

PROSTAGLANDIN $F_{2\alpha}$
AND LUTEAL REGRESSION IN THE EWE :
COMPARISON WITH 16 ARYLOXYPROSTAGLANDIN
(I. C. I. 80, 996)

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SUMMARY

The evidence supporting the hypothesis that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is « the » luteolytic hormone in the sheep is summarised. On the basis of the temporal relationships between the ovarian secretion of progesterone and oestradiol, and the uterine release of $PGF_{2\alpha}$, it is suggested that luteal regression in the sheep may involve two stages. *Functional* luteal regression as indicated by a fall in progesterone secretion commences on Day 13 in response to a relatively small release of $PGF_{2\alpha}$ and can be halted by hysterectomy as late as Day 15. The massive amounts of $PGF_{2\alpha}$ on Day 15 and 16 released from the uterus in response to secretion of oestradiol from the preovulatory follicle induces irreversible *structural* luteolysis. The mechanism by which $PGF_{2\alpha}$ causes luteal regression probably involves effects on the enzyme systems of the luteal cell as well as on the redistribution of blood within the ovary. The luteolytic effect of a synthetic 16-aryloxy prostaglandin of $PGF_{2\alpha}$ (I. C. I. 80, 996) is compared to that of $PGF_{2\alpha}$.

INTRODUCTION

In many species of domestic animals (e.g. sheep, cow, horse, pig and goat) the uterus plays a key role in regression of the corpus luteum of the cycle (ANDERSON *et al.*, 1969; HANSEL *et al.*, 1973 for review). The luteolytic influence of each uterine horn is exerted principally, and in some species (e. g. the sheep) exclusively, on the adjacent (ipsilateral) ovary (MOOR, 1968). Until 1969 the search for luteolytic activity in fractions prepared from the uterus at various stages of the oestrous cycle were unrewarding (see SHORT, 1967, for review).

One factor contributing to the difficulty of identifying potential uterine luteo-

lysins was the lack of suitable test systems (see CALDWELL, 1969, for review). In the ewe when the ovary was autotransplanted to the neck, it was noted that cyclical ovarian activity ceased (GODING *et al.*, 1967 *a*; BAIRD *et al.*, 1968). Instead the corpus luteum persisted due to a failure of the luteolytic influence of a locally adjacent uterine horn. This preparation which allows easy access to both the ovarian arterial and venous circulation provided an ideal model in which to test luteolytic activity (McCRACKEN *et al.*, 1971).

In 1969 a naturally occurring fatty acid, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was shown to be luteolytic when injected into the uterus of pseudopregnant rats (PHARRISS and WYNGARDEN, 1969). Pharriss argued that $PGF_{2\alpha}$ might be released from the uterus into the uterine vein and by causing veno-constriction of the common utero-ovarian vein impair venous return and eventually induce regression of the corpus luteum on the adjacent ovary (PHARRISS, 1970). Soon afterwards $PGF_{2\alpha}$ was shown to be luteolytic in the sheep (McCRACKEN *et al.*, 1970) and in other species (PHARRISS *et al.*, 1972). $PGF_{2\alpha}$ is much more effective as a luteolytic agent when delivered locally to the ovary either via the ovarian artery (McCRACKEN *et al.*, 1970) or into the uterine lumen (GODING *et al.*, 1971) than when injected systemically. The relative ineffectiveness of $PGF_{2\alpha}$ when injected intravenously is almost certainly related to its very rapid removal from the blood stream. Over 95 p. 100 is extracted from the blood during a single passage through the lungs (PIPER *et al.*, 1970) which of course limits its biological activity in the systemic circulation but does not prevent it from acting locally.

Thus in 1970 a naturally occurring compound ($PGF_{2\alpha}$) was available which had all the properties necessary for a uterine luteolysin. At the Laurentian Hormone Conference in 1970 it was suggested that $PGF_{2\alpha}$ might be synthesised in the uterus under the influence of progesterone secreted by the corpus luteum (McCRACKEN *et al.*, 1971). It was postulated that following release of $PGF_{2\alpha}$ into the uterine vein it might be transferred to ovarian arterial blood by direct diffusion from the utero-ovarian vein to the overlying ovarian artery. The evidence for and against this « counter-current exchange » hypothesis has been discussed in detail recently (summarised by McCRACKEN *et al.*, 1972 *a*; BAIRD *et al.*, 1973 *a*; GODING, 1973; COUDERT *et al.*, 1974 *a* and *b*).

It is the purpose of this paper to (*a*) summarise the evidence supporting the view that $PGF_{2\alpha}$ is the luteolytic hormone in sheep; (*b*) consider the mechanism by which $PGF_{2\alpha}$ might induce luteal regression; (*c*) compare the luteolytic action of $PGF_{2\alpha}$ with 16-aryloxy prostaglandin (I. C. I. 80, 996) a synthetic analogue with more selective properties.

