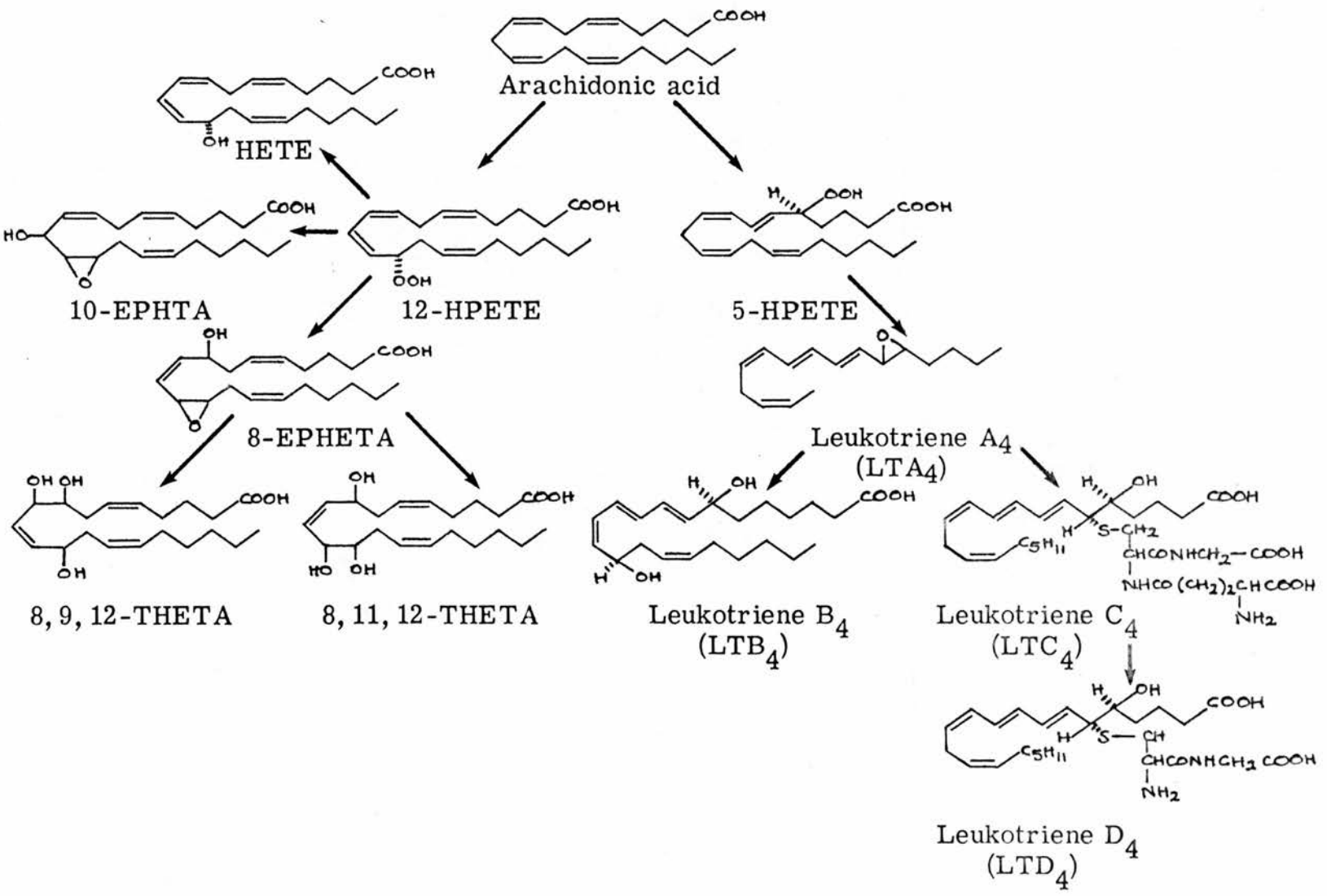
INTRODUCTION

The field of prostaglandin research has been closely linked with that of reproductive physiology since the 1930s. Kurzrok and Lieb (1930) showed that fresh human semen was capable of affecting the motility of the human uterus, *in vitro*, and that the response of the tissue depended on its reproductive state. The effects of human and sheep semen on various biological systems were examined by Von Euler (1934) and Goldblatt (1935), and in 1935, in the same journal in which the name 'progesterone' was suggested for the hormone produced by the corpus luteum (Allen, Corner, Butenandt and Slotta, 1935), Von Euler proposed that the name 'prostaglandin' be given to the constituents of human semen that are responsible for its vasodepresser and smooth-muscle stimulating activity. However, it was not until the early 1960s, following the isolation and determination of the absolute configuration of prostaglandin E_1 (PGE_1), prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Bergström, Ryhage, Samuelsson and Sjövall, 1962), that it was proved that these properties of seminal fluid were attributable to a new group of compounds.

Chemically, the primary prostaglandins are C_{20} unsaturated, hydroxy fatty acids comprising two chains joined by a cyclopentane ring. They are formed from certain essential fatty acids by the action of a membrane bound enzyme complex, prostaglandin synthetase. In mammalian tissues, the most prominent substrate for this enzyme complex is free arachidonic acid. The majority of arachidonic acid in tissues is in the form of phospholipids, cholesterol esters or triglycerides. The first stage in prostaglandin biosynthesis therefore requires the liberation of arachidonic acid by the action of a phospholipase, cholesterol esterase or triglyceride lipase.


Arachidonic acid
COOH
O_2 Cycloxygenase
COOH
.OO
O
O
PGG_2
COOH
OOH
COOH
O
O
OH
TXA_2
O
COOH
OH
COOH
PGI_2
OH
OH
OH
COOH
OH
O
OH
OH
Prostaglandin H_2
(PGH_2)
TXB_2
COOH
OH
OH
O
OH
PGD_2
6-keto-$PGF_{1\alpha}$
O
COOH
OH
COOH
COOH H
O
+ H
O
OH
OH
OH
OH
OH
PGE_2
$PGF_{2\alpha}$
HHT
MDA


Arachidonic acid
HETE
10-EPHTA
12-HPETE
5-HPETE
8-EPHETA
Leukotriene A4
(LTA4)
8, 9, 12-THETA
8, 11, 12-THETA
Leukotriene B4
(LTB4)
Leukotriene C4
(LTC4)
Leukotriene D4
(LTD4)

Since the original studies of Bergström and his co-workers in the early 1960s, several pathways in the oxidative biotransformation of arachidonic acid have been characterised. Some of these pathways are summarised in Figs 1 and 2.

Prostaglandins are not stored in tissues, so their release is preceeded by their immediate synthesis. It is possible that every cell in the body has the capacity to synthesis and release prostaglandins, and will do so if damaged. The release of prostaglandins from many intact tissues has been examined and one organ which has received much attention is the uterus. The main reason for this is that in many mammalian species the uterus influences ovarian function.

Uterine luteolytic hormone (luteolysin)

In 1923, Loeb showed that the removal of the uterus during the luteal phase of the cycle in the guinea-pig, extended the life span of the corpus luteum for a period equivalent to, or longer than, pregnancy. In 1927, Loeb wrote "It is possible that the uterus, in particular its mucosa, produces an internal secretion which exerts a specific abbreviating effect on the life of the corpus luteum." Since that time hysterectomy has been shown to prolong luteal function in the cycling sheep (Wiltbank and Casida, 1956), pig (Anderson, Butcher and Melampy, 1963), cow (Wiltbank and Casida, 1956; Anderson, Neal and Melampy, 1962) and mare (Hughes, Stabenfeldt and Evans, 1977) and in the pseudopregnant hamster (Duby, McDaniel, Spilman and Black, 1969), rabbit (Asdell and Hammond, 1933; Chu, Lee and You, 1946), rat (Melampy, Anderson and Kragt, 1964; Bradbury, 1937), vole (Eloff and Milligan, 1980) and mouse (Crister, Rutledge and Evans, 1980). However, in certain other species including the ferret

(Deanesly and Parkes, 1933), monkey (Burford and Diddle, 1936; TeLinde and Wharton, 1960) and human (Beling, Stewart, Marcus and Markham, 1970) hysterectomy does not appear to affect luteal function. This suggests that, in those species, the control of the life span of the corpus luteum is independent of the uterus.

Between 1927 and 1969 much evidence for the existence of a uterine luteolysin accumulated. Although attempts to isolate and identify the principle involved failed, several important characteristics of the uterine-luteal relationship were revealed. One of the most striking features of this relationship is that in many species it acts primarily on a local basis, each uterine horn exerting its influence over the ipsilateral ovary only. Inskeep and Butcher (1966) showed that in bilaterally ovulating ewes, hemihysterectomy only prolonged the oestrous cycle when combined with contralateral ovariectomy. Cycle length was not affected if the ipsilateral ovary was removed. However, if the remaining uterine horn was separated and retracted from its physical connections with the overy, luteal maintenance occurred. Similar results were reported in the cycling guinea-pig (Bland and Donovan, 1969a; Butcher, Barley and Inskeep, 1969; Fischer, 1974) and in the pseudopregnant rat (Barley, Butcher and Inskeep, 1966) and hamster (Orsini, 1968). In the cycling pig unilateral regression also occurs but only when all but one quarter of one uterine horn has been removed (Anderson, Butcher and Melampy, 1961).

Experiments involving the removal of the uterus at different stages of the luteal phase showed that the luteolytic factor was secreted around the time of luteal regression. The exact timing of onset and the duration of secretion of luteolysin needed to bring about

luteal regression shows considerable species variation. In the pseudopregnant hamster and cycling guinea-pig, hysterectomy will prolong luteal function if performed as late as the morning of Day 8 (pseudopregnancy 9 days) or Day 14 (cycle length approximately 16 days) respectively (Caldwell, Mazer and Wright, 1967; Bland and Donovan, 1969a). In sheep, the removal of the uterus as late as Day 15 (cycle length approximately 17 days), will arrest involutionary changes in the luteal cells providing the corpora lutea are still functional (Moor, Hay, Short and Rowson, 1970; Kiracofe and Spies, 1964). Uterine venous blood from donor ewes on Day 15 of the cycle will also cause a 50% decrease in progesterone levels in recipient animals with an autotransplanted ovary. (McCracken, Carlson, Glew, Goding, Baird, Green and Samuelsson, 1972). In the pig hysterectomy on Day 16 will maintain luteal function in 50% of animals (Anderson, Bowerman and Melampy, 1963).

Premature, uterine mediated luteolysis can also be induced by a variety of stimuli. The insertion of a foreign body into the uterine horn(s) adjacent to the functional corpus luteum in the guinea-pig (Bland and Donovan, 1965), cow (Chatterjee and Luktuke, 1961) and sheep (Ginther, Pope and Casida, 1966) is one such stimulus. The foreign body probably acts by causing local irritation or inflamation of the endometrium and thereby releasing the uterine luteolysin. Treatments which result in the destruction of the endometrium however, are often associated with luteal maintenance (Anderson *et al.*, 1961).

Oestrogen is luteolytic in the cow when given on Days 2 to 12 of the cycle, in the sheep when given after Day 10 and in the guinea-pig when administered on Days 4 to 6 (Greenstein, Murray and

Folley, 1958; Stormshak, Kelley and Hawk, 1968; 1969; Bland and Donovan, 1968; Choudary and Greenwald, 1968). Progesterone treatment begun on Day 1 of the cycle and continued for several days also decreases cycle length in the sheep, cow, and guinea-pig (Loy, Zimbleman and Casida, 1960; Woody, First and Pope, 1967) providing the uterus is intact (Bolt and Hawk, 1972a; Stormshak *et al.*, 1968; 1969; Bland and Donovan, 1970; Rowlands, 1962; Brunner, Donaldson and Hansel, 1969; Kaltenbach, Niswender, Zimmerman and Wiltbank, 1964). Oxytocin treatment will also induce precocious oestrus in the cow providing the uterine horn adjacent to the corpus luteum is left intact (Ginther, Woody, Mahajan, Janakiraman and Casida, 1967).

Before the role of a uterine luteolytic hormone can be ascribed to a compound, the pattern of secretion and metabolism of that compound by the uterus and other relevant tissues should be able to account for the above observations and satisfy the following criteria.

1) The exogenous administration of this compound should induce luteal regression and be accompanied by a decrease in the circulating plasma levels of progesterone.
2) The compound should be produced by the uterus, in amounts sufficient to cause luteolysis, at the time luteal regression occurs.
3) It should pass from the uterus to the ovary in a localised manner.
4) Factors which affect its synthesis, metabolism and/or mode of action, should affect luteal function in the appropriate manner.

These criteria will be considered separately :-

1) In 1969, Phariss and Wyngarden reported that the daily, subcutaneous administration of 1 mg kg^{-1} $PGF_{2\alpha}$ to pseudopregnant rats reduced the duration of pseudopregnancy from a mean length of 14 days to 7 days. Since this initial study $PGF_{2\alpha}$ has been shown to be luteolytic in many non-primate, mammalian species including the sheep (McCracken, Glew and Scaramuzzi, 1970), cow (Liehr, Marion and Olsen, 1972; Lauderdale, 1972), pig (Gleeson, 1974), guinea-pig (Blatchley and Donovan, 1969), mare (Noden, Oxender and Hafs, 1974), and pseudopregnant rabbit (Gutknecht, Duncan and Wyngarden, 1970).

The mechanism by which $PGF_{2\alpha}$ induces luteolysis is not fully known although much data has accumulated in the past 10 yrs. Because $PGF_{2\alpha}$ is a potent venoconstrictor (Du Charme, Weeks and Montgomery, 1968), Pharriss, Cornette and Gutknecht (1970) proposed that the luteolytic effect of $PGF_{2\alpha}$ was due to constriction of the uteroovarian vein, resulting in a reduced ovarian blood flow and luteolysis ensuing as a consequence of anoxia. This theory gained support when Speroff and Ramwell (1970) showed that, during a three hour incubation period, $PGF_{2\alpha}$ stimulated the *in vitro* production of progesterone by the ovary. This suggested that $PGF_{2\alpha}$ did not have a direct luteolytic effect on the ovary. However, subsequent experiments showed that when the incubation time was doubled, $PGF_{2\alpha}$ did inhibit progesterone production (O'Grady, Kohorn, Glass, Caldwell, Brock and Speroff, 1972). Prostaglandins of the E series stimulate ovarian progesterone production *in vitro* by increasing the intracellular levels of adenosine 3',5'-monophosphate (c-AMP). The stimulatory effect of $PGF_{2\alpha}$ *in vitro* is probably due to a PGE-like action. The initial increase in progesterone production followed by its inhibition is also seen *in vivo* when $PGF_{2\alpha}$ is infused into the

autotransplanted sheep ovary at a low dose level. If the dose of $PGF_{2\alpha}$ is increased the initial stimulant effect is not seen. Moreover, the decrease in progesterone production occurs without any corresponding reduction in the total ovarian blood flow (McCracken, *et al.*, 1970; Baird, 1974) suggesting that $PGF_{2\alpha}$ is capable of exerting a direct, biochemical effect on the luteal cell. Niswender, Diekman, Nett and Akbor (1973) have reported a selective reduction in the blood flow to the corpus luteum in the sheep ovary, and $PGF_{2\alpha}$ has been observed to selectively reduce blood flow to the corpus luteum of the sheep (Thorburn and Hales, 1972) and rabbit (Novy and Cook, 1973). However, Einer-Jenson and McCracken (1977) found that the decrease in progesterone output from the corpus luteum preceded the reduction in blood flow. Similar results have also been reported for pseudopregnant (Janson, Albrecht and Ahrén, 1975) and pregnant (Bruce and Hillier, 1974) rabbits. It is clear that pharmacological doses of $PGF_{2\alpha}$ will decrease ovarian blood flow but, at lower doses, the decrease in progesterone secretion appears to precede any decrease in blood flow. The latter may occur as a consequence, as opposed to a cause, of luteolysis.

Steroid production by the luteal cell is stimulated by luteinizing hormone (LH). LH binds to specific receptors in the plasmamembrane of the granulosa cell and stimulates the production of c-AMP by activation of the enzyme adenylate cyclase. The c-AMP then interacts with protein receptors to activate a protein kinase (Garren, Gill, Masui and Walton, 1971) which in turn activates various enzymes, including cholesterol esterase. The cholesterol esterase converts the cholesterol esters stored in the lipid droplets into free cholesterol which is then converted into progesterone.

The first indications that $PGF_{2\alpha}$ might be exerting its effect *via* antagonism of gonadotropin action arose from the work of Behrman, Yoshinaga and Greep (1971). They showed that in the Day 5 pseudo-pregnant rat, LH administration (20µg i.v.) could reverse the acute inhibition of progesterone secretion caused by the i.v. injection of 10µg $PGF_{2\alpha}$. Further studies demonstrated that $PGF_{2\alpha}$ also blocked luteal maintenance in hypophysectomised rats maintained on prolactin (Behrman, Macdonald and Greep, 1971). In the rat there is a significant reduction in LH receptor number at the time of luteal regression (Lee, Tateishi, Ryan and Jiang, 1975). Hichens, Grinwich and Behrman (1974) have shown that $PGF_{2\alpha}$ treatment causes a significant decrease in luteal LH receptor number within 24 hr in the rat but does not affect LH binding to isolated luteal membranes. Since similar effects can be induced by ergocryptine treatment (Grinwich, Hichens and Behrman, 1976) and by hypophysectomy (Behrman, Grinwich, Hichens and Macdonald, 1978) these authors concluded that $PGF_{2\alpha}$ acted *via* the inhibition of prolactin. This conclusion is supported by the fact that prolactin treatment prevents the loss in LH receptor number produced by $PGF_{2\alpha}$, ergocryptine or hypophysectomy (Berhman *et al.*, 1978; Grinwich *et al.*, 1976). The luteolytic effect of $PGF_{2\alpha}$ treatment is, however, unlikely to be mediated *via* the reduction in LH receptor number alone since the fall in serum progesterone precedes the fall in LH receptor binding capacity by some 6 hrs (Grinwich *et al.*, 1976). The latter, however, does coincide with a marked elevation in serum 20α-dihydroprogesterone (20α-ol) which is generally thought to signal the onset of functional luteolysis in the rat. The loss of LH receptors would ensure the final demise in luteal function.

The more rapid effect of $PGF_{2\alpha}$ on progesterone secretion could be mediated in a number of ways. Behrman and Hichens (1976) observed that following $PGF_{2\alpha}$ treatment there was a marked fall in the uptake of radiolabelled LH and radiolabelled prolactin by the rat corpus luteum, *in vivo*, within 30 min. The mechanism by which this occurs is, at present, unknown.

A direct antagonism of gonadotrophin action by $PGF_{2\alpha}$ *in vitro* has also been demonstrated. Henderson and McNatty (1975) suggested that the rapid effect of $PGF_{2\alpha}$ on progesterone secretion is due to the $PGF_{2\alpha}$ interfering with the coupling mechanism by which the LH receptor activation stimulates adenylate cyclase activity. This would result in a decrease in intracellular c-AMP which in turn would reduce the availability of cholesterol for conversion into progesterone. Behrman *et al.*, (1971) have shown that the treatment of rats *in vivo* with $PGF_{2\alpha}$ depresses cholesterol ester synthetase activity in the rat ovary. Cholesterol esterase activity was also reduced but to a lesser degree. Again this action appears to be mediated *via* the antagonism of the action of prolactin (Behrman *et al.*, 1971). Lahav, Freud and Lindner (1976) have shown that $PGF_{2\alpha}$ inhibits the accumulation of c-AMP induced by LH in slices in rat luteal tissue but has no effect on LH-stimulated adenylate cyclase activity in rat isolated luteal plasma membranes (Dorflinger and Behrman, 1978). The fact that $PGF_{2\alpha}$ only exerts its rapid anti-LH action in the intact cell supports the idea of Henderson and McNatty (1975) that it exerts its effect by interfering with the coupling mechanism by which LH receptor activation stimulates adenylate cyclase. The mechanism by which this occurs is not known but the calcium ionophore A23187 has been found to mimic the action of

$PGF_{2\alpha}$ in dispersed luteal cells. Also, the addition of calcium to isolated plasma membranes produced a dose dependent inhibition of LH-stimulated adenylate cyclase activity (Dorflinger and Behrman as reported in Behrman, 1979). The preliminary evidence therefore indicates that one of the initial actions of $PGF_{2\alpha}$ in the luteal cell is to increase the intracellular content of free calcium which in turn inhibits adenylate cyclase activity and thereby reduces progesterone production.

Binding sites for $PGF_{2\alpha}$ have been found in the corpora lutea of the sheep (Powell, Hammarström and Samuelsson, 1974a), cow (Powell *et al.*, 1974b), mare (Kimball and Wyngarden, 1977), rat (Wright, Luborsky-Moore and Behrman, 1979) and human (Powell, Hammarström, Samuelsson and Sjöberg, 1974). Whether the combination of $PGF_{2\alpha}$ with these binding sites is important for luteolysis is not known. Kimball and Wyngarden (1977) were unable to show any cyclical changes in the number of high affinity $PGF_{2\alpha}$-binding sites in equine corpora lutea. Similarly, Wright, Pang and Behrman (1980) could show no difference in the $PGF_{2\alpha}$ binding capacity of rat corpora lutea on Days 4 and 8 of pseudopregnancy. On the other hand Rao, Estergreen, Carmen and Moss (1979) have reported that there is a progressive increase in the specific binding of ^{3}H-$PGF_{2\alpha}$ by bovine corpora lutea between Day 13 and Day 20, the time at which functional luteolysis occurs, and that binding decreases between Days 21 and 24 when both structural and functional luteolysis are complete. The increase in $PGF_{2\alpha}$-binding between Days 13 and 20 was also accompanied by a 203-fold increase in receptor affinity. It appears, therefore, that there is a large degree of species variation in the ability of corpora lutea to bind $PGF_{2\alpha}$. In species in which $PGF_{2\alpha}$-receptor levels and/or affinity

do show cyclical changes, it is possible that these changes may also be under hormonal control.

2) $PGF_{2\alpha}$ is found as a major PG in the uterine tissues of a large number of animals. In the sheep the concentration of $PGF_{2\alpha}$ in the endometrial tissue is significantly higher on Day 14 of the cycle than on Days 3, 5 or 11 (Wilson, Cenedella and Butcher and Inskeep 1972). Similarly, in the cow the endometrial levels of $PGF_{2\alpha}$ are approximately three times higher on Days 15 to 20 of the cycle than on Days 1 to 14. (Shemesh and Hansel, 1975) In the guinea-pig, Poyser (1972) has shown that the level of $PGF_{2\alpha}$ in the uterine tissue on Days 13, 14 and 15 are higher than on earlier days of the cycle, while in the hamster the highest concentrations of $PGF_{2\alpha}$ occur on Day 8 of pseudopregnancy (Lukaszewska, Wilson and Hansel, 1972).

In all these species, therefore, there is a significant increase in the endogenous concentration of $PGF_{2\alpha}$ in uterine tissue immediately prior to the onset of luteolysis. This increase in uterine tissue levels is accompanied by an increase in the concentration of $PGF_{2\alpha}$ in the uterine venous plasma. In fact, since prostaglandins are not stored intracellularly but are synthesised *de novo*, the increase in uterine venous plasma concentrations of $PGF_{2\alpha}$ probably gives a more accurate measure of the synthetic activity of the uterus than measurements of endogenous tissue levels. In the sheep, uterine venous blood collected on Day 15 of the cycle induces luteal regression when infused into donor ewes in which the ovaries have been autotransplanted to the neck. Blood taken on earlier days of the cycle had no such effect (McCracken *et al.*, 1972). $PGF_{2\alpha}$ has been identified by combined gas chromatography-mass spectrometry, (g.c.-m.s.) in the uterine venous plasma of sheep (Bland, Horton and

Poyser, 1971; McCracken *et al.*, 1972; Harrison, Heap, Horton and Poyser, 1972), guinea-pigs (Blatchley, Donovan, Horton and Poyser, 1972) and pigs (Hunter and Poyser, as reported by Horton and Poyser, 1976). In all these species as well as in the cow (Shemesh and Hansel, 1975) and mare (Douglas and Ginther, 1976), the concentration of $PGF_{2\alpha}$ in the uterine vein begins to increase from about Day 11 of the cycle and tends to increase further until Days 16 to 18, by which time luteal regression is complete. $PGF_{2\alpha}$ release from the uterus occurs in a pulsatile fashion in the sheep (Thorburn *et al.*, 1973), cow (Nancarrow, Buckmaster, Chamley, Cox, Cumming, Cummings, Drinan, Findlay, Goding, Restall, Schneider and Thorburn, 1973) and pig (Gleeson and Thorburn, 1973). The peaks of $PGF_{2\alpha}$ vary from 8 to 25 ng ml^{-1}, they are of short duration and tend to increase in frequency and amplitude towards the end of the luteal phase. Although measuring $PGF_{2\alpha}$ in samples of utero-ovarian venous plasma allows the determination of sequential changes in the concentration of the prostaglandin in the plasma, these concentrations cannot really be related to uterine production rates without measuring the blood flow. The high levels of $PGF_{2\alpha}$ found in the uterine effluent at the time of luteolysis could arise as a consequence of a decrease in blood flow. Measurements of the peripheral plasma levels of $PGF_{2\alpha}$ are not open to this bias. However, peripheral plasma levels of $PGF_{2\alpha}$ tend to be low due to the rapid removal of prostaglandins from the circulation by the lung. One way of overcoming these problems is to measure the peripheral plasma levels of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (13, 14-dihydro-15-keto-$PGF_{2\alpha}$) the main circulating metabolite of $PGF_{2\alpha}$. In the sheep (Peterson, Tervit, Fairclough, Hawick and Smith, 1976), cow (Kindahl, Edqvist, Bane and Granström) and pig (Shille, Karlbom,

Einarsson, Larsson, Kindahl and Edqvist, 1979) the peripheral plasma levels of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ increase around the time of luteolysis in accordance with the proposed increase in uterine $PGF_{2\alpha}$ output. In the guinea-pig the urinary levels of 5α,7α-dihydroxy-11-oxotetraner-prostanoic acid increase at the end of the oestrous cycle (Granström and Kindahl, 1976). These results suggest that the increases in uterine venous plasma $PGF_{2\alpha}$ around the time of luteolysis reflect an increase in $PGF_{2\alpha}$ synthesis and secretion (providing that the pattern of prostaglandin metabolism does not vary greatly during the cycle).

In order to establish that $PGF_{2\alpha}$ acts as the luteolytic hormone in these species, it is necessary to establish that the amounts of $PGF_{2\alpha}$ released by the uterus are sufficient to bring about luteolysis. Thorburn and Nicol (1971) showed that the infusion of $PGF_{2\alpha}$ at a rate of 40 or 10μg h^{-1} into the ovarian artery on Day 5, 6 or 7 of the oestrous cycle resulted in a decrease in the peripheral plasma progesterone levels and a return to oestrøus 72h later in 50% of the animals treated. The authors calculated that an infusion rate of 40μg h^{-1} into the ovarian artery corresponds to a concentration of 10ng ml^{-1} blood. This probably represents a pharmacological dose in terms of the ovarian arterial blood supply, but a similar infusion into the utero-ovarian vein which represents physiological concentrations, also induced luteolysis in 3 out of 4 animals. When $PGF_{2\alpha}$ was infused into the jugular vein there was a slight, but transient fall in the circulating level of progesterone and cycle length was not affected. McCracken *et al.* (1972) confirmed these results. They calculated that immediately prior to oestrus the uterus releases 25μg h^{-1} $PGF_{2\alpha}$ into the uterine vein. When $PGF_{2\alpha}$ is infused at a

rate of 25μg h^{-1} into the right uterine vein of the uterus of a ewe on Days 6, 10 or 14 of the cycle, progesterone secretion decreased, reaching very low levels by the end of the infusion period. These results show that the luteolytic effect of $PGF_{2\alpha}$ occurs at physiological concentrations and acts in a highly localised manner. The failure of $PGF_{2\alpha}$ infusions into the jugular vein to induce luteolysis can probably be attributed in part to a dilution effect and, more importantly, to the fact that, following a single passage of blood through the lungs, 95-99% of the $PGF_{2\alpha}$ in the blood is metabolised (Ferriera and Vane, 1967). This highly efficient mechanism of removing prostaglandins from the systemic circulation probably accounts for the highly localised action of $PGF_{2\alpha}$.

3) Uterine ligation experiments have shown that the luteolytic substance in uterine vein blood reaches the ovary via the vascular system (Bland and Donovan, 1966; Butcher *et al.*, 1969; Kiracofe, Menzies, Gier and Spies, 1966). In the sheep the ovarian artery is highly convoluted and, before it enters the hilus of the ovary, it is closely apposed to the utero-ovarian vein . Hansel (personal communication quoted in McCracken *et al.*, 1972) considered that it would be possible for substances to diffuse from the vein into the artery and thereby pass directly into the ovary.

In the sheep, separation of the utero-ovarian vein and the ovarian artery prevented regression of the corpus luteum at the expected time (Kifacofe *et al.*, 1966; Baird and Land, 1973). McCracken *et al.* (1972) reported that following the infusion of tritiated $PGF_{2\alpha}$ (^3H-$PGF_{2\alpha}$) into the uterine vein of a sheep at a point proximal to its confluence with the utero-ovarian vein, there was a small but significant increase in ^3H-$PGF_{2\alpha}$ in the ovarian arterial

blood within 20 minutes. This increase continued over the next 60 minutes to reach peak levels approximately 20 to 30 minutes after termination of the infusion. The efficiency of transfer was estimated to be about 2%. Land, Baird and Scaramuzzi (1976) found that the concentration of ^{3}H-$PGF_{2\alpha}$ in the ovarian artery was proportional to the log of the concentration of ^{3}H-$PGF_{2\alpha}$ in the uterine vein, but estimated that the efficiency of transfer was in the order of 0.2%. There is also evidence to suggest that the vascular plexus in the ovarian pedicle may also be involved in the transfer of $PGF_{2\alpha}$ from the uterus to the ovary. Baird and Land (1973) found that luteal maintenance only occurred in 4 out of 10 ewes in which the main uterine vein alone had been ligated and severed, but occurred in 6 out of 7 ewes in which the tubal arcade of veins anastomosing between the uterine and ovarian veins was severed. Measurements of $PGF_{2\alpha}$ concentrations in the uterine vein and oviducal vein of sheep from Days 12 to 16 of the cycle are not significantly different from one another but are significantly higher than those recorded in the mammary vein and ovarian vein (Alwachi, Bland and Poyser, 1981). To what extent this system contributes to the transfer of $PGF_{2\alpha}$ from the uterus to the ovary *in vivo* is not known. There appears to be a substantial variation in the diameter of the oviducal veins from different animals and Ginther (1974) has suggested that the relative amount of venous effluent discharging through the oviducal vein, although probably small in normal ewes, may be considerable after ligation or constriction of the main uterine vein.

The existence of the counter-current mechanism in the sheep for the transfer of $PGF_{2\alpha}$ has been disputed by Coudert, Phillips, Faiman, Chernecki and Palmer (1974). They failed to demonstrate any transfer

of ^{3}H-$PGF_{2\alpha}$ from the utero-ovarian vein to the ovarian artery. However, the sensitivity of the experimental system employed by Coudert *et al.* (1974) had a maximum efficiency of 0.7% and this could explain their failure to detect an increase in ^{3}H-$PGF_{2\alpha}$ in the ovarian artery. Whether the low efficiency of transfer recorded by Baird *et al.* (1976) enables sufficient $PGF_{2\alpha}$ to enter the ovarian artery and initiate luteolysis is not clear. However, as Horton and Poyser (1976) point out, the ability of most tissues, with the exception of the lungs, liver and kidney, to metabolise prostaglandins is very low so that most of the $PGF_{2\alpha}$ leaving the ovary will pass into the utero-ovarian vein and mix with the freshly secreted $PGF_{2\alpha}$ from the uterus. A low efficiency of transfer would therefore help to maintain a steady and 'moderate' level of $PGF_{2\alpha}$ in the ovarian arterial blood. Any $PGF_{2\alpha}$ not captured by the ovarian artery/utero-ovarian vein counter-current loop, would pass into the systemic circulation and be inactivated by the lungs.

A similar counter-current mechanism has been reported to exist in the cow (Hixon and Hansel, 1974), but whether it operates in other species with a highly localised utero/ovarian relationship is not known. Del Campo and Ginther (1972, 1973) have shown that in species in which a unilateral pathway has been demonstrated, the uterus and the ovaries share common arteries and veins which would allow such a mechanism to operate. In species in which no unilateral pathway has been demonstrated, such as the rabbit (Hunter and Casida, 1967) the uterine vasculature is anatomically more independent from the ovarian vasculature and a counter-current mechanism is not feasible.

4) If $PGF_{2\alpha}$ is indeed the uterine luteolytic hormone, stimuli which induce premature luteal regression, with a concomitant shortening of

the oestrous cycle, should stimulate the early production of $PGF_{2\alpha}$. Using data on luteal size, Bland and Donovan (1966) showed that the insertion of glass beads into the uterine lumen of guinea-pigs on Days 2 to 4 of the cycle caused luteal regression to occur between Days 9 to 11. This is accompanied by an increase in utero-ovarian $PGF_{2\alpha}$ levels by Day 8, but this increase in the levels of utero-ovarian $PGF_{2\alpha}$ does not become significant until Day 12 when the utero-ovarian plasma progesterone levels are also greatly reduced (Blatchley, Donovan and Poyser, 1976). The pattern of $PGF_{2\alpha}$ secretion is similar to, and of the same order of magnitude as that found during the normal oestrous cycle (Earthy, Bishop and Flack, 1975). The delay between bead insertion and luteal regression which occurs in the guinea-pig is not observed in the sheep. Bead insertion between Days 2 to 4 of the cycle results in increased endometrial and uterine venous plasma levels of $PGF_{2\alpha}$, and in greatly reduced peripheral plasma progesterone levels 3 to 4 days later (Spilman and Duby, 1972; Pexton, Ford, Wilson, Butcher and Inskeep, 1975). Again, the levels of $PGF_{2\alpha}$ recorded were as high as those found at the end of the oestrous cycle. The presence of an intra-uterine device (I.U.D.) has also been reported to raise the endometrial PGF content in the rat, hamster and rabbit (Saksena, Lau and Castracane, 1974; Harper and Saksena, 1974).

If $PGF_{2\alpha}$ is the uterine luteolysin, then drugs which inhibit prostaglandin synthesis or inhibit the action of $PGF_{2\alpha}$ should prevent normal luteal regression. Unfortunately, no chemical antagonists, in the pharmacological sense of the word, to prevent the action of $PGF_{2\alpha}$ are yet available. However, much useful information has been gained by active immunisation against $PGF_{2\alpha}$ and by the

use of indomethacin, a potent inhibitor of the cycloxygenase enzyme (Vane, 1971).

Indomethacin inhibits the *in vitro* synthesis of prostaglandins by the guinea-pig uterus (Poyser, 1972) and increases cycle length when incorporated into a slow-release preparation and placed in the uterine lumen (Horton and Poyser, 1973; Marley, 1973). The increase in cycle length following indomethacin treatment was similar to that observed following hysterectomy and measurements of peripheral plasma progesterone levels show that the corpus luteum is well maintained throughout this period (Poyser and Horton, 1975). Since oral or parenteral administration of indomethacin to guinea-pigs, in near toxic doses, only increased cycle length by 3 to 4 days (Marley, 1972; Horton and Poyser, 1973), it seems likely that the increase in cycle length induced by indomethacin treatment results specifically from the inhibition of uterine prostaglandin synthesis. However, this has not been verified experimentally by measuring uterine venous plasma levels of $PGF_{2\alpha}$ following the local administration of indomethacin to the uterus. Mellin and Busch (1976) were unable to show an increased oestrous cycle length in sheep following the oral administration of indomethacin (20 mg kg^{-1}), aspirin (100 mg kg^{-1}) or flufenamic acid (10 mg kg^{-1}), whereas the daily, intrauterine administration of indomethacin, from Days 12 to 17 of the cycle, inhibited luteal regression (Lewis and Warren, 1975). In the pseudopregnant rat and rabbit, the systemic administration of indomethacin prolongs the duration of pseudopregnancy (O'Grady, Caldwell, Auletta and Speroff, 1972; Chaterjee, 1973).

Active immunisation against $PGF_{2\alpha}$ prolongs the oestrous cycle in both sheep (Scaramuzzi, Baird, Wheeler and Land, 1973) and guinea-

pigs (Horton and Poyser, 1974; Hildebrandt-Stark, Marcus, Yoshinaga, Behrman and Greep, 1975). In the sheep, following the primary immunisation, the concentration of antibodies directed against $PGF_{2\alpha}$ in the blood rose steadily over a period of 90 days and cyclic activity halted within the first month. Thereafter surgical removal of the corpus luteum resulted in a return to oestrus, within 3 days (Scaramuzzi *et al.*, 1973). These findings suggest that the corpus luteum was active during these elongated 'cycles'. In the guinea-pig, animals with high PGF_{α} titres had their oestrous cycles elongated to a greater extent than those animals with lower antibody titres. Measurements of the peripheral plasma progesterone levels again showed that the corpora lutea were still functional (Horton and Poyser, 1974). The most attractive explanation for these observations is that the PGF_{α} antibodies effectively neutralise the $PGF_{2\alpha}$ secreted by the uterus. However, there is evidence to suggest that prostaglandins are also involved in the process of ovulation both at the hypothalamic (Carlson, Barcikowski and McCracken, 1973; Harms, Oyeda and McCann, 1974; Tsafriri, Koch and Lindner, 1973) and ovarian level (Ainsworth, Baker and Armstrong, 1975; LeMaire, Yang, Behrman and Marsh, 1973; Tsafriri, Lindner, Zor and Lamprecht, 1972). The observed increase in cycle length could therefore arise from the inhibition of ovulation. Moreover, if the inhibition occurs at the ovarian level, the mature follicles can become luteinised and secrete progesterone. Under these circumstances, the levels of circulating progesterone would not accurately reflect luteal function. Hildebrandt-Stark *et al.* (1975) have reported the presence of luteinised, unovulated follicles, in the ovaries of animals immunised against $PGF_{2\alpha}$. It would be of interest to know whether the original

corpora lutea in such animals were still functional. Horton and Poyser (1976) reported that one guinea-pig immunised against $PGF_{2\alpha}$ did become pregnant when mated, indicating that ovulation had not been blocked completely. However, only one foetus was present and this did not survive beyond mid-term. The mother also failed to abort it.

The next point that must be considered is how the synthesis, metabolism and action of $PGF_{2\alpha}$ is normally regulated. The cyclical pattern of release suggests that this may be under hormonal control.

In the guinea-pig, administration of oestradiol (10μg day^{-1}) on Days 4 to 6 of the cycle increases the amount of $PGF_{2\alpha}$ measured in the utero-ovarian venous plasma on Day 7 (Blatchley, Donovan, Poyser, Horton and Thompson, 1971). This could explain the uterine mediated, luteolytic effect of oestradiol reported by Bland and Donovan (1970). Blatchley, Donovan, Horton and Poyser (1972) have suggested that oestradiol released from the maturing ovary after Day 10 of the cycle might form the physiological trigger for the increase in uterine $PGF_{2\alpha}$ secretion seen towards the end of the oestrous cycle. Blatchley and Poyser (1974) showed that following the *in vivo* treatment of ovariectomised guinea-pigs with either progesterone or oestradiol benzoate both PGF-like and PGE-like activity can be detected in the utero-ovarian venous plasma. The levels of PGF_{α} tended to be higher following oestradiol benzoate treatment than after progesterone treatment but the wide scatter of individual results meant that there was no significant difference between the two treatments. When oestradiol benzoate treatment was preceded by a period of progesterone priming however, the increase in utero-ovarian vein levels of $PGF_{2\alpha}$ was significantly higher than

when either hormone was administered alone. This suggests that a period of progesterone priming may also be important.

In the sheep, administration of oestradiol for a period of 2 to 4 days after the middle of the cycle also induces luteolysis in intact animals but not in hysterectomised (Hawk and Bolt, 1970; Stormshak *et al.*, 1968; Ginther, 1970) or in indomethacin-treated animals (Lewis and Warren, 1977). Injection of oestradiol or progesterone on Days 1 to 8 of the cycle has no effect on the weight or progesterone content of ovine corpora lutea. However, if progesterone is administered on Days 1 to 4 of the cycle, the injection of oestradiol as early as Day 5 or 6, induces luteolysis. Caldwell, Tillson, Brock and Speroff (1972) showed that in progesterone-primed, ovariectomised sheep, oestradiol treatment increases the peripheral plasma levels of $PGF_{2\alpha}$ to values similar to those observed at the end of the oestrous cycle. This effect was abolished by hysterectomy or by active immunisation against oestradiol. The importance of progesterone priming is further supported by the experiments of Barcikowski, Carlson, Wilson and McCracken (1974) who showed that the infusion of physiological amounts of oestradiol into the arterial supply of an autotransplanted uterus on different days of the cycle caused a significant increase in $PGF_{2\alpha}$ release only when the infusion was performed during the late luteal phase of the cycle. The increase in $PGF_{2\alpha}$ release could be blocked by the simultaneous infusion of indomethacin, demonstrating that oestradiol stimulated *de novo* synthesis of $PGF_{2\alpha}$. Further support for the role of oestradiol in controlling ovarian cyclicity comes from the observation that destruction of the follicles in the sheep ovary, either by X-irradiation or cauterisation, delays luteal regression (Karsch, Noveroske,

Roche, Norton and Nalbandov, 1970; Ginther, 1971).

In animals in which the oestrous cycle is characterised by a spontaneously active corpus luteum, progesterone levels generally rise rapidly at the beginning of the cycle and remain elevated until very near the end of the cycle when they decline sharply, often reaching almost undetectable levels immediately prior to oestrus. If the physiological requirement for an increase in the uterine output of $PGF_{2\alpha}$ does involve a period of progesterone priming, followed by oestrogen secretion, then plasma oestradiol levels should increase prior to $PGF_{2\alpha}$ release.

The utero-ovarian venous plasma levels of oestradiol are elevated on Days 3 to 4, 10 to 12 and 14 to 16 of the sheep oestrous cycle (Cox, Thorburn, Currie and Restall, 1974; Cox, Mattner and Thorburn, 1971) $PGF_{2\alpha}$ release first occurs around Days 12 to 13 and thereafter continues to increase until Days 14 and 15. There appears to be a good temporal relationship between oestradiol secretion and $PGF_{2\alpha}$ release from the uterus from Day 13 onwards (Barcikowski *et al.*, 1974). The peak in oestradiol secretion observed on Day 3 and 4 does not appear to be associated with an increase in utero-ovarian venous plasma levels of $PGF_{2\alpha}$. This may be because of the lack of adequate progesterone priming of the uterus at this time (Moore, Barrett, Brown, Schindler, Smith and Smyth, 1969). Similarly in the cow peripheral plasma oestrogen increases between Days 10 and 12 and in the 2-3 days preceding oestrus (Shemesh, Ayalon and Lindner, 1972) when $PGF_{2\alpha}$ levels also increase. Again there is a small increase in oestradiol secretion between Days 4 and 6 but, unlike that in the sheep, it is associated with increased levels of $PGF_{2\alpha}$ in the utero-ovarian vein and is accompanied by a

transient decrease in utero-ovarian venous plasma progesterone levels. This species difference may be due to the fact that, in the cow, the uterus is exposed to a high level of progesterone between Days 3 and 5. The transient decline in utero-ovarian and peripheral plasma progesterone levels may reflect the presence of a weak luteolytic stimulus (Nancarrow *et al.*, 1973). In the guinea-pig, oestradiol levels increase after Day 10 and again after Day 13(Joshi, Watson and Labhsetwar, 1973) while in the pig there is a small peak in oestradiol levels between Days 4 to 8 which is then followed by a steady increase from Day 12 until oestrus (Henricks, Guthrie and Handlin, 1972). In both species the increase in oestradiol levels after the middle of the cycle precedes the increase in $PGF_{2\alpha}$ in the utero-ovarian vein (Blatchley *et al.*, 1972; Gleeson and Thorburn, 1973). In the pig, as in the sheep, the earlier peak in oestradiol secretion is not associated with a significant increase in uterine venous plasma levels of $PGF_{2\alpha}$ (Hunter and Poyser, as reported by Horton and Poyser, 1976). In the pseudopregnant hamster, peripheral plasma oestradiol levels increase from Day 6 to reach peak values on Day 8. Again, this increase precedes the increase in peripheral plasma $PGF_{2\alpha}$ concentration observed on Days 8 and 9 (Shaikh, Birchall and Saksena, 1973).

It would appear, therefore, that the increase in $PGF_{2\alpha}$ in the utero-ovarian vein seen towards the end of the oestrous cycle requires a period of progesterone priming followed by a 2 to 3 day exposure to oestrogen. The duration of oestrogen exposure may also be important. Bolt and Hawk (1972b) have shown that the intravenous infusion of oestradiol ($10.4\mu g\ hr^{-1}$) will only induce luteolysis if infused for a minimum of 24 hr. This could also explain why small increases in

oestrogen secretion early in the cycle fail to stimulate adequate $PGF_{2\alpha}$ release to initiate luteolysis. It is also possible that the susceptibility of the corpus luteum to $PGF_{2\alpha}$ changes during the oestrous cycle and that this too is under hormonal control.

Although the above evidence suggests that the luteolytic capacity of the uterus is controlled by the sequential exposure of the uterus to progesterone and oestrogen, there are certain observations which are difficult to interpret on this basis. The most paradoxical of these is that in the pig (Gardner, First and Casida, 1963), Sheep (Piper and Foote, 1968; Denamur, Martinet and Short, 1970) and guinea-pig (Illingworth and Perry, 1973) daily administration of oestradiol towards the end of the cycle maintains luteal function. Also, like hysterectomy, oestradiol treatment will maintain luteal function in sheep following pituitary stalk transection (Denamur *et al.*, 1970). This suggests that the oestradiol counteracts the luteolytic presence of the uterus. Frank, Bazer, Thatcher and Wilcox (1977) have shown that in the pig the administration of oestradiol valerate (5mg day^{-1}) on Days 11 to 15 of the cycle decreases the number and amplitude of $PGF_{2\alpha}$ peaks' in the uterine venous plasma and maintains circulating progesterone levels above those observed in untreated animals.

In all instances, however, the dose of oestradiol needed to prolong luteal function is high and probably leads to plasma oestradiol levels in excess of those occurring naturally. Moreover, since oestradiol, in physiological doses, induces luteolysis in sheep when given after Day 10 of the cycle (Stormshak *et al.*, 1969; Barcikowski *et al.*, 1974) it seems likely that, *in vivo*, the secreted oestrogen will promote luteolysis rather than exert a luteotrophic effect.

More recently evidence has begun to accumulate to suggest that oxytocin may also be involved in the control of $PGF_{2\alpha}$ release from the uterus in the sheep. Fairclough, Moore, McGowan, Peterson, Smith, Tervit and Watkins (1980) examined the temporal relationship between plasma concentrations of oxytocin, as determined by measurement of plasma concentrations of oxytocin-associated neurophysins I/II, and plasma concentrations of 13,14-dihydro-15-keto-PGF in sheep over the time of expected luteolysis. They found that there was a close temporal relationship between these two parameters. The highest levels of neurophysin I/II were recorded on Days 14 to 16 but peaks of neurophysin I/II were associated with small peaks in 13,14-dihydro-15-keto-PGF from Day 13 onwards. These results would indicate that there is an increase in oxytocin release around the time of luteolysis in the ewe. The possible involvement of oxytocin in the control of luteolysis is also supported by the work of Sheldrick, Mitchell and Flint (1980) who found that active immunisation of sheep against oxytocin prolonged the luteal phase of the oestrous cycle, as judged by oestrus behaviour and the circulating levels of progesterone, by some 3·7 days.

Roberts, Barcikowski, Wilson, Skarnes and McCracken (1975) have shown that infusions of oxytocin into the arterial supply of the uterus evoke a substantial increase in $PGF_{2\alpha}$ release from the uterus when administered after Day 14 of the cycle. The ability of oxytocin to stimulate $PGF_{2\alpha}$ release closely parallels the ability of the endometrium to bind oxytocin (Roberts, McCracken, Gavagan and Soloff, 1976) and this, in turn is dependent on the hormonal state of the animal. In the anoestrous ewe oxytocin will not stimulate the release of $PGF_{2\alpha}$ from the uterus unless the animal has been primed with

oestrogen (Sharma and Fitzpatrick, 1974). In experiments with ovariectomised ewes with autotransplanted uteri, oestradiol-17β has been shown to increase the number of oxytocin receptors in the uterus within 6 hrs while the administration of progesterone (500μg hr^{-1}, i.v.) resulted in the uterus becoming refactory to oxytocin stimulation. When oestradiol-17β infusions were superimposed upon progesterone treatment the ability of oestradiol-17β to stimulate oxytocin receptor formation was not evident until after progesterone treatment had been continued for 10 days. The response of the uterus to oxytocin challenge was then found to be 50 to 100 fold greater than when oestradiol-17β was given alone (McCracken, Gammel, Glew and Underwood, 1978). The period of progesterone priming therefore appears to amplify the response of the uterus to oxytocin in terms of $PGF_{2\alpha}$ secretion. The mechanism by which this is achieved is not clear.