A. — SUMMARY OF EVIDENCE TO SUPPORT $PGF_{2\alpha}$ AS « THE » LUTEOLYTIC HORMONE IN SHEEP

1. Hysterectomy results in failure of luteal regression (WILTBANK and CASIDA, 1956).
2. Partial hysterectomy demonstrates the effect to be locally mediated (MOOR and ROWSON, 1966 *a*).

3. Separation of uterus and ovary by autotransplantation prevents luteal regression (GODING *et al.*, 1967 *a*; GODING *et al.*, 1967 *b*) while autotransplantation of ovary and uterus together results in normal cyclical ovarian activity (HARRISON *et al.*, 1968).

4. Uterine vessels involved in luteolysis (KIRACOFÉ *et al.*, 1966; INSKEEP and BUTCHER, 1966).

5. Uterine vein involved luteolysis (BAIRD and LAND, 1973).

6. Uterine venous blood and plasma has luteolytic properties (McCRACKEN *et al.*, 1971; CALDWELL and MOOR, 1971; BAIRD *et al.*, 1973 *c*).

7. Ovarian arterial blood at the time of luteal regression is luteolytic (GINTHER *et al.*, 1973).

8. Separation of ovarian artery from utero-ovarian vein results in failure of luteal regression (BARRETT *et al.*, 1971; RESTALL *et al.*, 1973).

9. PGF_{2α} is luteolytic when given into the ovarian artery (McCRACKEN *et al.*, 1970; THORBURN and NICOL, 1972) uterine vein (GODING *et al.*, 1971) or into the uterine lumen (GODING *et al.*, 1972).

10. PGF_{2α} is present in uterus (WILSON *et al.*, 1972), and uterine venous plasma (BLAND *et al.*, 1971).

11. PGF_{2α} is released into uterine vein in increased amounts at the time of luteal regression (THORBURN *et al.*, 1972 *c*; McCRACKEN *et al.*, 1973; BAIRD *et al.*, 1973 *c*).

12. ³HPGF_{2α} is transferred directly from uterine vein to ovarian artery (McCRACKEN *et al.*, 1971; McCRACKEN *et al.*, 1972 *a*).

A crucial part of the counter-current hypothesis is that uterine PGF_{2α} reaches the ovary by being transferred from the utero-ovarian vein to the ovarian artery. The concentration of PGF in ovarian arterial plasma is significantly higher than in aortic plasma collected simultaneously (mean concentration = 122 versus 85 pg/ml paired 't' test P < 0.05). Moreover the concentration of PGF in ovarian arterial plasma is higher on Day 14, 15 and 16 of the cycle than at any other stage of the cycle (LAND *et al.*, 1974 unpublished results). These data indicate that PGF is concentrated in ovarian arterial blood and that increased amounts are delivered to the ovary at a time when the secretion of progesterone from the corpus luteum is falling.

Recently we have obtained compelling evidence that PGF_{2α} is involved in normal luteal regression. When six Welsh mountain ewes were immunized to PGF_{2α} made antigenic by conjugating to a protein, antibodies specific to PGF developed (SCARAMUZZI *et al.*, 1973). In contrast to control ewes, oestrous behaviour ceased due to the failure of the luteum to regress. These data indicate that when the biological activity of endogenous circulating PGF_{2α} is neutralised, luteal regression is prevented and strongly supports the view that PGF_{2α} is involved in the mechanism of normal luteal regression. Moreover it is likely that at some time during luteal regression, PGF_{2α} enters the blood stream where its activity can be neutralised by circulating antibodies. Similar findings have recently been reported in the guinea pig (HORTON and POYSER, 1974).

A study involving measurement of PGF in sera during the oestrous cycle of the ewe claimed that « these observations tend to cast doubt on the physiological role of PGF as the uterine luteolytic factor » (COUDERT *et al.*, 1972). The concentration

of PGF was measured in serial samples collected daily by chronic catheterization of the inferior vena cava in eight ewes.

Although doubt has been cast on the technical competence of much of this study (GODING, 1973) in the majority of ewes two definite peaks of concentrations of PGF were observed about Day 12 and again on Day 15 and 16 of the cycle (Day 0 = day of oestrus). Because the first rise of PGF occurred before there was a significant decline in the concentration of progesterone it was concluded that luteal regression was unlikely to be causally related to increased secretion of PGF. However the frequency of sampling in this study (daily) was probably insufficient to define accurately the relationships between PGF and ovarian steroids.