Whether oxytocin also plays an important role in regulating uterine $PGF_{2\alpha}$ secretion in other species is not known although Armstrong and Hansel (1959) have reported that oxytocin will induce precocious oestrus in the cow when given on Days 2 to 6 of the cycle. The premature demise of the corpus luteum by such treatment is associated with increased concentrations of $PGF_{2\alpha}$ in the uterine venous blood (Milvae and Hansel, 1980).

As previously mentioned prostaglandins are not stored intracellularly but are synthesised *de novo* as required. This process involves the release of arachidonic acid from tissue stores followed by the enzymatic conversion of the precursor into the relevant prostaglandin. An increase in $PGF_{2\alpha}$ production could therefore be effected by increasing the availability of the precursor, by

increasing the amount and/or rate of enzyme conversion of substrate, either by activation of pre-existing enzyme or through *de novo* synthesis of new enzyme, or by a redirection of synthesis towards $PGF_{2\alpha}$ production (see Fig. I). The *in vitro* capacity of guinea-pig uteri to synthesise $PGF_{2\alpha}$ is approximately three fold higher on Days 14 and 15 than on earlier days of the cycle (Poyser, 1972). The addition of arachidonic acid (0.5 or 2.5µg ml^{-1}) or phospholipase (5µg ml^{-1}) to incubates of Day 6 uterine homogenates failed to increase the amount of $PGF_{2\alpha}$ synthesised by these tissues (Poyser, 1972). This suggests that the differences in the synthetic capacity of uterine tissue on Days 6 and 14 of the cycle cannot be accounted for in terms of lack of substrate. Using a partially purified prostaglandin synthetase enzyme complex prepared from sheep vesicular glands, Lands, Le Tellier and Vanderhoek (1973) observed that synthesis of the 'oxygenated' product from arachidonic acid ceased before all the substrate had been utilised, and that the reaction could be restarted if more enzyme was added. They therefore proposed that a self-catalysed destruction of the enzyme occurred and that it was this property of autocatalysis that determined the amount of product that could be synthesised by a given amount of enzyme. Oestrogen stimulates uterine growth and protein synthesis. The action of oestrogen on $PGF_{2\alpha}$ production could therefore be linked with fresh protein synthesis. Oestradiol is believed to enter the cell *via* a specific protein-mediated process (Milgrom, Atger and Baulieu, 1977) and to become bound to a cytosolic receptor (Gorski, Taft, Shyamala, Smith and Nolides, 1968). Once bound, the oestrogen-cytosol receptor complex undergoes translocation to specific nuclear sites where it stimulates RNA synthesis. The RNA in turn stimulates production of

small amounts of a 'specific induced' protein. The synthesis of specific induced protein occurs within one hour of oestrogen administration and is blocked by actinomycin D, but not by puromycin or cyclohexamide (De Angelo and Gorski, 1970). The induced protein then stimulates the production of general RNA which is followed by an increase in general protein levels and the accumulation of phospholipids (Mueller, Gorski and Aizawa, 1961). The intrauterine administration of actinomycin D (500μg hom^{-1}) to sheep on Day 11 of the cycle inhibits luteal regression in a highly localised manner and blocks the luteolytic effect of oestrogen (French and Casida, 1973). The initial DNA dependent synthesis of RNA therefore appears to be important for the expression of the luteolytic effect of oestrogen. However, whether oestrogen acts to increase prostaglandin synthesis by increasing the amount of prostaglandin synthetase-present is not clear. In the guinea-pig the intrauterine injection of actinomycin D also significantly prolongs luteal function and oestrous cycle length (Poyser, 1979). This effect is accompanied by a decrease in the ability of the Day 15 uterus to synthesise $PGF_{2\alpha}$, an effect which cannot be overcome by the addition of exogenous precursor to the incubation medium or by *in vivo* oestrogen treatment. Since the ability of the tissue to synthesise PGE is also decreased by actinomycin D treatment, these results suggest that, in the guinea-pig, oestradiol exerts its luteolytic affect by increasing prostaglandin synthetase activity, *via* a DNA dependent mechanism, either by inducing *de novo* prostaglandin synthetase synthesis or by activation of the existing enzyme.

Using an immunochemical technique Huslig, Fogwell and Smith (1979) have shown that there is a threefold increase in the specific

activity of the cyclo-oxygenase in caruncular microsomes from sheep uteri on Day 14 of the cycle. Whether this occurs as a consequence of the increase in oestradiol secretion from the ovary is not known. Alwachi *et al.* (1979) have shown that the ability of the uterine endometrium to synthesise $PGF_{2\alpha}$ varies with the presence or absence of the neighbouring ovary. In unilaterally ovariectomised sheep endometrial tissue from the uterine horn adjacent to the ovary synthesised significantly more $PGF_{2\alpha}$ than endometrial tissue from the contralateral horn. Naylor and Poyser (as reported in Horton and Poyser, 1976) found that the administration of oestradiol benzoate to guinea-pigs on Days 4 to 6 of the cycle did not increase the synthesising capacity of the uterus on Day 7 of the cycle despite the fact that this treatment stimulates $PGF_{2\alpha}$ release from the uterus (Blatchley *et al.*, 1971; 1972). Oestrogen may therefore have both a direct, immediate effect to initiate $PGF_{2\alpha}$ release and a more prolonged action to increase the synthetic capacity of the uterus.

In the rat, ovariectomy induces a ten fold increase in the uterine tissue levels of PGE and a three fold increase in PGF levels. Subsequent oestrogen treatment does not appear to affect the total prostaglandin content but it does result in a decrease in PGE levels and a concomitant elevation in PGF levels. The ability of isolated uterine microsomes to biosynthesise prostaglandins when incubated with arachidonic acid in the presence of co-factors, was also found to be enhanced by *in vivo* oestradiol treatment. $PGF_{2\alpha}$ was synthesised in preference to PGE (Ham, Cirillo, Zanetti and Kuehl, 1975). These results suggest that oestradiol exerts a dual action:-

a) to increase the synthetic capacity of the uterus

and

b) to exert a directional influence on the products formed.

A similar pattern of changes in the endogenous levels of PGF and PGE in the rat uterus throughout the oestrous cycle has been reported by Poyser and Scott (1980). However, they were unable to support the findings of Ham *et al.* (1975) that the increase in the ability of the uterus to synthesise PGF at pro-oestrus and oestrus occured at the expense of PGE synthesis and that, during dioestrus, the uterus synthesises PGE in preference to PGF. In their study Poyser and Scott (1980) found that the major prostaglandin to be synthesised by homogenates of the rat uterus *in vitro* was prostacyclin (PGI_2). Appreciable quantities of $PGF_{2\alpha}$, PGE_2 and PGD_2 were also formed but PGF production always exceeded PGE production and the ratio of the amounts of prostaglandins formed remained fairly constant. The reason for the differences in the findings of Ham *et al.* (1975) and Poyser and Scott (1980) may be due to differences in experimental design. Poyser and Scott (1980) measured the synthetic capacity in uterine tissue homogenates in the absence of added arachidonic acid whereas Ham *et al.* (1975) used a microsomal uterine tissue preparation incubated in the presence of exogenous arachidonic acid, and the co-factors hydroquinone and reduced glutathione which promote PGE synthesis. The ability of guinea-pig uterine microsomes to synthesise $PGF_{2\alpha}$ from arachidonic acid also increases towards the end of the cycle and after oestrogen treatment (Wlodawar, Kindahl and Hamberg, 1976). Again it is not clear whether this is due to an increase in prostaglandin synthetase or a directional influence, as is seen in the rat. However, Wlodawar *et al.* (1976) have also reported that both cow and guinea-pig uteri contain an endogenous inhibitor of cycloxygenase. The activity of this inhibitory factor also alters during the cycle but these changes could not be measured

accurately enough to relate them to changes in the synthetic capacity of the uterus during the oestrous cycle. The effect of oestrogen on the 'activity' of this inhibitory factor was not studied.

Relatively little work has been carried out to investigate the role of progesterone in controlling $PGF_{2\alpha}$ secretion by the uterus. In the castrate guinea-pig the *in vivo* administration of progesterone does not appear to affect the ability of uterine tissue homogenates to synthesise $PGF_{2\alpha}$ *in vitro* nor to modify the increase in uterine synthetic capacity induced by *in vivo* oestradiol treatment (Naylor and Poyser, 1975). These results contrast markedly with those obtained when exogenous oestradiol and progesterone, either alone or in combination, are added *in vitro* to the incubation medium containing homogenates of Day 7 guinea-pig uterine tissue. Oestradiol treatment significantly increased PGF production while progesterone treatment, which had no effect on its own, significantly reduced the response to oestrogen. However, the concentrations of oestradiol and progesterone used are high and may represent a pharmacological action. In the castrate rat, progesterone treatment also has little effect on prostaglandin synthesis when administered alone but substantially reduces the total prostaglandin content and blunts the increase in PGF levels induced by oestrogen treatment. This effect differs from that of indomethecin treatment in that indomethecin reduces the total endogenous tissue concentrations of PGE and PGF and completely abolishes the 'directional' effect of oestrogen treatment (Ham *et al.*, 1975).

Like oestrogen, progesterone also binds to a uterine cytosolic receptor which is then translocated to the nucleus. The ability of a tissue to respond to either oestrogen or progesterone is presumably

related to its ability to bind these hormones. In the human (Bayard, Damilano, Robel and Baulieu, 1978), rat (Bhakoo and Katzenellenbogen, 1977) and sheep (Koligan and Stormshak, 1977; Zelinski, Hirota, Keenan and Stormshak, 1980), oestrogen appears to stimulate the synthesis of its own receptor and of the cytosoloic progesterone receptor (CPR), while progesterone exerts the opposite effect (Milgrom, Thi, Atger and Baulieu, 1973). In the immature rat, the administration of progesterone causes an increase in uterine glucose metabolism and in the rate of DNA synthesis. When co-administered with oestrogen, progesterone decreases the uterine responsiveness to subsequent oestrogen administration. This action is accompanied by a decrease in the number of cytosolic oestrogen receptors (CER) but does not appear to interfere with the translocation of receptor-bound oestrogen to the nucleus. The administration of either cycloheximide or actinomycin D between 0 to 6 hrs following the injection of oestradiol induces a 60% decrease in CER (Bhakoo and Katzenellenbogen, 1977). It has therefore been proposed that CER replenishment is a dual process involving both receptor recycling and *de novo* synthesis. Progesterone appears to inhibit the latter process.

If oestradiol determines the amount of $PGF_{2\alpha}$ produced by the uterus by regulating the amount and/or expression of the prostaglandin synthetase enzymes, the increase in uterine secretion of $PGF_{2\alpha}$ towards the end of the oestrous cycle could arise in the following way. Increased oestradiol secretion from the ovary after Day 10 of the cycle would stimulate synthesis of CER and increase the amount of oestradiol translocated to the nucleus, so stimulating RNA and protein synthesis. This in turn would stimulate $PGF_{2\alpha}$ production

and the $PGF_{2\alpha}$ would then act on the corpus luteum to decrease progesterone secretion. The resulting fall in progesterone production would then enhance the production and/or replenishment of CER (Jacobson, Keyes and Bullock, 1972) and thereby further stimulate $PGF_{2\alpha}$ synthesis. As progesterone levels continue to fall and oestrogen levels rise, the uterine levels of CER and CPR would increase. Following ovulation oestradiol secretion would fall, $PGF_{2\alpha}$ synthesis would no longer be stimulated and progesterone levels would begin to rise and so bring about a decrease in uterine CER and CPR. This type of positive feedback could explain the cyclical changes in $PGF_{2\alpha}$ secretion observed *in vivo*. The pulsatile nature of the release could reflect the synthesis of a new 'batch' of enzyme produced in response to an oestrogen surge.

Although the above theory may account for the increase in $PGF_{2\alpha}$ secretion at the end of the oestrous cycle it does not explain the *in vivo* requirement for a period of progesterone priming. It is possible that progesterone is important in controlling the availability of substrate. Following progesterone treatment there is an increase in the number of lipid droplets in endometrial cells (Brinsfield and Hawk, 1973). The lack of effect of progesterone in many of the *in vitro* studies may be due to the fact that these have been performed in the presence of added substrate or in tissue homogenates where the coherence of the intracellular structure has been destroyed.

As previously mentioned there is now evidence to suggest that oxytocin is also involved in controlling the uterine production of $PGF_{2\alpha}$ in the sheep. The ability of oxytocin to evoke $PGF_{2\alpha}$ release from the uterus closely parallels changes in the endometrial oxytocin-

receptor concentration (Roberts *et al.*, 1976). By using the oxytocin induced release of $PGF_{2\alpha}$ as an *in vivo* marker for the appearance of the oxytocin receptor, McCracken *et al.*, 1978 have shown that in ovariectomised sheep, the infusion of oestradiol into the arterial supply of the autotransplanted uterus, results in an increase in uterine sensitivity to oxytocin within 6 hrs. Progesterone treatment on the other hand resulted in the uterus becoming refactory to oxytocin treatment and initially blocked the oestradiol-17β-induced increase in uterine sensitivity to oxytocin. This effect is probably due to the ability of progesterone to inhibit CER replenishment (Koligan and Stormshak, 1977). However, after 10 days of progesterone treatment, oestradiol-17β treatment regains its ability to stimulate oxytocin receptor formation and, following oxytocin challenge, the uterus secretes 50 to 100 fold more $PGF_{2\alpha}$ than when oestradiol-17β is infused alone. The return of uterine responsiveness to oestradiol is probably due to the fact that progesterone catalyses the destruction of its own receptor (Vu Hai, Logeat, Warembourg and Milgrom, 1977) so that progesterone can no longer inhibit CER replenishment. The reason for the amplification of the uterine response to oxytocin following progesterone priming and oestrogen treatment is not known but could be due to an increased supply of lipid precursor (Brinsfield and Hawk, 1973).

When progesterone (500μg hr^{-1}, i.v.) is infused systemically into sheep bearing utero-ovarian transplants from Day 1 to 10 of the cycle, spontaneous peaks of $PGF_{2\alpha}$ of low magnitude (3 to 5 ng ml^{-1}) occurred in the uterine venous blood around Day 9 and 10. Following cessation of the progesterone infusion, the magnitude of the peaks of $PGF_{2\alpha}$ began to increase in a fashion similar to that seen after the

onset of luteolysis in the normal cycle (McCracken *et al.*, 1978.) Ottobre, Lewis, Thayne and Inskeep (1980) have also reported increased levels of $PGF_{2\alpha}$ in the uterine venous plasma of ewes treated with progesterone 8 or 32 hrs after the onset of oestrus, from Day 8 onwards. McCracken *et al.* (1978) have proposed that the increase in $PGF_{2\alpha}$ secretion observed following the termination of progesterone secretion may be due to an increase in oxytocin levels following removal of the blockade of progesterone at the level of the CNS (Blank and De Bias, 1977). The mechanism by which oxytocin evokes the rapid release of $PGF_{2\alpha}$ is not known, although it has been proposed that it may precipitate $PGF_{2\alpha}$ synthesis by activating a phospholipase. Although there is no direct evidence to support this idea, such a mechanism seems possible in view of the evidence that thyroid stimulating hormone regulates PG synthesis in thyroid cells by stimulating phospholipase A_2 activity (Haye, Champion and Jacquemin, (1976). Obviously, the control of $PGF_{2\alpha}$ synthesis by the uterus is a complex process.

From the above evidence, $PGF_{2\alpha}$ fulfils many of the previously mentioned criteria for the role of a uterine luteolytic hormone in several species of mammal, with the sheep and guinea-pig having been studied in the greatest detail. $PGF_{2\alpha}$, secreted by the uterus in greater amounts towards the end of the cycle than at earlier times, acts directly on the corpus luteum to terminate its functional life span. The trigger for the increase in uterine $PGF_{2\alpha}$ production appears to depend on the exposure of a progesterone primed uterus to oestrogen and, in the sheep at least, to oxytocin. The question of whether $PGF_{2\alpha}$ also acts as the uterine luteolysin in other species such as the rabbit, in which hysterectomy extends the length of pseudopregnancy, still requires further clarification.

Prostaglandin production by the uterus during early pregnancy

In many species progesterone is necessary for the maintenance of pregnancy. In eutherian mammals, but not marsupials, the length of gestation exceeds that of the oestrous cycle. In the rat, hamster, rabbit, pig and cow, the maintenance of pregnancy is dependent on ovarian progesterone. In the sheep and guinea-pig, however, ovarian progesterone is only required for the first third of gestation, after which time the placenta produces sufficient progesterone to maintain pregnancy. In all instances, therefore, the functional life span of the corpus luteum in the pregnant animal must be prolonged. This means that, in those animals that secrete a uterine luteolytic hormone, the effect of the uterus must be negated if the pregnancy is to proceed to a successful conclusion. Logically, the signal for luteal maintenance should come from the 'products of conception' and could be either luteotrophic, and directly stimulate the corpus luteum, be anti-luteolytic, and inhibit the production and/or action of the luteolysin, or be a mixture of both.

In the guinea-pig, rat, rabbit and hamster, implantation occurs at a time when the corpora lutea are still functional. In the sheep, cow and pig, however, implantation occurs after the time at which the corpora lutea of the cycling animal would normally have regressed.

$PGF_{2\alpha}$ output from the uterus begins to increase from Day 11 in most species. If $PGF_{2\alpha}$ is the uterine luteolytic hormone, then it seems likely that the signal for luteal maintenance should occur about this time.

In the pregnant sheep, removal of the embryo up to and including Day 12, allows regression of the corpus luteum to occur at the normal time. Removal of the conceptuses after Day 12 however, results in a marked extension of luteal function (Moor and Rowson, 1966a). Similarly, the transfer of embryos to the non-pregnant sheep uterus up to Day 12 of the cycle will prolong luteal function. Transfers performed after this time do not (Moor and Rowson, 1966b). The transferred embryos are capable of maintaining the corpora lutea in both unilaterally and bilaterally ovulating ewes irrespective of their position in the uterus relative to the active corpus luteum, providing the lumen of the two horns are contiguous (Moor, 1968; Moor and Rowson, 1966b). In unilaterally ovulating ewes in which one horn has been transected at the cervix, embryo transfer only results in luteal maintenance when the corpus luteum is adjacent to the pregnant horn. This suggests that the 'signal' for luteal maintenance acts locally on the uterus, rather than systemically on the ovary. This idea is also supported by the fact that the daily intrauterine infusion of homogenates prepared from Day 14 and Day 15 embryos into the uteri of non-pregnant sheep, extends luteal function until Day 25. Extrauterine administration of Day 14 or Day 15 embryo homogenates, or the intrauterine infusion of Day 25 embryo homogenates is without effect. This indicates that the antiluteolytic effect of the embryo is extended for a limited period only (Rowson and Moor, 1967). In the guinea-pig, grafts of Day 11 or Day 12 conceptuses to the spleen of normally cycling animals lead to luteal maintenance in 13 out of 25 animals. Grafts of Day 9 or Day 10 conceptuses, however, were without effect (Bland and Donovan, 1969b). This suggests that, in the guinea-pig, the 'signal' for luteal maintenance occurs after

Day 12 but, unlike that of the sheep, the 'signal' has a systemic component. Unilateral pregnancy in the guinea-pig, as in the sheep, results in bilateral luteal maintenance. In the pig, however, unilateral pregnancy tends to result in pregnancy failure due to luteal regression (Anderson, Rathmaker and Melampy, 1966). The removal of the non-gravid horn permits pregnancy to continue and results in the bilateral maintenance of the corpora lutea. In bilaterally pregnant pigs, the removal of the embryos from one horn up to Day 10 or Day 11 of pregnancy results in bilateral luteal maintenance (Dhindsa and Dzuik, 1968). The 'signal' for luteal maintenance in the pig therefore appears to be released around Days 10 to 12. Longenecker and Day (1972) have shown that the infusion of saline extracts of Day 20 to Day 25 conceptuses into the non-pregnant horn of unilaterally pregnant pigs results in an increased incidence of pregnancy maintenance of 57.1% of the animals as compared with non-treated, unilaterally pregnant pigs. If the infusion was restricted to only a small area of the sterile horn, the pregnancy rate at slaughter was only 16.7% greater than in the controls. This suggests that the conceptuses and associated membranes act directly on the uterus, and also that the 'anti-luteolytic' effect of the embryo is still present after Day 20 of pregnancy. Interestingly, saline extracts of embryonic tissue collected on Days 26 to 40 of pregnancy, although capable of blocking the luteolytic effect of a non-gravid horn in a unilaterally pregnant animal, will not prevent luteal regression when infused into the uteri of non-pregnant pigs. The infusion of 14-day old pig embryos into the uteri of non-pregnant sheep also failed to prevent luteal regression (Rowson and Moor, 1967).

Prostaglandin secretion by the uterus in early pregnancy has been studied in the sheep (Thorburn *et al.*, 1973), mare (Douglas and Ginther, 1976), cow (Lukaszewska and Hansel, 1980), pig (Moeljono, Thatcher, Bazer, Frank, Owens and Wilcox, 1977) and guinea-pig (Blatchley, Maule Walker and Poyser, 1975a). Using a chronic sampling technique Thorburn *et al.* (1973) found that the large peaks of $PGF_{2\alpha}$ in utero-ovarian venous plasma observed on Days 15 and 16 of the oestrous cycle are not present in the uterine blood of pregnant sheep. These results were confirmed by Barcikowski *et al.* (1974). However, Pexton, Weems and Inskeep (1975), using an acute sampling technique, found no significant difference in the levels of $PGF_{2\alpha}$ in the utero-ovarian venous plasma of pregnant and non-pregnant sheep on Days 13 to 15. These results were confirmed by Nett, Staigmiller, Akbar, Diekman, Ellinwood and Niswender (1976) but, like Thorburn *et al.* (1973) these authors also found that the 'peak' frequency of $PGF_{2\alpha}$ secretion was significantly lower in the pregnant animal as compared to the cycling sheep. In an attempt to help to resolve the question of whether $PGF_{2\alpha}$ output from the ovine uterus is inhibited during early pregnancy Peterson *et al.* (1976) measured the peripheral plasma levels of 13,14-dihydro-15-keto-$PGF_{2\alpha}$. In the cycling animal, the levels of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ increase towards the end of the cycle with peaks being observed around the time of luteolysis. In the pregnant sheep however, the metabolite levels were found to be lower and no peaks were observed between Days 12 to 20. These results, therefore, support the opinion that in the early pregnant sheep $PGF_{2\alpha}$ output from the uterus is suppressed. In the cycling pig and cow the peripheral plasma concentrations of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ also increase and exhibit peaks around the time of

luteolysis (Shille *et al.*, 1979; Kindahl *et al.*, 1976). Again these increases are thought to reflect the increased output of $PGF_{2\alpha}$ from the uterus (Shemesh and Hansel, 1975; Moeljono *et al.*, 1977). As in the sheep, these increases in metabolite levels are not found in the pregnant animal (Kindahl *et al.*, 1976; Shille *et al.*, 1979) suggesting that $PGF_{2\alpha}$ release is also suppressed during early pregnancy. Lukaszewska and Hansel (1980) have recently reported that the concentration of $PGF_{2\alpha}$ in the uterine vein is significantly lower in the pregnant cow on Day 18 than in the non-pregnant animal. Similarly in the guinea-pig, the levels of the main urinary metabolite of $PGF_{2\alpha}$ are also depressed in early pregnancy when compared with those observed in the non-pregnant animal (Granström and Kindahl, 1976). These results are in agreement with those of Antonini, Turner and Paverstein (1976) and Blatchley, Maule Walker and Poyser (1975a) who have shown that the increase in utero-ovarian venous levels of $PGF_{2\alpha}$ seen on Days 14 to 16 of the cycle is not observed in the pregnant animal. However, urinary metabolite levels of $PGF_{2\alpha}$ do begin to increase from Day 25 of pregnancy when the anti-luteolytic effect of the conceptus is no longer evident (Bland and Donovan, 1969).

In several species therefore it is apparent that decreased secretion of $PGF_{2\alpha}$ into the uterine venous drainage occurs during early pregnancy at the time at which corpus luteum regression would normally be occurring in the non-pregnant animal. The mechanisms by which this occurs again shows considerable species variation.

Maule Walker and Poyser (1974) have shown that uterine tissue from bilaterally pregnant guinea-pigs synthesises significantly less $PGF_{2\alpha}$, *in vitro*, on Day 15 of pregnancy than uterine tissue on Day

15 of the cycle. In unilaterally pregnant guinea-pigs the amount of $PGF_{2\alpha}$ synthesised by the pregnant horn is the same as the amount synthesised by uterine tissue from a bilaterally pregnant animal. The non-gravid horn, however, synthesises approximately twice as much $PGF_{2\alpha}$ as either the unilaterally pregnant or bilaterally pregnant uterus but synthesises significantly less than the Day 15, non-pregnant uterus. The mechanism by which the conceptus maintains luteal function during early pregnancy therefore appears to be partly, if not fully, *via* an antiluteolytic effect which possesses both a systemic and local component. Further evidence for this suggestion comes from studies involving guinea-pigs in which the luminal continuity of the uterine horns has been disrupted by transection of one of the horns. Following mating the ability of uterine tissue from the transected horn to synthesise $PGF_{2\alpha}$, *in vitro*, on Day 15 of pregnancy, although lower than that of the Day 14 non-pregnant uterus, was three fold higher than that of the pregnant horn (cleared of conceptuses and attached uterine tissue) and was also significantly higher than $PGF_{2\alpha}$ production by the sterile horn of spontaneous, unilateral pregnancies. Peripheral plasma progesterone levels in these animals showed that the corpora lutea were still functional but the circulating level of progesterone was still significantly lower than those observed in spontaneous, unilateral pregnancies or in bilaterally pregnant guinea-pigs (Maule Walker, 1976). (These findings contrast with those observed in the sheep where transection of a sterile horn results in luteal regression in the adjacent ovary (Rowson and Moor, 1967).)

In the sheep Ellinwood, Nett and Niswender (1979) found that there was no difference in the amount of $PGF_{2\alpha}$ released from uterine

tissue, *in vitro*, on Day 13 of either the oestrous cycle or pregnancy while $PGF_{2\alpha}$ release on Days 15 and 17 of pregnancy was significantly greater than on Days 14 and 17 of the oestrous cycle. However, they also reported that there was a marked increase in the concentrations of $PGF_{2\alpha}$ and PGE_2 in the uterine flushings from pregnant animals on Days 13, 15 and 17 and in the utero-ovarian venous plasma levels of PGE_2 on Days 15 and 17. It is possible that the presence of the developing conceptus induces a shift in the direction of secretion of $PGF_{2\alpha}$ from the lamina propria to the uterine lumen. Bazer and Thatcher (1977) have proposed a similar hypothesis to explain the antiluteolytic effect of oestradiol in the pig and the decrease in uterine venous plasma concentrations of $PGF_{2\alpha}$ during early pregnancy. However, whether the high levels of PGE_2 and $PGF_{2\alpha}$ in the uterine lumen result from the accumulation of prostaglandins in blastocoelic fluid, binding of prostaglandins to embryonic or uterine proteins or *de novo* synthesis of prostaglandins by the embryo and/or endometrium is not known. The amount of protein recovered from uterine flushings on Day 13, 15 and 17 of pregnancy is greater than the amount recovered from uterine flushings on the same days of the oestrous cycle. This suggests that pregnancy-specific proteins may be present in the uterine lumen as early as Day 13, and therefore these could play a part in the mechanism of luteal maintenance which becomes operational at this time (Moor, Hay, Short and Rowson, 1970).

The enhanced capacity of endometrial tissue to synthesise PGE_2 on Day 15 and 17 of pregnancy (Ellinwood *et al.*, 1979) may also be significant in terms of luteal maintenance. Experiments performed by Mapletoft, Del Campo and Ginther (1975) suggest that, in the unilaterally pregnant ewe there is also a locally mediated luteo-

trophic effect of the uterus. PGE_2 counteracts the luteolytic effect of $PGF_{2\alpha}$ when infused simultaneously into the ovarian artery of cycling ewes (Henderson, Scaramuzzi and Baird, 1977). The intrauterine administration of PGE_2 to cycling ewes also overcomes both natural and oestradiol induced luteolysis (Magness, Huie and Weems, 1978; Colcord, Hoyer and Weems, 1978). The mechanism by which PGE_2 exerts this antiluteolytic/luteotrophic effect is not known. It could be due to a vascular effect resulting from its potent vasodilator properties (Bergström, Carlson and Weeks, 1968) and/or its ability to elevate intracellular c-AMP and stimulate progesterone production by luteal tissue (Marsh, 1971). It is possible that PGE_2 is the luteotrophic substance produced by the pregnant uterus.

In the sheep Martal, Lacroix, Loudes, Saunier and Wintenberger-Torrès (1979) have identified a protein-like material secreted by the trophoblast which is capable of maintaining luteal function when injected for periods of 4 to 11 days into the uterus of normally cycling ewes from Day 12 of the cycle. This principle, called trophoblastin, appears to be secreted for a limited period only and is no longer evident in homogenates of embryos and their membranes from Day 21 to 23 of pregnancy. Removal of embryos on Days 21 to 23 of pregnancy also results in luteal maintenance for periods in excess of 30 days in over 50% of the operated animals although luteal weight, as compared to normal pregnancy, is reduced. The latter effect probably reflects the absence of placental luteotrophic influences. The mechanism by which trophoblastin secures luteal maintenance is not known. Its action is essentially local (Moor and Rowson, 1966b) but whether it acts to inhibit $PGF_{2\alpha}$ synthesis and/or release or by stimulating the production of a locally acting luteotrophin is still

a matter of conjecture. Certainly it is present in the uterus at the time that $PGF_{2\alpha}$ output is reduced, but a purely antiluteolytic effect would not explain the prolonged effect of trophoblastin on luteal maintenance since the levels of progesterone in the peripheral venous plasma of these animals are higher than those observed following hysterectomy in the non-pregnant animal. Such an effect is more readily explained in terms of a luteotrophic effect and could involve stimulation of PGE_2 synthesis. In this respect it is interesting to note that during early pregnancy there is some evidence to suggest that the sensitivity of the uterus to luteolytic stimuli, such as oestrogen or $PGF_{2\alpha}$ treatment, is reduced (Kittock and Britt, 1977; Pratt, Butcher and Inskeep, 1977). As previously mentioned PGE_2 has been shown to overcome both natural and oestradiol induced luteolysis and to antagonise the effect of $PGF_{2\alpha}$ infusion (Magness *et al.*, 1978; Colcord *et al.*, 1978; Henderson *et al.*, 1977). Similarly, in the guinea-pig there is evidence to suggest that the conceptus also produces a luteotrophic hormone which acts locally between the uterus and the ovary (see Poyser and Maule Walker, 1979) although attempts to isolate such a factor have so far proved unsuccessful.

If oestrogen, acting in the presence of progesterone, forms part of the physiological stimulus for the increase in uterine secretion of $PGF_{2\alpha}$ which occurs towards the end of the oestrous cycle, then it is of interest to know how the advent of pregnancy affects oestrogen secretion. In the early pregnant sheep (Cox *et al.*, 1974), cow (Henricks, Dickey, Hill and Johnston, 1972), guinea-pig (Blatchley *et al.*, 1975), pig (Guthrie, Henricks and Handlin, 1972) and hamster (Shaikh *et al.*, 1973) the increases in oestradiol levels seen towards the end of the oestrous cycle or pseudopregnancy do not occur. It

is possible therefore, that the developing embryo maintains luteal function by inhibiting oestradiol output by the ovary.

In the guinea-pig, the administration of oestradiol (10μg day^{-1}) from Day 10 of pregnancy results in a fall in plasma progesterone from Day 12 onwards and termination of pregnancy occurs by Days 20 to 22 (gestation length 65 days). The uteri of these animals showed an increased ability to synthesise $PGF_{2\alpha}$ when compared to the uteri of untreated, pregnant animals (Poyser and Maule Walker, 1979). However, in one animal treated with oestradiol, the peripheral plasma levels of progesterone continued increasing, instead of falling, after Day 12. Abortion still occurred by Day 21 but in this animal the conceptuses were still present in the uterus whereas in the other animals they were in the vagina.

Oestradiol is also luteolytic in the pregnant cow (Wiltbank, 1966), sheep (Kittock and Britt, 1977) and the early pregnant hamster (Greenwald, 1965). However, in the pig the blastocyst is capable of synthesising oestrogens, principally oestrone, from Day 12 of pregnancy (Perry, Heap and Amoroso, 1973; Perry, Heap, Burton and Gadsby, 1976). Kraeling, Rampacek and Ball (1975) have reported that the administration of oestradiol benzoate on Days 10 to 15 of pregnancy, prolongs luteal function in unilaterally pregnant pigs and blocks the luteolytic effect of $PGF_{2\alpha}$ in hemi-hysterectomised, pregnant sows. The dose of oestradiol benzoate required to overcome the luteolytic effect of $PGF_{2\alpha}$ was high (5 mg) and so may represent a pharmacological effect rather than a physiological one. Treatment with oestradiol valerate in non-pregnant gilts however, maintains peripheral plasma progesterone levels above those of untreated animals. $PGF_{2\alpha}$ still appears to be secreted after Day 13 but the

peak frequency is more erratic (Frank, Bazer, Thai and Wilcox, 1977). Bazer and Thatcher (1977) have proposed that in the pregnant animal, oestradiol exerts a luteotrophic effect, not by decreasing the amount of $PGF_{2\alpha}$ synthesised but by redirecting its secretion from the vasculature to the uterine lumen, since the amount of $PGF_{2\alpha}$ in the uterine lumen of pregnant gilts, or in gilts treated with oestradiol valerate, is significantly higher than in the untreated, non-pregnant animal.

It is apparent therefore that in those species studied, the mechanisms by which $PGF_{2\alpha}$ secretion into the uterine vein during early pregnancy is reduced, compared with the non-pregnant animal, vary amongst the species (*e.g.* decreased uterine synthetase levels in the guinea-pig; a redirection of secretion in the pig). This decreased supply of $PGF_{2\alpha}$ to the ovary may be the cause of luteal maintenance during early pregnancy. However, it is apparent that in some species (*e.g.* sheep) the embryos secrete a luteotrophic hormone (or cause such a hormone to be secreted by the uterus) which also aids in maintaining the corpus luteum during early pregnancy. The presence of conceptuses therefore greatly affects prostaglandin synthesis by and/or release from the uterus.

Aims of the present Studies

The purpose of the experiments reported in this thesis was to examine whether $PGF_{2\alpha}$ could be the uterine luteolysin in the rabbit.

The rabbit, unlike the sheep, pig, cow and guinea-pig is a reflex ovulator and, as such, does not exhibit an ovarian cycle characterised by the presence of a spontaneously active corpus luteum. However, the doe does show seasonal variation in mating behaviour and during the breeding season shows oestrus activity. Oestrus lasts from 2 to

15 days. Cycles of oestrus activity of 4 to 6 days or 7 days, or multiples thereof, have been reported (Asdell, 1964). Ovulation occurs approximately 10 hrs after mating (Harper, 1961) and by Day 4 post coitus the corpus luteum is secreting substantial amounts of progesterone (Challis, Davies and Ryan, 1973). Pregnancy lasts approximately 32 days in the rabbit and is dependent upon pituitary support (Smith and White, 1931) and ovarian progesterone (Allen and Corner, 1930) throughout the whole of the gestational period. If the mating is infertile, an active corpus luteum still forms but the animal enters into a state of pseudopregnancy which lasts approximately half as long as pregnancy (17 days). Luteal regression, as determined by the appearance and weight of the corpora lutea and the histology of the endometrium begins to occur from Day 12 of pseudopregnancy. Ovarian progesterone secretion reaches peak values between Days 8 to 14 of pseudopregnancy and then declines steadily from Day 15 to Day 21. The decline in progesterone output is accompanied by a marked increase in 20α-hydroxypregn-4-en-3-one (20α-OH) and a significant increase in the 20α-OH : progesterone ratio (Hilliard, Spies and Sawyer, 1968). There is a good correlation between corpus luteum weight and progesterone secretion throughout pseudopregnancy. In the pregnant rabbit the concentration of progesterone in the peripheral venous plasma reaches peak levels on Days 10 to 14 of pregnancy, they fall abruptly on Day 15 to a lower level which is then maintained for about 12 days when the levels fall again to reach pre-ovulation values by the time parturition occurs (Fuchs, 1978). On the other hand, luteal weight increases to reach peak values between Days 15 to 20 and then maintains this weight until parturition, despite the fact that progesterone secretion begins to decline by Day 28 (Hilliard

et al., 1968). Also, unlike the pseudopregnant animal, the levels of 20α-OH are similar to those of progesterone throughout pregnancy and parturition and the ratio of 20α-OH : progesterone approaches unity (Fuchs, 1978).

Implantation occurs around Day 6.5 to 8 post coitus (Steven, 1975) and the placenta is established by Day 11. This coincides with the time at which differences in luteal progesterone output in pregnant and pseudopregnant animals occurs, suggesting that the placenta or developing embryo is responsible for maintaining luteal function in the pregnant animal.

Oestrogen appears to act as the ultimate luteotrophin in the rabbit and is essential for the development, persistence and function of the corpora lutea. Removal of the pituitary after ovulation results in the rapid involution of the corpora lutea. This can be prevented by the daily administration of 2μg oestradiol benzoate or by low doses of luteinizing hormone (10μg animal^{-1}) (Hilliard, Saldarini, Spies and Sawyer, 1971). As the luteotrophic effect of luteinizing hormone (LH) does not occur following destruction of the ovarian follicles (Rennie, 1968) the luteotrophic action of LH is thought to be mediated *via* its ability to stimulate oestrogen secretion from the follicles (Eaton and Hilliard, 1971).

The ovarian follicles are the principal source of oestrogen since their destruction by cautery (Rennie, 1968), X-irradiation (Keyes and Nalbandov, 1967) or luteinization in response to high doses of LH (Stormshak and Casida, 1965) results in the rapid regression of the corpora lutea unless exogenous oestrogen is administered.

However, the uterus also appears to play a role in regulating the life span of the corpus luteum in the pseudopregnant animal. Removal of the uterus prior to ovulation increases the luteal life

span by between 6 to 10 days (Asdell and Hammond, 1933) beyond its normal duration. The degree of lengthening of pseudopregnancy has been reported to be inversely related to the quantity of uterine tissue remaining. However, no well defined unilateral relationship was evident (Hunter and Casida, 1967). Scott and Rennie (1970) showed that following the transfer of 2-day old corpora lutea from donor rabbits to beneath the kidney capsule of recipient animals, the life span of the transferred corpora lutea depended on the day of pseudopregnancy of the recipient on which transfer took place. In oestrus recipients, the life span of the transferred corpora lutea was approximately 17 days whereas in corpora lutea transferred to Day 12 pseudopregnant recipients, the life span was reduced to approximately 11 days. In the latter experiment, both ovarian and transferred corpora lutea regressed at the same time. However, if the recipient animals were hysterectomised at the time of transfer, the two sets of corpora lutea regressed assynchronously and had life spans of between 23 to 27 days. This data suggests that the uterus secretes a luteolytic principle towards the end of pseudopregnancy which is responsible for initiating luteal regression. As regards the maintenance of luteal function beyond Day 12 in the pregnant rabbit the induction of uterine deciduomata by trauma in the pseudopregnant rabbit does not extend luteal function (Hammond, 1917). This is in contrast to the rat (Velardo, 1955 ; Hashimoto, Henricks, Anderson and Melampy, 1968). However, following the post implantation destruction of the rabbit blastocyst on Day 8 of pregnancy, the functional life span of the corpus luteum is extended to a similar extent as produced by hysterectomy (Hoffman, Davies and Davenport, 1973). The decidual reaction produced following removal of the blastocyst closely resembles the maternal placenta of normal

pregnancy whereas decidual tissue induced by trauma does not and so may account for the differences in the responses to these two treatments. However, neither hysterectomy or decidualisation results in a condition of luteal maintenance equivalent to pregnancy. In the rabbit therefore, it would appear that in the absence of a developing embryo and its associated tissues, the uterus will curtail the functional life-span of the corpus luteum. However, in the pregnant animal the presence of the developing embryo exerts both an anti-luteolytic as well as a luteotrophic effect.

The next question which arises is whether $PGF_{2\alpha}$ is the uterine luteolytic principle involved. $PGF_{2\alpha}$ is luteolytic in the pseudopregnant (Carlson and Gole, 1978; Scott and Rennie, 1970) and pregnant rabbit (Challis, Porter and Ryan, 1974), and inhibits the *in vitro* synthesis of progesterone by rabbit corpora lutea (O'Grady *et al.*, 1972). Treatment with $PGF_{2\alpha}$ is accompanied by a rapid decrease in the peripheral plasma concentration of progesterone and an increase in plasma levels of 20α-OH (Lau, Saksena and Chang, 1976). Morphological regression occurs within 24 hr of $PGF_{2\alpha}$ treatment (Koering, 1974).

Wilks, Hunter and Norland (1972) have shown that the rabbit uterus is capable of synthesising $PGF_{2\alpha}$ and that the amount of $PGF_{2\alpha}$ synthesised (per unit wt per hr) is higher in oestrus animals than on Day 9 of pseudopregnancy.

The main evidence which suggests that $PGF_{2\alpha}$ is the uterine luteolytic principle in the rabbit comes from studies involving the inhibition of prostaglandin synthesis and the 'neutralisation' of its effect. Subcutaneous administration of indomethacin (8 mg kg^{-1}, 12 hr^{-1}) from Day 12 of pseudopregnancy prolongs luteal function as

determined by the measurement of peripheral plasma progesterone concentrations until Day 24 post coitus (p.c.). The plasma levels in treated rabbits is substantially higher on Day 13 than in untreated animals. The concentration of progesterone in treated rabbits drops significantly between Days 13 and 15 to plateau at a lower level of about 4 ng ml^{-1} which is maintained until Day 22 when it then drops again to reach 2 ng ml^{-1} by Day 24 (O'Grady *et al.*, 1972). This stepwise decrease in progesterone secretion seen around Day 15 is similar to that observed in the pregnant animal although the concentration of progesterone in the peripheral plasma of indomethacin treated animals is still lower than that observed in the pregnant rabbit. Similar results are also obtained when the rabbit is immunised against $PGF_{2\alpha}$ (Caldwell, Auletta, Gordon and Speroff, 1972).

In the rabbit, therefore, $PGF_{2\alpha}$ fulfils several of the previously mentioned criteria for a luteolytic hormone. However, before such a role can be unequivocally established, it is also necessary to show that there is an increase in the uterine secretion of $PGF_{2\alpha}$ immediately prior to the onset of luteal regression. The following experiments were undertaken in an attempt to clarify the role of $PGF_{2\alpha}$ in controlling the function of the corpus luteum in the rabbit and to determine the hormonal factors controlling its synthesis. The effect of pregnancy on uterine prostaglandin production in the rabbit has also been studied.

SECTION 1. MATERIALS & GENERAL METHODS

The work presented in this thesis has involved the measurement of prostaglandin concentrations in rabbit plasma during pseudopregnancy and pregnancy, and the ability of uterine tissue from pseudopregnant and pregnant rabbits, and ovariectomised rabbits treated with progesterone and oestradiol-17β ($E_2$17β), either alone or in combination, and of placental tissue, to synthesise, and metabolise, prostaglandins. The methods of analysis involved the extraction of prostaglandins from biological samples by organic solvents, further purification by silicic acid, column chromatography and measurement by radioimmunoassay. Prostaglandin identification was confirmed whenever possible by combined gas chromatography and mass spectrometry (g.c.-m.s.). The experimental work has been divided into several sections but, as many of the analytical procedures used are common to each section the details of such procedures are given in Section 1.

Solvents

Chloroform (analar)	B.D.H. Chemicals Ltd.
*1,2-Dichloroethane (HPLC grade)	Rathbone Chemicals.
*Ethanol (absolute alcohol)	J. Boroughs Ltd., London.
*Ethyl Acetate (reagent grade)	B.D.H. Chemicals Ltd.
*Heptane (reagent grade)	Koch Light Laboratories Ltd.
*Methanol (reagent grade)	Koch Light Laboratories Ltd.
*Petroleum Spirit, b.pt 40-60°C, (reagent grade)	B.D.H. Chemicals Ltd.
*Petroleum Spirit, b.pt 60-80°C, (analar grade)	B.D.H. Chemicals Ltd.
*Toluene	Koch Light Laboratories Ltd.

All solvents were redistilled prior to use (*) unless otherwise stated.

Radioactive compounds

[5,6,8,11,12,14,15(n)-^{3}H] Prostaglandin E_2 (Sp. act. 160 Ci mmol^{-1})
Radiochemical Centre, Amersham.

[5,6,8,11,12,14,15n-^{3}H] Prostaglandin $F_{2\alpha}$ (Sp. act. 160 Ci mmol^{-1})
Radiochemical Centre, Amersham.

[1,2,6,7-^{3}H] Progesterone (St. act. 110 Ci mmol^{-1})
Radiochemical Centre, Amersham.

^{3}H-Progesterone was checked for purity on a column of Sephadex LH-20 (Pharmacia Fine Chemicals) run in Heptane : Chloroform : Ethanol (200:200:1) saturated with water. It was then stored at 5.0μCi ml^{-1}) in ethanol at -20°C.

Radioactivity was monitored on a Mark II Nuclear Chicago liquid scintillation counter.

Other Chemicals

Authentic prostaglandins E_2, $F_{2\alpha}$ and 6-keto-prostaglandin $F_{1\alpha}$ were the gift of Dr. J. Pike, Upjohn Company, Kalamazoo, U.S.A.

Arachidonic Acid — Sigma Chemical Company.

Authentic Progesterone, 4-Pregnen-3,20-dione, pure B.P.
Koch Light Laboratories.

Authentic Oestradiol-17β, $\Delta^{1,3,5}$-Estratrien-3,7β-diol
Sigma Chemical Company.

Human chorionic gonadotrophin (HCG)-Pregnyl B.P.
Organon Laboratories Limited.

Urethane, Ethyl Carbamate — May & Baker Limited

Heparin, Pularin Heparin inj B.P. Evans Medical Limited

Sagatal — May & Baker Limited

Krebs' Solution was made up daily as required in 2 quantities:-

*including deuterated 6-keto-Prostaglandin $F_{1\alpha}$.

13.8g Sodium Chloride

4.2g Sodium dihydrogen phosphate

4.0g Glucose

2.8ml 10% Magnesium Sulphate

3.2ml 10% Potassium dihydrogen orthophosphate

7.0ml 10% Potassium chloride

5.04ml 1 M Calcium chloride

All reagents were supplied by BDH Chemicals Ltd.

Chemicals and Solutions for Radioimmunoassay (RIA)

Tris(hydroxymethyl)methylamine)Analar B.D.H. Chemicals Limited

Sodium azide Hopkin and Williams Limited

Gelatin B.D.H. Chemicals Limited

PPO(2,5-diphenyloxazole) Fisons

Butyl PBD (2-(4-tert-Butylphenyl)-5-(4-biphenyl)-1,3,4-oxadiazole)
Koch Light Laboratories Ltd.

Napthalene May & Baker Limited

The above chemicals were used to make up the following solutions.

Diluent 1 0.05m Tris buffer pH 8.0
$0.1g\ l^{-1}$ Sodium azide
$1.0g\ l^{-1}$ Gelatin

Diluent 2 0.05m Phosphate buffer pH 7.5
$0.1g\ l^{-1}$ Sodium azide
$1.0g\ l^{-1}$ Gelatin

Diluent 3 0.05m Tris buffer pH 6.8
$0.1g\ l^{-1}$ Sodium azide
$1.0g\ l^{-1}$ Gelatin

Scintillant 1	10.5g PPO
	1.5 l Toluene
	0.9 l 2 Ethoxyethanol
Scintillant 2	10.0g Butyl PBD
	2.5 l Toluene
Scintillant 3	112.5g Napthalene
	10.5g PPO
	1.5 l Toluene
	0.9 l 2 ethoxyethanol
Donkey anti-rabbit serum (DARS) stored at 4°C in 25 ml aliquots until use	Wellcome Reagents Limited

Normal rabbit serum (NRS) - obtained from non-immunised New Zealand white rabbits (male) by the method of Dighe Emslie, Henderson and Simon, 1975. Stored in 1 ml fractions at -20°C. Prior to use the serum was thawed and thereafter stored at 4°C. Dilutions of NRS were made up in diluent 1 or diluent 2 as required.