In order to investigate these temporal relationships more closely the concentrations of PGF_{2α}, oestradiol-17β, progesterone, androstenedione and LH were measured in utero-ovarian venous plasma throughout seven oestrous cycles of four ewes with cervical utero-ovarian autotransplants (SCARAMUZZI *et al.*, 1974 *b*). The characteristic temporal relationships in the timing of the secretion of these hormones are summarised in figure 1 which illustrates a schematic mean of all seven cycles. The first

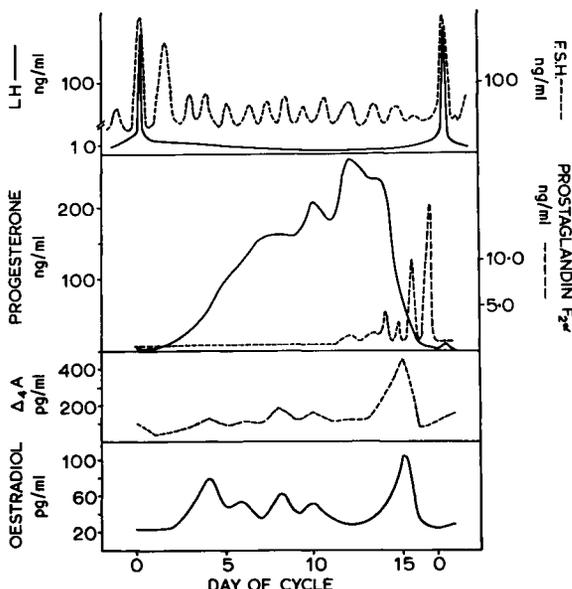


FIG. 1. — The concentration of ovarian steroids, prostaglandin F and gonadotrophins throughout the oestrous cycle of the ewe

The concentrations of oestradiol, androstenedione, progesterone and prostaglandin F_{2α} were measured in serial samples of utero-ovarian venous plasma collected from three ewes with cervical utero-ovarian transplants. The values are the mean of seven cycles adjusted for differences in length by dating back five days from the onset of oestrus (Day 0). The concentrations of LH are those of LAND *et al.*, 1973 expressed in ng of MI/ml and FSH (NIH-FSH-S6) are a schematic representation of SALAMONSEN *et al.*, 1973. The first fall in progesterone on Day 13 is preceded by a rise in the secretion of prostaglandin F_{2α} on Day 12. The magnitude of the pre-oestrus rise of oestradiol (and androstenedione) is obscured by the twofold increase in uterine blood flow at this time (BAIRD D. T., SCARAMUZZI R. J., LAND R. B., WHEELER A. G., unpublished results).

significant increase in release of PGF_{2α} occurs on Day 12 and is followed on Day 13 by a fall in the secretion of progesterone. At this time the secretion rate of both

hormones when measured serially at 3 hourly intervals is extremely variable from sample to sample. The secretion of oestradiol which in the ewe is an exclusive product of the follicle (BAIRD *et al.*, 1973 *a*) fluctuates apparently at random throughout the luteal phase. In six of the seven cycles the rise in secretion of oestradiol from the pre-ovulatory follicle started at least 24 hours after the decline in secretion of progesterone. The maximum release of PGF_{2α} occurs on Day 16 by which time luteal regression is virtually complete.

The timing of these events which is similar to those reported by COUDERT *et al.*, 1972 is compatible with the suggestion that luteal regression is initiated by the comparatively small amount of PGF_{2α} released on Day 12. The first histological evidence of luteal regression is detected at this time (DEANE *et al.*, 1966) and if an embryo is to prevent luteal regression it must be transferred to the uterus no later than Day 12 (MOOR *et al.*, 1966 *b*). Pituitary luteotrophic support is probably at a minimum on Day 12 the concentration of LH falling to its lowest level in the mid to late luteal phase of the cycle (LAND *et al.*, 1973) and may explain the fact that the corpus luteum is more sensitive to the luteolytic action of PGF_{2α} at this time than earlier in the cycle (HEARNshaw *et al.*, 1973).

The massive release of PGF_{2α} which occurs on Day 16 may be induced by the pre-ovulatory release of oestradiol. Oestrogen is known to produce premature regression of the corpus luteum if given on Day 10 of the cycle (STORMSHAK *et al.*, 1969). Oestradiol infused via the uterine artery will release PGF from the uterus during the luteal phase (McCRACKEN *et al.*, 1972 *b*) or from a uterine autotransplant primed for 14 days with progesterone (SCARAMUZZI *et al.*, 1974 *a*). A fall in the concentration of progesterone may also facilitate the release of PGF from the uterus. Thus the sequence of events occurring at the time of luteal regression may reinforce the effectiveness of the subsequent stimuli in a way similar to the cascade effect which occurs at the onset of parturition (LIGGINS *et al.*, 1972).

The function of the massive release of PGF_{2α} which occurs on Day 16 is not understood although it may be a fail safe mechanism which finally induces irreversible structural luteal regression. The regression of the functional activity of the corpus luteum which commences on Day 12 can be arrested by hysterectomy as late as Day 15 (MOOR *et al.*, 1970) presumably before structural regression has advanced. By Day 16 the corpus luteum cannot be rescued by removing the uterus indicating that irreversible structural luteolysis has occurred. Thus luteal regression may be considered as occurring in two phases (*a*) *functional* luteolysis commencing on Day 12, (*b*) irreversible *morphological* luteal-regression on Day 16.

B. — MECHANISM OF ACTION OF PROSTAGLANDIN F_{2α}

Although opinions may differ concerning the role played by endogenous PGF_{2α} in normal luteal regression, there is no question that exogenous PGF_{2α} will induce luteolysis in a variety of species (PHARRISS *et al.*, 1972). PHARRISS (1970) originally suggested that PGF_{2α} might induce luteal regression by its venoconstrictor action on the utero-ovarian-vein. In support of this hypothesis it was noted that the venous out flow from a catheter in the utero-ovarian vein of the rabbit was reduced follo-

wing an intravenous injection of 100 μg of $\text{PGF}_{2\alpha}$ (PHARRISS *et al.*, 1970). A mechanism involving an action on the vascular system is attractive because it explains both the local action of $\text{PGF}_{2\alpha}$ and the apparent paradox that $\text{PGF}_{2\alpha}$ may stimulate progesterone production when added to luteal tissue *in vitro* (SPEROFF and RAMWELL, 1970).

However further experimentation has made it unlikely that Pharriss's original suggestion is correct. Ovarian blood flow in the rabbit is virtually without autoregulatory control and is extremely sensitive to changes in arterial blood pressure. Systemic administration of 100 μg of $\text{PGF}_{2\alpha}$ results in a profound drop in aortic blood pressure which is reflected by a fall in ovarian blood flow (JANSON *et al.*, 1974). When luteal regression was induced in rabbits by intra aortic infusion of $\text{PGF}_{2\alpha}$ in amounts which had no effect on arterial blood pressure, capillary blood flow to the corpus luteum did not decrease until progesterone secretion had virtually ceased (BRUCE and HILLER, 1974). There was a significant increase in capillary flow to the stroma of the ovary with little if any resulting change in total ovarian blood flow. Thus in the rabbit at any rate functional regression of the corpus luteum can occur without any marked change in blood flow either to the corpus luteum or the whole ovary.

Minor changes in total ovarian flow have been reported when luteal regres-

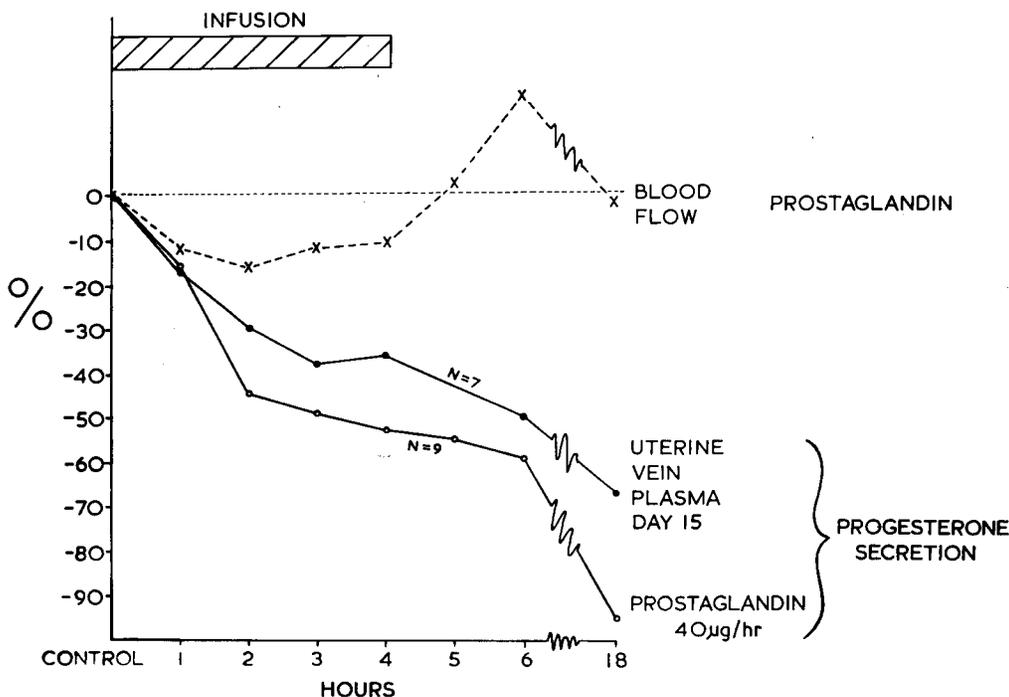


FIG. 2. — The change in ovarian blood flow and progesterone secretion rate during and following the infusion of $\text{PGF}_{2\alpha}$ in ewes with ovarian autotransplants