Section 1.1a Experiment to check the purity of tritiated prostaglandin E_2 and $F_{2\alpha}$ tracers.

Tritiated prostaglandin $F_{2\alpha}$ (^{3}H-PG$F_{2\alpha}$) and tritiated prostaglandin E_2 (^{3}H-PGE_2) were obtained from the Radiochemical Centre, Amersham in quantities of 250µCi.

On arrival the radioactive prostaglandins (tracers) were taken to dryness and resuspended in methanol to give a final dilution of 5.0µCi ml^{-1}. This was stored in sealed vials containing 2 to 3 ml

methanol and kept at -20°C until use.

Because of the high substitution of hydrogen atoms with tritium and because PGE_2 is liable to dehydration, the purity of the tracers was checked periodically.

Method

Stock solutions of authentic PGE_2 and $PGF_{2\alpha}$ at a concentration of 5.0µg ml^{-1} were made up in methanol. 0.1 ml Authentic PGE_2 or $PGF_{2\alpha}$ was spotted on to a 50 × 200 × 0.25 mm, precoated, silica gel thin layer chromatography plate (Merck, precoated TLC plates without fluorescence indicator) using a 100µl Hamilton syringe, to give an origin 30 mm from the end of the plate. The spot was allowed to dry and 0.1 ml of ^3H-PGE_2 or ^3H-$PGF_{2\alpha}$ (5µCi ml^{-1}) spotted over it. Control plates were spotted with 100µl of cold standard only. All plates were first developed in solvent F VI (Andersen 1969; a mixture of ethyl acetate : acetone : glacial acetic acid, 90:10:0.1) and then in solvent G.C.M. (Miller 1974; a mixture of ethyl acetate : methanol : glacial acetic acid, 100:10:1). The plates were developed to give a solvent front of 160 mm from the origin.

The radioactivity was localised using a Panax radio thin layer chromatographic (T.L.C.) plate scanner, the position of the radioactive peaks relative to the origin measured and the Rf values calculated. Control plates were visualised by spraying with a saturated solution of phosphomolybdic acid in ethanol and heating at 110°C for 15 min. The positions of the spots were located and the Rf values calculated.

Results

Compound	^{3}H-PG	Authentic Std Rf
$PGF_{2\alpha}$	0.33 ± 0.03	0.33 ± 0.01
PGE_2	0.51 ± 0.01	0.51 ± 0.03

Table 1. Comparison of the Rf values of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ and the unlabelled standard (mean ± standard error of the mean (SEM), n = 5)

When the tracer did not run as a single spot with the same Rf value as the authentic standard, the remaining ampoules of that tracer were pooled and the tracer purified by column chromatography on Lipidex 1000 using the method of Brash and Jones (1974).

Section 1.1b Purification of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ by Column Chromatography

Method

20g Lipidex 1000 was placed in an excess of running solvent of the following composition, Hexane : dichloroethane : ethanol : glacial acetic acid (100:100:15:0.1%) and allowed to swell overnight. A glass column of 300-400 mm length and 10 mm internal diameter, which had a teflon wool plug covered with acidic, washed sand at the bottom and a solvent reservoir to form a constant pressure head at the top was used. The swollen Lipidex was poured into the column to a height of 310 mm and left to wash overnight with a further 250 ml running solvent.

The pooled tracer was evaporated to dryness at 45°C under reduced pressure on a rotary evaporator, and drying was completed in

a vacuum dessicator. The ^{3}H-PGF$_{2\alpha}$ was redissolved in 0.5 ml of running solvent and applied to the column. The flask containing the tracer was washed twice with a further 0.25 ml running solvent and the washings applied to the column. The reservoir was filled with 500 ml of running solvent and the column developed. The column effluent was led *via* narrow bore Teflon tubing to the drop-counting head of a L.K.B. ultronic fraction collector. Fractions were collected on a fixed volume basis of 3 ml (150 drops) and the height of the column outflow adjusted to give flow rates of 10 to 15 ml h^{-1}.

20µl samples were removed from each fraction, transferred to scintillation vials containing 13 ml of scintillant 1 and monitored for radioactivity for 1 min. The relevant fractions containing the purified tracer were pooled, taken to dryness at 45°C on a rotary evaporator and drying was completed in a vacuum dessicator.

The purified tracer was redissolved in 10 ml methanol and three 20µl amounts were transferred to scintillation vials containing 13 ml scintillant I and monitored for radioactivity using the external standards channels/ratio method. The total amount of radioactivity present was calculated. The remaining tracer was taken to dryness and redissolved in methanol to give a final dilution of 5.0µCi ml^{-1}.

^{3}H-PGE$_{2}$ was purified by the same method but the running solvent had the following composition; Hexane : dichloroethane : ethanol : glacial acetic acid (100:100:10:0.1%).

Following purification by the above method the purified tracers were re-run on a TLC plate as described in section 1.1a and the Rf value of the tracer compared with the Rf value of the authentic

standard. Following purification tracers were reampouled and stored as previously described.

Results

Both ^{3}H-$PGF_{2\alpha}$ and ^{3}H-PGE_2 ran as single spots with Rf values comparable to the Rf values obtained for authentic standards following purification by the above procedure. ^{3}H-PGE_2 had to be purified more frequently than ^{3}H-$PGF_{2\alpha}$.

Conclusion

^{3}H-PGE_2 is not as stable as ^{3}H-$PGF_{2\alpha}$ when stored at -20°C in methanol and should be checked for purity on a regular basis.

Section 1.2a Extraction of Prostaglandins from Tissue Homogenates

Prostaglandins were extracted from rabbit uterine tissue homogenates using the method described by Poyser (1972). Tissue homogenates were adjusted to pH 4.5 by the dropwise addition of 0.5M hydrochloric acid and extracted three times with five volumes of ethyl acetate. The ethyl acetate fractions were combined and taken to dryness at 45°C on a rotary evaporator. The residue was redissolved in 20 ml of 67% aqueous ethanol (v/v), washed twice with two 20 ml fractions of petroleum spirit (b.pt 60-80°C) and the ethanolic fraction taken to dryness at 45°C on a rotary evaporator. Drying was completed by placing the extract in a vacuum dessicator for a minimum period of 5 min. The residue was redissolved in 10 ml ethyl acetate and samples stored at -20°C until assayed.

Section 1.2b Determination of the recovery of tritiated Prostaglandins E_2 and $F_{2\alpha}$ from tissue Homogenates

Method

Two female New Zealand white rabbits were killed by stunning and incising the neck. The uteri were removed and divided into twelve segments each weighing approximately 1 g. Each segment was homogenised in 5 ml Krebs' solution using a Fisons glass homogeniser and the homogenate poured into a 50 ml conical flask. The homogeniser was washed with two further 5 ml aliquots of Krebs' solution and the washings added to the flask. 50µl ^{3}H-$PGF_{2\alpha}$ (0.26µCi ml^{-1}) in saline was added to each of six flasks, and also to four liquid scintillation vials containing 13 ml scintillant I which acted as counting standards. 50µl ^{3}H-PGE_2 (0.26µCi ml^{-1}) in saline was added to each of the remaining six flasks and to another four scintillation vials containing 13 ml scintillant I. The homogenates were incubated for 90 min at 37°C in a Grant automatic shaker and gassed with a mixture of 95% O_2 and 5% CO_2. Following incubation the homogenates were extracted as described in section 1.2a.

After vacuum dessication each sample was dissolved in 2 ml ethyl acetate and transferred to a scintillation vial. The ethyl acetate was evaporated off at 45°C under a jet of nitrogen. 13 ml Scintillant I were added to each vial and all vials monitored for radioactivity using the external standard/channels ratio method. The percentage recovery was calculated from the expression

$$\% \text{ recovery} = \frac{\sum \frac{\text{disintegrations per minute (dpm) per vial}}{6}}{\text{Average dpm of counting standards}} \times 100$$

Results

The average percentage recovery of added ^{3}H-$PGF_{2\alpha}$ and ^{3}H-PGE_2 was 91.97 ± 2.96 and 89.68 ± 1.75 respectively (mean ± SEM, n = 6).

Conclusion

These results indicate that the method described in section 1.2a for the extraction of prostaglandins E and F_{α}, as judged by the recovery of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$, gives high recovery values and is very reproducable.

Concentrations of prostaglandins measured in tissue homogenates have not been corrected for procedural losses.

Section 1.2c Determination of the percentage recovery of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ from Krebs' solution

Method

Flasks containing 10.0, 100.0 or 1000.0 ng authentic $PGF_{2\alpha}$ were set up in quadruplicate. 0.013μCi ^{3}H-$PGF_{2\alpha}$ in saline were added to each flask and to 4 scintillation vials containing 13 ml scintillant I; these acted as counting standards. 15 ml Krebs' solution was added to each flask and the flask contents extracted as described in section 1.2a. After vacuum dessication each sample was dissolved in 2 ml ethyl acetate and transferred to a scintillation vial. The ethyl acetate was blown off at 45°C under a stream of nitrogen and 13 ml scintillant I added to each vial. All vials were monitored for radioactivity using the external standard channels/ratio method and the percentage recovery of ^{3}H-$PGF_{2\alpha}$ calculated.

The recovery of ^{3}H-PGE_2 from Krebs' was determined as for recovery of ^{3}H-$PGF_{2\alpha}$ except that authentic PGE_2 and ^{3}H-PGE_2 were substituted for authentic $PGF_{2\alpha}$ and ^{3}H-$PGF_{2\alpha}$ respectively

Results

Table 2 shows the percentage recovery of ^{3}H-PGF$_{2\alpha}$ and ^{3}H-PGE$_2$ from Krebs' solution over a range of concentrations of prostaglandin.

Cold PGE_2 ng	% Recovery ^{3}H-PGE_2	Cold $PGF_{2\alpha}$ ng	% Recovery ^{3}H-$PGF_{2\alpha}$
10	91.75 ± 4.92	10	97.00 ± 5.12
100	93.00 ± 4.74	100	96.25 ± 4.91
1000	97.25 ± 4.70	1000	92.00 ± 4.50

Table 2. Percentage recovery of ^{3}H-PGE_2 and ^{3}H-$PGF_{2\alpha}$ from Krebs' solution in the presence of different quantities of authentic PGE_2 and $PGF_{2\alpha}$ (mean ± SEM, n = 4)

Conclusion

The method described here for the extraction of prostaglandins from Krebs' solution gives a high percentage recovery of ^{3}H-$PGF_{2\alpha}$ and ^{3}H-PGE_2 which remains constant over a wide range of concentrations.

Section 1.2d Recovery of tritiated 6-keto-prostaglandin $F_{1\alpha}$ from Krebs' solution

A similar experiment to the one described above was performed in this laboratory by Miss F. Housman to determine the percentage recovery of tritiated 6-keto-prostaglandin $F_{1\alpha}$ (^{3}H-6-keto-$PGF_{1\alpha}$) from Krebs' solution. 0.5μCi ^{3}H-6-keto-$PGF_{1\alpha}$ was added to three flasks each containing 1μg 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) in 15 ml Krebs' solution and the flask contents extracted as described in section 1.2a.

The percentage recovery was 63.4 ± 2.5%. Lowering the pH of the extraction process did not affect the percentage recovery of ^{3}H-6-keto-$PGF_{1\alpha}$ but increasing the pH to pH 5.0 decreased the percentage recovery. It was decided therefore that extractions should be carried out at pH 4.5 as this yielded a high percentage recovery of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$.

Section 1.3a Solvent extraction of Prostaglandins from rabbit plasma

Method

Prostaglandins were extracted from rabbit plasma using a modification of the extraction procedure described by Bergström and Samuelsson (1964), and outlined in Fig. 3.

10 ml of rabbit plasma were acidified to pH 5 with 0.5M hydrochloric acid and washed three times with three volumes of ethyl acetate. The combined ethyl acetate fractions were taken to dryness at 45°C on a rotary evaporator, the residue dissolved in 20 ml ethyl acetate and extracted three times with an equal volume of 0.05 M phosphate buffer solution at pH 8.0. The aqueous phases were pooled and acidified to between pH 4 to pH 5 by the dropwise addition of concentrated hydrochloric acid. The aqueous phase was washed three times with an equal volume of ethyl acetate (60 ml), the ethyl acetate fractions pooled and evaporated to dryness at 45°C on a rotary evaporator. Drying was completed in a vacuum dessicator. The residue was redissolved in 20 ml 67% aqueous ethanol and washed twice with an equal volume of petroleum spirit (b.pt 60-80°C). The aqueous ethanol fraction was evaporated to dryness, dessicated and the residue dissolved in 0.15 ml of a mixture

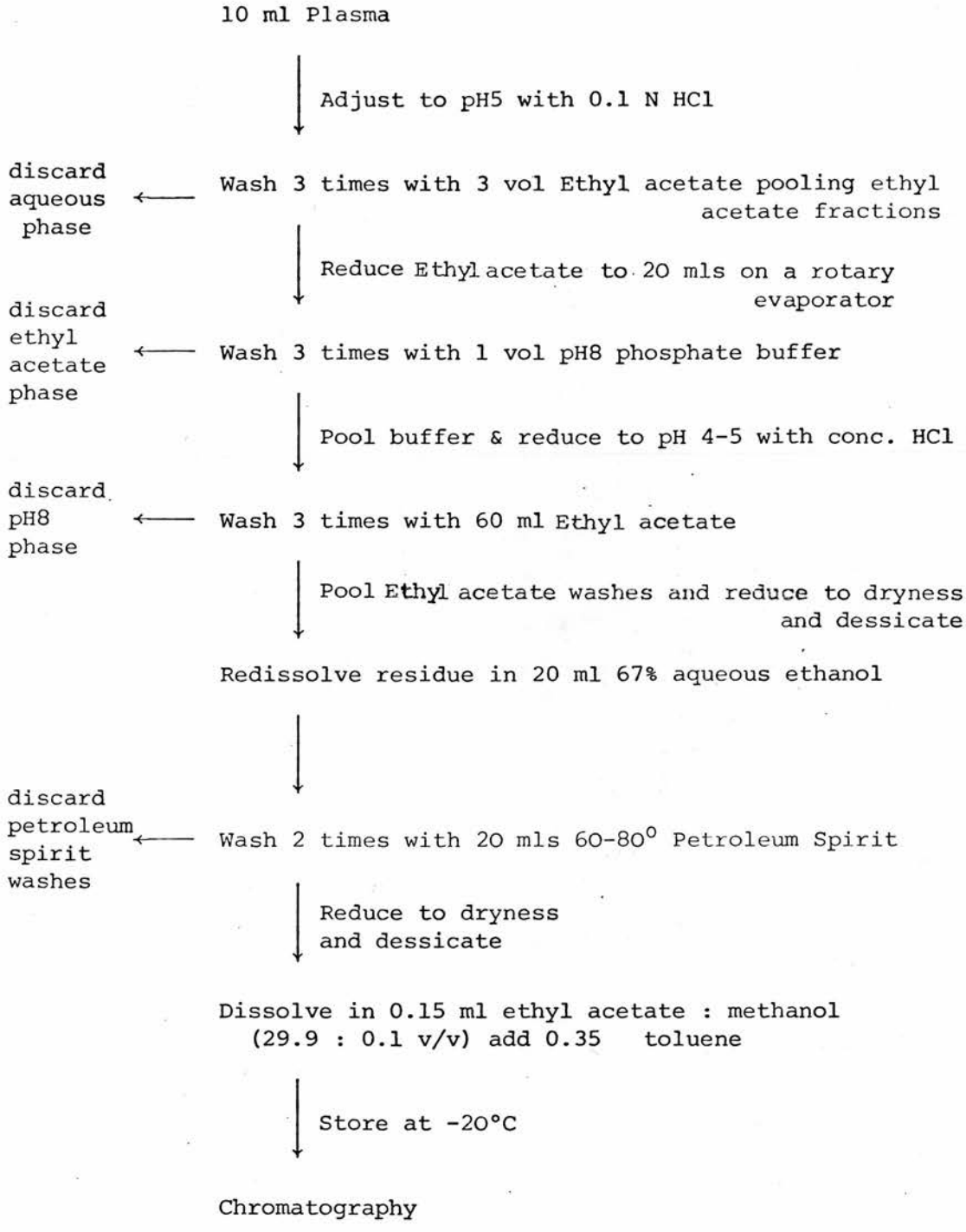


Fig. 3 Extraction of Prostaglandins from Rabbit Plasma

of ethyl acetate and methanol (29.9 : 0.1). 0.35 ml of toluene was then added and the sample stored at -20°C until chromatography.

Section 1.3b Separation of Prostaglandins of the E and F series by silicic acid column chromatography: Characterisation of silicic acid

Introduction

When rabbit plasma prostaglandin concentrations were measured by RIA following solvent extraction only, the levels of PGF_{α} recorded were approximately 50% higher than those obtained when the same plasma was assayed after solvent extraction followed by silicic acid chromatography and correction for procedural losses. This indicated that some constituent of the rabbit plasma was interfering with the assay. In order to avoid this interference all plasma samples were subjected to silicic acid column chromatography following solvent extraction.

The method used was first described by Samuelsson (1963) and is a further modification of the method used by Downie, Poyser and Wunderlich (1972). Batches of silicic acid were found to differ in their chromatographic characteristics, an observation confirmed by Kibbey, Brown and Minton (1971), such that the characteristics of each batch were determined prior to use.

Method

10 ml rabbit plasma were extracted as previously described. The residue was dissolved in 10 ml ethyl acetate and divided into two 5 ml fractions. 20 ng authentic $PGF_{2\alpha}$ (0.2 ml of 100 ng ml^{-1} in methanol) and 0.013µCi ^{3}H-$PGF_{2\alpha}$ (50µl of 0.26µCi ml^{-1} in methanol) were added to one fraction while the other received the same quantities

of cold and tritiated PGE_2. Each sample was taken to dryness at 45°C on a rotary evaporator. Drying was completed in a vacuum dessicator and the residue redissolved in 0.15 ml of a mixture of ethyl acetate and methanol (29.9 : 0.1). 0.35 ml toluene was added and the sample stored at -20°C overnight.

Two chromatography columns were prepared as follows:- 3.8 to 4.2g silicic acid, depending on the batch, were suspended in 10 ml of a mixture of methanol, ethyl acetate and toluene (0.1 : 29.9 : 70). Hereafter this mixture is referred to as Fl. The slurry was poured into a glass chromatography column of 100 mm length, 10 mm internal diameter and possessing a glass scinter at its base. Prior to use 0.25 mm acid washed sand was placed over the scinter to protect it from becoming blocked by fine particles of silicic acid. Columns were kept moist with Fl overnight. The following morning the excess Fl was removed and the extracted plasma added to the top of the column. The flask containing the extracted plasma was washed three times with 0.5 ml of Fl and the washings added to the top of the column.

Columns were eluted under reduced pressure at a rate of approximately 30 drops per minute using solvent mixtures of increasing polarity as detailed in Table 4. Each fraction was taken to dryness on a rotary evaporator and the residue dissolved in 0.5 ml methanol, transferred to a scintillation vial containing 13 ml scintillant 1 and monitored for radioactivity using the external standards channels/ ratio method. The percentage of radioactivity recovered was calculated using the formula

$$\% \text{ recovery} = \frac{\text{dpm fraction}^{-1}}{\sum \text{dpm of all fractions}} \times 100$$

The ratio of methanol to ethyl acetate in fractions 3,4 and fraction 5 was adjusted to maximise the separation of prostaglandins of the E and F series and to optimise recovery. The solvent composition of

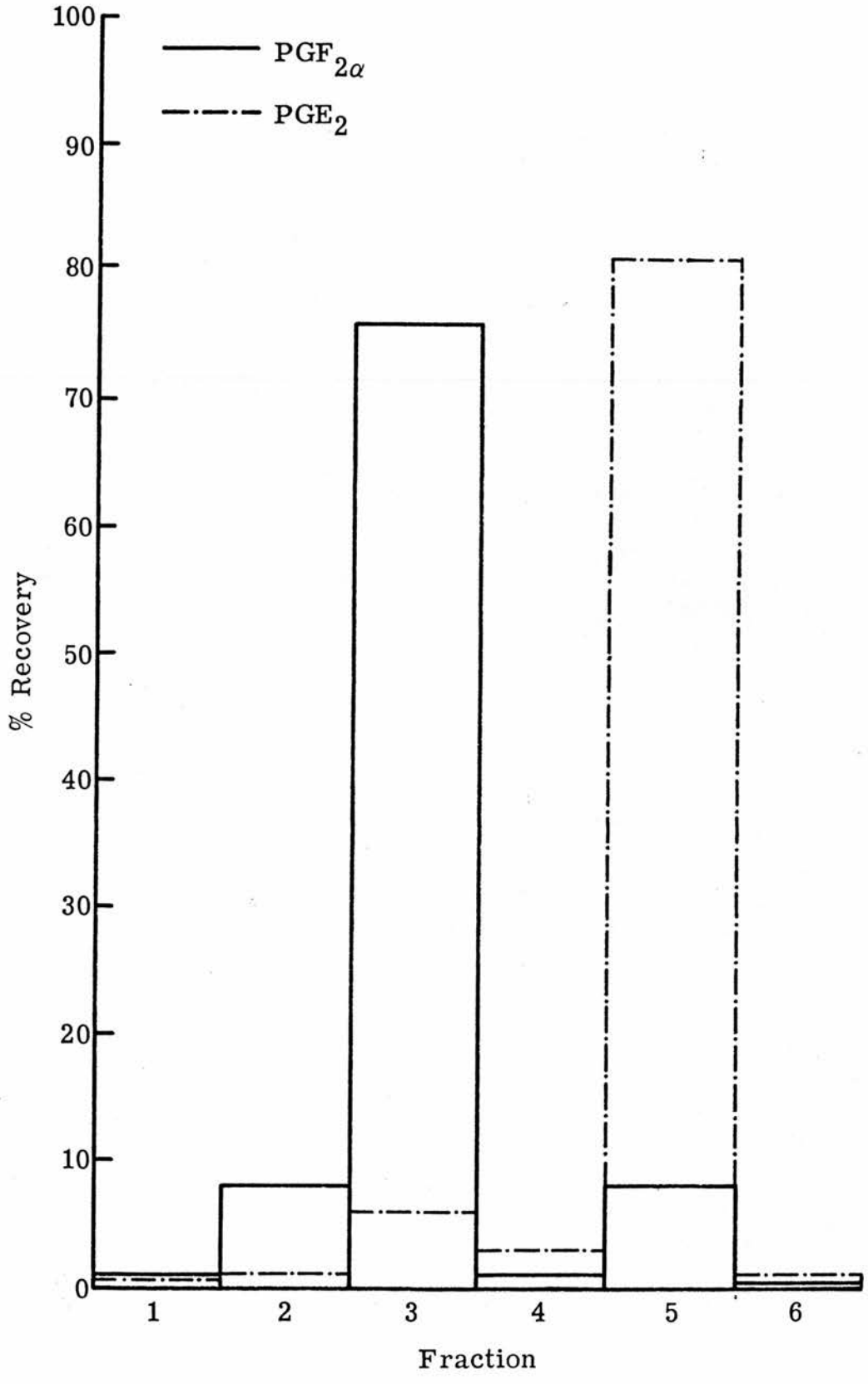
Fraction	Volume ml	Ethyl Acetate %	Toluene %	Methanol %
1	40	29.9	70	0.1
2	40	39.9	60	0.2
3	120	64.4	35	0.6
4	30	64.4	35	0.6
5	150	79.0	20	1.0
6	40	98.0	-	2.0

Table 4. The solvent composition and volumes of fluid applied to silicic acid columns made from silicic acid, Batch No. 2847.

the fractions in Table 4 fulfilled these requirements for Batch No. 2847. Fig. 4 gives a graphic representation of the separation of PGE_2 from $PGF_{2\alpha}$ using this system.

Conclusion

As shown in Fig. 4 this system provided good separation of prostaglandins of the E and F series, PGE_2 being almost completely eluted in fraction 3 and $PGF_{2\alpha}$ in fraction 5. When this system was used to purify plasma extracts prior to assay by RIA fraction 3 and 4 were run as a single fraction of 150 ml. New columns were prepared for each sample.


PGF2α
PGE2
100
90
80
70
60
50
40
30
20
10
0
% Recovery
1
2
3
4
5
6
Fraction

Section 1.3c Correction for procedural losses

Introduction

When a radioactive tracer is used as an internal standard to monitor losses incurred during the preparation of samples to be assayed by RIA it is important that the internal standard does not contribute significantly to the mass and/or the radioactivity of the tracer added for assay purposes. This requires that the internal standard have a high specific activity such that a small mass produces a sufficient number of counts.

$PGF_{2\alpha}$

In order to correct for losses of $PGF_{2\alpha}$ which occurred during the extraction and purification of plasma samples, 0.0013μCi ^{3}H-$PGF_{2\alpha}$ (3pg) was added to all plasma samples and to a counting standard before extraction. Following chromatography fractions 3 and fraction 5 were taken to dryness at 45°C on a rotary evaporator and drying was completed in a vacuum dessicator. The residue was redissolved in 10 ml ethyl acetate and stored at -20°C. Prior to assay, 1 ml of fraction 5 was transferred to a scintillation vial, the ethyl acetate blown off at 45°C under a jet of air and 13 ml scintillant 3 added. The sample was monitored for radioactivity for 100 min using the external standard channels/ratio method. The percentage recovery of ^{3}H-$PGF_{2\alpha}$ from the plasma sample was calculated by using the formula

$$\% \text{ recovery} = \left(\frac{\text{dpm per sample} \times 10}{\text{dpm counting standard}} \right) \times 100$$

The concentrations of $PGF_{2\alpha}$ in plasma given in the following sections have been corrected for procedural losses.

PGE_2

As ^3H-$PGF_{2\alpha}$ was used to correct for procedural losses of $PGF_{2\alpha}$ extracted from plasma, it was considered unsuitable to use ^3H-PGE_2 as an internal standard to monitor losses of PGE_2 as any incomplete separation of tracers on the silicic acid column would lead to spurious results concerning the calculation for correction for procedural losses for each plasma sample. The average efficiency of the extraction and purification procedure for PGE_2 was therefore determined and it was assumed that the recovery of PGE from subsequent plasma samples did not differ greatly from this average.

Method

A New Zealand white female rabbit was anaesthetized by the intravenous injection (i.v.) of urethane (7 ml kg^{-1} of a 25% solution of ethyl carbamate in 0.9% saline, w/v). A mid-line abdominal incision was made, the aorta exposed and cannulated. Blood was collected in a glass beaker surrounded by crushed ice and containing sufficient heparin to produce a minimum final concentration of 10 I.U. ml^{-1} of blood. Following collection the blood was centrifuged for 30 min at 4°C at 1300 × g in a MSE Coolspin centrifuge. The plasma was decanted.

Known amounts of cold PGE_2 in methanol were added to five conical flasks. 0.013μCi ^3H-PGE_2 in methanol was added to each flask, and also to four scintillation vials containing 8 ml scintillant 3. The methanol was evaporated off at 45°C under a stream of nitrogen and 10 ml of aortic plasma added to each flask. The plasma samples were extracted and purified by silicic acid column chromatography as previously described. The 10 ml of ethyl acetate, containing the redissolved residues of fraction 3, were transferred to scintillation

vials, the ethyl acetate evaporated off at 45°C under a stream of nitrogen and 8 ml scintillant 3 added to each vial. All vials were monitored for radioactivity using the external standard channels/ratio method. The percentage recovery of ^{3}H-PGE_2 was calculated from the formula

$$\% \text{ recovery} = \frac{\text{dpm per sample}}{\text{Average dpm of the counting standards}} \times 100$$

Results

The percentage recovery of ^{3}H-PGE_2 from plasma containing 0 to 1000 ng of added PGE_2 is shown in Table 5.

Cold PGE_2 ng	% Recovery
0	49.4 ± 0.9
10	51.4 ± 2.6
100	52.6 ± 2.1
1000	50.1 ± 1.3

Table 5. Percentage recovery of ^{3}H-PGE_2 from rabbit plasma after solvent extraction and silicic acid column chromatography (mean ± SEM, n = 3)

Conclusion

The average percentage recovery of ^{3}H-PGE_2 from rabbit plasma following solvent extraction and silicic acid chromatography is unaffected by the amount of cold PGE_2 present and is about 50%. In the following section the concentrations of PGE in plasma samples are uncorrected for procedural losses.

Section 1.3d Extraction of progesterone from rabbit plasma

Method

0.05 to 0.2 ml of rabbit plasma was made up to a volume of 0.2 ml if necessary by the addition of redistilled water. In samples in which the extraction was performed on undiluted plasma, a small volume of ethanol (10 vol plasma + 1 vol ethanol) was added to improve the consistency of progesterone recovery. Each sample was extracted twice with 2 ml petroleum spirit (b.pt 40-60°C). The two petroleum fractions were pooled and evaporated to dryness at 60°C under a jet of air. The residue was dissolved in 1 ml diluent 2 and the amount of progesterone per tube determined by RIA.

Section 1.4 Measurement of Prostaglandins and Progesterone by Radioimmunoassay

Section 1.4a PGE Radioimmunoassay

Introduction

The concentration of PGE in rabbit plasma and tissue extracts were routinely measured by RIA using an antibody supplied by Dr. K.K. Dighe. The production of antisera to PGE_2 from rabbits immunised with PGE_2-bovine thyroglobulin conjugate has been described by Dighe, Smith, Ungar and Whelpdale, (1978). The process is not repeated here but, as the particular bleed of antisera used had not been fully characterised the developmental details of the assay are described in detail.

Dilution Curve

The purpose of this experiment was to determine the dilution of antibody which will bind approximately 60% of the tritiated PGE_2 added. The mass of tracer used was chosen to give approximately 20,000 counts over a period of four minutes and was 30 pg per tube.

Method

^{3}H-PGE_2 (sp. act 160μCi $mmol^{-1}$) was checked for purity and stored as previously described. Before use the methanol was evaporated off at 45°C under a stream of nitrogen and redissolved in diluent 2 to give a final concentration of 0.26μCi ml (0.6 ng ml^{-1}). This solution is referred to as 'tracer' hereafter. 0.1 ml neat antibody serum (Rabbit 8, 6th boost) was diluted in diluent 2 to give a stock solution of a 1 in 100 dilution. This was then used to prepare a series of two-fold dilutions (0.5 ml each) ranging from 1 in 800 to 1 in 12800.

50μl of tracer (30 pg) was added to all dilutions, and to 4 counting standards and 4 non-specific binding standards containing 0.7 ml and 0.5 ml diluent 2 respectively. The solutions were whirlimixed and allowed to incubate for 2 hr at room temperature. After incubation 50μl of a 1 in 140 dilution of normal rabbit serum (NRS) in diluent 2 was added to all tubes except the counting standards and mixed. This ensures that at the higher dilutions of antiserum there is sufficient γ-globulin present for adequate precipitation. 50μl of a 1 in 2 dilution of Donkey anti-rabbit serum (DARS) in diluent 2 was added to the same tubes, the tubes whirlimixed and left to incubate for a minimum of 16 hrs at 4°C. All tubes were centrifuged at 1300 × g in an MSE Coolspin centrifuge for 30 min at 4°C, the supernatant, containing the unbound tracer, decanted into vials containing 13 ml scintillant 1 and counted for 4 min.

The percentage of tracer bound by a given dilution of antibody was calculated using the formula

$$\% \text{ bound} = 100 - \left(\frac{\text{sample counts}}{\text{Average counting standard counts}} \times 100 \right)$$

A graph of percentage tracer bound v. dilution was plotted and the dilution of antiserum binding 60% of the added tracer, calculated.

Results

Fig. 5 shows the dilution curve obtained from rabbit 8, 6th boost.

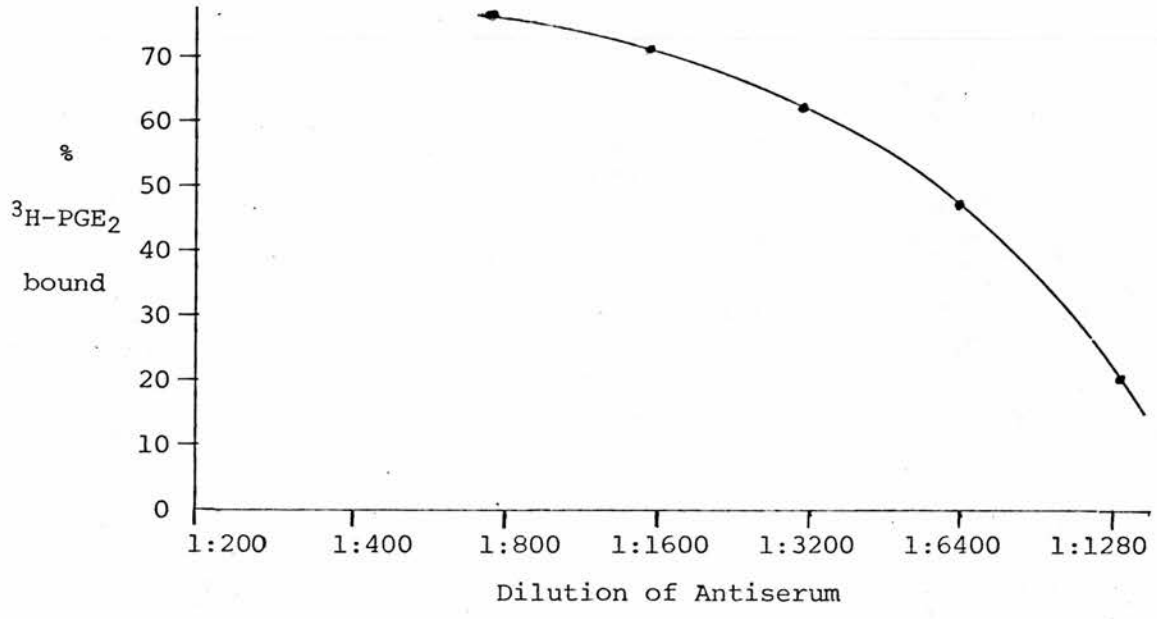


Fig. 5 Dilution curve for PGE_2 antiserum obtained from rabbit 8 (6th boost). Double antibody method using 0.013µCi ^{3}H-PGE_2 (30 pg)

Conclusion

The following conditions were found suitable. Final dilution of antiserum binding 60% tracer was 1 in 3200

Reaction volume for assay was 0.65 ml

Volume antiserum added was 0.05 ml

Working dilution of antiserum was 1 in 250

Non specific binding (NSB) was <10%.

PGE_2 Standard Curve

Method

A series of standard solutions of cold PGE_2 ranging from 0.2 ng ml^{-1} to 10.24 ng ml^{-1} were made up in diluent 2 from a stock solution of 1µg ml^{-1} in methanol. Tubes containing 0.5 ml of the above standards were set up in triplicate as detailed in Table 6.

Concentration PGE_2 Standards ng ml^{-1}	Volume $tube^{-1}$ ml	ng PGE_2 $tube^{-1}$ ng
10.24	0.5	5.12
5.12	"	2.56
2.56	"	1.28
1.28	"	0.64
0.64	"	0.32
0.32	"	0.16
0.16	"	0.08
0.08	"	0.04
0.04	"	0.02
0.02	"	0.01

Table 6. Concentrations of PGE_2 standards used in setting up the PGE_2 Standard Curve together with the volume of standard and amount of PGE_2 placed in each tube.

Counting standards and zero standards containing 0.6 ml and 0.5 ml of diluent 2 respectively, and non-specific binding standards containing 0.5 ml of 100 ng ml^{-1} PGE_2 in diluent 2, were set up in quadruplicate. All tubes were then treated as shown in Fig. 6.

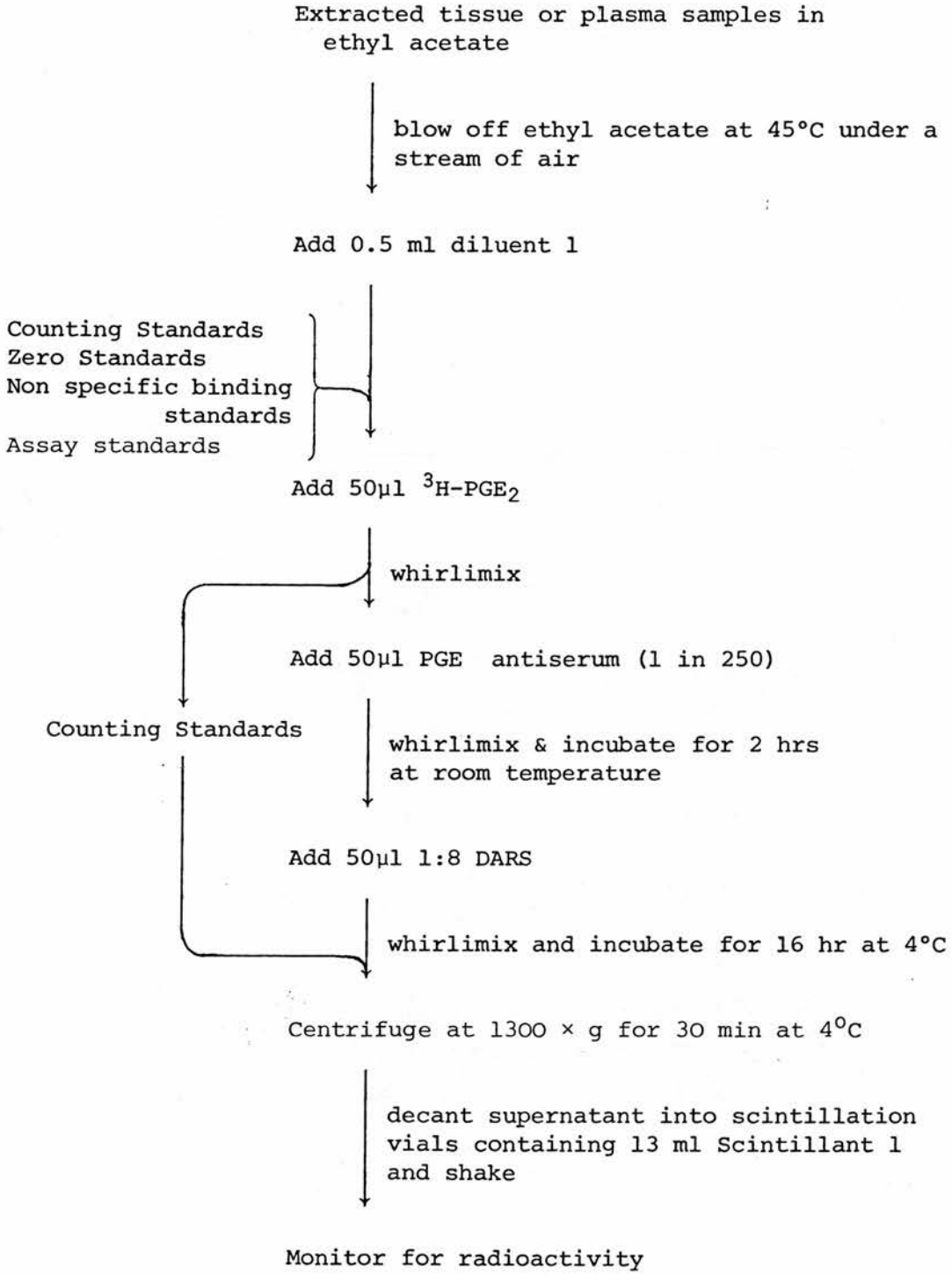


Fig. 6 Method for setting up a PGE-Standard Curve and Assay

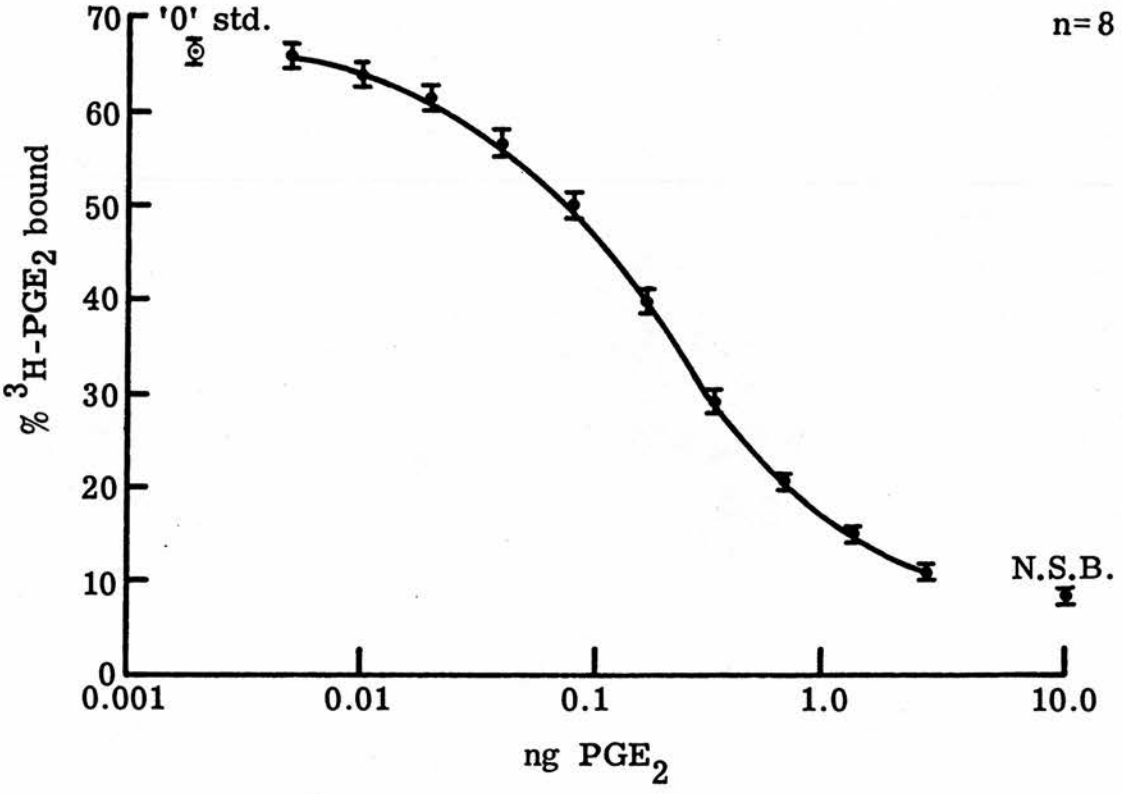
The counting standards give an estimate of the average number of counts added to each tube. The zero standards indicate the maximum binding of the tracer bound by the added antiserum in the presence of cold prostaglandin E_2. The non-specific binding standards, which contain sufficient cold PGE_2 to completely saturate the added antiserum, provide a measure of the degree of binding of the tracer by other components of the assay. If the non-specific binding was greater than ten per cent the assay was judged to be unacceptable.

The number of counts per vial were recorded on a paper punch tape which was fed into a PDP 8 computer programmed to calculate the percentage binding of the tracer as previously described. The computer then applied the logistic curve fit formula of Parker and Waud (1971) to the observed values of percentage tracer bound in the presence of different concentrations of cold PGE_2 and calculated the co-ordinates for the curve of best fit. The two sets of values, observed and calculated, were compared and, if the variation between them was less than 5% the calculated curve was judged to be acceptable.

Results

Fig. 7 shows the standard curve from eight consecutive assays (mean ± SEM). The limit of sensitivity was defined as the concentration of cold PGE_2 which produced a 10% decrease in the binding from the zero standards. The limit of sensitivity of this assay was 0.040 ng.

When this assay was used to measure the concentration of PGE in extracts of biological samples, samples were assayed in triplicate at two different volumes. Extracted samples were stored in ethyl acetate at -20°C until assay. Known volumes of each sample were


'0' std.
n=8
N.S.B.
% ^{3}H-PGE$_2$ bound
70
60
50
40
30
20
10
0
0.001
0.01
0.1
1.0
10.0
ng PGE$_2$

transferred to assay tubes using fixed volume Eppendorf pipettes. The ethyl acetate was evaporated off at 45°C under a stream of air. 0.5M diluent was added to each tube and all tubes treated as for the standard curve. The number of counts per vial were recorded on paper punch tape. Following calculation of the standard curve, the computer programme went on to calculate the amount of PGE in each tube and the coefficient of variation for each triplicate. Values of PGE which did not lie on the linear portion of the curve were reassayed using different volumes or dilutions of the sample. A standard curve was included in all assays and only assays in which the linear portion of the curve showed an 8.0 to 12.5% gradient, as measured over a doubling of the concentration of PGE_2 per tube, were accepted. When the gradient is less than 8.0%, small changes in the amount of PGE produced too small a change in the percentage binding of tracer for accurate measurement of sample differences in PGE concentrations to be made while too steep a gradient reduces the working range of the assay to an unacceptable degree. These criteria for the acceptance of a standard curve were applied to all assays.

The intra-assay coefficient of variation was calculated from the formula

$$\text{intra-assay coefficient of variation} = \frac{\sum\left(\frac{\text{Standard deviation of sample triplicate}}{\text{mean value of triplicate}}\right)}{\text{total number of triplicates}} \times 100$$

For this assay the intra-assay coefficient of variation was 8.51 ± 0.28% (mean ± SEM, $n = 8$)

The accuracy of this assay was calculated by incorporating three tubes containing an 'external standard' of 0.35 ng per tube. The mean calculated value of the 'external standard' for 5 assays was 0.35 ± 0.02 ng (mean ± SEM).

The inter-assay coefficient of variation for this assay was calculated using the formula below, and was found to be 8.7%.

$$\text{inter-assay coefficient of variation} = \sum \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Determination of the cross reactivity of the PGE antiserum of rabbit 8, 6th boost

Method

A standard curve for PGE_2 was set up in parallel with standard curves for several other prostaglandins. The percentage cross reactivity of the antiserum obtained from rabbit 8, 6th boost was determined by finding the concentration of prostaglandin which produced a 50% inhibition of binding of the tracer and then applying the following formula.

$$\% \text{ Cross reactivity} = \frac{\text{Concentration of prostaglandin binding 50\% of tracer}}{\text{Concentration of cold } PGE_2 \text{ binding 50\% of tracer}} \times 100$$

Results

Table 7 gives the percentage cross reactivity of antiserum from rabbit 8, 6th boost with various prostaglandins. As shown in Fig. 8 the dose response curves of other prostaglandins which react with the antiserum are not parallel to the PGE_2 standard curve, the degree of

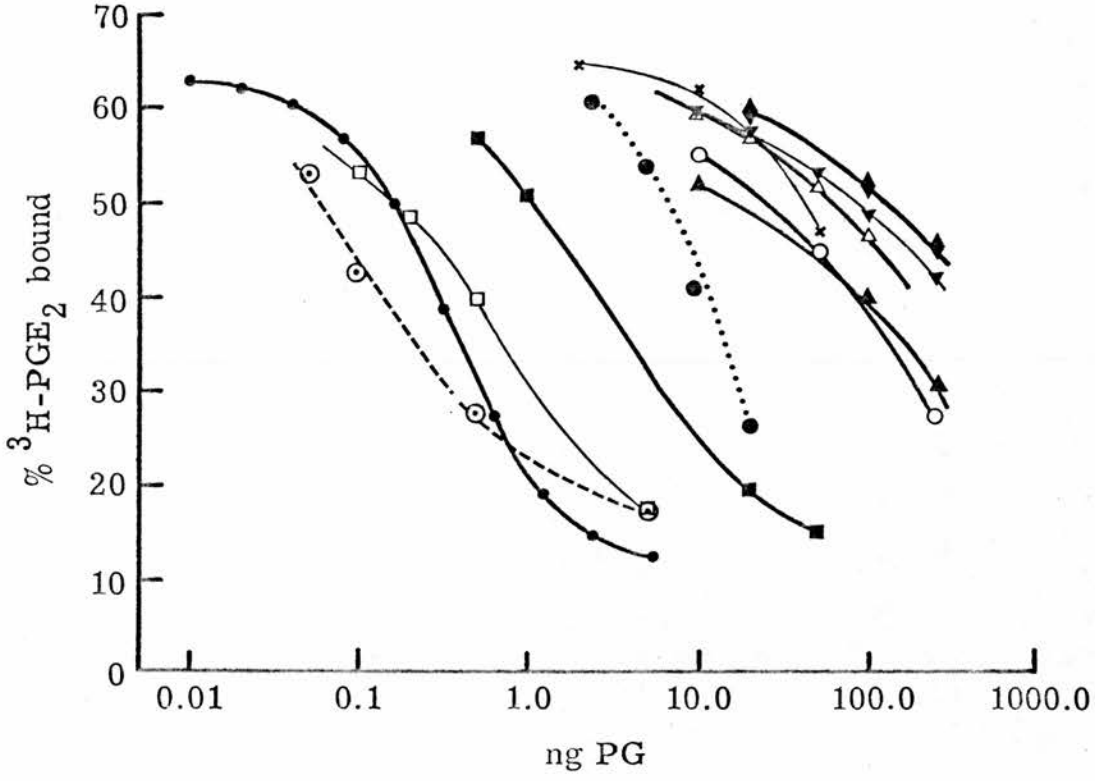
cross reaction differs over its response curve. By measuring the cross reactivity at 50% the values quoted represent the upper limits of cross reaction.

Compound	% Cross Reactivity at 50% binding of tracer
PGE_2	100
PGE_1	100
PGA_2	13.64
PGB_2	260.00
$PGF_{2\alpha}$	2.5
PGD_2	0.23
15-oxo-PGE_2	0.63
13,14-dihydro-15-oxo-PGE_2	0.23
6-keto-$PGF_{1\alpha}$	0.65
TXB_2	0.08
Arachidonic Acid	0.23

Table 7. Cross reactivity of antiserum from rabbit 8, 6th boost, measured at 50% inhibition of binding of tracer.

Conclusion

The antibody showed high cross reactivity with prostaglandin B_2 (PGB_2) and prostaglandin E_1 (PGE_1). As all plasma samples were subjected to silicic acid column chromatography prior to radioimmunoassay, any PGB_2 present in the plasma would have been removed from the extracted plasma prior to assay (Davies and Horton, 1972). The chromatographic system used, however, did not permit separation of



PGE$_2$

Arachidonic acid

PGA$_2$

PGB$_2$

PGD$_2$

PGE$_1$

15-keto-PGE$_2$

13,14-dihydro-15-keto-PGE$_2$

PGF$_{2\alpha}$

6-keto-PGF$_{1\alpha}$

TXB$_2$

PGE_1 and PGE_2 and the results obtained following measurements made on samples of extracted plasma using this assay have been quoted as concentrations of PGE present.

When this assay was used to measure the PGE content in tissue extracts the chromatography step was omitted. However, in samples, subjected to analysis by combined gas liquid chromatography - mass spectrometry no PGB_2 was detected consequently the measurements made on extracted tissue homogenates are also expressed in terms of PGE concentration.

Section 1.4b $PGF_{2\alpha}$ Radioimmunoassay

Antisera to $PGF_{2\alpha}$ were raised in rabbits immunised with a prostaglandin $F_{2\alpha}$-bovine serum albumin conjugate as described by Dighe *et al.* (1975).

Reagents

Diluent 1

Scintillant 1

^{3}H-$PGF_{2\alpha}$ 0.26μCi ml^{-1} in diluent 1

Antiserum R6 - 5th boost at a dilution of 1 in 1700 in diluent 1

1 in 140-dilution of NRS in diluent 1

1 in 15-dilution of DARS in diluent 1

Method

A series of standard solutions of $PGF_{2\alpha}$ ranging from 0.1 ng ml^{-1} to 5.12 ng ml^{-1} were made up in diluent 1. Tubes retaining 0.5 ml of the above standards were set up in triplicate. Counting standards, non-specific binding standards and zero standards containing 0.65, 0.55 and 0.5 ml of diluent 1 respectively were set up in quadruplicate. All tubes were treated as shown in Fig. 9.

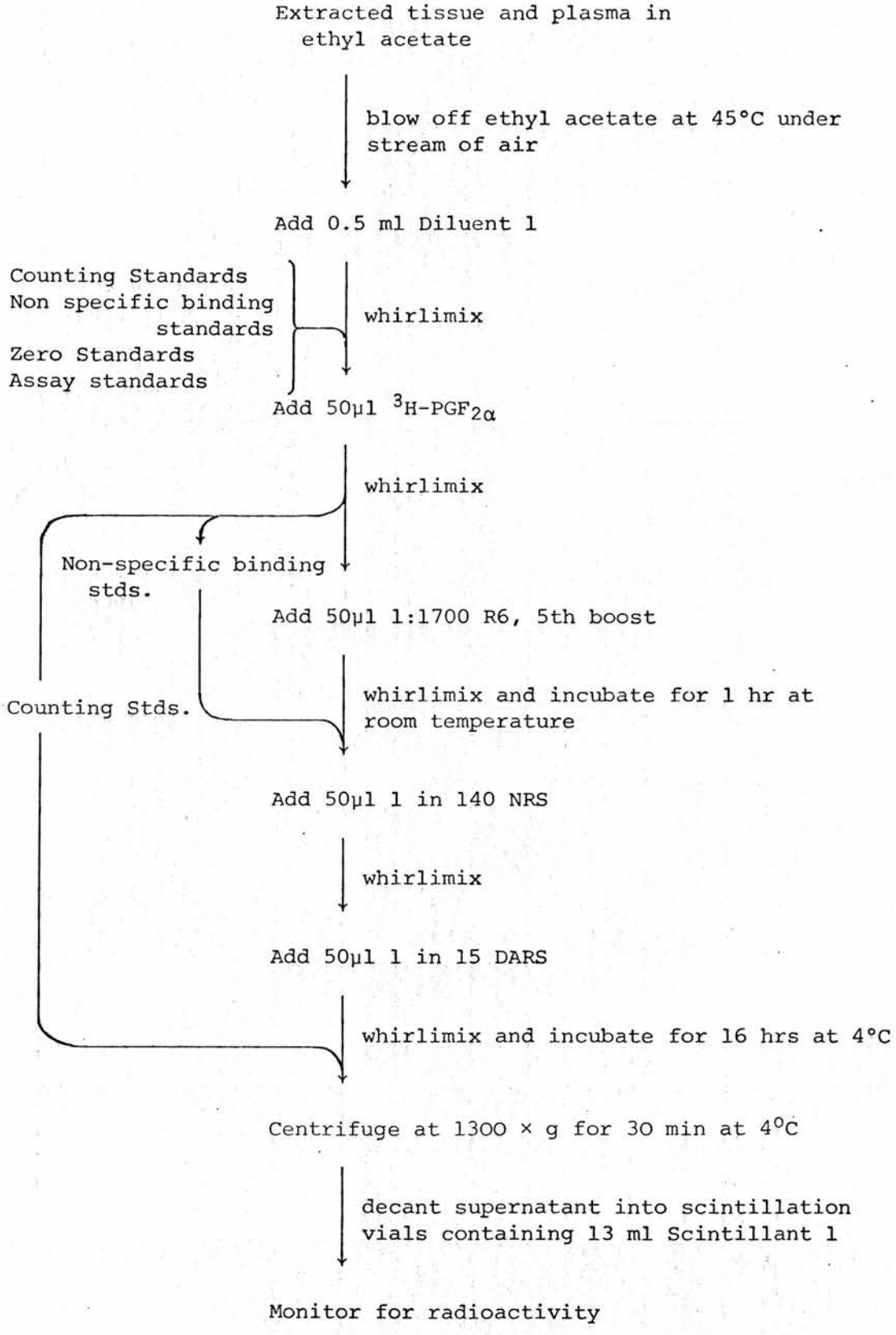
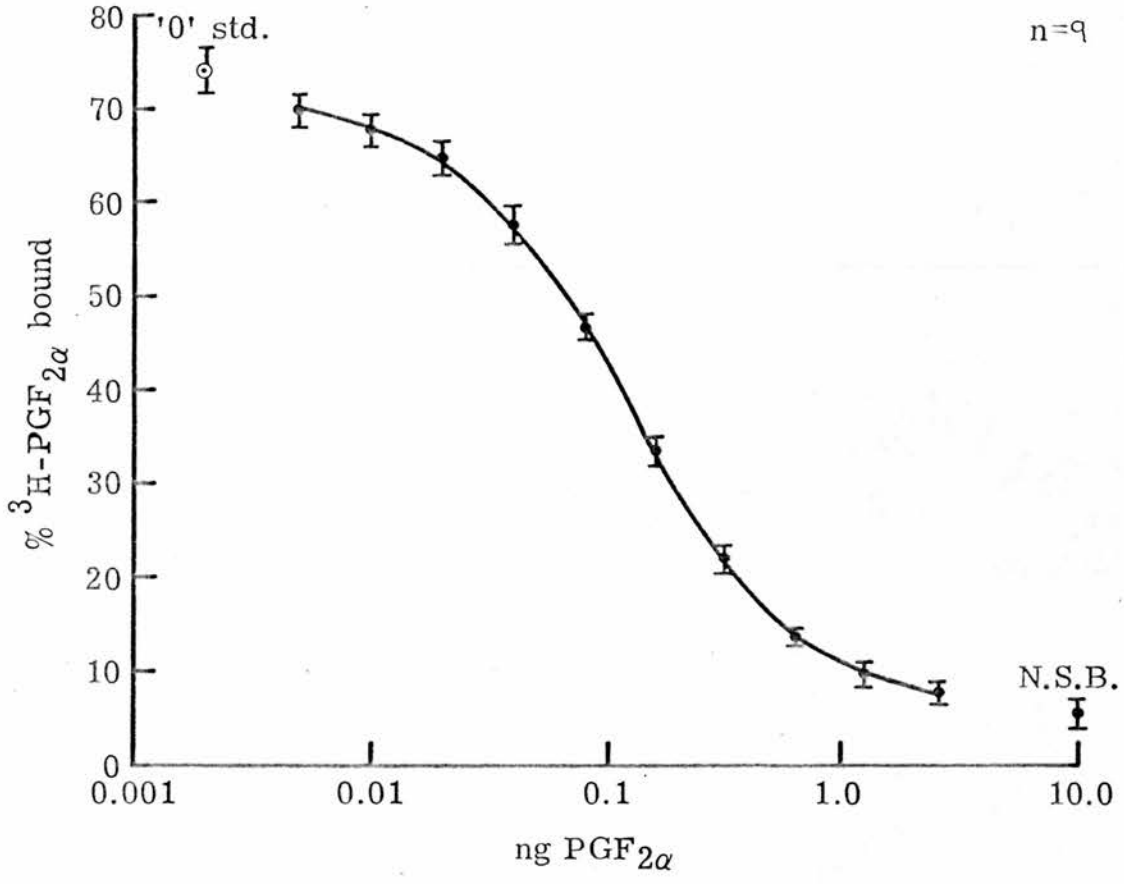


Fig. 9 Method of setting up $PGF_{2\alpha}$ Standard Curve and Assay


'0' std.
n=9
N.S.B.
% ^{3}H-PGF$_{2\alpha}$ bound
ng PGF$_{2\alpha}$
80
70
60
50
40
30
20
10
0
0.001
0.01
0.1
1.0
10.0

Results

Fig. 10 shows the standard curve from nine consecutive assays (mean ± SEM).

The limit of sensitivity was 0.024 ng.

The intra-assay coefficient of variation was 7.87%.

The inter-assay coefficient of variation was 7.90%.

An 'external standard' of 0.25 ng per tube was incorporated in all assays. The mean calculated value for this standard as determined over 9 assays = 0.247 ± 0.019 (mean ± SEM).

The cross reactivities of antiserum R-6, 5th boost with other prostaglandins was determined by Ms. L. Simon and are shown in Table 8.

Compound	% Cross reactivity
$PGF_{2\alpha}$	100
$PGF_{1\alpha}$	100
$PGF_{2\beta}$	0.83
PGE_2	0.80
PGE_1	0.65
PGA_2	0.04
PGB_2	0.14
PGD_2	3.4
15-oxo-$PGF_{2\alpha}$	2.8
13,14-dihydro,15-oxo-$PGF_{2\alpha}$	0.67
6-keto-$PGF_{1\alpha}$	1.2
TXB_2	0.07

Table 8. Cross reactivity of Antiserum obtained from rabbit 6, 5th boost.

Conclusion

The antiserum showed negligible cross reaction with all prostaglandins tested except $PGF_{1\alpha}$ with which it showed 100% cross reaction. All measurements made using this assay are quoted as concentrations of PGF_{α} present.

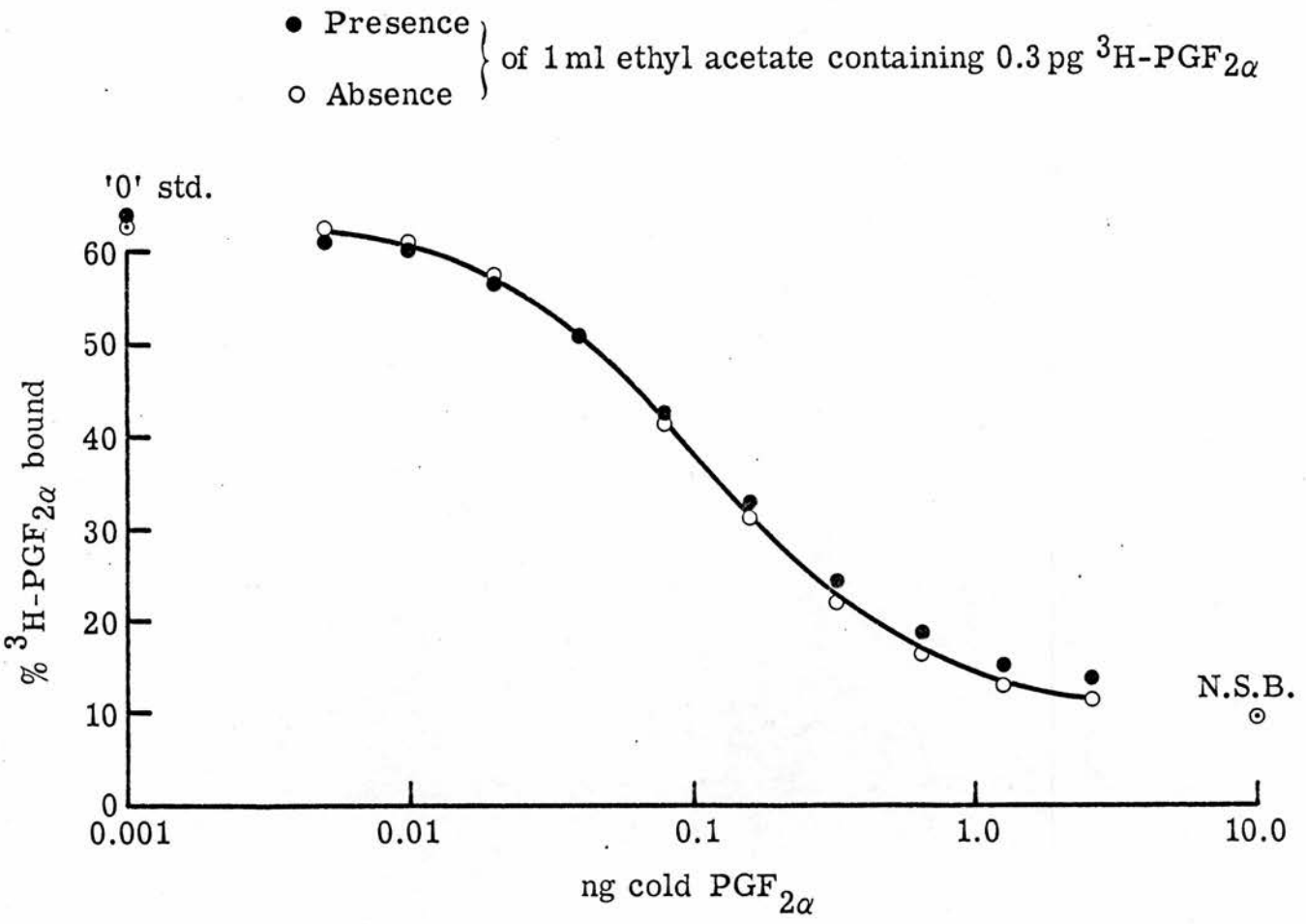
Experiment to determine the effect of the addition of 0.0013μCi ^{3}H-$PGF_{2\alpha}$ on the $PGF_{2\alpha}$ Standard curve

Introduction

The measurement of antigen by radioimmunoassay relies on the observation that a given amount of antigen produces a given percentage inhibition of binding of radioactive antigen to antiserum providing the amount of radioactive antigen and the dilution of antiserum are kept constant. It is important therefore that when a radioactive tracer is used as an internal standard in samples to be assayed by RIA, the internal standard does not contribute significantly to the mass and/or radioactivity of the tracer added for assay purposes.

Method

The maximum mass of ^{3}H-$PGF_{2\alpha}$ present in extracted plasma samples is 0.3 pg ml^{-1}. A solution of 0.3 pg ml^{-1} ^{3}H-$PGF_{2\alpha}$ in ethyl acetate was prepared. 1 ml of this solution was placed in each of 42 assay tubes. The ethyl acetate was blown off at 45°C under a jet of air and the tubes used to set up a $PGF_{2\alpha}$ standard curve as previously described. A second standard curve was set up in parallel using untreated assay tubes. Both standard curves were counted, the curves calculated and each set of corresponding points analysed for differences using Students' 't' test.


● Presence
○ Absence
of 1 ml ethyl acetate containing 0.3 pg ^{3}H-PGF$_{2\alpha}$
'0' std.
N.S.B.
% ^{3}H-PGF$_{2\alpha}$ bound
0
10
20
30
40
50
60
0.001
0.01
0.1
1.0
10.0
ng cold PGF$_{2\alpha}$

Results

Fig. 11 shows the effect of the addition of 0.0013μCi ^{3}H-$PGF_{2\alpha}$ on the $PGF_{2\alpha}$ standard curve. Although there was a slight decrease in the percentage binding of the zero standards and a slight increase in binding of the tracer at the lower end of the curve, the two curves did not differ significantly ($p > 0.05$) from one another.

Conclusion

The addition of 0.0013μCi ^{3}H-$PGF_{2\alpha}$ to rabbit plasma samples prior to extraction does not interfere with the assay of these samples by RIA.

Section 1.4c 6-Keto-$PGF_{1\alpha}$ Radioimmunoassay

The antiserum to 6-keto-$PGF_{1\alpha}$, a stable metabolite of prostaglandin I_2 (PGI_2), was raised in rabbits immunised against a conjugate of 6-keto-$PGF_{1\alpha}$-thyroglobulin as described by Dighe, Jones and Poyser (1978).

Reagents

Diluent 3

Scintillant 1

^{3}H-6-keto-$PGF_{2\alpha}$ - was synthesised from octa-tritiated arachidonic acid incubated with a sheep uterus microsomal enzyme preparation and purified as described by Dighe, Jones and Poyser (1978). The tracer was made up in diluent 3 at a concentration of 0.23μCi ml^{-1} (sp. act 150μCi $mmol^{-1}$)

Antibody - Rabbit NP-1, 6th boost was used at a dilution of 1 in 650 in diluent 3

DARS - 1 in 8 dilution in diluent 3.

Method

A series of standard solutions of cold 6-keto-$PGF_{1\alpha}$ ranging from 0.2 ng ml^{-1} to 10.24 ng ml^{-1} were made up in diluent 3. The assay was set up using the method previously described for the PGE_2 standard curve.

Results

Fig. 12 shows the standard curve from seven consecutive assays.

The sensitivity was 70 pg.

The intra-assay coefficient of variation was 9.01%, as measured over 7 consecutive assays.

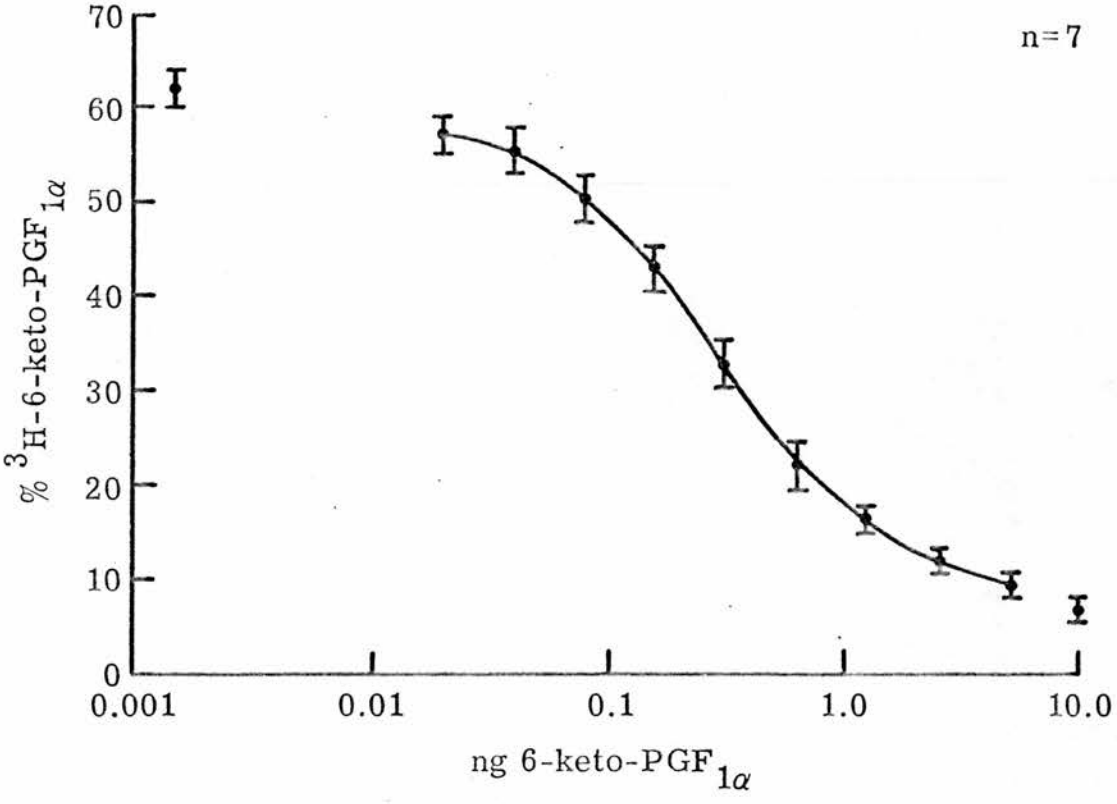
The inter-assay coefficient of variation was 12.01%.

An 'external standard' of 0.2 ng per tube was incorporated in all assays. The mean calculated value of the 'external standard' was 0.198 ± 0.240 (mean ± SEM). This value also reflects the accuracy and precision of the assay.

The cross reactivities of antiserum NP-1, 6th boost with other prostaglandins were determined by Ms. I. Ramsay and are as shown in Table 9.

Conclusion

Antiserum NP-1, 6th boost shows negligible cross reactivity with all other prostaglandins tested and this together with the high precision and accuracy make this a suitable assay for the determination of 6-keto-$PGF_{1\alpha}$.


n=7
70
60
50
40
30
20
10
0
% ^{3}H-6-keto-PGF$_{1\alpha}$
0.001
0.01
0.1
1.0
10.0
ng 6-keto-PGF$_{1\alpha}$

Compound	% Cross Reaction
6-keto-$PGF_{1\alpha}$	100
PGE_2	4.2
PGE_1	1.1
$PGF_{2\alpha}$	4.0
$PGF_{1\alpha}$	0.43
PGA_2	0.065
PGB_2	0.03
PGD_2	< 0.01
TXB_2	< 0.01
15-oxo-PGE_2	0.08
13,14-dihydro,15-oxo-PGE_2	0.09
13,14-dihydro,15-oxo-$PGF_{2\alpha}$	0.065

Table 9. Percentage Cross Reaction of NP-1, 6th boost

Section 1.4d Progesterone Radioimmunoassay

The progesterone antiserum was supplied by Dr. K. Dighe and Dr. W.M. Hunter and was raised in rabbits immunised with a conjugate of 11α-hydroxyprogesteronehemisuccinate-bovine serum albumin. Details of the preparation of conjugate and testing of the antisera have been described by Dighe and Hunter (1974).

Reagents

Diluent 2

Scintillant 2

^{3}H-Progesterone - 0.07μCi ml^{-1} in diluent 2

N.R.S. - 1 in 140 dilution in diluent 2

Reagents (continued)

D A R S - 1 in 15 dilution in diluent 2

Antibody R353, 5th bleed, was used at 1:2000 dilution in diluent 2

Method

Five standard solutions of progesterone, ranging from 0.1 ng ml^{-1} to 12.8 ng ml^{-1} were made up in ethanol and used to set up a series of tubes as detailed in Table 10. Each standard was set up in triplicate. Counting standards, non-specific binding standards and zero standards containing 1.15 ml, 1.05 ml and 1.0 ml respectively were set up in quadruplicate. The standard curve was set up as shown in Fig. 13.

Progesterone standards in ethanol ng ml^{-1}	Vol ml $tube^{-1}$	Progesterone ng $tube^{-1}$
12.8	0.2	2.56
	0.1	1.28
0.32	0.2	0.64
	0.1	0.32
0.08	0.2	0.16
	0.1	0.08
0.02	0.2	0.04
	0.1	0.02
0.05	0.2	0.01
	0.1	0.005

Table 10. Concentration of Progesterone standards used in setting up the Progesterone standard curve together with the volumes and amounts of each standard used.

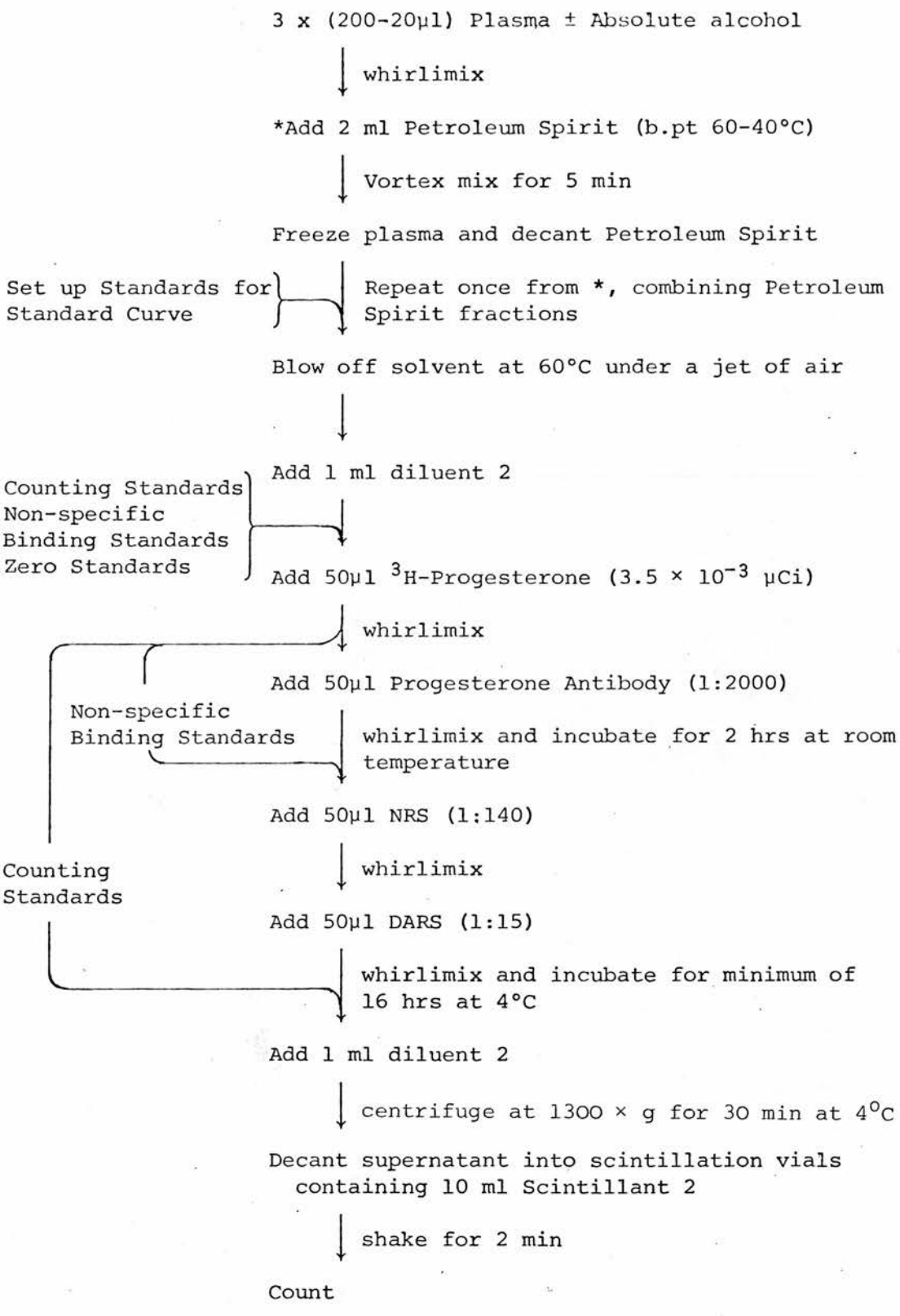


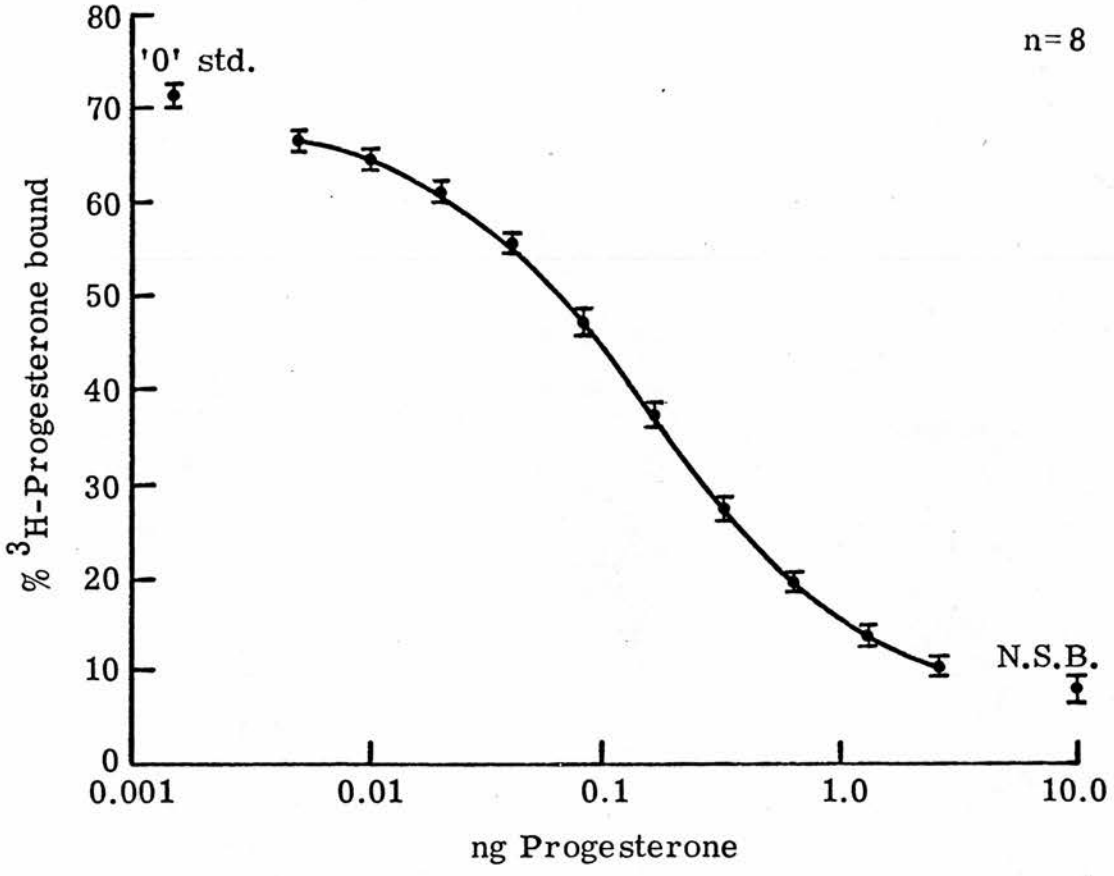
Fig. 13 Method for Progesterone extraction and setting up of Standard Curve and Assay

Results

The standard curve from eight consecutive assays is shown in Fig. 14. The sensitivity of the assay was 18 pg. The intra-assay coefficient of variation was 8.55% (n = 8). The inter-assay coefficient of variation was 9.8% (n = 8). The cross reactivities of this antiserum were determined at 50% inhibition of binding of the tracer and were performed by Dr. K.K. Dighe; they are as shown in Table 11.

Compound	% Cross Reactivity
Progesterone	100%
11α-OH-Progesterone	29.0
11β-OH-Progesterone	12.0
17α-OH-Progesterone	1.0
11-deoxycorticosterone	1.0
11-deoxycortisol	0.2
Cortisol	0.01
Pregnenolone	0.2
Cholesterol	0.002
Androstenedione	0.03
20α-hydroxypregn-4-ene-3-one	0.9
20β-hydroxypregn-4-ene-3-one	0.05
5α-Pregnane-3,20-dione	12.0
5β-Pregnane-3,20-dione	7.0

Table 11. Cross-reactivity of progesterone antiserum as determined by K.K. Dighe using the method of Dighe & Hunter (1974).


80
70
60
50
40
30
20
10
0
'0' std.
n=8
% ^{3}H-Progesterone bound
N.S.B.
0.001
0.01
0.1
1.0
10.0
ng Progesterone

Conclusion

The progesterone antiserum shows high cross reactivity with 11α-OH-progesterone, 11β-OH-progesterone and 5α-Pregnane-3,20-dione. However, it shows low cross reaction with 20α-hydroxypregn-4-en-3-one which is the only other progestin found in the rabbit in any appreciable amounts (Simmer, Hilliard and Archibald, 1963). The progesterone antibody was therefore considered to be suitable for the measurement on progesterone in rabbit plasma samples.

Measurement of the precision and accuracy of the progesterone RIA.

The efficiency of the extraction procedure was determined by adding known amounts of progesterone to 0.2 ml of ear vein plasma collected from an ovariectomised rabbit, and estimating the amount of progesterone received.

Method

Two sets of tubes were set up in triplicate containing different amounts of progesterone in ethanol. The ethanol was evaporated off at 60°C under a jet of air and 0.2 ml plasma was added to each tube. In addition 0.07μCi ^{3}H-Progesterone was added to one set of tubes and to four scintillation vials containing 13 ml scintillant 3 which acted as counting standards. Both sets of tubes were extracted as previously described in section 1.3d and the petroleum spirit blown off. The residue from the tubes containing added tracer was redissolved in 0.5 ml methanol, transferred to liquid scintillation vials containing 13 ml scintillant 3 and counted. The residue from the remaining samples was redissolved in 1 ml diluent 2, and assayed by RIA.

Amount added Progesterone ng	% ^{3}H-Progesterone recovered	Progesterone recovered as determined by RIA		No. determinations
		ng	%	
0	93.38 ± 1.23	0	-	3
0.64	92.13 ± 0.43	0.62 ± 0.02	96.87 ± 3.1	3
10.00	92.09 ± 1.53	10.58 ± 1.12	103.00 ± 11.10	3
50.00	92.63 ± 0.43	48.88 ± 6.14	97.7 ± 12.23	3
100.00	94.85 ± 0.30	97.18 ± 4.93	97.18 ± 4.93	3
150.00	93.23 ± 1.25	141.93 ± 11.54	95.00 ± 7.69	3

Table 12. Comparison of percentage recovery of progesterone as determined by RIA and recovery of ^{3}H-progesterone

Results

The amount of Progesterone in 1 ml of plasma was 0.57 ng ml^{-1}. Table 11 shows the amount of Progesterone recovered as measured by RIA following solvent extraction. All values have been corrected for a 'blank' value of 0.57 ng ml^{-1}.

Conclusion

The percentage recovery of ^{3}H-Progesterone is in good agreement with the percentage recovery of progesterone as determined by RIA and is unaffected by the amount of progesterone present. Moreover the amounts of progesterone present, as determined by RIA show that this assay exhibits a high degree of accuracy.

Section 1.5 Gas chromatography - Mass spectrometry

Introduction

Although radioimmunoassay offers a very sensitive method for measuring small amounts of biologically active material on a routine basis, it has the disadvantage that it does not provide a definitive identification of the compounds measured. Within certain limits the ability of the antisera to discriminate between the antigen to which it has been raised and other structurally similar molecules is determined from cross-reactivity studies. However, such studies are confined to compounds already known to exist and which are available in a pure form. Moreover, even when the cross reactivity of the antiserum is known, the ability of the antisera to measure the required antigen will depend on the relative amounts present of the antigen and other substances with which it cross reacts. Selective extraction procedures help to minimise this type of error but cannot eliminate it. A more positive identification of a

compound can be obtained from its mass spectrum.

As a detection system the mass spectrometer offers both high sensitivity and high specificity especially when coupled to a gas liquid chromatography system. Consequently following measurement by RIA, extracts of plasma and tissue homogenates were deriv tized and subjected to further analysis by combined gas-chromatography - mass spectrometry (g.c. - m.s.).

The g.c. - m.s. data given in the subsequent sections were obtained using a VG-Micromass 70-70F mass spectrometer coupled to a Pye 204 gas chromatograph by means of a single stage glass separator. The gas chromatograph was equipped with a 3m spiral glass column of 4 mm internal diameter, packed with 3% OVI on supelcoport 100-120 mesh (Supelco Inc., Bellefonte, Pennsylvania, U.S.A.). The oven temperature was 265°C, and the carrier gas was Helium which flowed at a rate of 30 ml min^{-1}.

Typical operating conditions for the mass spectrometer were :-

Separator temperature	250°C
Ion source temperature	250°C
Electron Energy	22-70 eV
Accelerating Voltage	4 kV
Signal Amplification	5×10^{-6} amps

Mass spectra were recorded using a light beam oscillograph (S.E. Labs. (EMI) Ltd., Feltham, England). When there was insufficient material present to obtain a full mass spectrum the V.G.-Micromass 70-70F was used in the selected ion monitoring mode (MID) with fixed magnet current and voltage scanning. The exact mass measurement of the ion fragments from the OVI phase column bleed, determined

by using perfluorokerosene as a calibration standard, were used for reference purposes. The mass spectrometer was tuned to the required ion fragments for selected ion monitoring by reference of their precise masses to the precise mass of the nearest column bleed ion fragment. Single ion chromatograms were recorded on a multi-pen recorder (Rikadenki, Mitsui Electronics (U.K.) Ltd.). A mixture of straight chain saturated fatty acid methyl esters (1µg μl^{-1}) were used to calibrate the g.c. column. The retention time of each standard fatty acid was plotted, on a logarithmic scale, against the number of carbon atoms in the fatty acid used (Bergström, Ryhage, Samuelsson and Sjövall, 1963) and the graph obtained used to convert the observed retention times of the prostaglandins into carbon values. This fatty acid mixture also provided an indication of the chromatographic efficiency of the column and of the sensitivity of the mass spectrometer prior to analysis of the samples.

Section 1.5a Derivatization of prostaglandins for combined gas liquid chromatography - mass spectrometry

Introduction

The presence of carboxyl and hydroxyl groups in the free prostaglandins make these compounds too polar to be run directly on a gas chromatograph. Various derivatives therefore have been used to reduce the polarity of these functional groups. Carboxcyclic acid groups on the prostaglandin molecules were protected by methyl ester formation, free hydroxyl groups were protected by trimethylsilyl ether formation and free ketone groups were protected by O-butyloxime formation. 6-Keto-$PGF_{1\alpha}$ with four deuterium atoms substituted at positions 4 and 5 (d_4-6-keto-$PGF_{1\alpha}$) was used as an

internal standard and also served to improve the sensitivity of the g.c. - m.s. system.

Method

All reactions were performed in 1 ml Eppendorf tubes which had previously been rinsed in chloroform and methanol. Extracted samples and authentic prostaglandin standards in methanol were transferred to the Eppendorf tubes and 500 ng d_4-6-keto-$PGF_{1\alpha}$ (ie 100µl of a 5µg d_4-6-keto-$PGF_{1\alpha}$ ml^{-1} in methanol) was added to each tube. The methanol was evaporated off at 45°C under a jet of air. Two drops of methanol were added to each tube followed by the addition of approximately 1 ml diazomethane solution (9 parts diethyl-ether : 1 part methanol). The contents were allowed to react for 5 min at room temperature. The solvent was removed at 45°C under a stream of air and the process repeated. Following removal of the solvent again, the residue was vacuum dessicated for 10 min.

n-Butyloxime formation

3-5 drops of 5 mg ml^{-1} O-butylhydroxylamine hydroxylchloride in dry pyridine were added to all tubes, the tubes stoppered and left at room temperature overnight. The following morning the samples were heated for 15 min at 60°C to complete the reaction. The pyridine was evaporated off under a jet of air and the sample vacuum desiccated for 15 min.

Trimethylsilyl ether formation

10-30µl bis(trimethylsilyl)-trifluoroacetamide (BSTFA) were added to each tube, the tubes stoppered and heated for 15 min at 60°C. Samples were injected in the BSTFA into the gas chromatograph.

Results

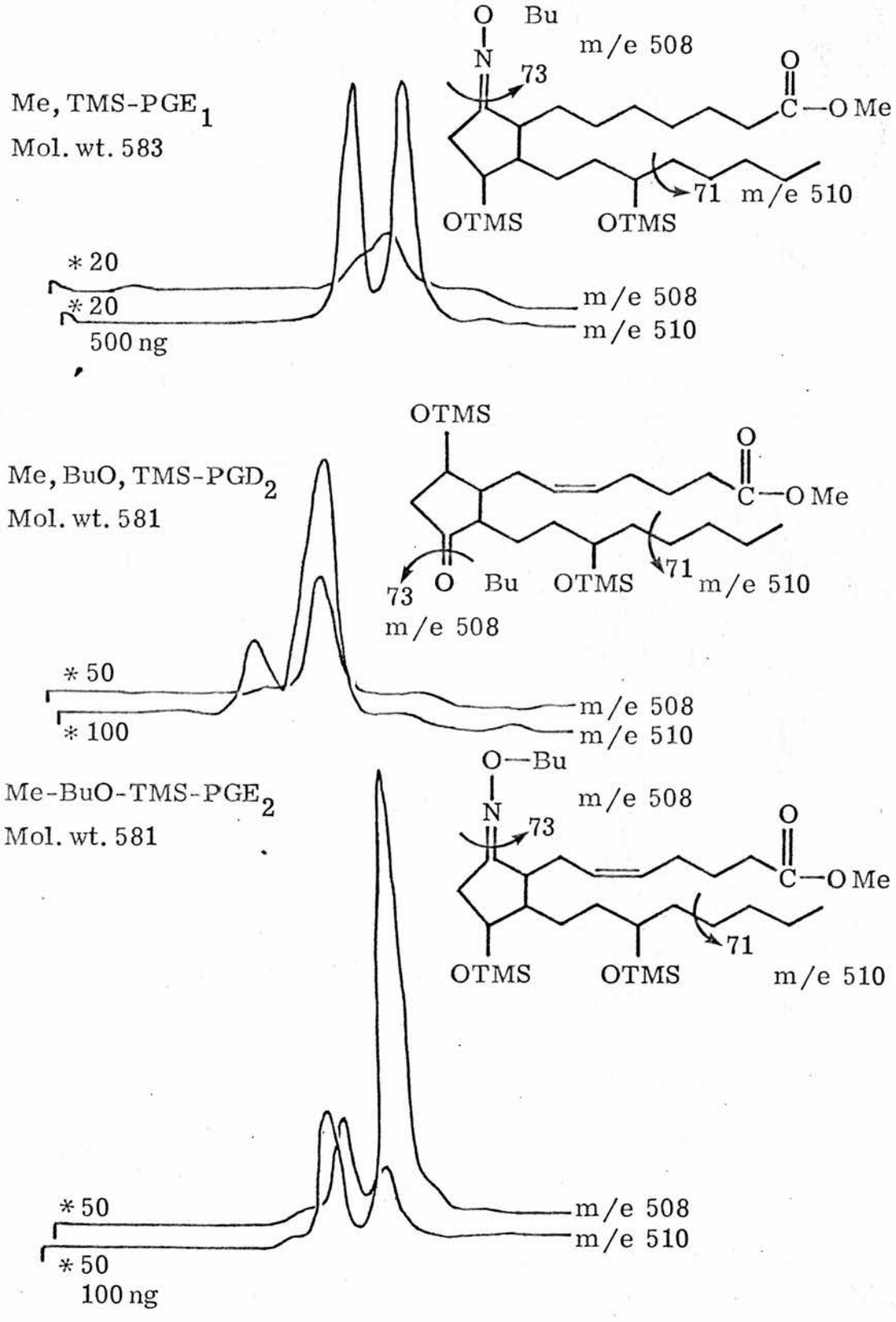
Fig. 15 and Fig. 16 show typical chromatograms obtained by selected ion monitoring of authentic prostaglandin standards following derivatization and using the 503 column bleed ion fragment as the "lock" peak. The carbon values of the various standards used are shown in Table 13.

Compound	Derivative	Ion Monitored	Carbon value Cv
$PGF_{2\alpha}$	Me,TMS	423	23.8
$PGF_{1\alpha}$	Me,TMS	425	24.4
d_4-6-keto-$PGF_{1\alpha}$	Me,BuO,TMS	495	26.4
6-keto-$PGF_{1\alpha}$	"	508	26.4
PGE_1 1st isomer	"	510	25.95
PGE_1 2nd isomer	"	510	26.25
PGE_2 1st isomer	"	508	25.52
PGE_2 2nd isomer	"	508	26.1
PGD_2 1st isomer	"	510	25.1
PGD_2 2nd isomer	"	510	25.8

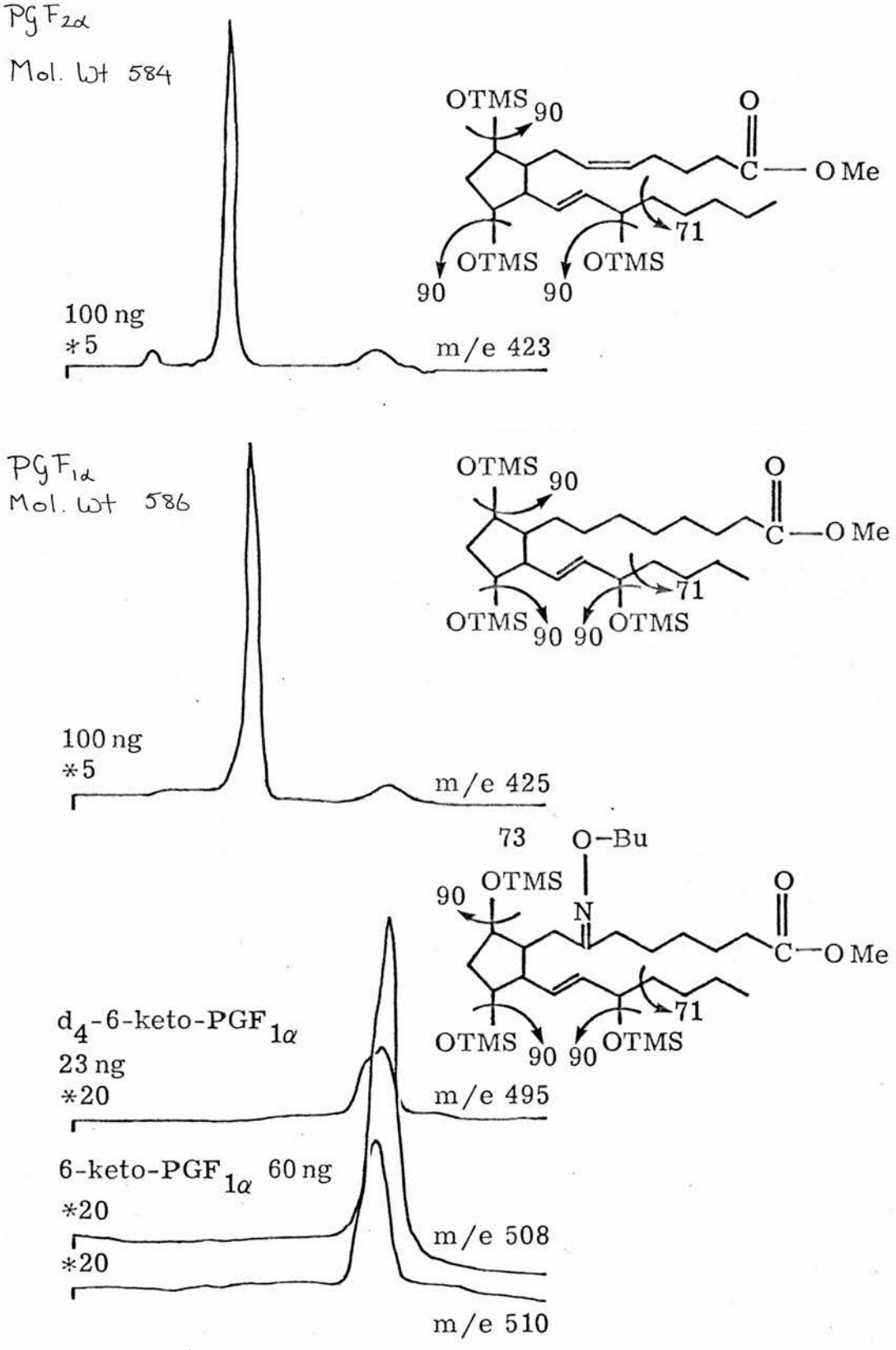
Table 13. Carbon values for the methyl trimethylsilyl ether (Me,TMS) and methyl, trimethylsilyl butoxime (Me,BuO,TMS) derivatives of prostaglandin standards

Conclusion

The derivatization procedure and conditions of operation of the g.c. - m.s. used gave a good separation of the different prostaglandins in the gas chromatograph. Therefore, the detection in a sample of the selected ions, at the correct retention time for a particular



* Voltage gain



* Voltage gain

prostaglandin provides further evidence for the presence of that prostaglandin in the sample. The lower limit of sensitivity of this system for the prostaglandin standards was approximately 20 ng.