The means of nine experiments are illustrated and the results expressed as a percentage of the control values. $\text{PGF}_{2\alpha}$ was infused via the ovarian artery at a rate of 40 $\mu\text{g}/\text{hour}$ as indicated (BAIRD, 1974). The mean change in progesterone secretion following infusion of uterine vein plasma collected on Day 15 infused via the ovarian artery is included for comparison (from BAIRD *et al.*, 1973 c).

sion is induced by the infusion of a wide range of doses of PGF_{2α} through the auto-transplanted ovary of the sheep (McCRACKEN *et al.*, 1970; CHAMLEY *et al.*, 1972; BAIRD, 1974). The fall in ovarian blood flow is small when compared to the change in secretion of progesterone which drops to 50 p. 100 of the control value within two hours of starting the infusion (fig. 2). When allowance is made for the skin contribution to total flow measured in the preparation (5.1 ± 0.7 S. E. ml/min $n = 11$ BAIRD *et al.*, 1973 *b*) it can be calculated that the fall in total ovarian flow during the infusion of PGF_{2α} does not exceed 25 p. 100. These findings indicate that in the sheep functional luteolysis (as indicated by a fall in progesterone secretion) can occur without a marked fall in total blood flow and make it unlikely that PGF_{2α} exerts its effect on either the ovarian artery or utero-ovarian vein.

Ovarian blood flow in the sheep per unit weight of tissue (about 600 ml/100 g/min) as measured by cannulation of the ovarian vein *in situ* (MATTNER and THORBURN, 1969) or when autotransplanted to the neck (GODING *et al.*, 1971) is much higher than that in other tissues in the body. Even higher estimates have been reported for capillary flows to the corpus luteum and pre-ovulatory follicles (THORBURN and HALES, 1972; NETT *et al.*, 1974; BRUCE and MOOR, 1975). It has been suggested that the high PO₂ and oxygen content of ovarian venous blood in the sheep relative to that of jugular venous blood might indicate the presence of arteriovenous shunts within the ovary or its pedicle (BAIRD *et al.*, 1973 *b*). The PO₂ in ovarian arterial and venous blood remains relatively constant during infusion of PGF_{2α}.

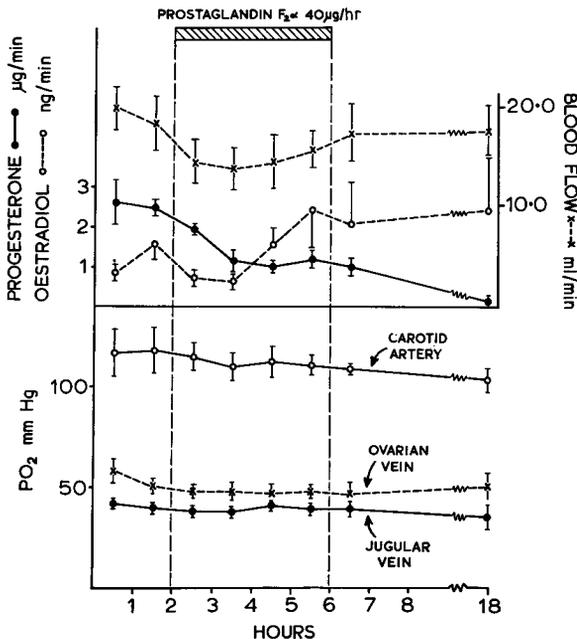


FIG. 3. — Ovarian blood flow, steroid secretion and oxygen tension (PO₂) during luteal regression

Prostaglandin F₂₂ was infused through the ovary via the ovarian artery as indicated in four ewes with cervical ovarian autotransplants. Each point represents the mean \pm S. E. of five experiments. There is no significant change in oxygen tension during or following the infusion. Progesterone secretion fell to 50 p. 100 of the control level during the infusion and luteal regression was complete by 18 hours. Note the temporary fall in ovarian blood flow during the infusion. The change in secretion of oestradiol was variable as indicated by the high S. E. of the mean.

(fig. 3) at a time when presumably the capillary flow to the corpus luteum is reduced drastically (THORBURN and HALES, 1972). The relatively small changes in blood flow and oxygen tension following infusion of $\text{PGF}_{2\alpha}$ could be explained if a significant and variable fraction of the total flow by-passed the capillary bed (BAIRD *et al.*, 1973 *b*). Although functional arterio-venous shunts within the ovary of the rabbit have not been demonstrated (AHREN *et al.*, 1975) it is possible that they only become functional at the time of luteal regression.