Ideally, for absolute identification of a prostaglandin in an extracted sample, a full mass spectrum should be taken and compared with the mass spectrum produced by the authentic compound. However, this requires the presence of about 300 ng of the extracted prostaglandin, especially when injected into the g.c. as an extract rather than the pure compound. Consequently, it was not always possible to obtain a full mass spectrum of the extracted prostaglandin in all instances.

SECTION 2. CONCENTRATIONS OF $PGF_{2\alpha}$ AND PGE IN THE UTERINE VENOUS PLASMA OF PSEUDOPREGNANT AND PREGNANT RABBITS

Section 2.1 Pseudopregnancy

Introduction

In certain sub-primate, mammalian species, removal of the uterus prolongs the functional life span of the corpus luteum of the oestrous cycle or of pseudopregnancy (Anderson, Bland and Melampy, 1969). In the cycling sheep and guinea-pig there is strong evidence to suggest that $PGF_{2\alpha}$ acts as a uterine luteolytic hormone as discussed in the general introduction. In both species hysterectomy prolongs luteal function (Wiltbank and Casida 1956, Loeb, 1923) while the administration of exogenous $PGF_{2\alpha}$ induces luteolysis (Blatchley and Donovan, 1969; McCracken *et al.*, 1970). Furthermore, the concentration of $PGF_{2\alpha}$ in the uterine venous plasma increases immediately prior to the onset of luteal regression at the end of the oestrous cycle (Blatchley, *et al.*, 1972; Earthy *et al.*, 1975; Bland, Horton and Poyser, 1971; Thorburn *et al.*, 1972). Experiments designed to negate the luteolytic effect of the uterus, either by inhibiting prostaglandin synthesis with indomethacin (Lewis and Warren,1975; Marley 1973; Horton and Poyser, 1973) or, more specifically, by immunisation against $PGF_{2\alpha}$ (Horton and Poyser 1974, Scaramuzzi *et al.*, 1974) result in a lengthening of the oestrous cycle.

Unlike the sheep and guinea-pig, the rabbit is a reflex ovulator and does not have an ovarian cycle. Under normal conditions mating results in pregnancy, however, if the mating is infertile the rabbit enters into a state of pseudopregnancy which lasts for between 16 to 18 days, approximately half the length of the normal gestation period. Chu *et al.* (1944) have shown that when hysterectomy is

performed between Days 11 to 14 of pseudopregnancy the life span of the corpora lutea is extended by some 11 to 18 days, and that this effect is abolished by the presence of endometrial implants. $PGF_{2\alpha}$ is luteolytic in rabbits when administered either subcutaneously or intravenously on 4 consecutive days of pseudopregnancy (Gutknecht, Duncan and Wyngarden 1970). Following treatment with indomethacin (8 mg kg^{-1} every 12 hr) from Day 12 of pseudopregnancy, the peripheral plasma progesterone levels are significantly higher than those observed in untreated rabbits from this time onwards and do not return to basal levels until Day 26, approximately 10 days later than in normal pseudopregnancy (O'Grady *et al.*, 1972). It has therefore been suggested that $PGF_{2\alpha}$ may also act as a uterine luteolysin in the pseudopregnant rabbit. The purpose of the following experiments was to investigate this hypothesis further and to determine whether the onset of luteolysis is immediately preceded by an increase in the concentration of $PGF_{2\alpha}$ in the uterine venous plasma.

Section 2.1a Experiment to determine whether the onset of luteolysis in the pseudopregnant rabbit is preceded by an increase in the concentration of $PGF_{2\alpha}$ in the uterine venous plasma

Method

Collection of blood samples

Forty-eight, female, New Zealand white rabbits were made pseudopregnant by the intravenous (i.v.) injection of 500 IU Human chorionic gonadotrophin (HCG) into the marginal ear vein, between 15.00 and 15.30 hr. The day of injection was designated Day 0 of pseudopregnancy. The animals were divided into 12 groups of 4 animals per group, and each group was sacrificed on a specific day of pseudopregnancy.

On the selected day rabbits were anaesthetised by i.v. injection of urethane (7 ml Kg^{-1} of 25% solution of ethyl carbamate in 0.9% saline) and fully heparinised by the concomitant injection of 1500 IU Heparin Kg^{-1}. Blood samples were collected from the marginal ear vein, the left and right uterine veins and the aorta. A mid line abdominal incision was made to expose the uterus, and the uterine veins were dissected free of fat. Care was taken to avoid undue handling of the uterus and surrounding tissues. In the rabbit the uterine vein has several prominent branches. The vessels draining the caudal part of one uterine horn also drain the vagina and show a large degree of anastomosis with vessels draining the caudal part of the second horn (Del Campo and Ginther, 1972). Blood samples were therefore collected from branches of the uterine vein draining the cranial end of the uterine horn in an attempt to avoid sampling the venous effluent from the vagina and the second horn. Once exposed, therefore, an appropriate branch of the uterine vein was cannulated using a 2 G 1½" B.D. Yale microlance needle attached to a 3" piece of narrow bore, teflon tubing. The blood was allowed to flow freely and was collected in a glass vial surrounded by crushed ice. In certain instances following cannulation, no blood flowed through the cannula, possibly because the tip of the needle became blocked during entry through the vessel wall or because the resistance to flow offered by the cannula was too great. Under these circumstances the cannula was flushed with warm saline, but if this failed to induce blood flow the cannula was withdrawn and the vein recannulated. 20 ml blood was collected from the uterine vein on each side. The ear vein sample (20ml) was collected by incising the marginal ear vein with a scalpel and collecting the venous

effluent, while the aortic sample (20 ml) was obtained by cannulation of the aorta. In both instances the blood was collected in glass vials surrounded by crushed ice. The animal was then killed by exsanguination.

Blood samples were stored in crushed ice until the last sample had been collected. They were then centrifuged at 4°C for 20 min at 1300 × g on a MSE Coolspin centrifuge. The plasma was transferred to universal bottles by means of a pasteur pipette and stored at -20°C until extraction. Plasma samples were extracted within 1 week of collection.

All rabbits had their ovaries checked for the presence of corpora lutea and if these were not present in both ovaries the results from that animal were discarded and the animal replaced.

Extraction of blood samples

Prior to assay by RIA plasma samples underwent solvent extraction followed by silicic acid column chromatography as described in sections 1.3a and 1.3b. Following chromatography, fraction 3 (PGE) and fraction 5 (PGF) were taken to dryness at 45°C on a rotary evaporator, and drying was completed in a vacuum dessicator. The residue in each fraction was redissolved in 10 ml ethyl acetate and stored at -20°C until assayed for PGE and PGF_{α}, as described in Sections 1.4a and 1.4b respectively (see Fig. 4 and Fig. 7). 1 ml of ear vein plasma from each animal was extracted and assayed for progesterone as described in sections 1.3d and 1.4d. All determinations were performed in triplicate at two dilutions.

Identification of extracted plasma prostaglandins by g.c. - m.s.

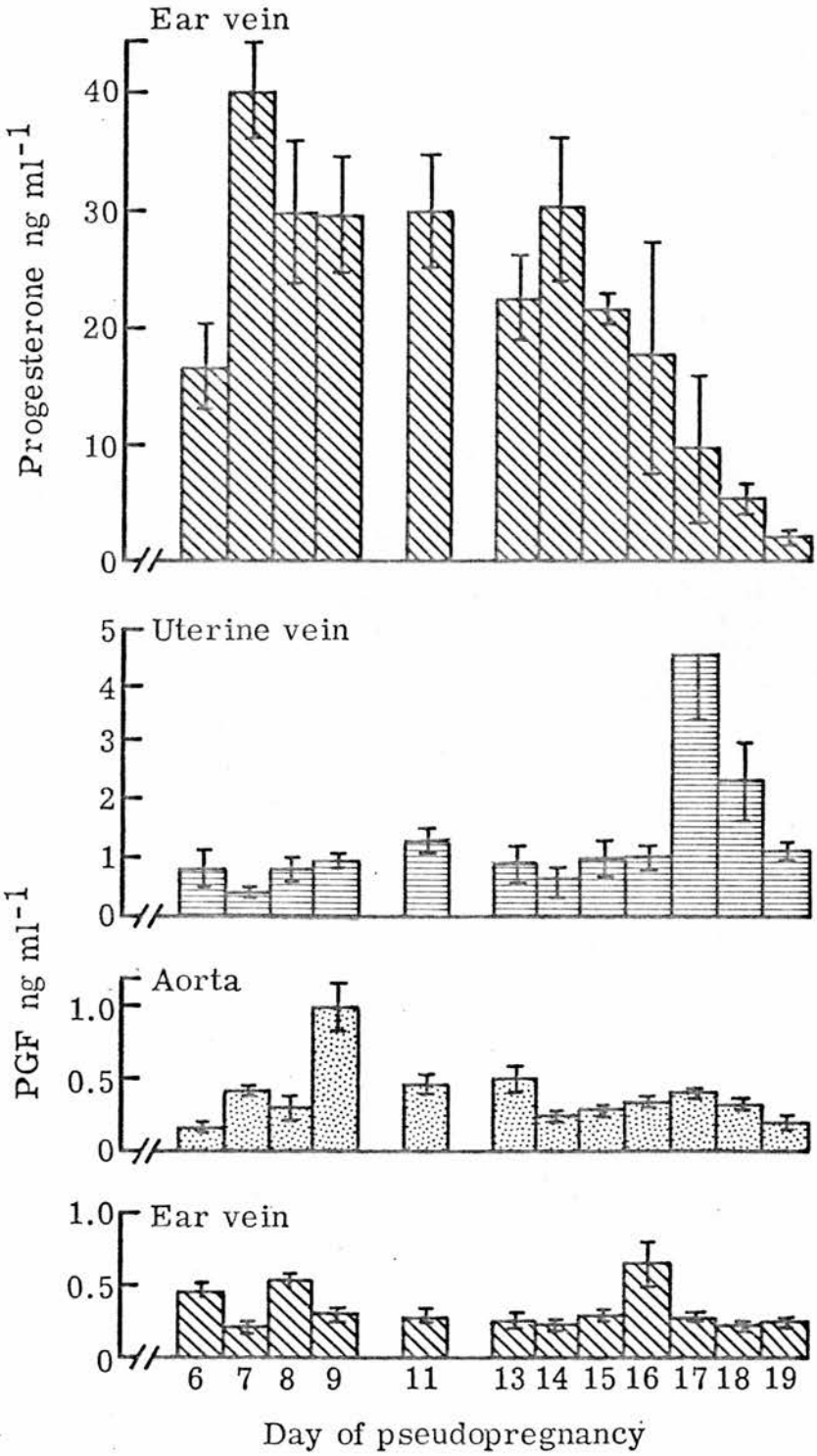
Fractions 3 of the extracted ear vein plasma samples collected throughout pseudopregnancy were pooled and taken to dryness on a rotary evaporator. The residue was redissolved in 0.5 ml methanol and transferred to an Eppendorf vial. The flask was washed with a further 0.5 ml methanol and the wash added to the Eppendorf vial. The methanol was blown off at 45°C under a jet of air. 500 ng d_4-6-keto-$PGF_{1\alpha}$, in methanol, was added to each vial, the methanol blown off and the sample derivatized as described in section 1.5a to form the methyl ester, n-butyloxime, trimethylsilyl ether (Me,BuO,TMS derivative). Similarly the following samples were pooled and derivatized.

1) Fraction 3 extracted uterine vein plasma (left and right sides)
2) Fraction 5 extracted uterine vein plasma (left and right sides)
3) Fraction 3 extracted aortic plasma
4) Fraction 5 extracted aortic plasma
5) Fraction 5 extracted ear vein plasma

Trimethylsilyl ether formation was performed using 30µl BSTFA and samples were injected into the g.c. in volumes of 5µl. The m.s. was set up for MID as described in section 1.5a, using the 503 ion fragment from the column bleed as the lock ion. Samples were monitored for $PGF_{2\alpha}$ (m/e 423), $PGF_{1\alpha}$ (m/e 425), 6-keto-$PGF_{1\alpha}$ (m/e 491), PGE_2 (m/e 508), PGE_1 (m/e 510), PGD_1 (m/e 510).

Results

The concentrations of progesterone in the peripheral venous plasma increased up to Day 7, remained elevated until Day 14, and then declined steadily to reach pre-injection levels by Day 19 (Fig. 17). The mean concentrations of PGF_{α} in the aortic and

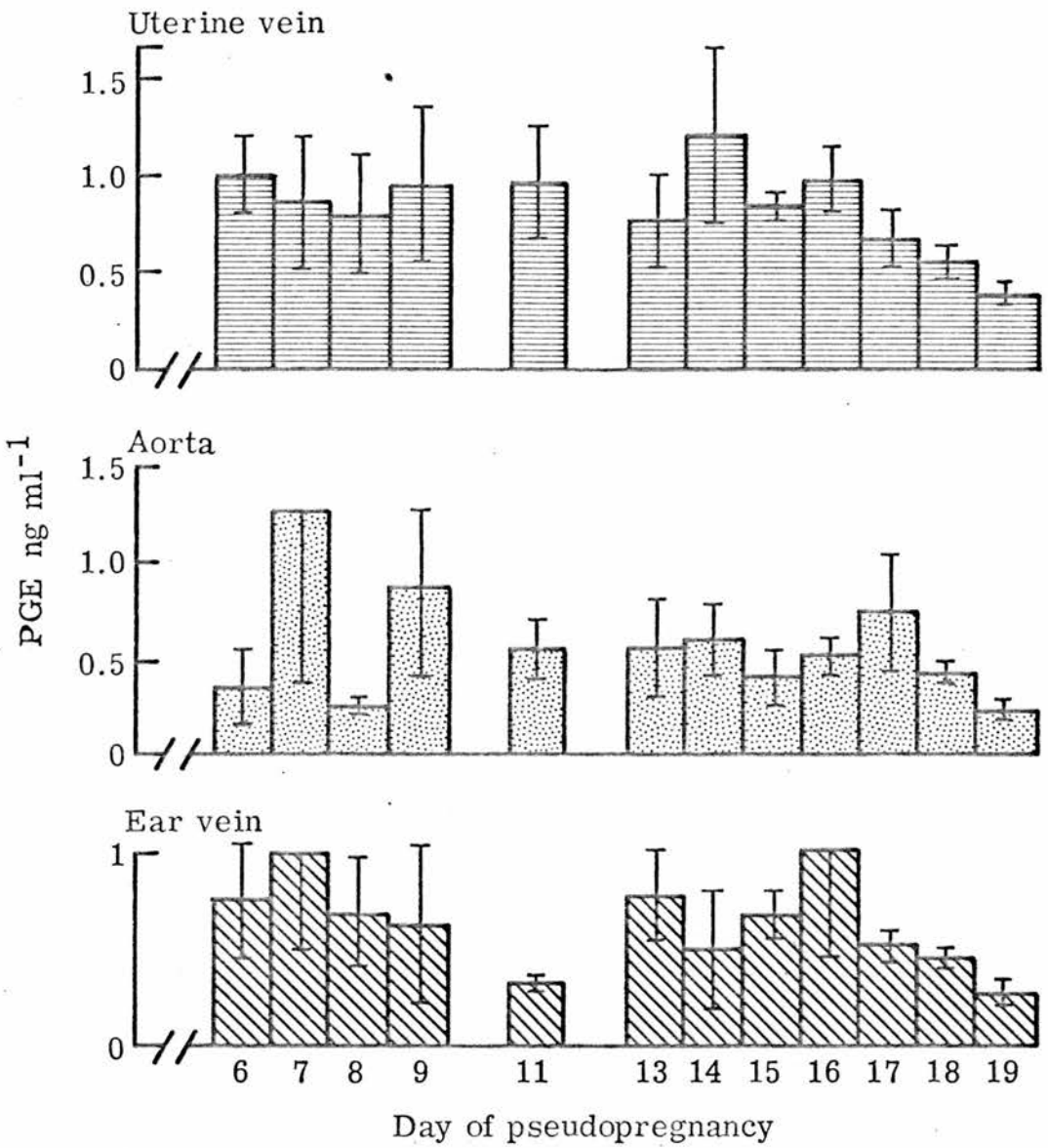

Ear vein
Progesterone ng ml-1
40
30
20
10
0
Uterine vein
5
4
3
2
1
0
PGF ng ml-1
Aorta
1.0
0.5
0
Ear vein
1.0
0.5
0
6 7 8 9 11 13 14 15 16 17 18 19
Day of pseudopregnancy

peripheral venous plasmas showed little variation, remaining below 0.5 ng ml^{-1} throughout most of pseudopregnancy (Fig. 17). The concentration of PGF_{α} in the uterine venous plasma was higher than that observed in either the aortic or peripheral venous plasma but remained low, approximately 0.75 ng ml^{-1} until Day 17 when it rose significantly ($p < 0.05$) to a concentration of 4.51 ± 1.55 ng ml^{-1}. The concentration of PGF_{α} on Day 18 of pseudopregnancy (2.34 ± 0.73 ng/ml) was significantly higher ($p < 0.05$) than on Day 16 but significantly lower ($p < 0.05$) than on Day 17. The mean concentration of PGF_{α} had returned to 'basal' levels by Day 19.

The concentrations of PGE in plasma from the ear vein, uterine vein and aorta, remained low throughout pseudopregnancy and showed no significant differences from each other although the mean concentration of PGE in the uterine venous plasma tended to be higher (Fig. 18). Analysis of the derivatized plasma samples by g.c. - m.s. was inconclusive. The amounts of PGE and PGF_{α} in the pooled, extracted plasma samples of the marginal ear vein, the left and right uterine veins and the aorta were found to be too low to be detected against the background of impurities even when MID was used.

Conclusion

The concentration of progesterone in the peripheral venous plasma had significantly decreased by Day 17, the time at which PGF_{α} levels in the uterine venous plasma increased. PGF_{α} released on Day 17, therefore, may accelerate the decrease in progesterone production and so contribute to the final demise of luteal function at this time. The low amounts of $PGF_{2\alpha}$ present in the pooled uterine venous plasma samples prevented the unequivocal identification of the extracted prostaglandin as $PGF_{2\alpha}$.


Uterine vein
1.5
1.0
0.5
0
Aorta
1.5
1.0
0.5
0
PGE ng ml-1
Ear vein
1
0
6 7 8 9 11 13 14 15 16 17 18 19
Day of pseudopregnancy

Section 2.1b Experiment to determine the effect of hysterectomy on the maintenance of the corpus luteum in pseudopregnant rabbits

Introduction

If the decline of progesterone in the peripheral venous plasma from Day 14 is independent of the release of PGF_{α} from the uterus then the removal of the uterus should not prevent the decline in plasma progesterone observed at this time. However, if the release of PGF_{α} on Days 17 and 18 of pseudopregnancy contributes to the cessation of luteal function then removal of the uterus should prolong pseudopregnancy. The effect of hysterectomy on peripheral plasma progesterone concentrations in pseudopregnant rabbits was therefore examined.

Method

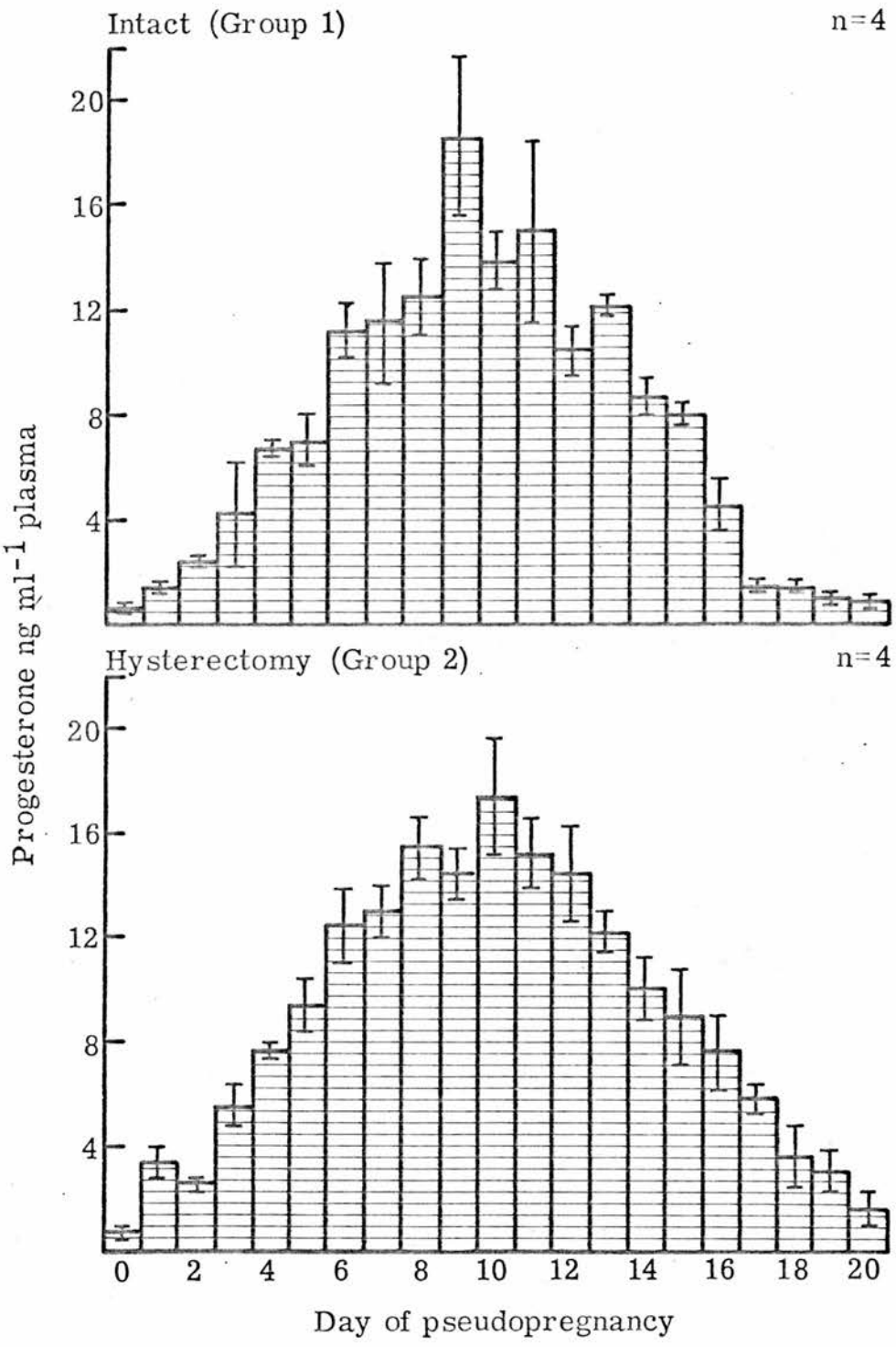
Eight New Zealand white, female, rabbits were divided into two groups of four animals per group. Animals in Group 1 were used intact while animals in Group 2 were hysterectomised prior to use. Hysterectomy was performed under Nembutal anaesthesia (35 mg Kg^{-1}). Following the opening of the abdominal wall by a mid-line, abdominal incision ligatures were placed round the oviducts, proximal to the utero-oviductal junction, round the vagina, immediately below the cervix and round the major blood vessels. The uterus was then removed, the abdominal wall closed with cat gut sutures and the skin with silk sutures. Animals were allowed to recover for 4 weeks before use. Rabbits in both groups were made pseudopregnant by the i.v. administration of HCG as previously described in section 2.1a. Blood samples were collected from alternate ears, every second day throughout pseudopregnancy. All animals underwent 2 pseudo-

pregnancies. The ear was shaved and the blood vessels made to dilate by the application of a small amount of xylene to the dorsal surface. The marginal ear vein was incised with a scalpel and the blood (2 ml) collected in a heparinized glass tube (10 IU Heparin ml^{-1} blood). Immediately after collection blood samples were centrifuged for 15 min at 4°C and 1300 × g in a MSE Coolspin centrifuge. The plasma was transferred to universal bottles by pasteur pipette and stored at -20°C until assayed for progesterone as described in sections 1.3d and 1.4d. Determinations were performed in triplicate at two dilutions.

Comparisons of the concentration of progesterone on different days of pseudopregnancy in intact (Group 1) and hysterectomised (Group 2) animals were made using the two tailed Students' 't' test.

Results

Fig. 19 shows the peripheral plasma profile throughout pseudopregnancy in intact and hysterectomised pseudopregnant rabbits. Results are expressed as the mean ± standard error of the mean of four pseudopregnancies. In the intact animals the concentrations of progesterone in the peripheral venous plasma increased steadily until Day 8, remained elevated until Day 13 and then began to decline steadily. By Day 20 they were no longer significantly ($p > 0.05$) elevated above pre-injection levels. In fact there were no significant differences between the concentrations of progesterone on Days 17, 18, 19 and 20. The mean plasma progesterone level observed on Day 16 was significantly higher than on the following day ($p < 0.05$). A similar, but not identical, profile was observed in hysterectomised animals. Plasma concentrations again increased steadily until Day 8 and declined from Day 13 but the concentrations


Intact (Group 1)
n=4
Hysterectomy (Group 2)
n=4
Progesterone ng ml-1 plasma
20
16
12
8
4
0
2
4
6
8
10
12
14
16
18
20
Day of pseudopregnancy

of progesterone observed in the hysterectomised animals were consistently higher than those observed in intact animals from Day 14 onwards. However, this difference was not statistically significant until Day 17, 18 and 19 of pseudopregnancy ($p < 0.05$), the time at which, as found in the previous experiment, PGFα release from the uterus is increased. The sharp decline in plasma progesterone levels on Day 17 in the intact animal is not observed. Instead there is a steady but gradual decline from Day 16 until Day 20 when, although the mean concentration of progesterone is not significantly different from that observed on Day 20 in intact animals or from pre-injection levels, it is still above 1 ng ml^{-1}.

Conclusions

When the concentration of progesterone in the peripheral venous plasma is used as an index of luteal function hysterectomy does not appear to affect the time at which luteal function begins to decline. However, the presence of the uterus does cause a more abrupt fall in plasma progesterone concentration, especially on Days 17 and 18 of pseudopregnancy.

These results support the earlier contention that the increase in PGFα observed in the uterine venous plasma at the end of pseudopregnancy is not responsible for the initiation of luteolysis but contributes to the more rapid and final demise of luteal function after Day 16.

Section 2.2 Pregnancy

Introduction

As already described in the general introduction and Section 2.1 there is strong evidence that $PGF_{2\alpha}$ acts as a uterine luteolytic hormone in the cycling sheep and guinea-pig. In both these species the maintenance of pregnancy is dependent upon progesterone secreted from the corpus luteum until such time as the placenta can take over (Deanamur and Martinet 1955; Heap and Deansley 1966). In both species this change-over occurs after the time at which the corpus luteum in the non-pregnant animal would normally have regressed. This implies that, in the pregnant animal, a mechanism operates to maintain luteal function during the early part of pregnancy. This could be achieved, a) by the production of a luteotrophin which overrides the effect of the luteolytic hormone, b) by inhibition of luteolysin production and/or its release into the uterine vein or c) by a combination of a) and b). Thorburn *et al.*, (1973) have shown that in the pregnant sheep the concentration of PGF in the uterine venous plasma does not increase after Days 12 to 14 while, in pregnant guinea-pigs the concentration of $PGF_{2\alpha}$ in the utero-ovarian venous plasma on Day 15 of pregnancy is significantly lower than in the non-pregnant animal at this time (Blatchley *et al.*, 1975a). These observations suggest that, in these species, luteal maintenance during early pregnancy is, at least in part, attributable to a suppression of $PGF_{2\alpha}$ release into the uterine venous blood.

In the rabbit the presence of a functional corpus luteum is required throughout pregnancy. If $PGF_{2\alpha}$ acts as a uterine luteolytic hormone in the pseudopregnant animal, then some mechanism must operate to neutralise its action during pregnancy. Therefore, the concen-

tration of PGF_{α} in the uterine venous blood of the rabbit during pregnancy was measured. The concentrations of progesterone in the peripheral venous plasma and the concentrations of PGE in the uterine venous plasma, aortic and peripheral venous plasma was also measured.

Section 2.2a Experiment to measure the concentrations of PGF_{α} and PGE in ear vein, aorta and uterine vein plasmas throughout pregnancy.

Method

Mature, female, New Zealand white rabbits, all of which had undergone at least one successful pregnancy prior to this experiment were made pregnant by mating to buck rabbits of proven fertility. The day of mating was designated Day 0 of pregnancy. Animals were anaesthetised on selected days throughout pregnancy and blood samples collected from the marginal ear vein, the left and right uterine veins and the aorta as previously described in Section 2.1a. The blood was centifuged and the plasma extracted and assayed for PGF_{α} and PGE content as previously described (Section 2.1a). 1 ml of ear vein plasma was extracted and assayed for progesterone as described in sections 1.3d and 1.4d. Rabbits used on Days 6, 7 and 8 of pregnancy had their ovaries checked for the presence of corpora lutea while in animals used after Day 8, the number and state of the foetuses in each uterine horn was noted. Results from rabbits in which the corpora lutea were absent or in which no foetuses were present were discarded and, whenever possible, the animals replaced.

Identification of extracted prostaglandins by g.c. - m.s.

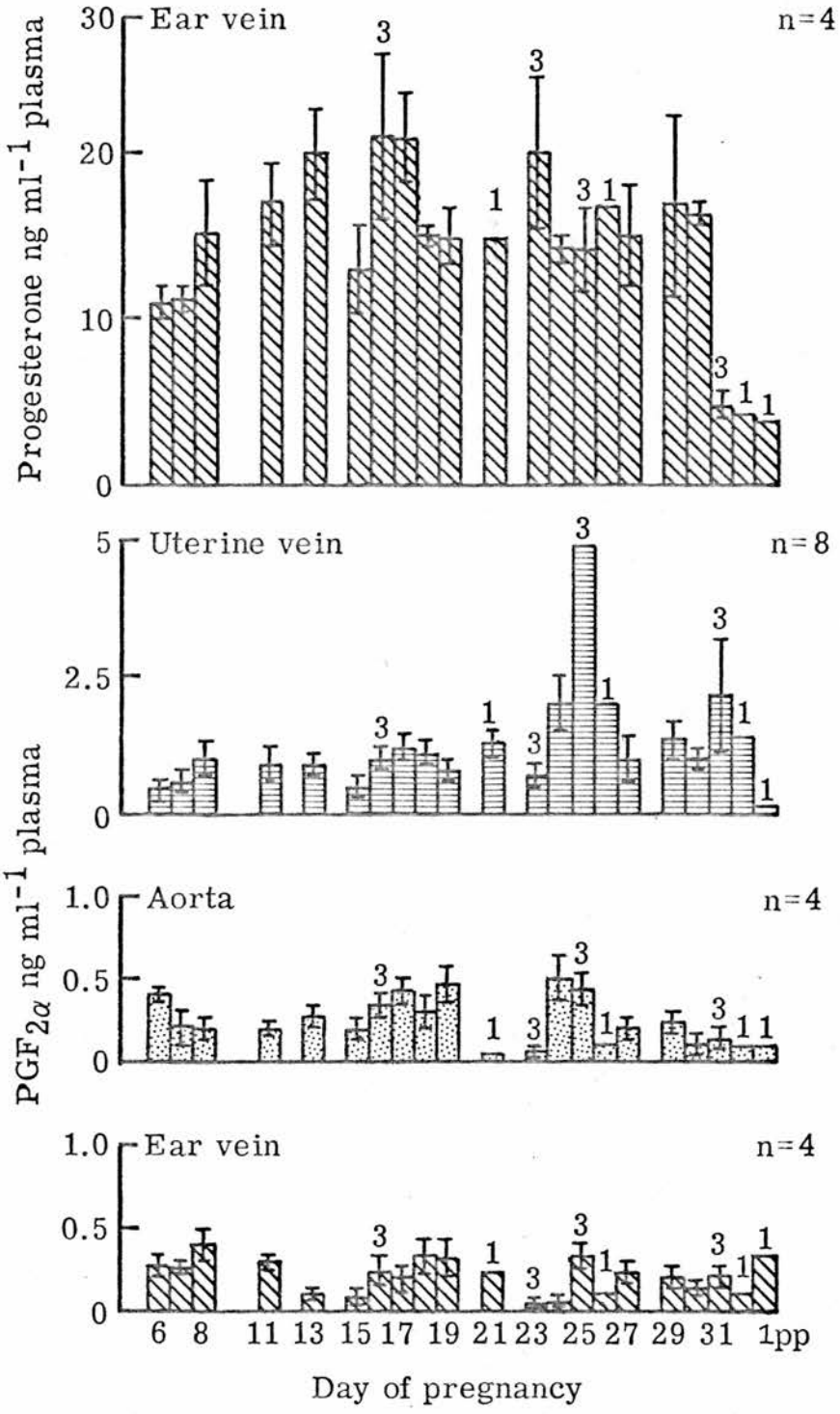
Fractions 3 and fractions 5 from extracted plasma obtained from the ear vein, the aorta and the left and right uterine veins were

pooled and derivatised as described in Section 2.1a. Samples were injected into the gas chromatograph in volumes of 5µl. The mass spectrometer was run in the MID mode of operation but full mass spectra were obtained when sufficient material was shown to be present.

Results

Figure 20 shows the concentration of progesterone in the peripheral venous plasma throughout pregnancy. The results are expressed as the mean ± SEM of four animals unless otherwise stated (numbers in parenthesis over relevant column). There is one value per day from each animal for ear vein and aorta, and two values per day from each rabbit for the uterine vein (i.e. left and right sides). The concentration of progesterone reached high values by Day 8, as observed in the pseudopregnant animal and peaked by Day 16 to Day 17. It fell on Day 18 to a lower level which was maintained until Day 30, and then declined sharply on Day 31. A similar progesterone profile has been reported by Fuchs (1978) in the ear vein plasma of pregnant rabbits. The concentration of progesterone in the peripheral venous plasma of one rabbit, Day 1 p.p., from which blood was collected within 1 hr after delivery, was similar to that observed on Days 30 and 31.

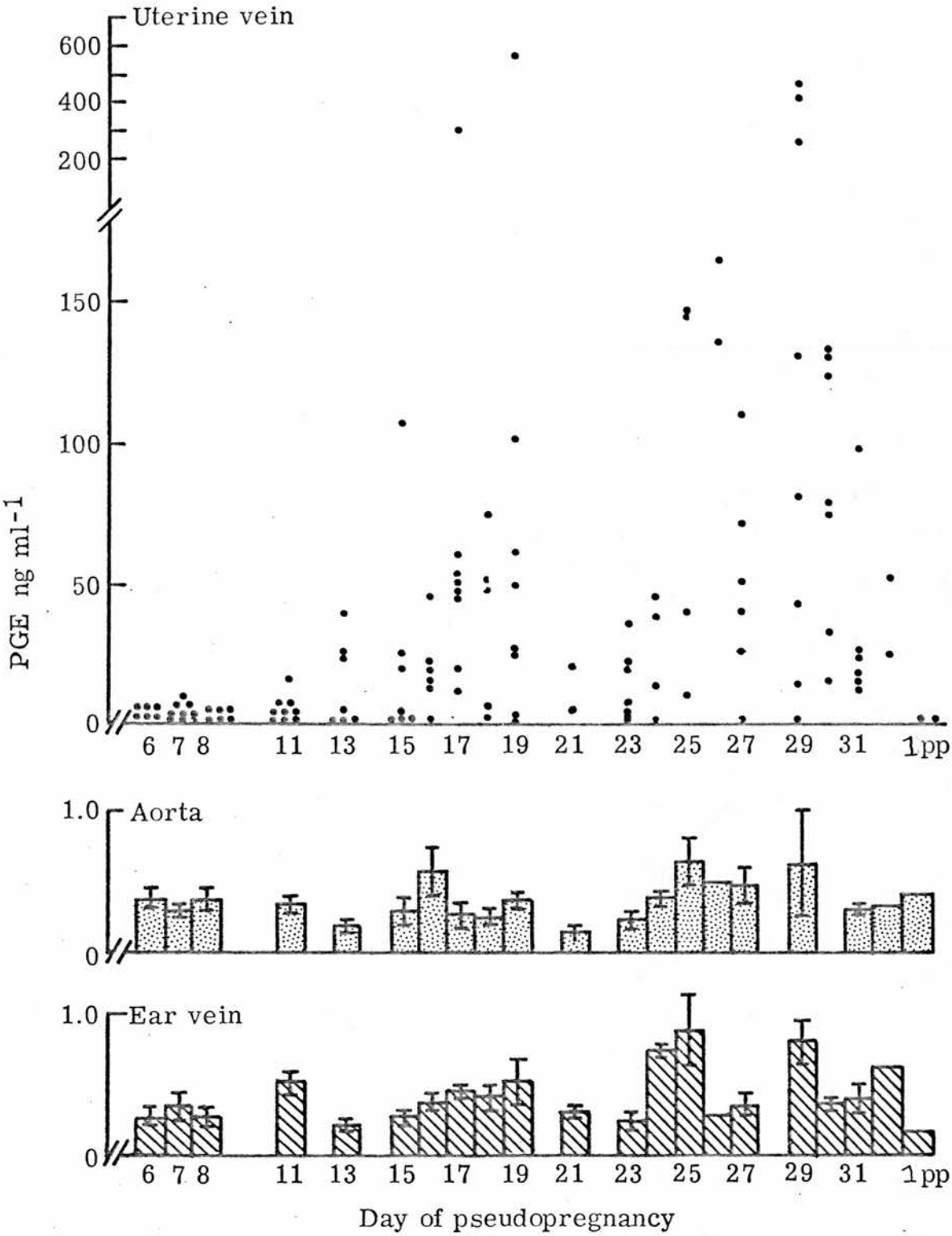
The concentration of PGF_{α} in the aortic and ear vein plasma did not differ significantly from the concentrations observed in similar samples collected during pseudopregnancy. This was not true of the concentration of PGF_{α} in the uterine venous plasma. The increased concentrations of PGF_{α} observed on Days 17 and 18 of pseudopregnancy were not present on Days 17 and 18 of pregnancy. Instead PGF_{α}


Progesterone ng ml-1 plasma
PGF2α ng ml-1 plasma
Ear vein
Uterine vein
Aorta
Ear vein
n=4
n=8
n=4
n=4
30
20
10
0
5
2.5
0
1.0
0.5
0
1.0
0.5
0
6 8 11 13 15 17 19 21 23 25 27 29 31 1pp
Day of pregnancy

concentrations remained low, approximately 1 ng ml^{-1}, until Day 24 when they rose significantly ($p < 0.05$). They reached peak values on Day 25 and, although still elevated on Day 26, had returned to 'basal' levels by Day 27. The concentration of PGF_{α} on Day 25 (4.97 ± 0.22) was significantly higher ($p < 0.05$) than that observed on Days 24 and 26 of pregnancy. Uterine venous PGF_{α} levels increased again on Day 31 (2.30 ± 1.01) but this increase was not significant.

The concentrations of PGE in aortic and peripheral venous plasma (Fig. 21) did not differ significantly from those observed during pseudopregnancy (Fig. 18). The concentrations of PGE in the uterine venous plasma on Days 6, 7 and 8, were similar to those observed in the pseudopregnant animal at this stage. From Day 11 onwards, however, the concentration of PGE in the uterine venous plasma rose significantly. There was a wide variation among PGE levels in animals of the same gestational age and even in the same animal between the left and right uterine veins. Due to the wide scatter, it was not considered valid to present the results as the mean ± SEM. Therefore, each point in Fig. 21 (a) represents an individual measurement from either the left or right uterine vein of each rabbit. Analysis of the number of foetuses per uterine horn and the concentration of PGE in the uterine vein draining that horn using the χ^2 test, shows that there is a positive correlation ($p < 0.05$) between these two parameters. In the single post partum rabbit studied (1 p.p.) the concentration of PGE in the left and right uterine venous plasma was not significantly different from that observed on Days 6, 7 and 8 of pregnancy and throughout pseudopregnancy.

The amounts of PGE (fraction 3) and PGF_{α} (fraction 5) in the pooled, extracted plasma samples obtained from the marginal ear vein


Uterine vein
600
400
200
150
100
50
0
PGE ng ml-1
6 7 8 11 13 15 17 19 21 23 25 27 29 31 1pp
1.0
Aorta
0
1.0
Ear vein
0
6 7 8 11 13 15 17 19 21 23 25 27 29 31 1pp
Day of pseudopregnancy

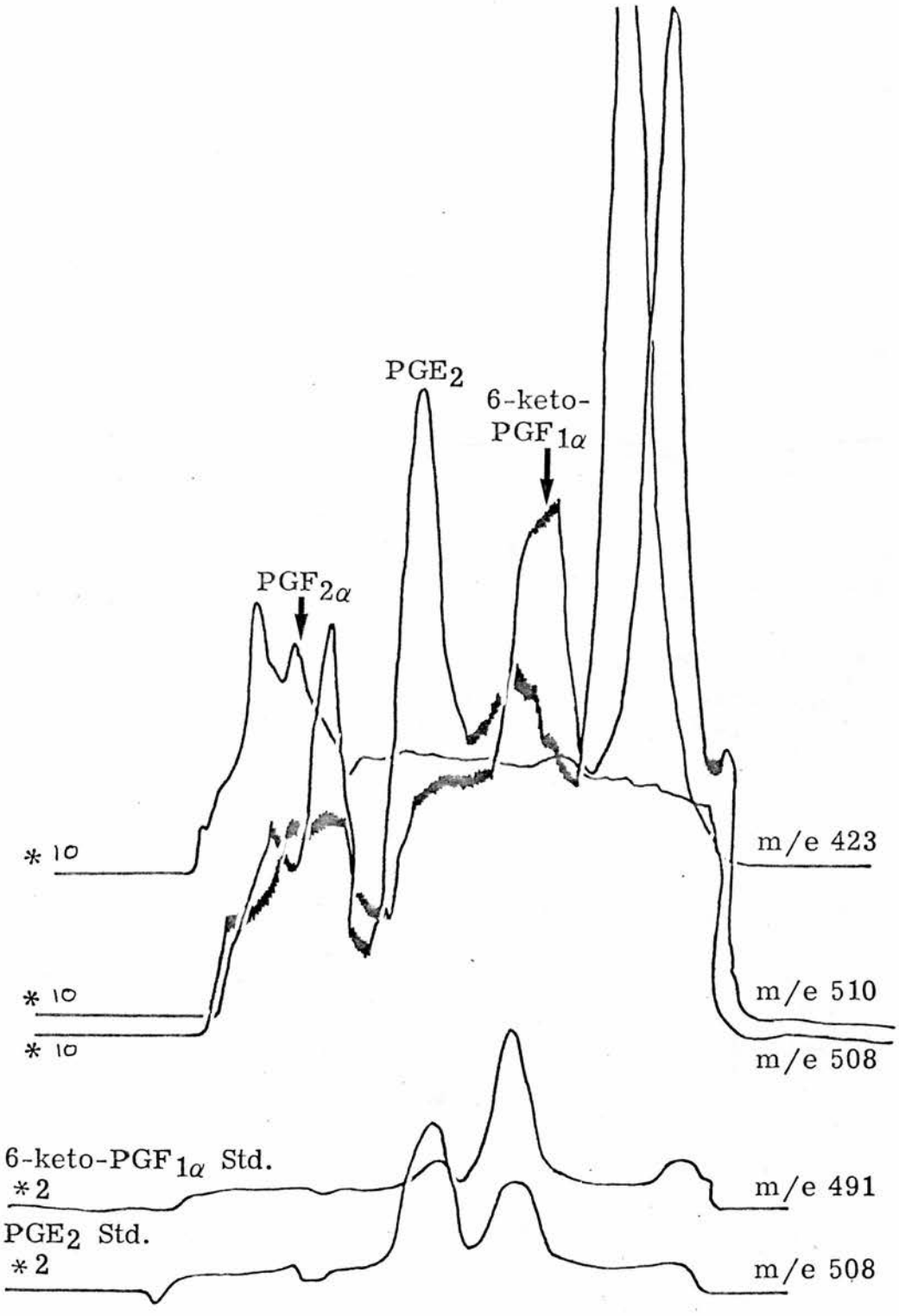
and aorta were found to be too low to be detected by MID in the g.c. - m.s. The presence of PGE_2, but not PGE_1, in the extracted uterine venous plasma was confirmed both by MID (Fig. 22) and by a full mass spectrum taken at the relevant retention time. In fraction 5 of the pooled uterine venous plasma extracts a m/e peak, corresponding to the 423 ion of $PGF_{2\alpha}$, was observed at the correct retention time for this compound. Similarly a m/e peak, corresponding to the 491 ion of 6-keto-$PGF_{1\alpha}$, was also observed. In the silicic acid column chromatography system described in Section 1.3b, 6-keto-$PGF_{2\alpha}$ elutes in both fraction 3 and fraction 5. However, no peak corresponding to 6-keto-$PGF_{1\alpha}$ was found in the pooled fraction 3 of the uterine venous extracts from pregnant rabbits. The low amounts of $PGF_{2\alpha}$ present precluded a full mass spectra of this compound from being obtained.

The pooled extracts of fraction 3 and fraction 5 showed no contamination by PGF_{α} and PGE respectively.

Conclusion

In the pregnant rabbit, the increase in PGF_{α} observed on Days 17 and 18 of pseudopregnancy is absent. This finding supports the idea that the increase in PGF_{α} observed at the end of pseudopregnancy contributes to luteal demise and that its absence in the pregnant animal may aid in luteal maintenance. The significance of the increase in the uterine venous plasma concentration of $PGF_{2\alpha}$ on Days 24 to 26 of pregnancy is not known. However, it did not lead to a fall in the peripheral plasma concentration of progesterone at this time.

The high concentrations of PGE observed in the uterine venous plasma of pregnant rabbits from Day 11 onwards are in direct contrast



* Voltage gains

with those observed in the pseudopregnant animal. This finding, together with the positive correlation between the number of foetuses per uterine horn and the concentration of PGE in the uterine vein draining that horn suggest that the rabbit foetal-placental unit may be synthesising and releasing large amounts of PGE. This is further supported by the observation that, in the post partum rabbit, the concentration of PGE in the uterine venous plasma is similar to that observed in the uterine venous plasma on Days 6, 7 and 8 of pregnancy, prior to the formation of the foetal-placental unit. Also, the concentration of PGE in the uterine venous plasma did not increase until after implantation had occurred and the placenta had become established. The decline in peripheral plasma progesterone concentration on Day 31 of pregnancy occurred independently of any significant increase of PGF_{α} in the uterine venous plasma at this time, although PGF_{α} levels did tend to be higher on Day 31. In two animals studied, one on Day 31 and one on Day 32 of pregnancy, the cervix was dilated and a foetus had already begun to move down into the vagina at the time the blood samples were collected. In both animals the peripheral plasma progesterone was approximately 4 ng ml^{-1}. The concentrations of PGF_{α} in the uterine venous plasma from both the left and right uterine veins were higher (2.75 and 4.92 ng ml^{-1} respectively on Day 31 and 1.67 and 1.21 ng ml^{-1} respectively on Day 32) than those observed in Day 31 (0.31 and 1.19 ng ml^{-1} respectively) and in Day 32 (.35 and .41 ng ml^{-1} respectively) rabbits in which labour had not yet started. This finding suggests that there may be an increase in PGF_{α} output by the uterus at the time of delivery.

SECTION 3. MEASUREMENT OF THE ENDOGENOUS CONCENTRATIONS AND THE ABILITY OF UTERINE TISSUE TO SYNTHESISE PGF_{α}, PGE AND 6-KETO-$PGF_{1\alpha}$, *IN VITRO*, ON SELECTED DAYS OF PSEUDOPREGNANCY AND DAY 17 OF PREGNANCY

Section 3.1a Experiment to determine the effect of homogenisation on uterine tissue production of PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$

Introduction

In this section the synthetic capacity of rabbit uterine tissue to synthesise prostaglandins at different stages of pseudopregnancy and pregnancy has been investigated using incubates of uterine tissue homogenates. As mechanical stimulation of many tissues is known to cause prostaglandin synthesis and release, the following preliminary experiment was performed to determine the effect of the process of homogenisation on uterine tissue prostaglandin production.

Method

Three female, New Zealand white rabbits were killed by stunning and incising the neck. The uteri were removed, and one horn from each animal was divided into three segments and each segment weighed. Segment 1 was used to determine the 'endogenous' prostaglandin content of the uterine tissue. It was homogenised in 5 ml ethanol using a Fisons glass homogeniser and the homogenate was poured into a 100 ml round bottom flask. The homogeniser was washed with two further 5 ml portions of ethanol and the washings added to the homogenate, which was then allowed to stand for 90 min, with occasional shaking. At the end of this period the ethanol was evaporated off under reduced pressure at 45°C on a rotary evaporator. The residue was resuspended in 15 ml Krebs' solution and extracted as described

in Section 1.2a. Segments 2 and 3 of the uterine horn were homogenised separately in 15 ml Krebs' solution as described in Section 1.2b. Following homogenisation, segment 2 was immediately solvent-extracted, using the method described in Section 1.2a, while segment 3 was incubated for 90 min at 37°C, as described in Section 1.2b and then solvent-extracted. All samples were assayed for PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ by RIA.

Results

The concentrations of PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ in uterine tissue samples of ethanolic homogenates (endogenous concentration) and of Krebs' solution homogenates, pre- and post-incubation, are shown in Table 14.

	Ethanolic homogenate	Krebs' solution homogenate	
Compound	Endogenous ng/100 mg tissue	Pre-incubation ng/100 mg tissue	Post-incubation ng/100 mg tissue
PGF_{α}	2.83 ± 0.51	5.42 ± 1.47	20.86 ± 0.74
PGE	8.22 ± 0.08	8.62 ± 0.22	14.52 ± 0.22
6-keto-PGF	2.74 ± 0.29	3.52 ± 0.24	49.14 ± 17.54

Table 14. Amount of PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ in uterine tissue samples following homogenisation in ethanol and in Krebs' solution and following incubation of the Krebs' solution homogenates (mean ± SEM, n = 3)

Comparisons of the amounts of PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ in ethanolic homogenates and Krebs' solution homogenates, pre- and post-incubation, were made using Students' 't' test for paired data. There was no significant difference between the concentrations of PGE

and 6-keto-$PGF_{1\alpha}$ in ethanolic homogenates and pre-incubation, Krebs' solution homogenates. The increase in the concentration of PGF_{α} following homogenisation in Krebs' solution, however, was significant ($p < 0.05$). Following incubation of Krebs' solution homogenates the concentrations of PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ were significantly higher than those observed in pre-incubation, Krebs' solution homogenates, or ethanolic homogenates.

Conclusion

Although PGF_{α} concentrations increased significantly following the homogenisation of uterine tissue in Krebs' solution the increase was low when compared to the increase observed after these homogenates had been incubated for 90 min. The process of homogenisation did not appear to stimulate PGE and 6-keto-$PGF_{1\alpha}$ synthesis to any significant extent. It was, therefore, considered that the difference between endogenous PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ concentrations and the concentrations of PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ following incubation, reflected the synthetic capacity of the uterine tissue for each of these prostaglandins and was not attributable to the effects of mechanical stimulation alone.

Section 3.1b Experiment to investigate the ability of the rabbit uterus to synthesise PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ on selected days of pseudopregnancy

Introduction

In the cycling sheep, guinea-pig and cow the endogenous concentration of $PGF_{2\alpha}$ in the endometrium is higher at the end of the cycle, when luteal regression occurs, than earlier in the cycle (Wilson *et al.*, 1972; Poyser, 1972; Shemesh and Hansel, 1975). Moreover in the

guinea-pig and sheep the ability of the uterus to synthesise prostaglandins, *in vitro*, is also increased at this time (Poyser 1972; Wlodawar *et al.*, 1976; Alwachi *et al.*, 1979). In the guinea-pig this increase appears to be related to a genuine increase in the synthetic capacity of the uterus, as opposed to reflecting a decrease in metabolism or an increase in the availability of substrate (Mitchell, Poyser and Wilson, 1977; Maule Walker and Poyser, 1978), and is probably responsible for the increased levels of $PGF_{2\alpha}$ observed in the uterine vein at this time.

In the following experiment, therefore, the endogenous prostaglandin content and the synthetic capacity of uterine tissue homogenates on selected days of pseudopregnancy has been measured. The effect of the addition of excess arachidonic acid to the incubation medium has also been studied.

Method

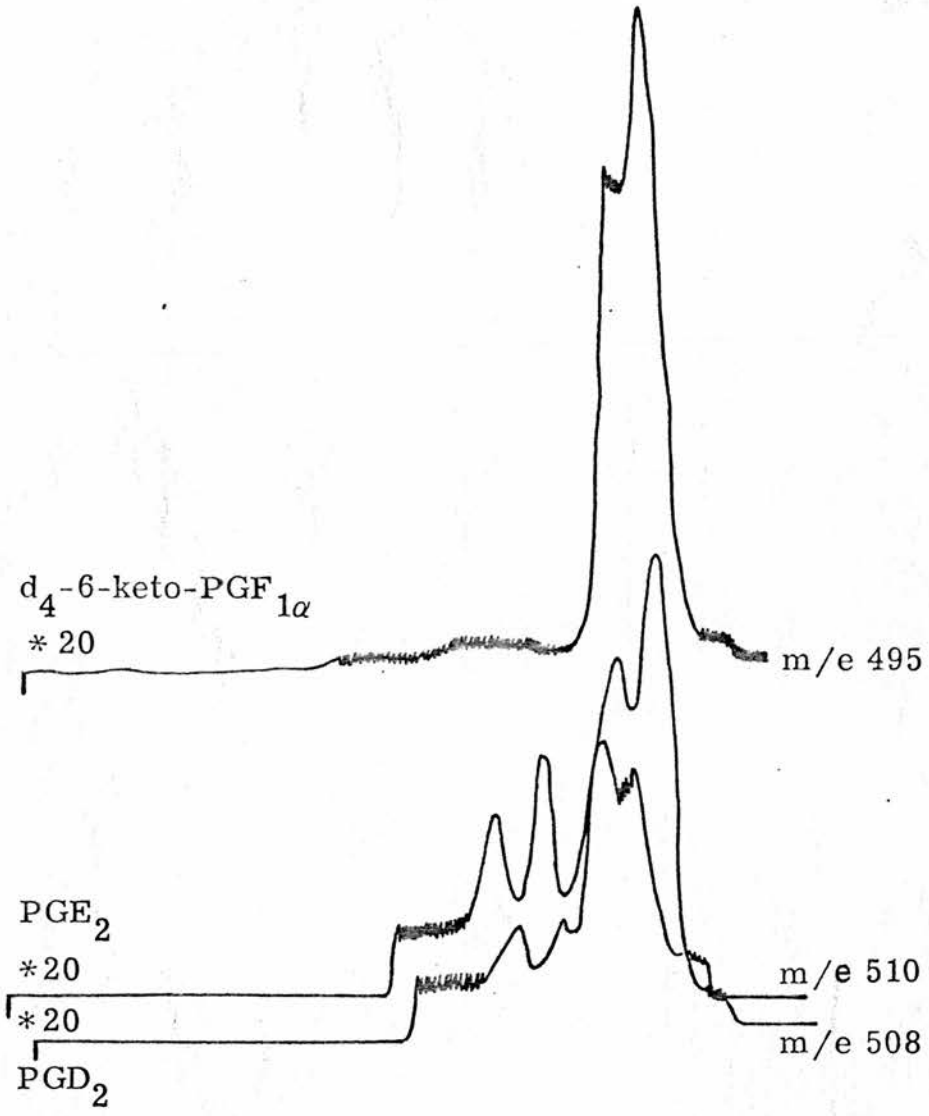
Twenty, mature, New Zealand white female rabbits, of proven fertility, were divided into five groups of four animals per group. Animals in the first group acted as controls, and received 0.5 ml saline into the marginal ear vein, while animals in the remaining four groups were made pseudopregnant by the intravenous injection of 500 IU of HCG in 0.5 ml saline into the marginal ear vein between 15.00 to 15.30 hr. The day of injection was designated as Day 0 of pseudopregnancy. On the day of sacrifice 2 ml of blood was collected from the marginal vein as previously described in section 2.1b. The blood was centrifuged and the plasma solvent extracted and assayed for progesterone as described in Section 1.4d. Animals were killed by stunning and incising the neck. Control animals

were sacrificed the day after injection and animals in the remaining four groups were sacrificed on Days 4, 7, 11 and 17 of pseudopregnancy. The uterus was exposed by making a mid-line, abdominal incision. The two uterine horns were severed on the uterine sides of the utero-oviductal junction and cervix, and transferred to a beaker of ice cold Krebs' solution. Each uterine horn was opened longitudinally and cut into segments each of approximately 1g, wet weight. Three segments were then blotted dry and weighed and cut into small pieces with a pair of scissors. The remaining tissue was left on ice cooled Krebs' solution and retained for further incubation studies and metabolism studies (see Section 5).

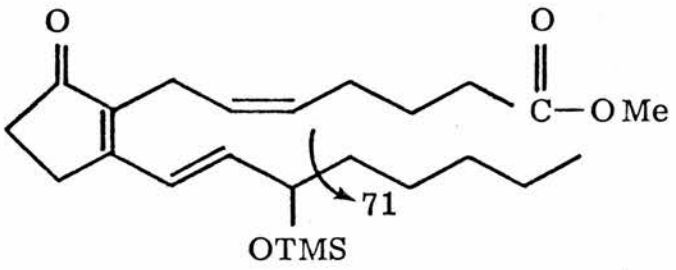
Segment 1 was used to determine the endogenous prostaglandin content of the uterine tissue as described in Section 3.1a. Segment 2 was used to measure the ability of homogenised, uterine tissue to synthesise prostaglandins *in vitro*, and was homogenised in 15 ml Krebs' solution and incubated for 90 min as described in Section 1.2b. The third segment was used to determine the effect of the presence of excess arachidonic acid on prostaglandin production by the uterus and was treated as segment 2, except that 75µg arachidonic acid was added to the homogenate (final concentration 5.0µg ml^{-1}) immediately prior to incubation. Following incubation both segments were extracted as described in Section 1.2a. All samples were assayed for PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ content by RIA, as previously described (Sections 1.4a, b and c). Results were compared using Students' 't' test for unpaired data unless otherwise stated.

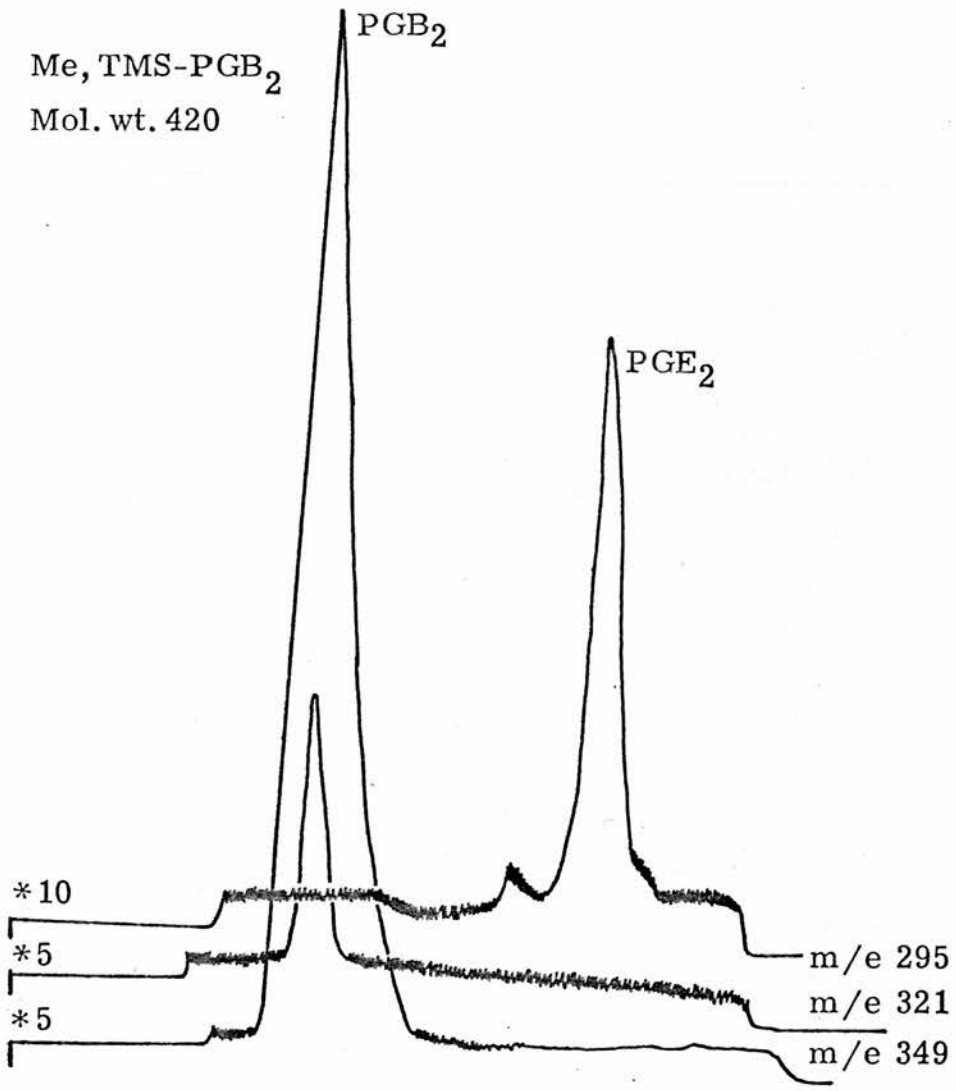
Identification of extracted prostaglandins by g.c. - m.s.

Following the measurement of PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ by RIA the extracts from ethanolic homogenates of the 4 control animals were pooled and taken to dryness on a rotary evaporator. The residue was redissolved in 1 ml methanol, transferred to an Eppendorf vial and derivatised to form the Me,BuO,TMS derivative as described in Section 2.1a. The extracts from the uterine incubates of the control animals were also pooled and derivatised as were the extracts from uterine homogenates, incubated with excess arachidonic acid. Extracts from ethanolic homogenates, and homogenates incubated in the absence and presence of arachidonic acid from Days 4, 7 and 11 of pseudopregnancy were similarly pooled and derivatised to form the Me,BuO,TMS derivatives. Trimethylsilyl ether formation was performed using 30µl BSTFA and samples were injected into the gas chromatograph in volumes of 2 or 3µl. The mass spectrometer was set up for MID using the m/e 503 ion from the column bleed as the lock ion as previously described (Section 1.5a). Samples were monitored for $PGF_{2\alpha}$ (m/e 423), $PGF_{1\alpha}$ (m/e 425), 6-keto-$PGF_{1\alpha}$ (m/e 508), d_4-6-keto-$PGF_{1\alpha}$ (m/e 495), PGE_2 (m/e 508), PGE_1 (m/e 510) and PGD_2 (m/e 510). Because of the high cross reactivity of the antisera used in the PGE RIA with PGB_2, samples were also monitored for the presence of PGB_2. The mass spectrometer was again set up for MID as previously described (Section 5.1a) but using the m/e 281 fragment of the column bleed as the lock ion. PGB_2 was monitored using the m/e 321 and m/e 349 ions. When the m/e 508 and m/e 510 ions were used to monitor for the presence of PGE_2, PGD_2 and 6-keto-$PGF_{1\alpha}$ in samples it became difficult to monitor PGE_2 as the first isomer of the Me,BuO,TMS derivative of PGE_2 had the same retention



*Voltage gain


O
O
C—O Me
71
OTMS


Me, TMS-PGB2
Mol. wt. 420
PGB2
PGE2
*10
*5
*5
m/e 295
m/e 321
m/e 349

time as the second isomer of the Me,BuO,TMS derivative of PGD_2, and the second isomer of the Me,BuO,TMS derivative of PGE_2 had the same retention time as the first isomer of the Me,BuO,TMS derivative of 6-keto-$PGF_{1\alpha}$ (Fig. 23). The presence of PGE_2 was, therefore, verified by monitoring the m/e 295 ion, which is not present in either the Me,BuO,TMS derivative of PGD_2 or in the Me,BuO,TMS derivative of 6-keto-$PGF_{1\alpha}$, at the same time as monitoring for PGB_2 (Fig. 24).

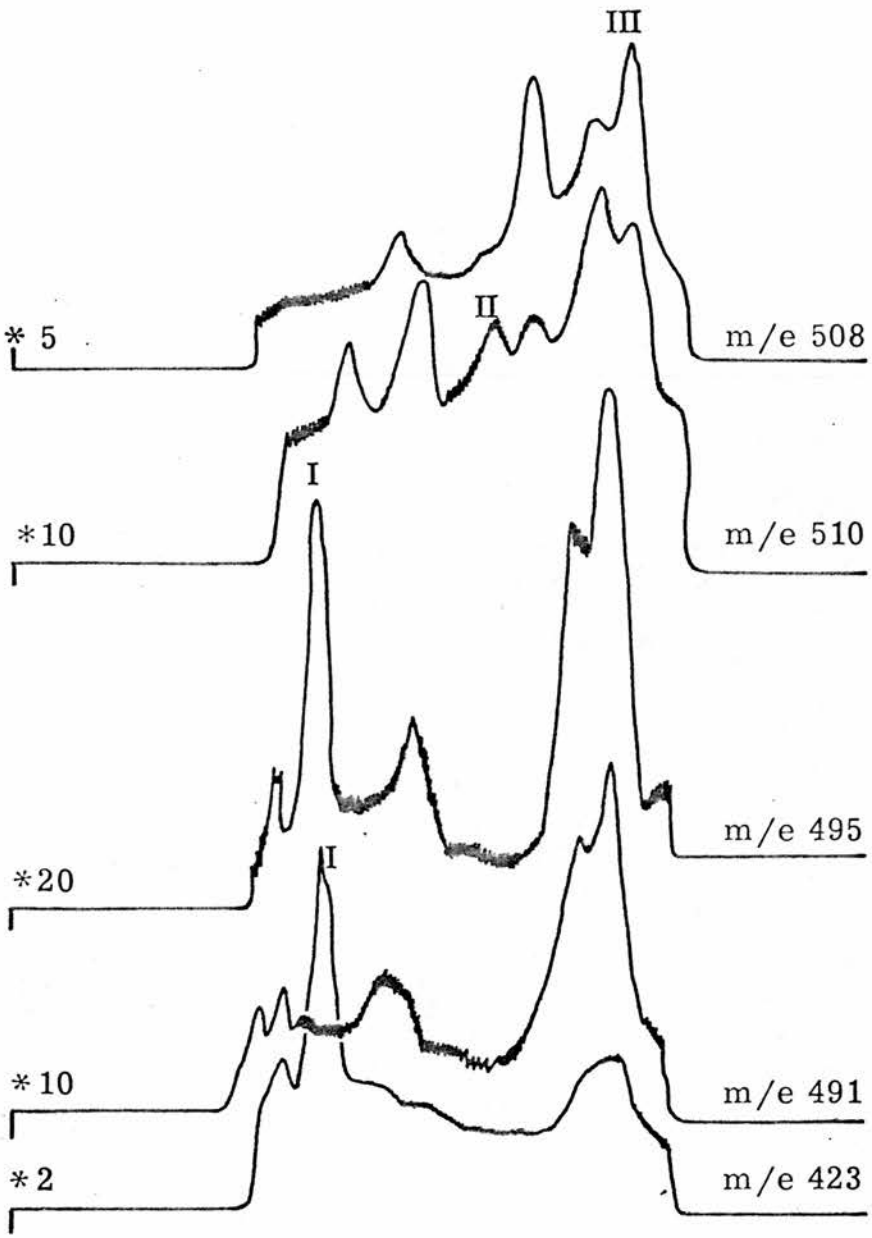
Results

The concentration of progesterone in the peripheral venous plasma on different days of pseudopregnancy is shown in Table 15. Progesterone increased until Day 11 of pseudopregnancy but had returned to pre-injection levels by Day 17.

Day of Pseudopregnancy	Concentration of Progesterone ng ml^{-1} of plasma
Control	.553 ± .039
4	10.60 ± 0.50
7	27.03 ± 3.29
11	38.21 ± 2.57
17	0.793 ± 0.053

Table 15. Concentration of progesterone in peripheral venous plasma (ear vein) on selected days of pseudopregnancy (mean ± SEM, n = 4)

The analysis of samples incubated in the absence or presence of arachidonic acid by g.c. - m.s. using MID showed that each sample contained $PGF_{2\alpha}$, PGE_2, PGD_2 and 6-keto-$PGF_{1\alpha}$. No PGB_2 or $PGF_{1\alpha}$ was detectable. Fig. 25 shows a typical trace obtained when recor-



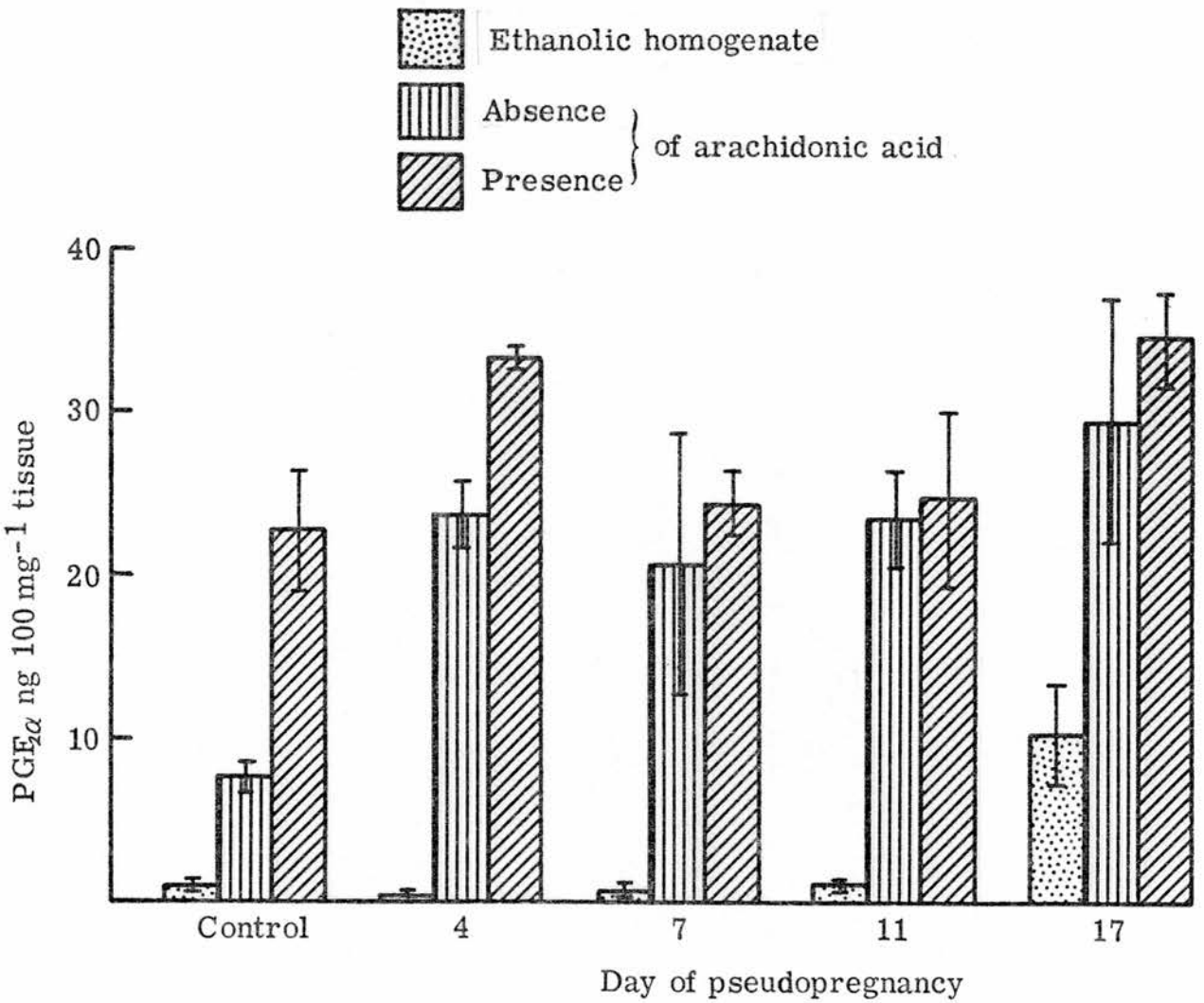
* Voltage gain

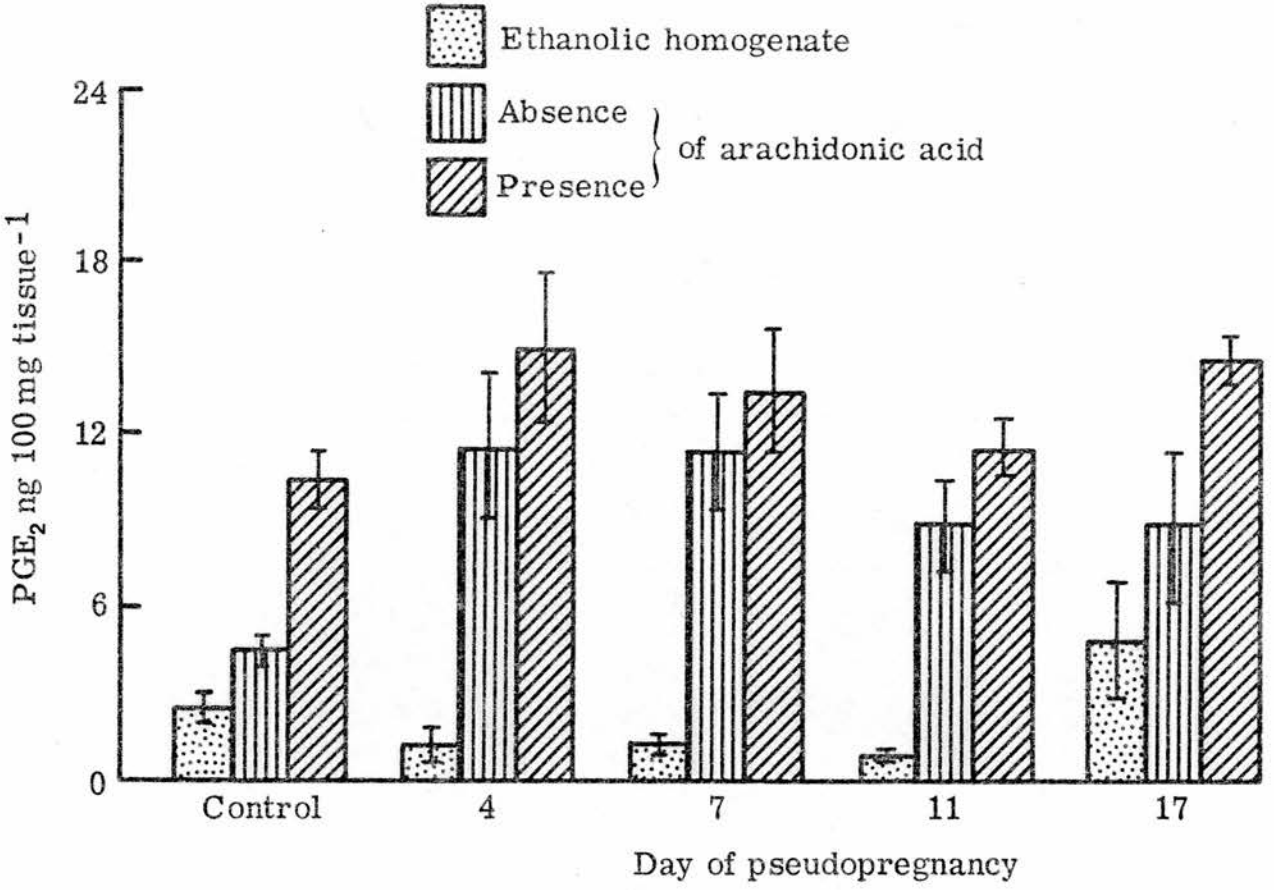
I = $PGF_{2\alpha}$, II = PGD_2, III = 6-keto-$PGF_{1\alpha}$

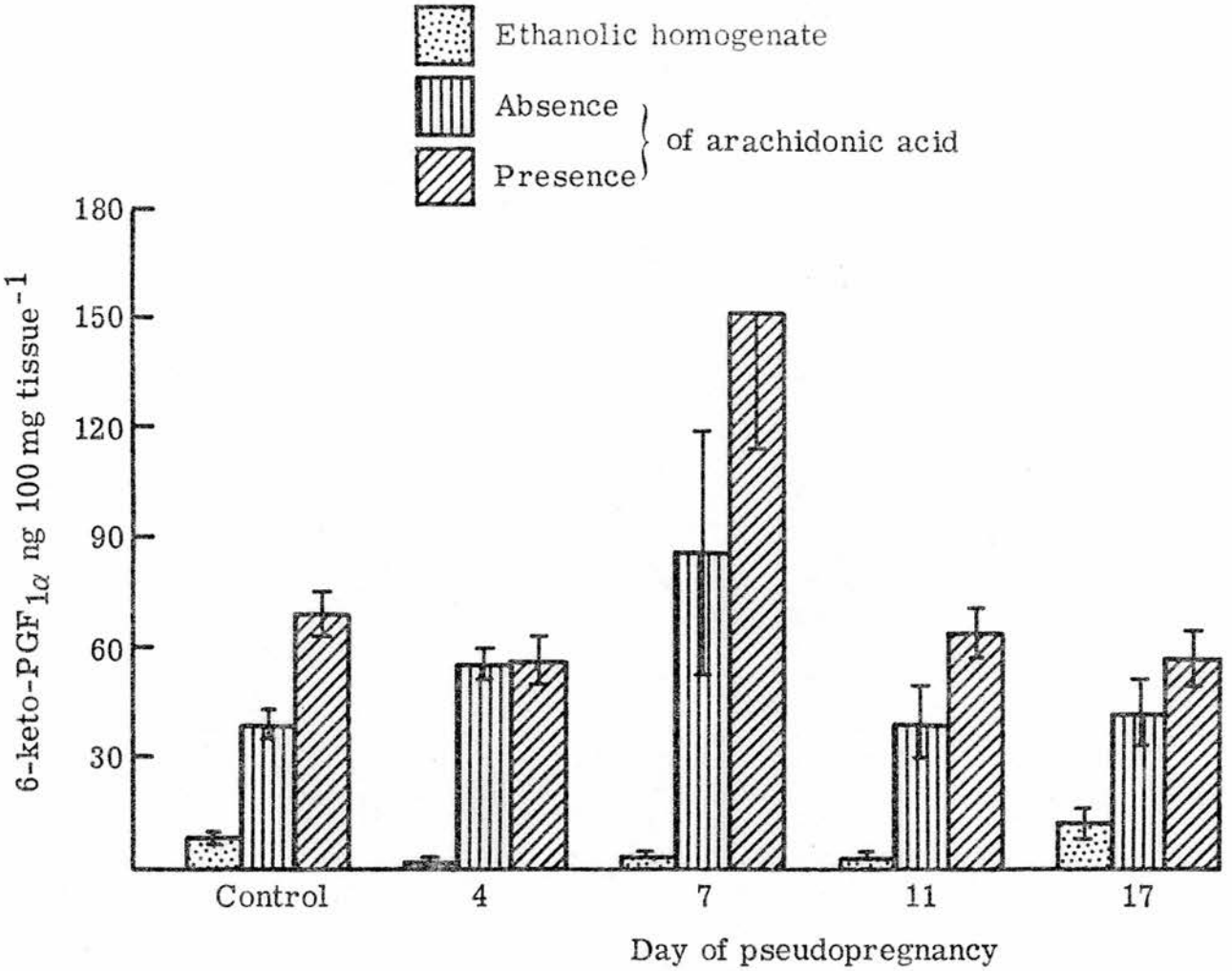
ding the m/e ion at 423, 491, 495, 508 and 510. The peaks shown confirm the presence of $PGF_{2\alpha}$ (I), PGD_2 (II) and 6-keto-$PGF_{1\alpha}$ (III). For reasons stated in the introduction the presence of PGD_2 and 6-keto-$PGF_{1\alpha}$ masked the presence of PGE_2. Fig. 24 shows the mass fragmentation of authentic PGE_2 monitored by the ion at m/e 295, and also of authentic PGB_2 by monitoring of the ions at m/e 321 and m/e 349. By subjecting the samples to this further analysis, the presence of PGE_2 was confirmed but PGB_2 was not detected. Since no $PGF_{1\alpha}$ or PGB_2 could be detected it is probable that the RIA's for PGE and PGF_{α} were measuring PGE_2 and $PGF_{2\alpha}$ respectively. Results from these studies will henceforward be described in terms of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$.

The endogenous concentration of $PGF_{2\alpha}$ in uterine tissues was higher on Day 17 of pseudopregnancy than on any other day studied ($p < 0.05$) (Fig. 26). The endogenous levels of PGE_2 (Fig. 27) and 6-keto-$PGF_{2\alpha}$ (Fig. 28) were also significantly higher ($p < 0.05$) on Day 17 of pseudopregnancy than on Days 4, 7 and 11 of pseudopregnancy, but did not differ significantly from those observed in control animals, although they did tend to be higher.

Following homogenisation and incubation in Krebs' solution there was a significant increase in the prostaglandin concentration in all uterine tissue homogenates when compared to uterine tissue homogenised in ethanol. The amounts of PGE_2 and $PGF_{2\alpha}$ produced on Days 4, 7, 11 and 17 of pseudopregnancy, showed no significant differences but all were significantly higher than the amounts of PGE_2 and $PGF_{2\alpha}$ produced during incubation of uterine tissue homogenates from control animals. The mean concentration of $PGF_{2\alpha}$ observed on Day 17 of pseudopregnancy tended to be higher than on earlier days of pseudo-


Ethanolic homogenate
Absence
Presence
of arachidonic acid
40
30
20
10
PGE$_2\alpha$ ng 100 mg^{-1} tissue
Control
4
7
11
17
Day of pseudopregnancy


Ethanolic homogenate
Absence
Presence
of arachidonic acid
24
18
12
6
0
PGE_2 ng 100 mg tissue^{-1}
Control
4
7
11
17
Day of pseudopregnancy


Ethanolic homogenate
Absence
Presence
of arachidonic acid
6-keto-PGF1α ng 100 mg tissue-1
180
150
120
90
60
30
Control
4
7
11
17
Day of pseudopregnancy

pregnancy but this is probably due to the high endogenous concentration of $PGF_{2\alpha}$ observed in uterine tissue at this time. The major prostaglandin produced during incubation was 6-keto-$PGF_{1\alpha}$. The amount of 6-keto-$PGF_{1\alpha}$ produced during incubation of uterine homogenates from pseudopregnant animals tended to increase up to Day 7 and then decline until Day 17 but the only significant difference was between control and Day 4 values ($p < 0.05$). The addition of 5μg ml^{-1} arachidonic acid to the incubation medium tended to increase the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ formed during incubation on all days studied, but these increases were only significant ($p < 0.05$) in control animals and also for $PGF_{2\alpha}$ on Day 4 of pseudopregnancy. In control animals the addition of arachidonic acid to uterine tissue homogenates increased the amounts of $PGF_{2\alpha}$ and PGE_2 formed during incubation to the amounts produced by uterine tissue homogenates from pseudopregnant animals when incubated in the absence of added arachidonic acid. There was no significant difference between the amounts of 6-keto-$PGF_{1\alpha}$ formed by uterine tissue from control animals and the amount produced by uterine tissue from pseudopregnant rabbits when incubated in the presence of excess arachidonic acid. The mean amount of 6-keto-$PGF_{1\alpha}$ produced by uterine tissue incubated in the presence of 5μg ml^{-1} arachidonic acid on Day 7 of pseudopregnancy was greater than on any other day studied but, because of the wide variation between individual animals, this difference was not significant.

Conclusion

The presence of $PGF_{2\alpha}$, PGE_2, PGD_2 and 6-keto-$PGF_{1\alpha}$ in uterine tissue homogenates incubated in the absence or presence of excess arachidonic acid was confirmed by g.c. - m.s. using MID.

The endogenous concentration of $PGF_{2\alpha}$ in the rabbit uterus was significantly higher on Day 17 of pseudopregnancy than on any other day studied. The endogenous concentrations of PGE_2 and 6-keto-$PGF_{1\alpha}$ were also significantly higher on Day 17 than on any other day of pseudopregnancy but did not differ significantly from the endogenous concentrations of PGE_2 and 6-keto-$PGF_{1\alpha}$ observed in uterine tissue of control animals. On each day studied the major prostaglandin formed during incubation was 6-keto-$PGF_{1\alpha}$. The ability of the uterine tissue to synthesise $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ did not appear to change significantly throughout pseudopregnancy although uterine tissue from pseudopregnant rabbits produced significantly more $PGF_{2\alpha}$, and PGE_2 than uterine tissue from control animals. This difference could be due to a lack in the availability of precursors as the addition of excess arachidonic acid to the incubation medium increases the amount of PGE_2 and $PGF_{2\alpha}$ formed, to that produced by uterine incubates from pseudopregnant animals. Incubates of rabbit uterus were also found to synthesise PGD_2 but this was not realised until after samples had been analysed by g.c. - m.s. using MID. The significance of this observation is not known, however it would be interesting to measure the amounts of PGD_2 produced by the rabbit uterus. Since g.c. - m.s. data of uterine tissue homogenates and uterine-venous plasma (Section 2.2a) show that only $PGF_{2\alpha}$ and PGE_2 are present, the amounts of PGF_{α} and PGE measured by RIA will be expressed as $PGF_{2\alpha}$ and PGE_2 hereafter.

Section 3.2a PGF_{α}, PGE and 6-keto-$PGF_{1\alpha}$ production by the Day 17 pregnant rabbit uterus.

Introduction

In both the pregnant sheep and guinea-pig, the increase in $PGF_{2\alpha}$ concentration in the uterine venous plasma around Day 15 of the oestrous cycle is not observed on Day 15 of pregnancy (Thorburn *et al.*, 1973; Blatchley *et al.*, 1975a). In the previous section, it was similarly found that the increase in the uterine venous plasma concentration of $PGF_{2\alpha}$ in the pseudopregnant rabbit on Day 17 is absent on Day 17 in the pregnant animal.

Maule Walker and Poyser (1974) have shown that the Day 15 pregnant, guinea-pig uterus synthesises significantly less $PGF_{2\alpha}$ than uterine tissue on Day 15 of the oestrous cycle. This decrease in $PGF_{2\alpha}$ production was not due to increased metabolism, lack of substrate or the redirection of synthesis towards PGE (Maule Walker and Poyser, 1978). The authors, therefore, suggest that the lower level of $PGF_{2\alpha}$ production by the pregnant uterus may account for the reduced output of $PGF_{2\alpha}$ observed at this time and thereby result in the maintenance of the corpora lutea beyond Day 15 in the pregnant animal. In the following experiment, therefore, the amounts of $PGF_{2\alpha}$, as well as PGE_2 and 6-keto-$PGF_{1\alpha}$, present in, and produced by, uterine tissue on Day 17 of pregnancy, have been measured.

Method

Four, female, New Zealand white rabbits of proven fertility were mated with a fertile, buck rabbit. The day of mating was designated Day 0 of pregnancy. On Day 17 of pregnancy, 2 ml blood were collected from the marginal ear vein of each rabbit as previously described (Section 2.1b). The blood was centrifuged, and the plasma was

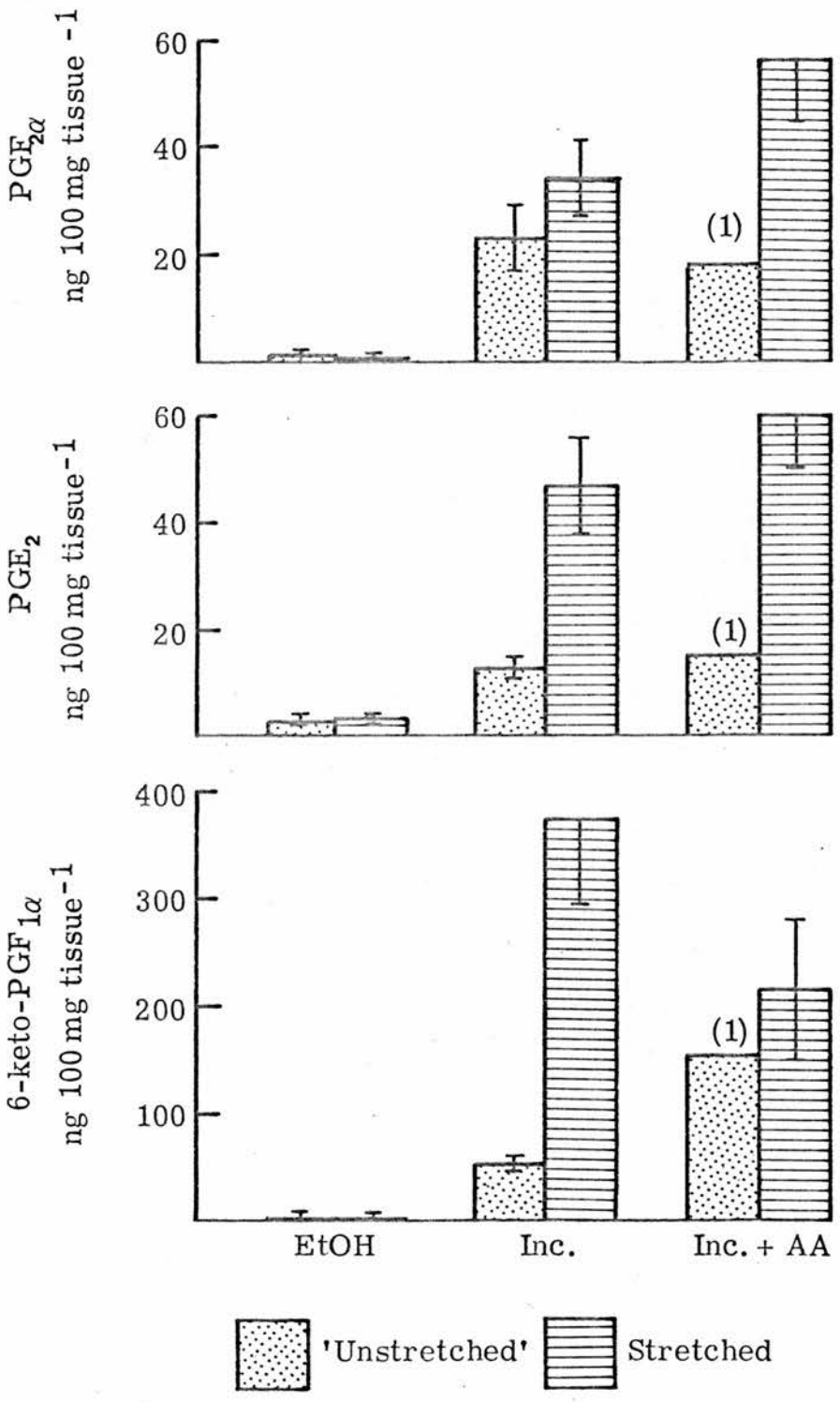
solvent-extracted and assayed for progesterone as previously described in Sections 1.3d and 1.4d. Each animal was then killed by stunning and incising the neck. The uterus was exposed and extirpated as described in Section 3.1b. The number and state of the foetuses in each horn was noted. The uterus was opened longitudinally and freed of any placental and embryonic tissue. These tissues were placed in ice-cold, Krebs' solution and retained (Sections 4.1a, 4.1b and 5). The uterine tissue appeared to consist of two 'types'; tissue which had surrounded a developing foetus, which had a 'stretched' appearance and an apparently thin layer of endometrium, and tissue from between developing foetuses, which appeared 'unstretched' and had a thick, spongey, endometrium. The uterine tissue was, therefore, divided into 'stretched' and 'unstretched' tissue. Each type of tissue was further divided into segments of approximately 1g, wet weight. Each segment was blotted dry and weighed. One segment from each tissue type was used a) to determine the endogenous prostaglandin content of the tissue, b) the, *in vitro*, synthetic ability of the uterine tissue to produce prostaglandins and c) the effect of the presence of excess arachidonic acid on the synthetic capacity of the tissue, as previously described in Section 3.1b. Any remaining uterine tissue was replaced in ice-cold, Krebs' solution and retained (Sections 4.1b and 5). Following extraction, samples were assayed by RIA for $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ as previously described (Sections 1.4a, b and c).

Results

The average litter size was 9.3 ± 1.3 foetuses per rabbit. The large litter size meant that the amount of 'unstretched' uterine tissue available was limited. In three of the four rabbits studied

it was not possible to measure the effect of excess arachidonic acid on prostaglandin production by incubates of 'unstretched' uterine tissue. The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ present in alcoholic homogenates of 'unstretched' and 'stretched' uterine tissue and in homogenised tissues, incubated in the absence and presence of added arachidonic acid are shown in Fig. 29. Results are expressed as the mean ± SEM of 4 animals, unless otherwise stated (number over relevant columns).