$\text{PGF}_{2\alpha}$ may cause a local redistribution of blood within the ovary so that any reduction in luteal flow is masked by an increase in flow to other compartments. In three ewes the capillary flow to the corpus luteum as measured by radio actively labelled microspheres after an initial temporary increase fell markedly following infusion of $\text{PGF}_{2\alpha}$ into the ipsilateral uterine vein (THORBURN and HALES, 1972). By 5 1/2 hours after the start of the infusion the capillary flow to the corpus luteum was 30 p. 100 of that of the control values (200-300 ml/100 g/mn) and by 28 hours it had fallen to 5 p. 100. There was no change in capillary flow to the rest of the ovary or to the contralateral ovary. Similar selective reduction in the capillary blood flow to the corpus luteum has been reported during spontaneous luteal regression (BRUCE and MOOR, 1975) and after infusion of $\text{PGF}_{2\alpha}$ into the uterine lumen (NETT *et al.*, 1974). The total ovarian blood flow as measured by cannulation of the utero-ovarian vein *in situ*, falls from 662 ± 71 (SE) ml/100 g/mn during the luteal phase to 340 ± 280 ml/100 g/mn during the peri-ovulatory period (MATNER and THORBURN, 1969). The change in flow to the corpus luteum may be masked in measurements of total flow by the increase in flow, both relative and absolute, to the follicles (BRUCE and MOOR, 1975). Unfortunately in none of these studies was it possible to determine whether blood flow to the corpus luteum fell before the change in secretion of progesterone. Thus the question remains as to whether these alterations in blood flow are a cause or a consequence of structural luteal regression.

$\text{PGF}_{2\alpha}$ has been shown to have profound effects on the biochemistry of the ovarian cell (BEHRMAN *et al.*, 1971). In the rat $\text{PGF}_{2\alpha}$ inhibits cholesterol ester synthetase and hence reduced the pool of « steroidogenic cholesterol » available for synthesis of progesterone. It also stimulates 20α steroid dehydrogenase activity an effect which can be reversed by prolactin (PHARRISS *et al.*, 1968). Although LH is unable to prevent luteal regression when infused at the same time as $\text{PGF}_{2\alpha}$ (CERINI *et al.*, 1972), preliminary evidence suggests that prolactin may protect the sheep corpus luteum (McCRACKEN *et al.*, 1972 *b*) although this claim has not been substantiated (CHAMLEY *et al.*, 1973). Receptors of $\text{PGF}_{2\alpha}$ have been demonstrated within the corpus luteum of women, cow and the sheep (POWELL *et al.*, 1974). These findings need to be confirmed but support the view that $\text{PGF}_{2\alpha}$ may exert its initial luteolytic effects by altering the biochemistry of the luteal cell.

In summary therefore it seems unlikely that $\text{PGF}_{2\alpha}$ exerts its luteolytic action by reducing total ovarian blood flow. Careful studies relating fractional blood flow and progesterone secretion at the time of luteal regression are required to determine whether a reduction in capillary flow to the corpus luteum precedes or is a consequence of functional luteolysis. The well defined biochemical effects of $\text{PGF}_{2\alpha}$ on luteal cells may be responsible for the initiation of functional luteal regression. Irreversible structural changes in the corpus luteum may be mediated via a selective effect of $\text{PGF}_{2\alpha}$ on its blood supply.

C. — COMPARAISON OF LUTEAL REGRESSION INDUCED BY PGF_{2α}
AND 16-ARYLOXYPROSTAGLANDIN (I. C. I. 80, 996)

Although PGF_{2α} will reliably induce luteal regression in the ewe when infused via the ovarian artery at between 2 and 100 µg per hour (GODING *et al.*, 1971), much larger doses (10-20 mg) are required if given systemically (McCRACKEN *et al.*, 1972 *b*) or into the uterine lumen (GODING *et al.*, 1972). Synthetic analogues such as 15-methyl PGF_{2α} were developed as a rational attempt to suppress the rapid metabolic degradation of PGF_{2α} (YANKEE and BUNDY, 1972). Unfortunately the increased potency of these compounds on the reproductive tract is matched by a corresponding increase in biological activity on other tissues. In particular the side effects due to their action on the smooth muscle of the vascular system and gastrointestinal tract has limited their use in medical and veterinary practice.

More recently a group of 16-aryloxy derivatives of PGF_{2α} with more selective biological properties have been developed (BINDER *et al.*, 1973). I. C. I. 80, 996 is said to be approximately 100 fold more potent than PGF_{2α} inducing abortion in rats whereas as a smooth muscle stimulant (e.g. gerbil colon) it is equipotent with the parent compound (DUKES *et al.*, 1974). In the following section the luteolytic effect of a single intramuscular injection of 50 µg of I. C. I. 80, 996 was compared to that of a continuous infusion of 180 µg of PGF_{2α} via the ovarian artery.