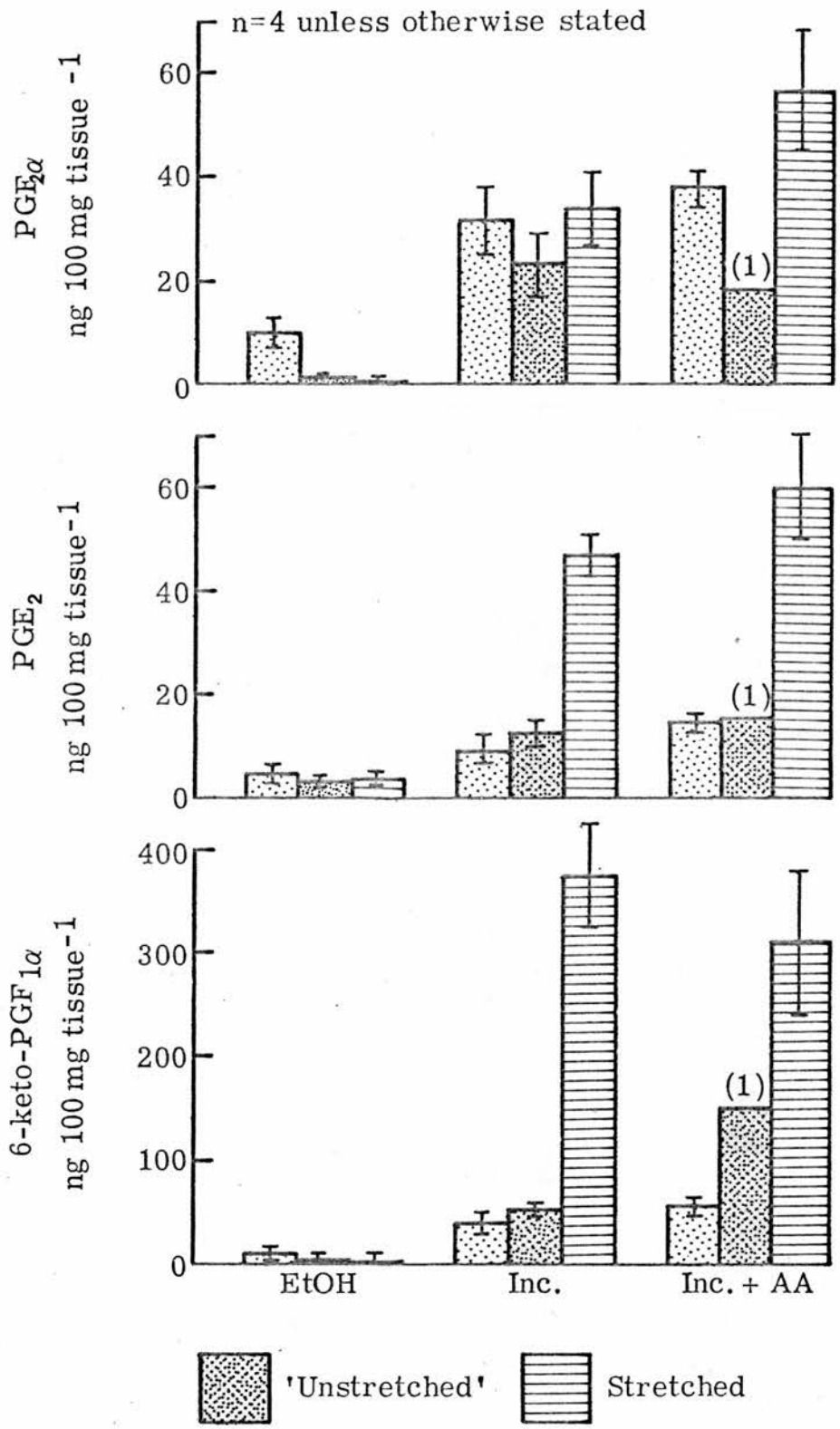
The endogenous concentrations of $PGF_{2\alpha}$ in 'unstretched' and 'stretched' uterine tissue on Day 17 of pregnancy showed no significant differences. Similarly, the endogenous concentrations of PGE and 6-keto-$PGF_{1\alpha}$ in 'unstretched' and 'stretched' uterine tissue did not differ significantly. The endogenous concentration of PGE_2 and 6-keto-$PGF_{1\alpha}$ in 'unstretched' and 'stretched' Day 17 pregnant uterine tissues were not significantly different from those in Day 17 pseudopregnant, uterine tissue. However, the endogenous concentration of $PGF_{2\alpha}$ in both 'unstretched' and 'stretched' uterine tissue on Day 17 of pregnancy was significantly lower ($p < 0.05$) than the endogenous concentration of $PGF_{2\alpha}$ in uterine tissue on Day 17 of pseudopregnancy (Fig. 30). Following incubation of both 'unstretched' and 'stretched' Day 17 pregnant, uterine tissue, there was a significant increase in the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ in each homogenate ($p < 0.05$) when compared with endogenous levels. The amounts of $PGF_{2\alpha}$ formed were not significantly different and did not differ significantly from the amount produced during incubation of Day 17 pseudopregnant, uterine tissue. There was no significant difference between the amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$ produced by 'unstretched' Day 17 pregnant, uterine tissue and Day 17 pseudopregnant, uterine


PGF2α
ng 100 mg tissue -1
60
40
20
(1)
PGE2
ng 100 mg tissue-1
60
40
20
(1)
6-keto-PGF1α
ng 100 mg tissue-1
400
300
200
100
(1)
EtOH
Inc.
Inc. + AA
'Unstretched'
Stretched

tissue, but both were significantly lower than the amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$ produced during incubation of 'stretched', Day 17 pregnant, uterine tissue (Fig. 30). The addition of 5µg ml^{-1} arachidonic acid to incubates of 'stretched' uterine tissue incubates on Day 17 of pregnancy, increased the amounts of $PGF_{2\alpha}$ and PGE_2 formed and slightly decreased the amount of 6-keto-$PGF_{1\alpha}$ formed. These changes, however, were not significant (Fig. 29). The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ formed during incubation of 'stretched', Day 17 pregnant, and Day 17 pseudopregnant, uterine tissue in the presence of 5µg ml^{-1} arachidonic acid did not differ significantly from the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ formed by these tissues when incubated in the absence of added arachidonic acid.

Conclusions

The endogenous concentration of $PGF_{2\alpha}$ in both 'unstretched', and 'stretched' uterine tissue homogenates on Day 17 of pregnancy is significantly lower than in uterine tissue homogenates on Day 17 of pseudopregnancy. This lower, endogenous level of $PGF_{2\alpha}$ in the Day 17 pregnant animal is unlikely to be due to a decrease in the synthetic capacity of the tissue as the amounts of $PGF_{2\alpha}$ produced by both 'unstretched', and 'stretched', Day 17 pregnant, and Day 17 pseudopregnant, uterine tissue are not significantly different. Instead, it may reflect a decrease in the synthesis of $PGF_{2\alpha}$, *in vivo*, when the cellular integrity of the tissue may be an important feature in controlling prostaglandin biosynthesis. "Stretched', Day 17 pregnant, uterine tissue synthesises significantly more PGE_2 and 6-keto-$PGF_{1\alpha}$ than either 'unstretched', Day 17 pregnant, or Day 17 pseudopregnant, uterine tissue. This increase may be related to the close proximity of the 'stretched', uterine tissue, with the tissues of the developing


n=4 unless otherwise stated
PGE2α
ng 100 mg tissue -1
60
40
20
0
(1)
PGE2
ng 100 mg tissue-1
60
40
20
0
(1)
6-keto-PGF1α
ng 100 mg tissue-1
400
300
200
100
0
(1)
EtOH
Inc.
Inc. + AA
'Unstretched'
Stretched

foetus and may contribute to the increase in PGE_2 and 6-keto-$PGF_{1\alpha}$ observed in the uterine venous plasma of pregnant rabbits.

SECTION 4. ENDOGENOUS LEVELS AND *IN VITRO* PRODUCTION OF $PGF_{2\alpha}$, PGE_2 AND 6-KETO-$PGF_{1\alpha}$ BY PLACENTAL TISSUE ON DAY 17 OF PREGNANCY AND THE EFFECT OF THE PRESENCE OF PLACENTAL TISSUE ON PROSTAGLANDIN PRODUCTION BY UTERINE TISSUE

Section 4.1a Experiment to determine the endogenous levels and *in vitro* production of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ by placental tissue on Day 17 of pregnancy.

Introduction

In the pseudopregnant rabbit the concentration of $PGF_{2\alpha}$ in the uterine venous plasma remains low until Day 17 of pseudopregnancy when it increases significantly. It is still elevated on Day 18, but has returned to basal levels by Day 19. The concentration of PGE_2 in the uterine venous plasma however, remains low throughout pseudopregnancy. In the pregnant animal the increase in $PGF_{2\alpha}$ output by the uterus on Days 17 and 18 of pseudopregnancy is not observed, but there is a significant increase in the uterine venous plasma concentration of PGE_2 from Day 11 of pregnancy onwards. This increase in the concentration of PGE_2 in the uterine vein occurs at the time when the placenta becomes established (Lutwak-Mann, 1971) and is positively correlated with the number of foetuses present in the uterine horn.

When extracts of uterine venous plasma from pregnant rabbits were derivatised and analysed by g.c. - m.s. using MID, they were found to contain an ion fragment, at the correct retention time, which corresponded to the m/e 491 ion of 6-keto-$PGF_{2\alpha}$. No such ion fragment was detected in extracts of uterine venous plasma from pseudopregnant rabbits.

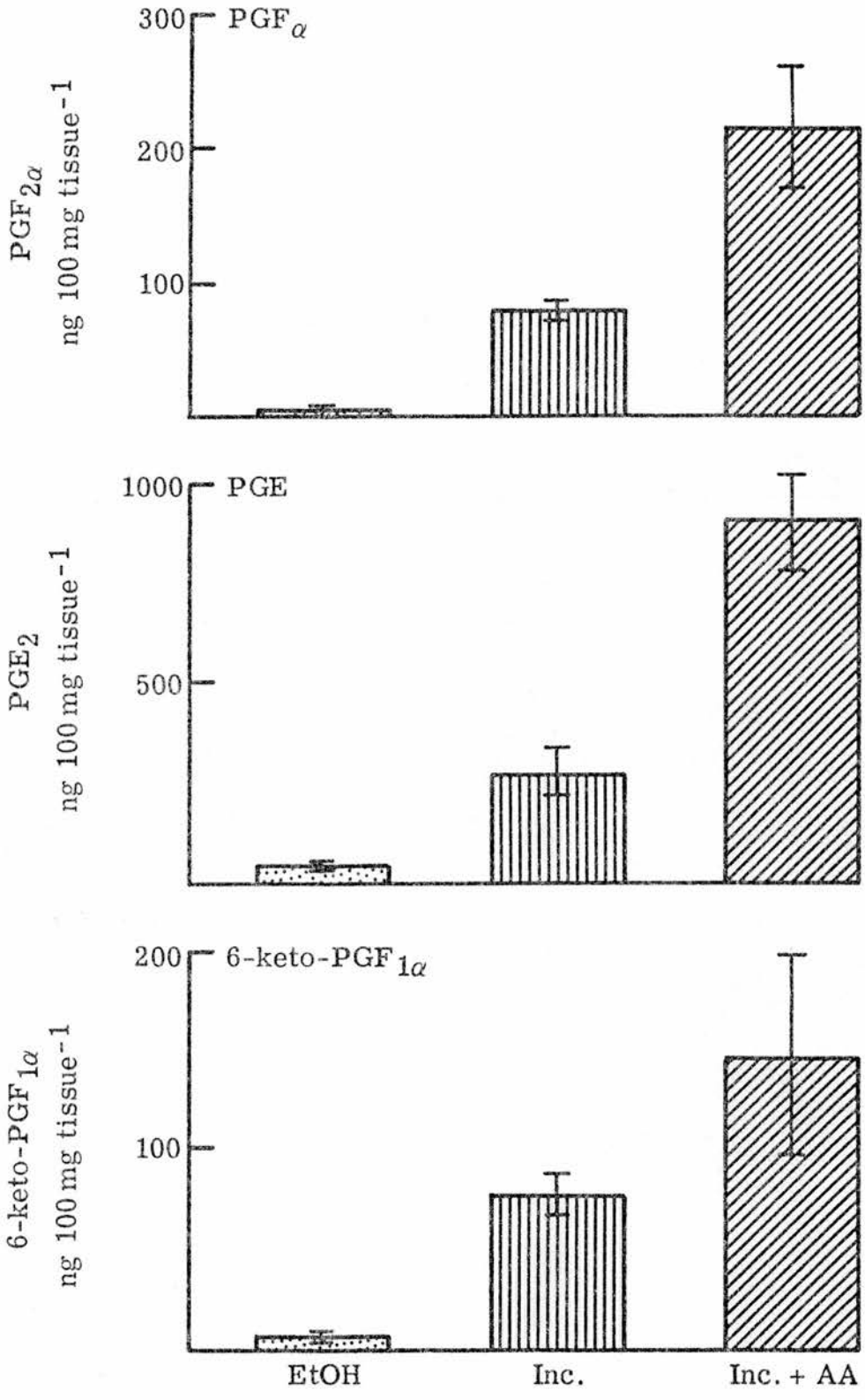
These findings suggest that the foetal-placental unit may synthesise PGE_2 and 6-keto-$PGF_{1\alpha}$. In the following experiment the ability of placental tissue homogenates, from Day 17 pregnant rabbits, to synthesise $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ has been measured.

Method

Placental tissue was obtained from the animals used in Section 3.2a as previously described. The placenta, consisting of the maternal decidua and the foetal-placenta, was dissected free of the myometrium and freed of the embryonic sac and its contents. The placental tissue from each animal was divided into segments of approximately 1g weight. Each segment was blotted dry and weighed. The endogenous prostaglandin content of the tissue and the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ produced during a 90 min incubation period, in the absence, or presence, of 5μg ml^{-1} arachidonic acid, was measured as described in Section 3.1b. In three animals there was sufficient placental tissue present for some of it to be divided into its maternal and foetal components. The endogenous concentration and the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ produced by the maternal-decidua and foetal-placenta, when incubated in Krebs' solution, was measured as described in Section 3.1b. The placental tissue remaining was replaced in ice-cold Krebs' solution and retained (Sections 4.2a and 5). The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ in the solvent-extracted tissue homogenates were measured by RIA as previously described (Section 1.4a, b and c).

Results

The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ in ethanolic homogenates of placental tissue, and in homogenates of placental tissue incubated in Krebs' solution, in the absence or presence of 5μg ml^{-1}


300
PGF$_\alpha$
200
100
PGF$_{2\alpha}$
ng 100 mg tissue^{-1}
1000
PGE
500
PGE$_2$
ng 100 mg tissue^{-1}
200
6-keto-PGF$_{1\alpha}$
100
6-keto-PGF$_{1\alpha}$
ng 100 mg tissue^{-1}
EtOH
Inc.
Inc. + AA

n=4

arachidonic acid, are shown in Fig. 31. The results are expressed as the mean ± SEM of placentae from 4 animals. The endogenous levels of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ were 1.57 ± 0.31, 44.33 ± 23.12 and 5.72 ± 1.92, ng 100 mg^{-1} tissue, respectively. Following incubation there was a significant increase in the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ in each homogenate ($p < 0.01$). Approximately 3.5 times as much PGE_2 was produced as $PGF_{2\alpha}$ or 6-keto-$PGF_{1\alpha}$. The addition of 5µg ml^{-1} arachidonic acid to the incubation medium significantly increased the amount of $PGF_{2\alpha}$ and PGE_2 formed ($p < 0.05$) but had no significant effect on the amount of 6-keto-$PGF_{1\alpha}$ produced, although the mean amount of 6-keto-$PGF_{1\alpha}$ synthesised was higher. The ratio of $PGF_{2\alpha}$ to PGE_2 to 6-keto-$PGF_{1\alpha}$ synthesised was 1.5 : 5.9 : 1.0.

The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ in ethanolic homogenates of the maternal-decidua and foetal-placenta, and the amounts of these compounds produced by these tissues during incubation, are shown in Table 16. The results are expressed as the mean ± SEM of 3 animals.

The endogenous levels and the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ produced during incubation of the maternal-decidua, were consistently lower than those of the foetal placenta. In both tissues the endogenous concentration of PGE_2 was higher than the endogenous concentration of $PGF_{2\alpha}$ or 6-keto-$PGF_{1\alpha}$. The foetal-placenta produced 7.8 times more $PGF_{2\alpha}$, 18.3 times more PGE_2 and 1.7 times more 6-keto-$PGF_{1\alpha}$ than the maternal-decidua during a 90 min incubation period. The major prostaglandin produced by the foetal-placenta was PGE_2 while the maternal-decidua produced approximately twice as much 6-keto-$PGF_{1\alpha}$ as $PGF_{2\alpha}$ or PGE_2.

Compound	Maternal-decidua		Foetal-Placenta	
	Endogenous ng 100 mg^{-1} tissue	Post-Incubation ng 100 mg^{-1} tissue	Endogenous ng 100 mg^{-1} tissue	Post-Incubation ng 100 mg^{-1} tissue
$PGF_{2\alpha}$	1.52 ± 0.08	31.82 ± 4.44	2.68 ± 0.01	246.60 ± 77.95
PGE_2	9.42 ± 6.51	28.82 ± 5.28	56.66 ± 13.6	527.61 ± 181.67
6-keto-$PGF_{1\alpha}$	1.96 ± 0.64	58.67 ± 6.32	5.22 ± 0.85	98.75 ± 57.07

Table 16. Concentration of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ in ethanolic homogenates (endogenous) of maternal-decidua and foetal-placenta on Day 17 of pregnancy, and the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$, produced by these tissues following homogenisation and incubation in Krebs' solution (mean ± SEM, n = 3).

Conclusion

Placental tissue, on Day 17 of pregnancy, has a high endogenous concentration of PGE_2. During incubation placental tissue homogenates synthesise approximately 3.5 as much PGE_2 as $PGF_{2\alpha}$ or 6-keto-$PGF_{1\alpha}$. The addition of 5µg ml^{-1} arachidonic acid to the incubation medium had little effect on the ratio of the amounts of $PGF_{2\alpha}$ to PGE_2 produced but increased the ratio of $PGF_{2\alpha}$ to 6-keto-$PGF_{1\alpha}$ from 1 to 1.5 and of PGE_2 to 6-keto-$PGF_{1\alpha}$ from 3.5 to 5.9. This suggests that, in the presence of excess arachidonic acid, PGE_2 and $PGF_{2\alpha}$ are synthesised in preference to 6-keto-$PGF_{1\alpha}$.

The foetal component of the placenta synthesises larger amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$, *in vitro*, then the maternal-decidua. The major prostaglandin to be synthesised by the foetal-placenta is PGE_2 while 6-keto-$PGF_{1\alpha}$ is the major prostaglandin to be synthesised by the maternal-decidua.

The high endogenous levels of PGE_2 and the large amounts of PGE_2 and $PGF_{2\alpha}$ produced during incubation of the placenta as a whole, are probably due to the presence of the foetal-placenta. The high level of PGE_2 in the uterine venous plasma of pregnant animals from Day 11 of pregnancy onwards is also probably due to the production of PGE_2 by the foetal placenta, although 'stretched' uterine tissue from round the developing foetus may also contribute (see previous section). The presence of 6-keto-$PGF_{1\alpha}$ in uterine venous plasma of pregnant, but not pseudopregnant, rabbits, is probably due a) to the presence of the placenta and b) to the increased ability of 'stretched', pregnant uterus, to synthesise larger amounts of 6-keto-$PGF_{1\alpha}$ than pseudopregnant, uterine tissue.

Section 4.1b Experiment to determine the effect of the presence of placental tissue homogenates on the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ formed by uterine tissue homogenates on Day 17 of pregnancy

Introduction

Maule Walker and Poyser (1974) have shown that uterine tissue from bilaterally pregnant guinea-pigs synthesises significantly less $PGF_{2\alpha}$ on Day 15 of pregnancy than uterine tissue on Day 15 of the oestrous cycle. In the unilaterally pregnant guinea-pig the amount of $PGF_{2\alpha}$ synthesised by the pregnant horn is the same as the amount synthesised by uterine tissue of the bilaterally pregnant animal while the non-pregnant horn synthesises approximately twice as much $PGF_{2\alpha}$ as either the unilaterally pregnant, or bilaterally pregnant, uterus. The amount of $PGF_{2\alpha}$ synthesised by the non-pregnant horn in the unilaterally pregnant animal however, is still significantly lower than the amounts synthesised by the Day 15, non-pregnant uterus (Blatchley *et al.*, 1975b). These results suggest that the conceptus supresses prostaglandin production by the uterus and that this effect has both a local and systemic component. In the pregnant sheep, removal of the embryo up to Day 12 of pregnancy results in a return to oestrus by Day 18, while removal of the embryo on Days 13, 14 and 15 of pregnancy prolongs the functional life span of the corpus luteum until Day 25 (Moor and Rowson, 1966a). Similarly, if embryos are transferred to the uteri of normally cycling sheep by Day 12 of the cycle the corpus luteum is maintained and becomes established as the corpus luteum of pregnancy (Moor and Rowson, 1966b). Therefore, if the corpus luteum of the oestrous cycle is to become established as the corpus luteum of pregnancy, the embryo, or its secretions

(Moor and Rowson, 1967) must be present in the uterus at the time at which, in the non-pregnant animal, the levels of $PGF_{2\alpha}$ in the uterine venous plasma would normally begin to increase (Thorburn *et al.*, 1972). In the pregnant sheep, however, the increase in $PGF_{2\alpha}$ in the uterine venous plasma seen on Days 14 to 16 of the oestrous cycle, is not observed. This suggests that in this species, as in the guinea-pig, the presence of the developing embryo in some way inhibits the synthesis and/or release of $PGF_{2\alpha}$ into the uterine vein.

In the pregnant rabbit the increase in $PGF_{2\alpha}$ in the uterine venous plasma observed on Days 17 and 18 of pseudopregnancy is absent. In the following experiment the effect of incubating pregnant uterine tissue in the presence of placental tissue, on Day 17 of pregnancy, has been measured to determine whether the presence of the placental tissue affects the synthetic capacity of the uterine tissue.

Method

'Stretched' uterine tissue and placental tissue from Day 17 pregnant rabbits was obtained from animals used in Section 3.2a. Placental tissue (i.e. maternal-decidua and foetal-placenta) was disected free of adhering uterine tissue and of the embryonic sac and its contents. Segments of 'stretched' uterine tissue and placental tissue, each weighing approximately 1g wet weight were prepared, blotted dry and weighed. 1.0g 'stretched' uterine tissue, and 1.0g placental tissue, from the same animal were homogenised together in a total of 15 ml Krebs' solution using a Fissons glass homogeniser as previously described in Section 1.2b. All homogenates were incubated for 90 min at 37°C as previously described (Section 1.2b). Homogenates were then solvent-extracted for prostaglandins (Section 1.2a) and assayed for $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ by RIA as

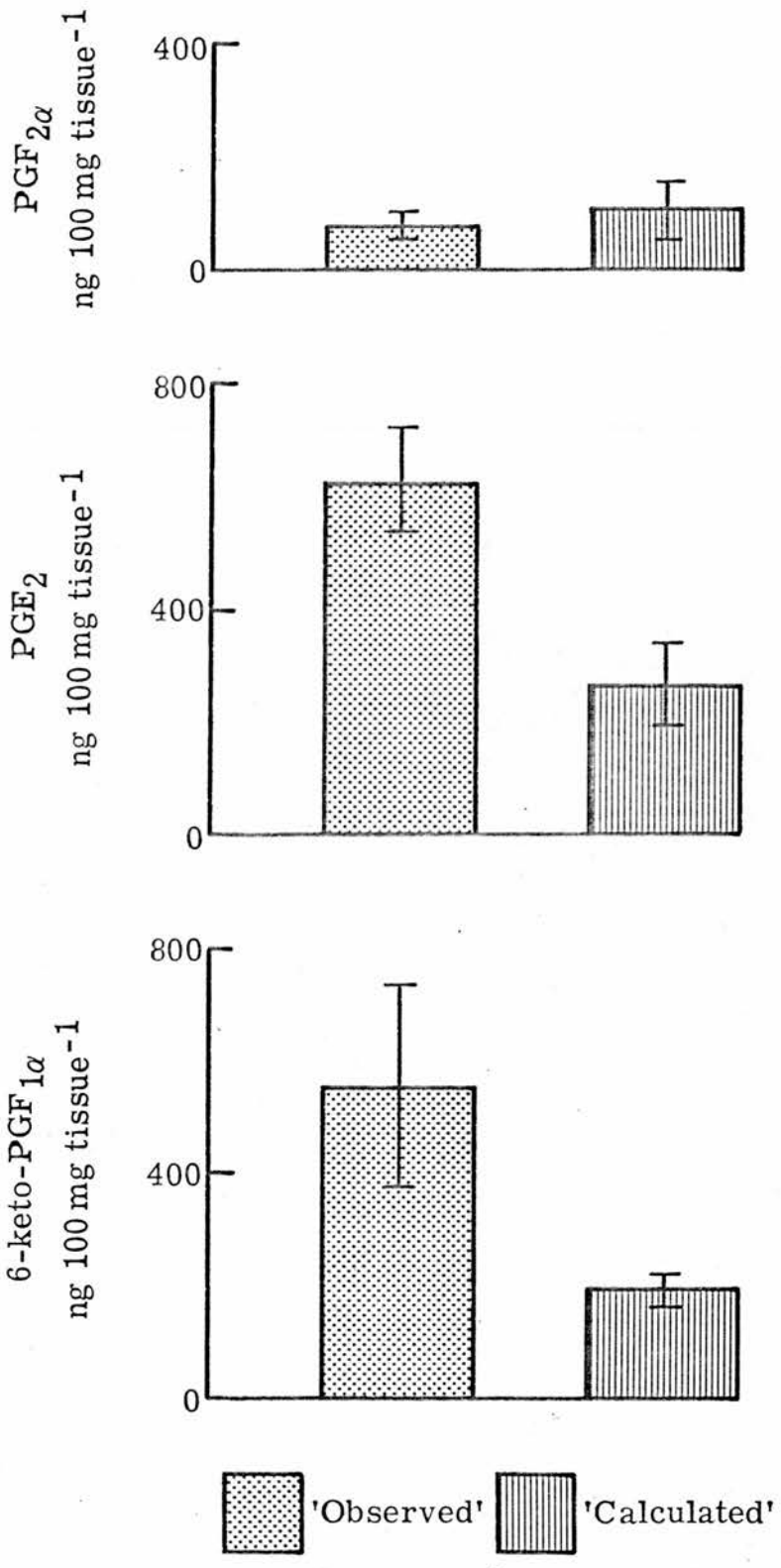
previously described. All determinations were performed in triplicate at two dilutions.

Results

The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ produced when homogenates of uterine and placental tissues, from Day 17 pregnant rabbits, are incubated together are shown in Fig. 32. Open bars represent the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ which were produced during the incubation period, expressed as ng 100 mg^{-1} of uterine/placental tissue. The amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ which would have been expected to be produced had the two tissue types been incubated separately and the products of the two incubations combined were calculated from the results of Section 3.2a and Section 4.1a (and, expressed as ng 100 mg^{-1} of uterine/placental tissue) are represented by the hatched bars in Fig. 32. When the 'observed' and 'expected' amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ were compared using Students' 't' test for both paired and unpaired data, there were no significant differences between the 'observed' and 'expected' amounts of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ formed although the mean amount of 6-keto-$PGF_{1\alpha}$ formed was higher than the calculated mean amount of 6-keto-$PGF_{1\alpha}$. The amount of PGE_2 formed during incubation of uterine tissue with placental tissue was significantly higher than the calculated amount of PGE_2.

Conclusion

The addition of placental tissue homogenates to incubates of uterine tissue from Day 17 pregnant rabbits does not significantly affect the amounts of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ produced but does appear to increase the *in vitro* ability of these tissues to synthesise PGE_2.


PGF2α
ng 100 mg tissue-1
400
0
PGE2
ng 100 mg tissue-1
800
400
0
6-keto-PGF1α
ng 100 mg tissue-1
800
400
0
'Observed'
'Calculated'
n=4

The reason for this increase in PGE_2 output is not clear. The addition of arachidonic acid to incubates of placental homogenates significantly increased the amounts of $PGF_{2\alpha}$ and PGE_2 formed during incubation but had no significant effect on the amounts of $PGF_{2\alpha}$, PGE_2 or 6-keto-$PGF_{1\alpha}$ produced during incubation of 'stretched', uterine tissue homogenates. The addition of homogenised, 'stretched', uterine tissue to the placental tissue homogenate may, therefore, increase the amount of arachidonic acid available and thereby contribute to the increase in PGE_2 production.

SECTION 5. EXPERIMENT TO MEASURE THE ABILITY OF UTERINE TISSUE ON SELECTED DAYS OF PSEUDOPREGNANCY AND OF UTERINE AND PLACENTAL TISSUE ON DAY 17 OF PREGNANCY, TO METABOLISE ^{3}H-$PGF_{2\alpha}$.

Introduction

In the previous sections the ability of pseudopregnant and pregnant uterine tissue, and of placental tissue to synthesise $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ have been measured. In all these studies the amounts of prostaglandins produced represent the total amount of prostaglandin synthesised minus the amount of prostaglandin metabolised by these tissues during the incubation period. In the following experiment the ability of pseudopregnant and pregnant uterine tissue and placental tissue on Day 17 of pregnancy to metabolise $PGF_{2\alpha}$ has been measured.

Method

Segments of uterine tissue (approximately 1g wet weight) from control and pseudopregnant rabbits (Section 3.1b) and from Day 17 pregnant rabbits (Section 3.2a) and placental tissue from Day 17 pregnant rabbits (Section 3.2a) were used. Each segment was blotted dry, and weighed and homogenised in 15 ml Krebs' solution as previously described (Section 3.1a). Nicotinamide adenine dinucleotide (NAD^+, to give a final concentration of 2.0 mM), 0.5µCi ^{3}H-$PGF_{2\alpha}$ (sp. act 160µCi $mmol^{-1}$) and 10µg authentic $PGF_{2\alpha}$ were added to each homogenate. All homogenates were incubated for 90 min at 37°C as previously described (Section 1.2b). Two further homogenates, one of uterine tissue from a Day 7 pseudopregnant rabbit, and one of placental tissue from a Day 17 pregnant rabbit, were also prepared as described above except that no NAD^+ was added to the incubation

medium. Following incubation all homogenates were solvent-extracted as previously described (Section 1.2a) and the residue redissolved in 200µl methanol and subjected to thin layer chromatography. ^{3}H-$PGF_{2\alpha}$ and non-radioactive marker standards (10µg each of $PGF_{2\alpha}$, 15-oxo-$PGF_{2\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$) were applied to 5 separate control plates. All plates were run first in solvent F VI (Andersen, 1969) and then in solvent system GCM (Millar, 1974) as previously described (Section 1.1a). Non-radioactive control plates were visualised by spraying with a saturated solution of phosphomolybdic acid in ethanol and heating at 110°C for 10 min, and the Rf values of the standards were calculated. Samples and marker standards containing radioactive material were scanned using a Panax radio thin-layer chromatographic plate scanner. The positions of the radioactive bands were identified from the scans and their positions relative to the solvent front recorded. The radioactive bands were then each scraped off and washed three times with 3 ml methanol. The washings were combined and transferred to scintillation vials. The methanol was evaporated off under a jet of air at 45°C and the dry residue was redissolved in 13 ml Scintillant. All samples were then monitored for radioactivity as previously described (Section 1.1a).

Results

The Rf values for the non-radioactive marker standards are shown in Table 17. Metabolism was calculated as a percentage of the total radioactivity recovered from each plate. Correction was made for the loss of tritiated hydrogen atoms. The results are expressed as the mean ± SEM of four animals and are shown in Table 18. The

major products of metabolism of $PGF_{2\alpha}$ had Rf values corresponding to

Compound	Rf value
$PGF_{2\alpha}$	0.33
15-oxo-$PGF_{2\alpha}$	0.51
13,14-dihydro-15-oxo-$PGF_{2\alpha}$	0.57

Table 17. Rf values for non-radioactive marker standards

15-oxo-$PGF_{2\alpha}$, 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ and a third, unidentified product with an Rf value of 0.16. The percentage conversion of ^{3}H-$PGF_{2\alpha}$ to this last product is included in the total percentage metabolism and is also shown separately in Table 18. The placenta

DAY of pseudopregnancy	% ^{3}H-$PGF_{2\alpha}$ unchanged	% Total Metabolism	% Metabolite Rf value .16
Control	68.29 ± 3.25	31.71 ± 3.25	n.d.*
Day 4	70.55 ± 6.06	29.65 ± 6.06	n.d.
Day 7	66.67 ± 7.92	35.70 ± 2.14	3.5 ± 2.14
Day 11	59.29 ± 5.02	40.54 ± 5.06	27.18 ± 12.86
Day 17	71.53 ± 4.78	28.47 ± 4.78	19.53 ± 1.01
Day 17 Pregnancy	66.80 ± 5.16	33.20 ± 6.06	16.00 ± 2.98
Day 17 Placenta	35.29 ± 3.09	64.72 ± 3.09	14.23 ± 0.17

Table 18. Percentage metabolism of ^{3}H-$PGF_{2\alpha}$ by uterine tissue on selected days of pseudopregnancy, and by uterine and placental tissue on Day 17 of pregnancy (mean ± SEM, n = 4) *n.d. non-detectable.

also produced another metabolite at Rf 0.24 which is included in Table 18.

Approximately 60-70% of added ^{3}H-$PGF_{2\alpha}$ was recovered unchanged following incubation with uterine tissue homogenates on selected days of pseudopregnancy and on Day 17 of pregnancy. In control animals and on Days 4 and 7 of pseudopregnancy the major radioactive metabolites had Rf values corresponding to 15-oxo-$PGF_{2\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$. However, from Day 7 onwards there was a steady increase in the percentage metabolism of ^{3}H-$PGF_{2\alpha}$ which was recovered as the unidentified metabolite (Rf value 0.16). The ratio of the percentage of ^{3}H-$PGF_{2\alpha}$ converted into 15-oxo-$PGF_{2\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ to the percentage of ^{3}H-$PGF_{2\alpha}$ recovered as the unidentified metabolite changed from 9.2 : 1 on Day 7, to 1 : 2 on Days 11 and 17 of pseudopregnancy, and to 1 : 1 on Day 17 of pregnancy.

Following incubation with placental tissue only, 35% of the added ^{3}H-$PGF_{2\alpha}$ was recovered in the unchanged form. The percentage of ^{3}H-$PGF_{2\alpha}$ metabolised by placental tissue incubates on Day 17 of pregnancy was significantly higher than that metabolised by any other tissue studied ($p < 0.05$). Approximately 14.23% of the metabolised ^{3}H-$PGF_{2\alpha}$ was recovered as the unidentified metabolite (Rf value 0.16) and 11.92% as another unidentified metabolite with an Rf value of 0.24. The ratio of the percentage of ^{3}H-$PGF_{2\alpha}$ converted to 15-oxo-$PGF_{2\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ to the percentage of ^{3}H-$PGF_{2\alpha}$ recovered as the unidentified metabolites, Rf values 0.16 and 0.24 respectively was 3.2 : 1.2 : 1.

In the two incubates, one of placental tissue and the other of uterine tissue, in which no NAD^{+} was added to the incubation medium, the radioactive material recovered ran as single spots on the TLC

plates, both spots having Rf values corresponding to $PGF_{2\alpha}$.

Conclusion

There is no significant difference in the ability of uterine tissue homogenates from control animals and on Days 4, 7, 11 and 17 of pseudopregnancy, and Day 17 of pregnancy, to metabolise ^{3}H-$PGF_{2\alpha}$. The increase in $PGF_{2\alpha}$ concentration in the uterine venous plasma of pregnant rabbits on Days 17 and 18 of pseudopregnancy, therefore, cannot be attributed to a decrease in the ability of the uterus to metabolise $PGF_{2\alpha}$. However, although the total percentage metabolism of ^{3}H-$PGF_{2\alpha}$ by uterine tissue homogenates did not change throughout pseudopregnancy or on Day 17 of pregnancy, there was a steady increase in the amount of ^{3}H-$PGF_{2\alpha}$ converted into the unidentified metabolite, Rf value 0.16, and a steady decrease in the percentage of ^{3}H-$PGF_{2\alpha}$ recovered as 15-oxo-$PGF_{2\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$. Powell (1978) found that following incubation of microsomal fractions of rabbit lung or liver with $PGF_{2\alpha}$ in the presence of reduced nicotinamide adenine dinucleotide (NADH), a compound was produced which was more polar than $PGF_{2\alpha}$ and which was identified as 20-hydroxy-$PGF_{2\alpha}$. The 20-hydroxylation activity of rabbit lung and, to a lesser extent, liver microsomes, increased dramatically during pregnancy, pseudopregnancy and following progesterone treatment of virgin female rabbits. The increase in 20-hydroxylation activity lags several days behind the increase in ovarian progesterone production and lasts several days longer. Powell has also reported that the rabbit placenta can convert $PGF_{2\alpha}$ to its 20-hydroxy derivative, although it is not as active as the lung or liver in this respect. The lung homogenates from pregnant rabbits also convert 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ to its 20-hydroxy derivative in the presence of NADH (Powell &

Solomon, 1978). Although the identity of the two polar metabolites, Rf values 0.16 and 0.24 respectively, formed during incubation of ^{3}H-$PGF_{2\alpha}$ with homogenates of uterine or placental tissue in the presence of NAD^{+} were not identified, it is probable that they are the 20-hydroxylation products of ^{3}H-$PGF_{2\alpha}$ and of 13,14-dihydro-15-oxo-$PGF_{2\alpha}$.

When uterine or placental tissue is incubated in the absence of NAD^{+}, there is no detectable metabolism of ^{3}H-$PGF_{2\alpha}$. As the same enzymes are responsible for the metabolism of $PGF_{2\alpha}$ and PGE_{2}, the amounts of $PGF_{2\alpha}$ and PGE_{2} synthesised by uterine tissue and placental tissue homogenates in Sections 2, 3, 4 and 6 reflect the total synthetic capacity of these tissues as the amount of loss of $PGF_{2\alpha}$ and PGE_{2} through metabolism is negligible.

SECTION 6. ENDOGENOUS LEVELS AND *IN VITRO* PRODUCTION OF $PGF_{2\alpha}$, PGE_2 AND 6-KETO-$PGF_{1\alpha}$ BY UTERINE TISSUE FROM OVARIECTOMISED RABBITS TREATED WITH PROGESTERONE AND OESTRADIOL-17β, EITHER ALONE OR IN COMBINATION

Introduction

In the normally cycling sheep, cow and guinea-pig, treatment with oestrogen or progesterone can result in a shortening of the oestrous cycle. Oestrogen is luteolytic on the cow when given on Days 2-12 of the cycle, in sheep when given after Day 10, and in the guinea-pig when given on Days 4 to 6 (Greenstein *et al.*, 1958; Stormshak *et al.*, 1968; 1969; Bland and Donovan, 1968; Choudary and Greenwald, 1968). Progesterone treatment, begun on Day 1 of the cycle and continued for several days, also decreases cycle length in the sheep, cow and guinea-pig (Loy *et al.*, 1960; Woody *et al.*, 1967). The luteolytic action of both these hormones appears to be mediated via the uterus as hysterectomy abolishes their luteolytic effect (Stormshak *et al.*, 1968, 1969; Bland and Donovan 1970; Rowlands, 1962; Brunner *et al.*, 1969; Kaltenbach *et al.*, 1964;). Moreover, in the hemi-hysterectomised sheep and cow, and hemi-hysterectomised and hemi-ovariectomised guinea-pig, progesterone and oestrogen will only shorten the length of the oestrous cycle when the retained horn is ipsilateral to the ovary containing the active corpora lutea (Stormshak *et al.*, 1968, 1969, Bland and Donovan 1970; Brunner *et al.*, 1969; Ginther 1968, 1969; Woody, Ginther and Casida 1967; Woody, Ginther and Pope, 1968).

Blatchley *et al.*, (1972) showed that the concentration of $PGF_{2\alpha}$ in the utero-ovarian vein of the guinea-pig fluctuates during the oestrous cycle. The highest concentrations of $PGF_{2\alpha}$ in the utero-

ovarian venous plasma were recorded on Day 15 when the levels of progesterone were low but those of oestradiol are high. In contrast, in the pregnant animal in which the levels of progesterone are still elevated, the utero-ovarian venous plasma levels of $PGF_{2\alpha}$ and oestradiol are low (Blatchley *et al.*, 1975a). The *in vivo* treatment of normally cycling guinea-pigs with 10µg oestradiol benzoate on Days 4 to 6 of the cycle results in an increase in the concentration of $PGF_{2\alpha}$ in the utero-ovarian venous plasma, as measured on Day 7 of the cycle (Blatchley *et al.*, 1971). The injection of oestradiol benzoate, either alone or in combination with progesterone into ovariectomised guinea-pigs also increases the concentration of $PGF_{2\alpha}$ in the utero-ovarian venous plasma and also increases the ability of the uterine tissue to synthesise $PGF_{2\alpha}$ (Blatchley and Poyser, 1974; Naylor and Poyser, 1975). These findings suggest that the increased ability of the uterus to synthesise $PGF_{2\alpha}$ at the end of the oestrous cycle is responsible for the increase in the uterine venous plasma levels of $PGF_{2\alpha}$ observed at this time and is under the hormonal control of oestrogen and progesterone.

In the sheep there is an increase in the concentration of oestradiol in the ovarian plasma between Days 10 and 12 and Days 15 and 16 of the cycle. Both increases precede an increase in $PGF_{2\alpha}$ output by the uterus (Cox *et al.*, 1974). The infusion of oestradiol into the uterine arterial blood supply of ewes in which the uterus has been transplanted to the neck, also increases the level of $PGF_{2\alpha}$ in the uterine venous effluent providing the animal has previously been primed with progesterone (Barcikowski *et al.*, 1974; Scaramuzzi, Boyle, Wheeler, Land and Baird, 1973). During the cycle $PGF_{2\alpha}$ synthesising capacity increases three days before oestrus

in the sheep, at a time when plasma oestradiol concentrations are increasing (Alwachi *et al.*, 1979).

In the following experiment, the endogenous content and the ability of the rabbit uterus to synthesise $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ following the *in vivo* treatment of ovariectomised rabbits with oestradiol and progesterone, either alone or in combination, has been measured, to investigate whether ovarian steroid hormones regulate uterine prostaglandin synthesising capacity in the rabbit.

Method

Twenty mature female New Zealand white rabbits were anaesthetised with sodium pentobarbitone (35 mg kg^{-1}). Following the opening of the abdominal wall by a mid-line, abdominal incision, ligatures were placed round the major ovarian blood vessels and the ovaries excised and removed. The abdominal wall was closed with cat-gut sutures and the skin closed with silk sutures. A month after ovariectomy, the rabbits were randomly divided into five groups of four animals per group and received daily injections of 0.5 ml arachis oil (vehicle), containing steroids, as follows:-

Group 1 - 0.5 ml vehicle for 10 days

Group 2 - 0.5 ml vehicle containing progesterone (10 mg) s.c. for 10 days

Group 3 - 0.5 ml vehicle containing progesterone (10 mg) s.c. twice daily for 10 days

Group 4 - 0.5 ml vehicle containing oestradiol-17β (E_2-17β, 2.0μg) for 10 days

Group 5 - 0.5 ml vehicle containing progesterone (10 mg) twice daily for 7 days followed by 0.5 ml vehicle containing E_2-17β (2.0μg) for 3 days

All injections were given subcutaneously (s.c.). Single injections of vehicle alone or vehicle containing progesterone or E_2-17β were given between 09.00 hr and 09.30 hr. Animals in Group 3, receiving two injections of progesterone (10 mg per injection) were injected between 09.00 and 09.30 hr and between 16.45 hr and 17.15 hr. The single daily injection of progesterone (10 mg) produced a mean concentration of progesterone in the marginal ear vein plasma which was similar to the physiological concentrations of progesterone observed during pseudopregnancy and pregnancy. Animals in Group 3 received a pharmacological dose of progesterone. The amount of E_2-17β injected (2.0μg per day) is the amount required to sustain pregnancy in hypophysectomised rabbits, or in pregnant rabbits which have had their follicles destroyed (Keyes & Nalbandov, 1967).

On the 11th day after starting treatment, 2 ml blood were collected from the marginal ear vein of each rabbit as previously described (Section 2.1b). The blood was centrifuged, and the plasma was solvent-extracted and assayed for progesterone as previously described (Sections 1.3d and 1.4d). Each animal was killed by stunning and incising the neck. The uterus was removed, blotted dry and weighed. The two uterine horns were opened longitudinally and three segments, of approximately 1g wet weight each, were cut. Each segment was weighed and then used to determine one of the following:

(a) the endogenous uterine tissue prostaglandin level

(b) the *in vitro* ability of the uterine tissue to synthesise prostaglandins or

(c) the effect of the presence of excess arachidonic acid on the synthetic capacity of the tissue,

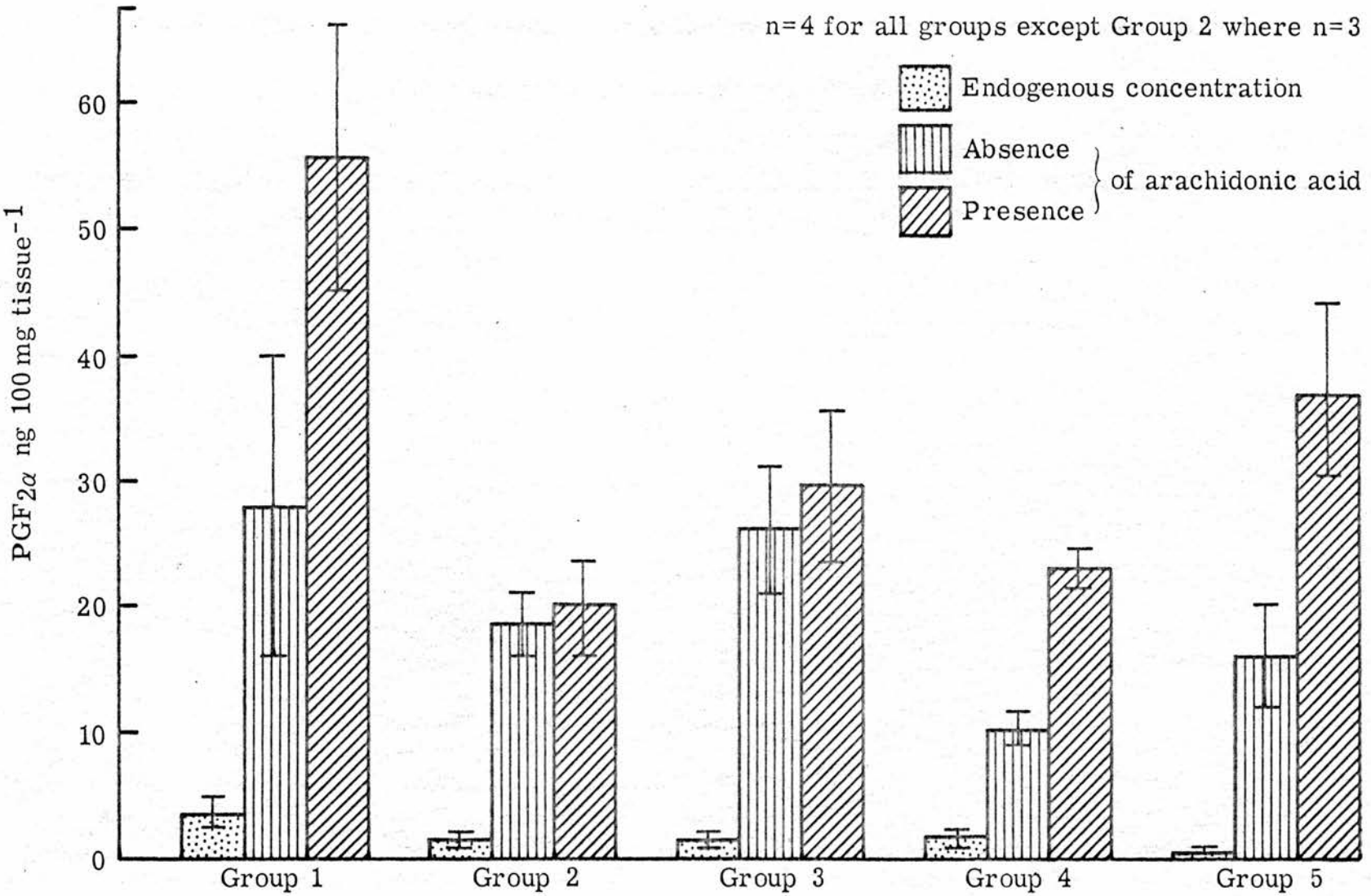
as previously described in Section 3.1b. Following solvent-extraction, samples were assayed by RIA for $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ as described in Sections 1.4a, 1.4b and 1.4c. Comparisons of endogenous prostaglandin levels and the amounts of prostaglandin synthesised by uterine tissue, in the absence and presence of added arachidonic acid, in the five treatment groups studied, were performed using Students' 't' test for unpaired data unless otherwise stated.