Eight ewes with the left ovary autotransplanted to a cervical carotid-jugular skin loop were used for this experiment (GODING *et al.*, 1967 *a*). The ovary of each of these used contained a corpus luteum which had persisted due to physical separation from the uterus (BAIRD *et al.*, 1968). In the first group of six ewes luteal regression was induced by a single intramuscular injection of aryloxy prostaglandin F_{2α} (I. C. I. 80, 996). In the second group PGF_{2α} was infused in six separate experiments in four ewes with ovarian autotransplants. Timed samples of ovarian venous blood were collected as previously described (GODING *et al.*, 1967 *a*). The concentration of progesterone in ovarian venous plasma was measured by competitive protein binding (COLLETT *et al.*, 1973) or by radioimmunoassay (SCARAMUZZI *et al.*, 1974 *c*). The secretion rate of progesterone was calculated from a knowledge of the plasma concentration, haematocrit and blood flow.

PGF_{2α} was administered as a continuous infusion in 0.9 p. 100 sodium chloride solution via the ovarian artery (COLLETT *et al.*, 1973). The rate of infusion was adjusted so that 180 µg PGF_{2α} in 45 ml of solution were administered over 4 1/2 hours. I. C. I. 80, 996 was administered as a single intramuscular injection of 50 µg. Control samples were taken before the prostaglandin administration and at 6 hourly intervals for up to 6 days afterwards.

The secretion of progesterone and ovarian blood flow before and after the administration of PGF_{2α} and I. C. I. 80, 996 are illustrated in fig. 4. The pattern of decline in both groups was very similar with progesterone secretion falling to about 50 p. 100 of the control value by 6 hours after the administration of prostaglandin. By 24 hours luteal regression was complete as indicated by virtual cessation of progesterone secretion. In the group treated with analogue the secretion of pro-

gesterone started to rise 90 hours after injection indicating ovulation and subsequent development of a corpus luteum. In the group treated with $\text{PGF}_{2\alpha}$ the collections were only continued for 78 hours after the injection.

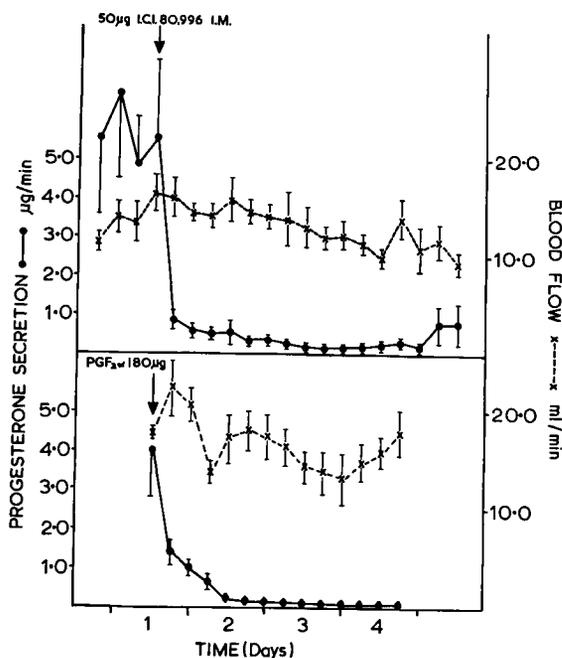


FIG. 4. — Changes in ovarian blood flow and progesterone secretion during luteal regression induced by prostaglandin $\text{F}_{2\alpha}$ and a synthetic analogue (I. C. I. 80, 996)

Each point represents the mean \pm S. E., of six experiments in ewes with cervical ovarian autotransplants. 180 μg of prostaglandin $\text{F}_{2\alpha}$ was infused via the ovarian artery at the rate of 40 μg per hour; I. C. I. 80, 996 as a single intramuscular injection. In both groups luteal regression was complete 24 hours after the injection. The rise in progesterone secretion 90 hours after the injection of the analogue indicates ovulation and subsequent formation of a fresh corpus luteum.

In this group of experimental ewes, oestrous behaviour was not examined. A group of intact ewes injected with I. C. I. 80, 996 in early pregnancy all returned to oestrus within two or three days (BAIRD-unpublished). In the experimental group a peak of LH was observed 36-72 hours after the injection of prostaglandin and was preceded by a rise in secretion of oestradiol (BAIRD and SCARAMUZZI, unpublished data). These data together with the rise in secretion of progesterone after 90 hours strongly suggest that ovulation followed by subsequent formation of the corpus luteum occurred in all animals. The timing of these events is similar to those observed following luteal regression induced by $\text{PGF}_{2\alpha}$ in ewes with ovarian autotransplants (CHAMLEY *et al.*, 1972).