Results

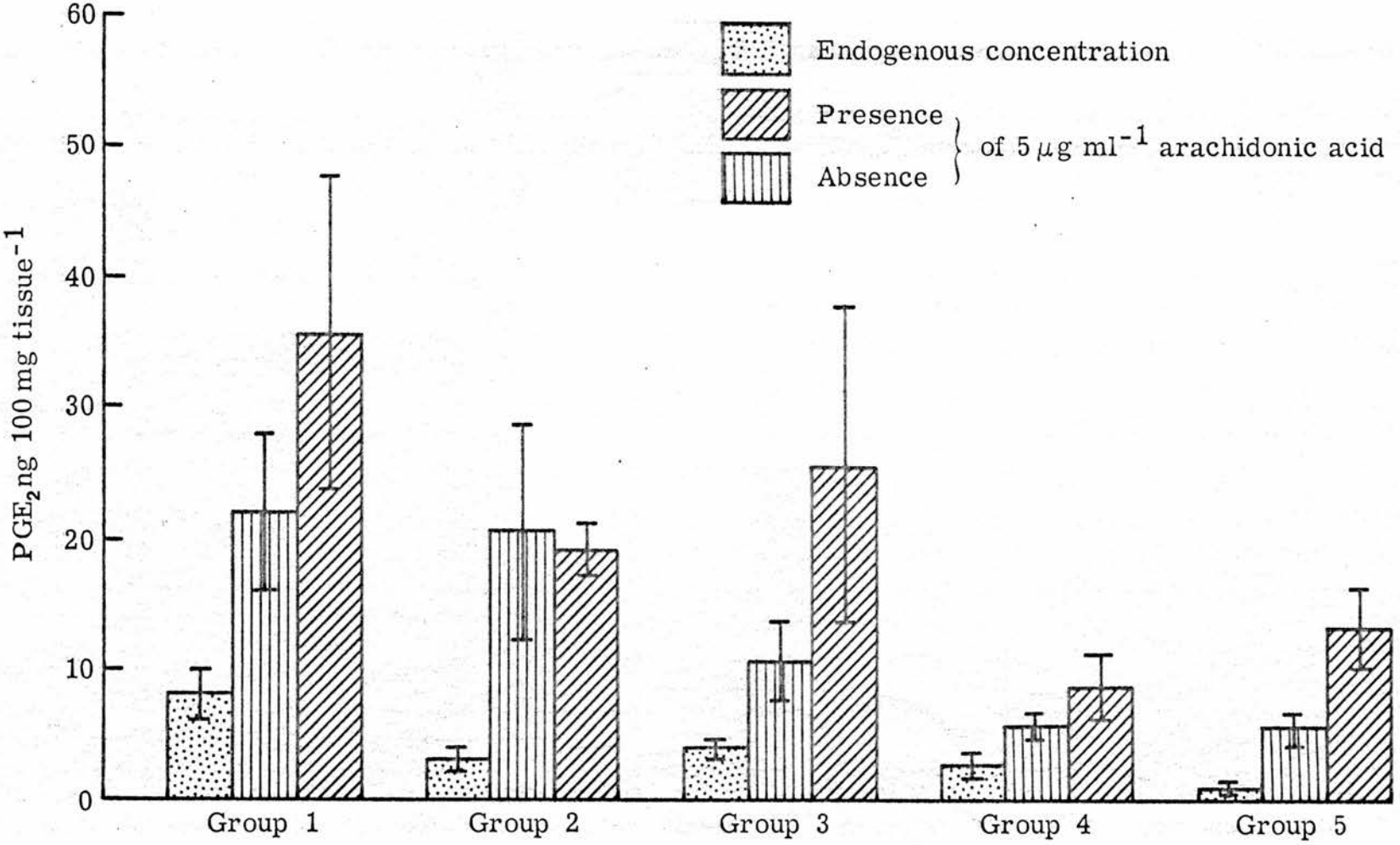
The concentration of progesterone in the marginal ear vein plasma and the effect of the various hormonal treatments on the weight of the uterus, in the different groups, are shown in Table 19. Uterine tissue from animals in Group 4 and 5 weighed significantly more ($p < 0.001$) than uterine tissue from animals in Groups 1 and 3, but did not differ significantly from each other. Treatment with progesterone alone (Group 3) also significantly increased the weight of the uterus when compared to uteri from control animals ($p < 0.05$).

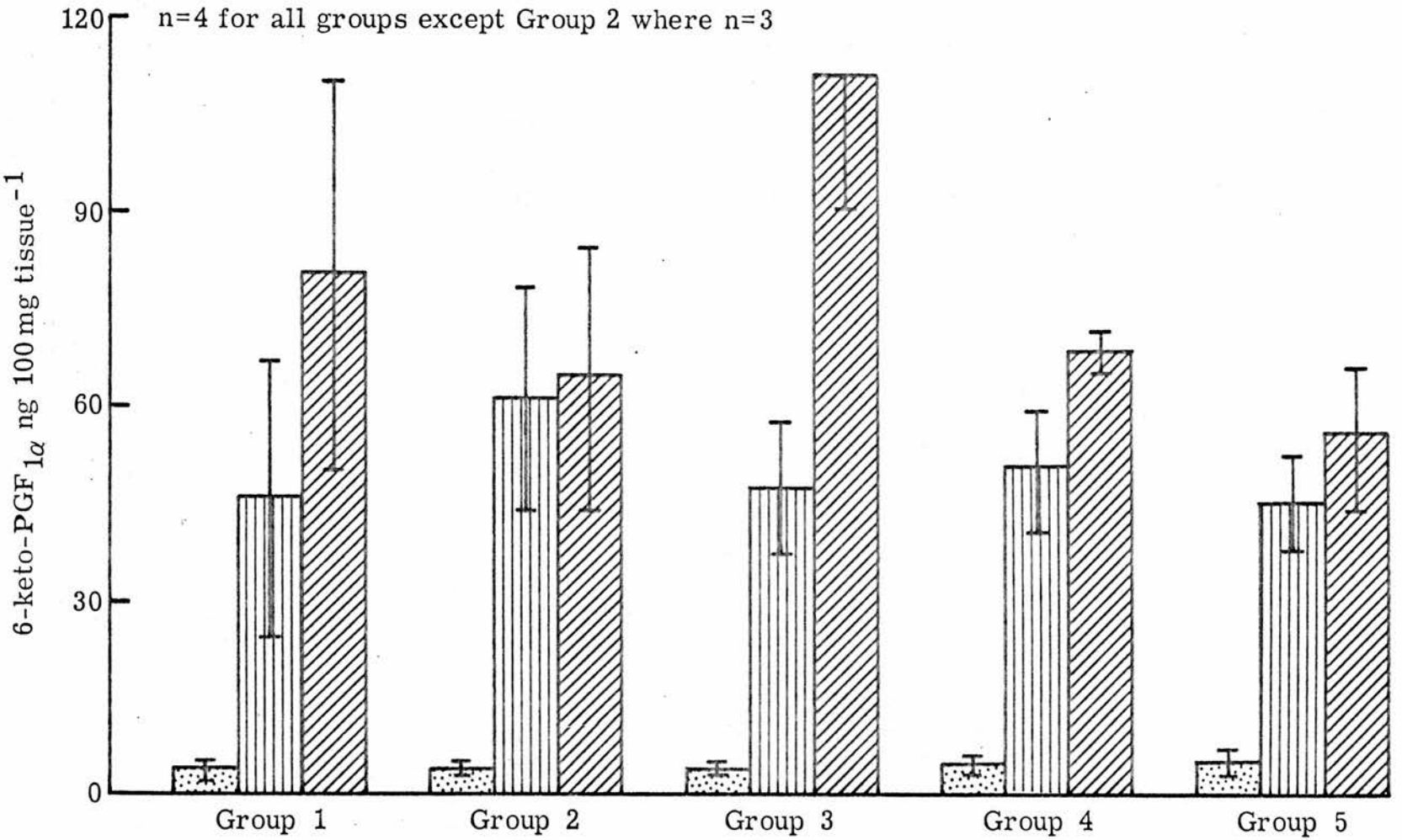
The endogenous levels of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$, and the *in vitro* capacity of the uterine tissue from animals in Groups 1 to 5 to synthesise these prostaglandins, in the absence or presence of excess arachidonic acid, are shown in Figs. 33, 34 and 35. Results are expressed as the mean ± SEM of four animals ($n = 4$) except in Group 2 where one animal died before treatment was completed ($n = 3$).

The endogenous level of PGE_2 in uterine tissue in animals in Group 1 (control) were significantly higher than in Group 2 ($p < 0.02$), Group 3 ($p < 0.05$), Group 4 ($p < 0.05$) and Group 5 ($p < 0.02$). The endogenous level of PGE_2 in uterine tissue from animals receiving


n=4 for all groups except Group 2 where n=3
Endogenous concentration
Absence
Presence
of arachidonic acid
PGF2α ng 100 mg tissue-1
0
10
20
30
40
50
60
Group 1
Group 2
Group 3
Group 4
Group 5

n=4 for all groups except Group 2 where n=3




n=4 for all groups except Group 2 where n=3
120
90
60
30
0
6-keto-PGF 1α ng 100 mg tissue -1
Group 1
Group 2
Group 3
Group 4
Group 5

Group No	Wt. of uterus g	Concentration of Progesterone in ear vein plasma ng ml^{-1}
1	1.79 ± 0.26	0.64 ± 0.07
2	n.m.	19.08 ± 5.09
3	3.82 ± 0.45	60.32 ± 7.04
4	11.12 ± 1.40	less than 0.4
5	11.95 ± 1.62	28.82 ± 7.60

Table 19. Concentrations of progesterone in ear vein plasma, and the weight of uteri in ovariectomised rabbits treated in the following way (mean ± SEM).

Group 1 - 0.5 ml arachis oil (vehicle) s.c. for 10 days

Group 2 - 0.5 ml vehicle containing progesterone (10 ml) s.c. for 10 days

Group 3 - 0.5 ml vehicle containing progesterone (10 mg) s.c. twice daily for 10 days

Group 4 - 0.5 ml vehicle containing E_2-17β (2μg) s.c. for 10 days

Group 5 - 0.5 ml vehicle containing progesterone (10 mg) s.c. twice daily for 7 days followed by

0.5 ml vehicle E_2-17β (2μg) s.c. for 10 days

n.m. = not measured

n = 4 for all Groups except Group 2 where n = 3.

progesterone followed by E -17β (Group 5), was also significantly lower than the endogenous level of PGE_2 in animals receiving progesterone only (Groups 2 and 3), but did not differ significantly from the endogenous level of PGE_2 in uterine tissue from animals treated with E_2-17β alone (Group 4). The endogenous concentration

of $PGF_{2\alpha}$ in uterine tissue from animals in Group 1 was significantly higher than the endogenous levels of $PGF_{2\alpha}$ in uterine tissue from animals in Group 3 and Group 5. There were no significant differences between the endogenous levels of $PGF_{2\alpha}$ in uterine tissues of animals in Groups 2, 3, 4 and 5. There were no significant differences in the endogenous uterine tissue levels of 6-keto-$PGF_{1\alpha}$ in any of the five treatment groups studied.

Following incubation there was a significant increase in the amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ produced per unit weight of uterine tissue compared to the endogenous tissue concentrations of these three compounds. The amounts of PGE_2 synthesised by uterine tissue from control animals (Group 1) were significantly higher ($p < 0.05$) than the amounts of PGE_2 synthesised by animals receiving E_2-17β, either alone or following progesterone pre-treatment (Groups 4 and 5), but did not differ significantly from the amounts of PGE_2 produced by uteri from animals receiving progesterone alone (Groups 2 and 3). The amounts of PGE_2 synthesised by uterine tissue from Groups 2 and 3 also tended to be higher than the amounts synthesised by uterine tissue from Groups 4 and 5, but these differences were not significant. Uterine tissue from control animals also tended to synthesise more $PGF_{2\alpha}$ than uterine tissue from the other treatment groups but the large variation between individual animals from the other treatment groups and the large viariation between individual animals in Group 1 meant that the results were not significant. The amount of $PGF_{2\alpha}$ produced by uterine tissue from animals in Groups 2 and 3 were significantly higher ($p < 0.05$) than the amounts synthesised by uterine tissue from animals in Groups 4 or 5. There were no significant differences between the amounts of 6-keto-$PGF_{1\alpha}$

synthesised by uterine tissue from any of the five treatment groups studied.

When Students' 't' test for paired data was used to compare results the addition of 5μg ml^{-1} arachidonic acid to the incubation medium significantly increased the amounts of $PGF_{2\alpha}$ synthesised by uterine tissue from animals in Group 1 ($p < 0.02$), Group 4 ($p < 0.01$) and Group 5 ($p < 0.01$) but, although it tended to increase the amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$ formed by uterine tissue from all five groups, these increases were not significant. The mean amounts of $PGF_{2\alpha}$ and PGE_2 synthesised by uterine tissue in the presence of exogenous arachidonic acid from animals in Group 1 were higher than in any other treatment group. However, the results were only significant when comparing $PGF_{2\alpha}$ production in Group 1 with $PGF_{2\alpha}$ production by uterine tissue from animals in Group 2 ($p < 0.05$) and Group 4 ($p < 0.05$).

The mean amounts of PGE_2 produced by animals receiving progesterone only (Groups 2 and 3) were higher than the amounts of PGE_2 synthesised by uterine tissue from animals receiving E_2-17β, either alone (Group 4) or following a period of progesterone priming (Group 5) but, because of the wide variation in animals in Groups 2 and 3, this difference was only significant when comparing values in Group 2 with values in Group 4 ($p < 0.05$). There were no significant differences in the amounts of $PGF_{2\alpha}$ produced by uterine tissue from Groups 2, 3, 4 and 5.

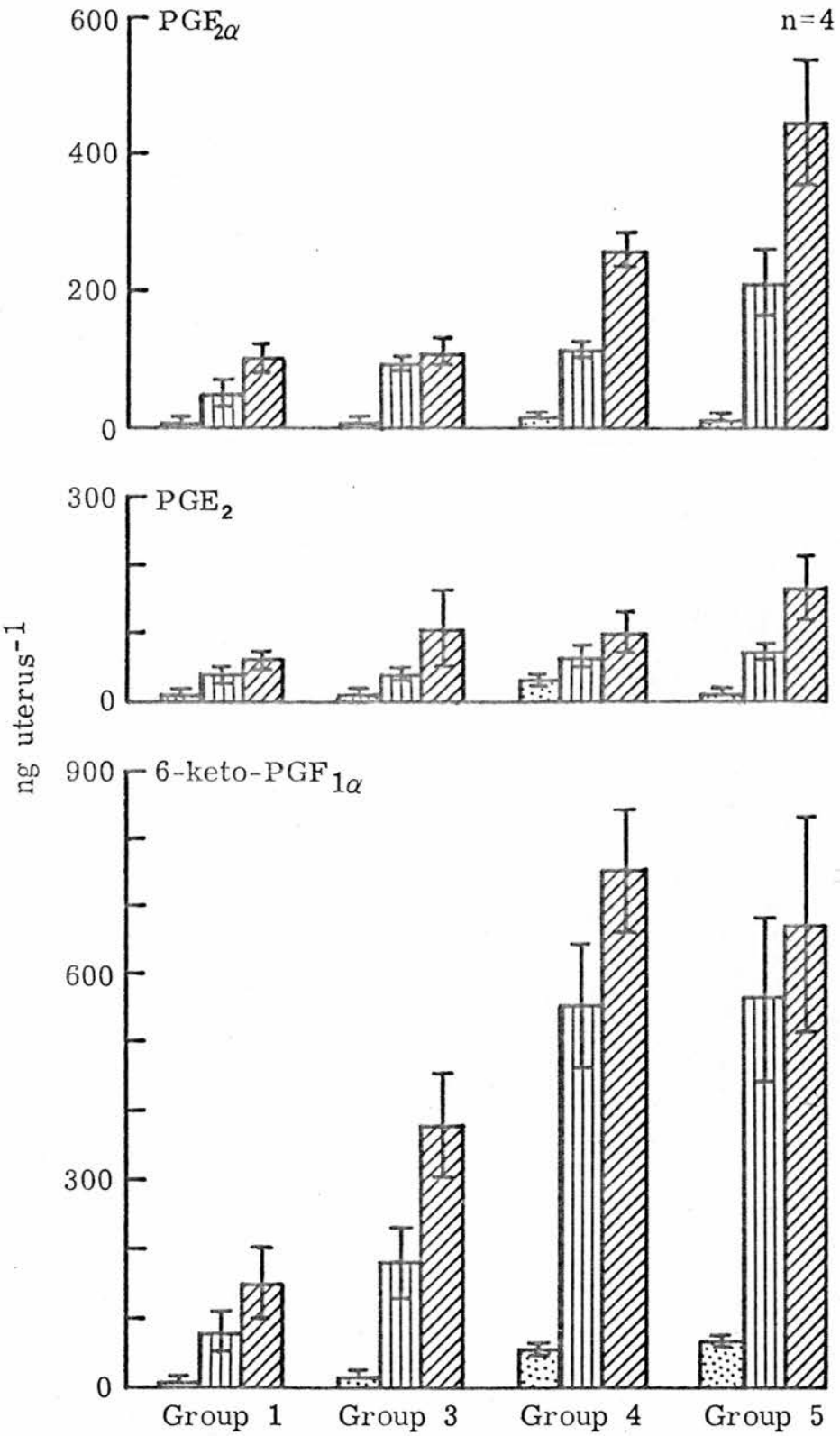
The addition of 5μg ml^{-1} arachidonic acid to the incubation medium did not significantly affect the amounts of 6-keto-$PGF_{1\alpha}$ formed by uterine tissue in all treatment groups. The mean amount of 6-keto-$PGF_{1\alpha}$ synthesised by uterine tissue from animals in Group 3

was higher than in any other group studied, but this difference was not significant, probably because of the wide variation in individual animals within many of the groups. The major prostaglandin to be produced during incubation in all groups studied, however, was 6-keto-$PGF_{1\alpha}$.

Conclusion

In the ovariectomised rabbit, the uterine tissue is capable of synthesising quite large amounts of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$. *In vivo* treatment with progesterone, at both physiological or pharmacological doses, did not significantly affect the capacity of the uterine tissue to synthesise $PGF_{2\alpha}$, PGE_2 or 6-keto-$PGF_{1\alpha}$, *in vitro*, whereas the administration of E_2-17β (2.0μg per day) for 10 days decreased the amounts of $PGF_{2\alpha}$ and PGE_2 synthesised per unit weight of uterine tissue. E_2-17β administered after a period of progesterone priming also decreased the amount of PGE_2, but not $PGF_{2\alpha}$, synthesised by uterine tissue *in vitro*. This decrease in $PGF_{2\alpha}$ and PGE_2 production following treatment with E_2-17β could be related to a decrease in the availability of precursor as the addition of excess arachidonic acid to the incubation medium increased the amount of $PGF_{2\alpha}$ and, to a lesser extent, the amount of PGE_2 produced by uterine tissue from animals treated with E_2-17β alone or following a period of progesterone priming. The *in vivo* treatment of rabbits with progesterone and E_2-17β, either alone or in combination, did not affect the capacity of uterine tissue to synthesise 6-keto-$PGF_{1\alpha}$.

Although the *in vivo* treatment of ovariectomised rabbits with E_2-17β, either alone or after a period of progesterone priming, decreased the amounts of $PGF_{2\alpha}$ and PGE_2 formed per 100 mg uterine tissue, the increase in uterine weight following E_2-17β treatment,


600
PGE2α
n=4
400
200
0
300
PGE2
0
ng uterus-1
900
6-keto-PGF1α
600
300
0
Group 1
Group 3
Group 4
Group 5

means that the total amount of prostaglandin synthesised by the uterus as a whole is highest in those animals receiving E_2-17β. Fig. 36 shows the total amount of $PGF_{2\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesised by the whole uterus in Groups 1, 2, 3, 4 and 5. The amount of PGE produced by the uterus as a whole was not altered significantly by any of the hormone treatments studied. However, the amounts of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ synthesised by whole uteri in Groups 4 and 5 were significantly higher than the amounts synthesised by uteri from control animals ($p < 0.01$). The mean amount of $PGF_{2\alpha}$ synthesised by uteri from animals receiving both progesterone and E_2-17β (Group 5) was higher than the amount synthesised by uteri from animals receiving only progesterone or E_2-17β. The amount of 6-keto-$PGF_{1\alpha}$ synthesised by uteri from animals in Group 3 was significantly lower than the amount synthesised by uteri from animals in Group 4 ($p < 0.02$) and Group 5 ($p < 0.05$). The addition of 5μg ml^{-1} arachidonic acid to the incubation medium significantly increased the amounts of $PGF_{2\alpha}$ produced by uteri from animals in Groups 4 and 5. It is possible that the increased amount of $PGF_{2\alpha}$ synthesised by uteri in Group 5 (when compared to Group 4) could be caused by an increase in arachidonic acid availability since progesterone treatment has been reported to increase the number of lipid droplets in endometrial cells (Brinsfield and Hawk, 1973). The mean amount of 6-keto-$PGF_{1\alpha}$ synthesised by uteri from animals receiving progesterone only (Group 3) was still significantly lower than the amounts synthesised by uteri from animals in Group 4 and 5 in the presence of added arachidonic acid. These results suggest that, as in the sheep and guinea-pig, the maximum potential for uterine $PGF_{2\alpha}$ synthesis occurs after a period of progesterone priming followed by exposure to oestrogen.

DISCUSSION

Scott and Rennie (1970) have shown that in the pseudopregnant rabbit, as in the cycling sheep, cow, pig and guinea-pig, and in the pseudopregnant rat and hamster, the functional lifespan of the corpus luteum is determined, at least in part, by the uterus. This effect is mediated *via* a uterine luteolytic hormone and, in the sheep and guinea-pig, there is now strong evidence to suggest that $PGF_{2\alpha}$ is the luteolytic principle involved.

As outlined earlier, in order for a compound to be ascribed the role of a uterine luteolysin it must fulfil certain minimum criteria. More specifically, if $PGF_{2\alpha}$ is the agent involved then these criteria can be stated as follows.

1) $PGF_{2\alpha}$ should be luteolytic *in vivo* when administered in doses that fall within the physiological range.
2) $PGF_{2\alpha}$ should be present in the uterine vein at the time of luteal regression.
3) Indomethacin, at low doses (0.17 - 38.0μM) which specifically inhibit the cycloxygenase enzyme in the prostaglandin synthetase system (Flower, 1974), should prolong luteal function.
4) Immunisation against $PGF_{2\alpha}$ should also prolong luteal function.

$PGF_{2\alpha}$ is luteolytic in both the pseudopregnant and pregnant rabbit when administered *in vivo*, either subcutaneously at doses of 1 to 5 mg kg^{-1} day^{-1} (Gutknecht *et al.*, 1970; Carlson and Gole, 1978) or *via* an intra-arterial or intravenous infusion of 10μg hr^{-1} (Challis *et al.*, 1974; Laudański, Batra and Åkerlund, 1979). Subcutaneous administration of indomethacin (8 mg kg^{-1} $12h^{-1}$) or immunisation against $PGF_{2\alpha}$ also prolongs luteal function in the pseudopregnant rabbit (O'Grady *et al.*, 1972; Caldwell *et al.*, 1972). $PGF_{2\alpha}$

therefore possesses several of the properties of a uterine luteolytic hormone in this species. However, before $PGF_{2\alpha}$ can be identified as a uterine luteolytic hormone in the rabbit it is necessary to show that the onset of luteolysis is immediately preceded by an increase in the uterine secretion of $PGF_{2\alpha}$.

In the rabbit the corpus luteum is the major source of progesterone (Keyes and Nalbandov, 1967; Eaton and Hilliard, 1971). During pseudopregnancy and pregnancy the metabolic clearance rate of progesterone remains fairly constant (Thau and Lanman, 1976) so that peripheral plasma concentrations reflect progesterone secretion by the luteal tissue and can be used as an index of the functional state of the corpus luteum. In the pseudopregnant animal the concentration of progesterone in the peripheral venous plasma was found to increase up to Day 7, remain elevated until Day 14 and then decline steadily to reach pre-injection levels by Day 19 (Fig. 17). A similar profile of peripheral plasma progesterone levels has been reported by other workers although the absolute values reported by them tend to be lower (Harrington and Rothermel, 1977; Miller and Keyes, 1976; Browning, Keyes and Wolf, 1980). These differences are most probably due to the higher dose of HCG used to induce pseudopregnancy in this study. Harrington and Rothermel (1977) have reported that following the induction of pseudopregnancy with 50-100 i.v. HCG, the plasma progesterone levels observed on Days 8 through to Day 13 were significantly higher than the levels recorded in animals mated to vasectomised males. The dose of HCG used does not appear to alter the initial number of ovulation points but higher doses of HCG do appear to cause the subsequent luteinization of non-ovulated follicles, possibly because of the long half life of HCG. It is also possible

that the HCG could affect the 'absolute' secretion rate of progesterone by the corpora lutea. In experiment 2.1b, however, in which the same dose of HCG was used to induce pseudopregnancy in intact and hysterectomised animals, the range of progesterone concentrations in the peripheral venous plasma is lower than in Fig. 17 and is similar to that reported elsewhere (Miller and Keyes, 1977). The reason for this discrepancy is not known.

The concentration of $PGF_{2\alpha}$ in the uterine venous plasma remained low until Day 17 of pseudopregnancy when it rose significantly to reach peak values of 4.51 ± 1.55 ng ml^{-1}. It was still elevated on Day 18 (2.34 ± 0.73 ng ml^{-1}) but had declined to 'basal' levels by Day 19. As this increase in uterine venous $PGF_{2\alpha}$ concentration was not seen in either the aortic or peripheral venous plasma levels, it seems likely that the increase is due to a specific release of $PGF_{2\alpha}$ from the uterus and is not a consequence of a general increase in prostaglandin synthesis and/or release within the animal as a whole. The increase in uterine $PGF_{2\alpha}$ output occurs 2 to 3 days after the mean peripheral plasma progesterone levels have begun to decline. This suggests that although $PGF_{2\alpha}$ released from the rabbit uterus after Day 16 may contribute to luteal demise, it is unlikely to initiate it. Failure to detect an increase in uterine $PGF_{2\alpha}$ secretion prior to Day 17 may have been due to the sampling technique used. The large volumes of blood needed for assay purposes precluded the taking of serial blood samples. Therefore, if $PGF_{2\alpha}$ release from the rabbit uterus occurs in a pulsatile fashion, as has been reported in the sheep (Thorburn *et al.*, 1973), cow (Nancarrow *et al.*, 1973) and pig (Gleeson and Thorburn, 1973), it is possible that an increase in uterine $PGF_{2\alpha}$ output would escape detection until such time as pulse

frequency and height led to gross changes in the uterine venous plasma concentration of $PGF_{2\alpha}$. Another point that should be taken into consideration is that the concentrations of $PGF_{2\alpha}$ recorded here do not take into account changes in uterine venous blood flow. During collection of the blood samples it was noted that the diameter of the uterine vein increased in size as pseudopregnancy progressed and was accompanied by a tendency to shorten the time required to collect 20 ml of blood. Measurements of uterine blood flow were not made, however, since the method of collection and the anatomy of the uterine vasculature would have made it difficult to relate these to the overall output of $PGF_{2\alpha}$ from the uterus. It is possible that, had $PGF_{2\alpha}$ release been expressed in terms of ng ml^{-1} min^{-1}, an increase in uterine $PGF_{2\alpha}$ output may have been observed prior to Day 17 of pseudopregnancy. If such a release did occur, and if it was important for the initiation of luteolysis, then the decline in peripheral plasma progesterone levels observed after Day 14 of pseudopregnancy should not occur in the hysterectomised, pseudopregnant rabbit. As can be seen from Fig. 19, this decline did occur and the concentration of progesterone in the peripheral venous plasma declined from Day 13 onwards in both intact and hysterectomised rabbits and, although the concentrations of progesterone in the hysterectomised animal tended to be higher than those observed in the intact animal, this difference was not significant except on Days 17, 18 and 19 of pseudopregnancy ($p < 0.05$), the time at which $PGF_{2\alpha}$ release from the uterus was found to increase in the intact animal. These results, therefore, support the earlier hypothesis that $PGF_{2\alpha}$ of uterine origin is unlikely to be responsible for the initiation of luteolysis but does contribute to the more rapid and final demise of luteal function after Day 16.

The increase in $PGF_{2\alpha}$ on Day 17 and 18 of pseudopregnancy coincides with the onset of the morphological regression of the corpora lutea reported by Scott and Rennie (1970).

Carlson and Gole (1978) have also tried to correlate changes in the plasma progesterone levels with changes in the levels of PGF in the posterior vena cava. As in the present study, evidence of functional regression of the corpus luteum, as determined by measurement of the venous plasma progesterone levels, occurred between Days 14 and 15 of pseudopregnancy. However, they found no significant increase in PGF concentrations in the posterior vena cava, either at this time or on later days of pseudopregnancy. Their failure to detect the increase in $PGF_{2\alpha}$ secretion observed in the present study may have been due to a dilution effect since blood from the vena cava is comprised of the venous effluents of a number of tissues besides that of the uterus. This could also explain why the levels of PGF recorded by Carlson and Gole (1978) tend to be slightly higher than the basal levels observed in the uterine venous plasma in this study. Moreover, Carlson and Gole (1978) measured the levels of PGF by radioimmunoassay of solvent-extracted blood samples. As mentioned in Section 1.3b, it was found that rabbit plasma contained a constituent which interfered with the assay. In order to avoid this interference, all plasma samples in the present study were subjected to silicic acid column chromatography following solvent extraction prior to being assayed by radioimmunoassay. The omission of a chromatographic step could also help to explain why the levels of PGF in the posterior vena cava reported by Carlson and Gole (1978) were higher than those observed in the uterine vein, aorta and ear vein in this study between Days 6 to 16 of pseudopregnancy.

In the pregnant rabbit, the presence of a functional corpus luteum is required throughout pregnancy. If $PGF_{2\alpha}$ is responsible for terminating luteal function at the end of pseudopregnancy, then some mechanism should operate to negate its effect in the pregnant animal. In the sheep and guinea-pig, the presence of the developing embryos results in a decrease in $PGF_{2\alpha}$ output from the uterus in the early pregnant animal (Thorburn *et al.*, 1973; Nett *et al.*, 1976; Poyser and Maule Walker, 1979; Antonini *et al.*, 1976). In the pregnant sow, $PGF_{2\alpha}$ output from the uterus is still fairly high during early pregnancy but is still lower than in the non-pregnant animal and appears to be accompanied by a redirection of $PGF_{2\alpha}$ secretion away from the vasculature towards the uterine lumen (Moeljono *et al.*, 1977).

The increase in $PGF_{2\alpha}$ concentrations in the uterine venous plasma seen on Days 17 and 18 of pseudopregnancy is not observed in the pregnant rabbit. The concentrations of $PGF_{2\alpha}$ remains low, approximately 1 ng ml^{-1}, until Day 24 when they rise significantly ($p < 0.05$). They reached peak values on Day 25 (4.97 ± 0.22 ng ml^{-1}) and, although still elevated on Day 26 (2.0 ng ml^{-1}) had returned to 'basal' levels by Day 27. The suppression of uterine $PGF_{2\alpha}$ secretion on Days 17 and 18 of pseudopregnancy may be important in maintaining the function of the corpora lutea of pregnancy. However, other factors of embryonic and placental origin are also involved (Laudanski *et al.*, 1979; Holt and Ewing, 1974; Fuchs and Beling, 1974; Singh and Adam, 1978).

It would appear, therefore, that $PGF_{2\alpha}$ does act as a uterine luteolytic hormone in the pseudopregnant rabbit. However, the rabbit differs from other species in which $PGF_{2\alpha}$ acts as a uterine luteolysin

in several respects :-

(1) The release of $PGF_{2\alpha}$ from the rabbit uterus, although responsible for terminating luteal function, does not appear to initiate it.

(2) The rabbit lacks the well defined unilateral, utero-ovarian pathway observed in such species as the sheep, guinea-pig, cow and pseudopregnant rat and hamster. Del Campo and Ginther (1972, 1973) have shown that in those species in which there is a well defined 'unilateral' effect of the uterus on the ovary, the anatomy of the uterine vasculature is such as to permit a counter-current mechanism of transfer to operate, similar to that proposed for the sheep and cow. In the rabbit, however, the anatomy of the uterine vasculature is sufficiently independent from the ovarian vasculature to make such a mechanism of transfer unlikely. It is possible that some $PGF_{2\alpha}$ of uterine origin could reach the ovary *via* the ovarian artery since branches of the ovarian artery and vein, which are closely apposed to one another, also supply and drain the cranial section of the uterine horn. The amount of $PGF_{2\alpha}$ which could be transferred in this way, however, is likely to be small and, since such a system would give rise to a 'unilateral' effect, it is probable that $PGF_{2\alpha}$ transferred by this route is insufficient to terminate luteal function. Scott and Rennie (1970) have shown that when corpora lutea are grafted to beneath the kidney capsule of a pseudopregnant rabbit, they regress at the same time as the ovarian corpora lutea unless the recipient doe has been previously hysterectomised. These results suggest that the mechanism by which the uterus controls the functional life span of the corpus luteum has a systemic component. The ability of the lungs to metabolise prostaglandins makes it unlikely that such an

effect is mediated *via* the circulation but it is possible that $PGF_{2\alpha}$ from the uterus could reach the ovaries by way of the lymphatic system. The possible involvement of the lymphatic system is the transfer of $PGF_{2\alpha}$ from the uterus to the ovary has so far been ignored and is in need of investigation. At present, however, it is difficult to see how $PGF_{2\alpha}$ from the uterus reaches the ovary in sufficient amounts to induce luteolysis in the rabbit.

(3) In the cycling sheep, guinea-pig and cow, there is an increase in the endogenous tissue levels of $PGF_{2\alpha}$ towards the end of the oestrous cycle at the time luteal regression occurs (Wilson *et al.*, 1972; Poyser, 1972; Shemesh and Hansel, 1975). Since this increase appears to coincide with the increase in utero-ovarian plasma levels of $PGF_{2\alpha}$ (McCracken *et al.*, 1972; Blatchley *et al.*, 1972, Shemesh and Hansel, 1975) it is probable that the endogenous tissue levels reflect the synthetic activity of the uterine tissue *in vivo*. Similarly in the rabbit the endogenous uterine tissue levels of $PGF_{2\alpha}$ on Day 17 of pseudopregnancy are significantly higher than in uterine tissue from rabbits on Days 4, 7 and 11 of pseudopregnancy and from control rabbits not injected with HCG. The endogenous uterine levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ are also significantly higher on Day 17 of pseudopregnancy than on any other day studied suggesting that there is a general increase in prostaglandin synthesised at this time. In the guinea-pig (Poyser, 1972) and sheep (Huslig *et al.*, 1979; Alwachi *et al.*, 1979) the increase in uterine venous plasma levels and uterine tissue levels of $PGF_{2\alpha}$ is correlated with an increase in prostaglandin synthetase levels. However, experiments with uterine tissue homogenates showed that although uterine tissue from Day 17 pseudopregnant rabbits does tend

to synthesise slightly more $PGF_{2\alpha}$ than uterine tissue on Days 4, 7 and 11 of pseudopregnancy, there is no significant change in the ability of the uterine tissue to synthesise (or metabolise) $PGF_{2\alpha}$ during pseudopregnancy. The increase in uterine $PGF_{2\alpha}$ output on Day 17 of pseudopregnancy, therefore, does not correlate with changes in the prostaglandin synthesising capacity of the uterus. A similar observation has been made in the rat where increased levels of PGF in the uterine venous plasma during dioestrus appear to be unrelated to changes in the uterine capacity to synthesise prostaglandins (Saksena, Shaikh and Shaikh, 1973; Poyser and Scott, 1980). Also there is no increase in the prostaglandin synthesising capacity of the rat uterus at the end of pseudopregnancy (Fenwick, Jones, Naylor, Poyser and Wilson, 1977).

(4) Uterine tissue homogenates from pseudopregnant rabbits synthesises significantly more $PGF_{2\alpha}$ and PGE_2 *in vitro* than uterine tissue from non-pseudopregnant rabbits. Since the addition of exogenous arachidonic acid to the incubation medium increases the amount of PGE_2 and $PGF_{2\alpha}$ formed to that produced by uterine incubates from pseudopregnant animals the reduced ability of uterine tissue from non-HCG injected rabbit to synthesis prostaglandins may be due to a lack in substrate availability. The increase in uterine $PGF_{2\alpha}$ output on Days 17 and 18 of pseudopregnancy, however, cannot be due to an increase in substrate availability since the addition of arachidonic acid to the incubation medium does not increase the amount of $PGF_{2\alpha}$ synthesised to any significant extent. However, it is possible that when the cellular integrity of the uterus is maintained the tissue may preferentially synthesise $PGF_{2\alpha}$ towards the end of pseudopregnancy. It should also be pointed out that in the rabbit

there is a substantial increase in uterine weight during pseudo-pregnancy and the increase in uterine mass could also help to account for the increase in uterine $PGF_{2\alpha}$ output on Days 17 and 18 of pseudo-pregnancy.

(5) In the pregnant sheep (Thorburn *et al.*, 1973), guinea-pig (Blatchley *et al.*, 1975a), cow (Kindahl *et al.*, 1976; Lukaszewska and Hansel, 1980) and pig (Moeljono *et al.*, 1977; Shille *et al.*, 1979) the increase in uterine venous $PGF_{2\alpha}$ observed towards the end of the oestrous cycle is not observed in the pregnant animal. Similarly in the pregnant rabbit the increase in uterine venous plasma levels of $PGF_{2\alpha}$ seen on Days 17 and 18 of pseudopregnancy is not observed. Unlike the guinea-pig, however, this decrease in uterine $PGF_{2\alpha}$ output is not associated with a decrease in the ability of the uterine tissue to synthesise $PGF_{2\alpha}$, *in vitro*, or with an increase in its ability to metabolise $PGF_{2\alpha}$. However, since the endogenous concentration of $PGF_{2\alpha}$ in both 'unstretched' and 'stretched' uterine tissue on Day 17 of pregnancy is significantly lower than in uterine tissue on Day 17 of pseudopregnancy, it is possible that, *in vivo*, the pregnant uterus does in fact synthesise less $PGF_{2\alpha}$ than the pseudopregnant uterus. On the other hand, it is also possible that, as has been proposed for the pregnant pig (Bazer and Thatcher, 1977) and sheep (Ellinwood *et al.*, 1980) that $PGF_{2\alpha}$ released by the pregnant rabbit uterus instead of entering the vascular system, is secreted into the uterine lumen. The concentration of $PGF_{2\alpha}$ in uterine flushings from pregnant rabbits has not been measured.

(6) The rabbit uterus also shows some differences in its response to oestrogen and progesterone when compared to such animals as the sheep and guinea-pig. In the ovariectomised guinea-pig

the injection of oestradiol, either alone or in combination with progesterone, increased the ability of the uterus to synthesise $PGF_{2\alpha}$, *in vitro* (Naylor and Poyser, 1975). In the ovariectomised rabbit, however, oestradiol treatment significantly reduced the ability of uterine tissue to synthesise $PGF_{2\alpha}$ when compared to progesterone treatment. The reduction in $PGF_{2\alpha}$ synthesising ability induced by oestrogen treatment is probably due to lack of substrate since it is abolished by the addition of exogenous arachidonic acid. In the progesterone primed, oestrogen treated rabbit, the ability of the uterus to synthesise $PGF_{2\alpha}$ from endogenous arachidonic acid is similar to that observed in progesterone only treated animals. However, unlike progesterone only treated animals, the addition of arachidonic acid to the incubation medium significantly increases the amount of $PGF_{2\alpha}$ synthesised. This suggests that, as in other species (see Introduction) the maximum potential for uterine $PGF_{2\alpha}$ synthesis occurs after a period of progesterone priming followed by exposure to oestrogen. In the pseudopregnant rabbit, the peripheral plasma concentration of oestradiol-17β increases steadily from Day 12 of pseudopregnancy until oestrus (Browning *et al.*, 1980), while in the pregnant rabbit, although the circulating levels of oestrogen remain fairly constant there is a steady increase in the ratio of progesterone to oestradiol-17β from Day 21 (Challis Davies and Ryan, 1973). This increase in oestrogen and/or in the progesterone : oestrogen ratio may be important in controlling uterine prostaglandin synthesis.

It is clear, therefore, that the factors responsible for controlling prostaglandin production by the uterus in the pseudopregnant and 'early' pregnant rabbit are different from those observed to

operate in other species such as the sheep and guinea-pig.

The significance of the increase in the uterine venous plasma concentration of $PGF_{2\alpha}$ on Day 24, 25 and 26 of pregnancy is not known. It does not appear to induce luteolysis since the increase in $PGF_{2\alpha}$ levels is not accompanied by a decrease in peripheral plasma progesterone levels. Chiboka, Casida and First (1977) have shown that foetectomy in Day 25 pregnant rabbits (leaving the placentae *in situ*) does not prevent delivery of the placentae from occurring at the normal time. When foetectomy is performed on Day 21, however, placental delivery is inhibited. These results imply that between Days 21 and 25 of pregnancy, the placentae receive a signal from the foetus which confers autonomy on the placentae to initiate their own delivery. Whether the increase in uterine venous plasma $PGF_{2\alpha}$ levels on Days 24 to 26 of pregnancy forms part of the signal or arises as a consequence of it requires further investigation. Elzayat and Stylos (1974) have reported that when rabbits immunised against $PGF_{2\alpha}$ are made pregnant, abortion occurs around Day 24. In these animals pregnancy progressed as normal until Day 14 but thereafter signs of retarded embryonic growth and placental insufficiency were evident. The reasons for these observations is not known but the results imply that $PGF_{2\alpha}$ may be involved in maintaining placental function. Challis, Davies and Ryan (1975) have shown that when indomethacin is administered either orally, from Day 20 to term, or subcutaneously from Days 23 to 28 of pregnancy, parturition is delayed by up to two days whereas indomethacin treatment on Days 29 to 31 does not prolong gestation to any significant extent. These results also imply that prostaglandin secretion between Days 23 to 28 of pregnancy is important in determining the time of delivery. As the above treatment did not affect

the decline of peripheral plasma progesterone levels, the effect on the time of delivery is unlikely to be related to the luteolytic properties of $PGF_{2\alpha}$. As in several other species, glucocorticoid treatment during the latter stages of pregnancy has been reported to shorten the length of gestation in the rabbit. In this respect it is interesting to note that the single injection of dexamethasone into the amniotic sac will only induce premature delivery when given on Day 25 of pregnancy (Kendall and Liggins, 1972). Whether this is related to the 'signal' which passes from the foetus to the placenta at this time and/or to the increase in uterine $PGF_{2\alpha}$ output is not known and requires further investigation. Premature delivery can also be induced by the continuous infusion of cortisol (Nathanielsz and Abel, 1973) or by the daily administration of dexamethasone (Challis *et al.*, 1975) from Day 21 of pregnancy and does not appear to be affected by indomethacin treatment (Davies, Yoshinaga and Ryan, 1976). It is clear that further work must be performed if the importance of the increase in uterine $PGF_{2\alpha}$ secretion on Days 24 to 26 of pregnancy on the parturient process is to be elucidated.

Although this study was not concerned with a detailed investigation into the uterine output of $PGF_{2\alpha}$ at term, it was noted that the uterine venous plasma concentrations of $PGF_{2\alpha}$ were higher in the two rabbits which were in the early stages of labour than in rabbits of the same gestational age in which labour had not yet started (3.84 ± 1.53 *vs* 0.75 ± 0.62 ng ml^{-1}, Day 31; and 1.44 ± 0.33 *vs* 0.38 ± 0.04 ng ml^{-1}, Day 32). Challis *et al.* (1973) found that there was a significant increase in the peripheral plasma concentration of PGF between Days 21 and 30 of pregnancy, and this increase occurred

immediately prior to, or coincident with the decline in progesterone levels. The levels of PGF in the peripheral venous plasma reported by Challis *et al.* (1973) are in good agreement with those reported here. However, no significant increase in the peripheral plasma levels of $PGF_{2\alpha}$ towards the end of pregnancy was observed.

In the pregnant rabbit the uterus remains relatively quiescent throughout gestation until about 48 hr before delivery. The increase in uterine activity which occurs at this time is coincident with an increase in uterine sensitivity to oxytocin. Characteristically, delivery is achieved with a sudden outburst of strong uterine contractions and the entire litter is delivered within 11.5 ± 2 min (Fuchs, 1964; Fuchs, 1978). It is possible that the uterine output of $PGF_{2\alpha}$ does increase during delivery. However, it is unlikely that such an increase would be detected in the peripheral venous plasma because of the rapid inactivation of prostaglandins by the lung. If measurements of uterine $PGF_{2\alpha}$ output during the delivery period are to be made, it would be more sensible to look at the peripheral plasma levels of $PGF_{2\alpha}$ metabolites in plasma obtained from conscious animals at this time. Fuchs (1978) has suggested that $PGF_{2\alpha}$ may play a role in the parturient process by virtue of its luteolytic properties. The experimental data on which this 'hypothesis' is based relies on the fact that, following indomethacin treatment, plasma progesterone levels have been claimed to decrease more slowly than in untreated animals (Davies *et al.*, 1976). The results reported in Section 2.2a do not support this idea since the decline in peripheral plasma progesterone levels occurred independently of any increases in uterine $PGF_{2\alpha}$ output. Moreover, since indomethacin also blocks phosphodiesterase activity at similar

concentrations to those at which it inhibits cyclo-oxygenase activity, it is possible that the slowed decrease in plasma progesterone levels observed by Davies *et al.* (1976) is due to the accumulation of intracellular c-AMP. $PGF_{2\alpha}$ may contribute to the activation of the myometrium during the delivery period since the levels of $PGF_{2\alpha}$ in the uterine venous plasma of the rabbit were found to be elevated during the early stages of labour.

The concentrations of PGE_2 were low and varied little in plasma from the aorta and ear vein during pseudopregnancy and pregnancy, and from the uterine vein during pseudopregnancy. In the pregnant rabbit, however, the concentrations of PGE_2 in the uterine venous plasma were markedly elevated after Day 11. Similarly high levels have been reported by Venuto, O'Dorisio, Stein and Ferris (1975) and by Meese, Fischer, Hoffman and Frolich (1980). The PGE_2 appears to be of foetal-placental origin since in rabbits made unilaterally pregnant by ligation of the oviduct prior to mating, the levels of PGE_2 in the uterine venous plasma on the sterile side are apprixi-mately 77% lower than the levels of PGE_2 in the uterine venous plasma collected from the intact, pregnant side (Frolich, Whorton, McKenna and Hoffman, 1978). Moreover, in the present study there is a positive correlation between the uterine venous plasma levels of PGE_2 and the number of foetuses present in the uterine horn. The endogenous levels of PGE_2 in the foetal-placental tissue, and the ability of this tissue to synthesise PGE_2, *in vitro* is also very high when compared to the endogenous levels and synthetic ability of the maternal decidua and Day 17 pregnant uterine tissue to synthesise PGE_2.

The significance of this large output of PGE_2 has not been studied in any detail. Elzayat and Stylos (1974) have shown that

when rabbits immunised against PGE are made pregnant, the doe dies shortly after mid-pregnancy. Post mortem studies have shown that the corpora lutea and ovaries appear to be functioning normally but that the placentae show infarcts, thromboses and haemolysis. This suggests that, like $PGF_{2\alpha}$, PGE_2 may be important in maintaining placental growth and function.

Since PGE_2 is known to elevate ovarian c-AMP levels and to stimulate progesterone production in a number of species, including the sheep (Marsh, 1971), pig (Kolera and Channing, 1972), human (McNatty, Henderson and Sawyers, 1975) and cow (Speroff and Ramwell, 1970), it is possible that PGE_2 may form part of the placental luteotrophic stimulus in the rabbit. However, since plasma progesterone levels decline at the end of pregnancy at a time when PGE_2 output is still high, it seems unlikely that ovarian progesterone secretion is controlled by PGE_2. Also, in rabbits immunised against PGE_2, the corpora lutea did not regress (Elzayat and Stylos, 1974). It seems more likely that PGE_2 released locally by the placenta is important in maintaining placental vascular patency. A similar mechanism for PGE_2 in regulating the ventillation-perfusion ratios in the lungs of dogs, swine and lambs has been proposed (Kadowitz, Joiner and Hyman, 1975; Orehek, Douglas, Lewis and Bouhuys, 1973). Whatever the action of PGE_2 it is presumably of a local nature since the large increases found in uterine venous plasma are not observed in the aortic plasma. This implies that the lungs remove PGE_2 from the venous blood. The enhanced ability of the rabbit lung to inactivate PGE_2 during pregnancy (Bedwani and Marley, 1975), due to a 20-fold increase in lung prostaglandin dehydrogenase activity (Sun and Armour, 1974), may be of importance in this respect.

In summary, therefore, the results of the experiments presented in this thesis suggest that $PGF_{2\alpha}$ of uterine origin is important in terminating luteal function in the pseudopregnant rabbit. The increased output of $PGF_{2\alpha}$ from the uterus on Day 17 of pseudopregnancy does not appear to be related to an increase in the ability of uterine tissue homogenates to synthesise $PGF_{2\alpha}$ *in vitro*, nor to the availability of substrate. However, it is possible that *in vivo* $PGF_{2\alpha}$ may be synthesised preferentially at this time. The capacity of the uterus to synthesise PGE_2 and $PGF_{2\alpha}$ is affected by the hormonal status of the animal and, as in other species in which $PGF_{2\alpha}$ acts as a uterine luteolysin, the synthetic potential of the uterus is greatest following the sequential exposure of uterine tissue to progesterone followed by oestrogen.

In the pregnant rabbit, the increase in the uterine venous plasma levels of $PGF_{2\alpha}$ seen at the end of pseudopregnancy is not observed. This cannot be attributed to a decrease in the ability of uterine tissue to synthesise prostaglandins, although the reduction in the endogenous levels of $PGF_{2\alpha}$ at this time may reflect a decrease in $PGF_{2\alpha}$ synthesis *in vivo*. However, prostaglandin production by the pregnant uterus and/or its contents does appear to be important in maintaining placental function, and is probably also involved in the process of parturition.

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