The luteolytic effect of intramuscular I. C. I. 80, 996 was identical to that of $\text{PGF}_{2\alpha}$ infused locally to the ovary via the ovarian artery. Intravenous infusion of $\text{PGF}_{2\alpha}$ at the dose level used in this experiment (40 μg per hour) is without effect on progesterone secretion (McCRACKEN *et al.*, 1972 *a*). The effectiveness of 16-aryloxy prostaglandin $\text{F}_{2\alpha}$ is due partially to its longer half life but also to its

more selective action on the corpus luteum (DUKES *et al.*, 1974). Clearly the ability to induce luteal regression reproducibly with a single injection of a potent luteolytic agent has enormous commercial importance for the synchronisation of oestrus.

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RÉSUMÉ

PROSTAGLANDINE F_{2x} ET RÉGRESSION DU CORPS JAUNE CHEZ LA BREBIS COMPARAISON AVEC LA 16-ARYLOXYPROSTAGLANDINE (I. C. I. 80, 996)

Dans cet article, les preuves en faveur de l'hypothèse que la prostaglandine F_{2x} (PGF_{2x}) est l'hormone lutéolytique chez la brebis sont passées en revue. Les relations chronologiques entre la sécrétion ovarienne de la progestérone et de l'œstradiol d'une part et la sécrétion utérine de la PGF_{2x} d'autre part suggèrent que la régression du corps jaune chez la brebis est réalisée probablement en deux phases. La première phase, ou phase de régression « fonctionnelle » qui commence le 13^e jour du cycle, est caractérisée par une réduction dans la sécrétion ovarienne de la progestérone, provoquée par une sécrétion utérine de la PGF_{2x} relativement faible ; jusqu'au 15^e jour du cycle, il est possible d'arrêter cette régression fonctionnelle par l'hystérectomie. Pendant les 15^e et 16^e jours du cycle, la sécrétion d'œstradiol par le follicule préovulatoire entraîne la sécrétion par l'utérus de quantités massives de PGF_{2x} qui induisent la régression « structurale » irréversible du corps jaune. Le mécanisme par lequel PGF_{2x} provoque la régression lutéale réside probablement dans des actions sur les systèmes enzymatiques des cellules lutéales aussi bien que dans une redistribution du sang dans l'ovaire. L'effet lutéolytique d'un 16-aryloxy dérivé synthétique de la PGF_{2x} (I. C. I. 80, 996) est comparé à celui de la PGF_{2x}.

REFERENCES

- AHREN K., JANSON P. O., SELSTAN G., 1975. Search for arterio-venous shunts in the rabbit ovary *in situ* using perfusion of microspheres. *J. Reprod. Fert.*, **41**, 133-142.
- ANDERSON L. L., BLAND K. P., MELAMPY R. M., 1969. Comparative aspects of uterine-luteal relationships. *Recent Prog. Horm. Res.*, **35**, 57-99.
- BAIRD D. T., GODING J. R., ICHIKAWA Y., MCCracken J. A., 1968. The secretion of steroids from the ewe ; spontaneously and in response to systemic gonadotrophins. *J. Endocrin.*, **42**, 283-299.
- BAIRD D. T., LAND R. B., 1973. Division of the uterine vein and the function of the adjacent ovary in the ewe. *J. Reprod. Fert.*, **33**, 393-397.
- BAIRD D. T., MCCracken J. A., GODING J. R., 1973 a. Studies in steroid synthesis and secretion with the autotransplanted sheep ovary and adrenal. In : PIERREPOINT C. G., *The Endocrinology of Pregnancy and Parturition*, 5-16. Alpha Omega Alpha, Cardiff.
- BAIRD D. T., GILES M., COCKBURN F., 1973 b. The PO₂, PCO₂, pH and oxygen content of ovarian venous blood of sheep. *J. Endocr.*, **57**, 405-411.

- BAIRD D. T., COLLETT R. A., FRASER I. S., KELLY R. W., LAND R. B., WHEELER A. G., 1973 *c*. Progesterone secretion from the ovary in the ewe following infusion of uterine venous plasma. *J. Reprod. Fert.*, **35**, 13-22.
- BAIRD D. T., 1974. Prostaglandin F_{2x} and ovarian blood flow in sheep. *J. Endocr.* **62**, 413-414.
- BARRETT S., BLOCKEY M. A., BROWN J. M., CUMMING I. A., GODING J. R., MOLE B. J., OBST J. M., 1971. Initiation of the oestrous cycle in the ewe by infusions of PGF $_{2x}$ to the autotransplanted ovary. *J. Reprod. Fert.* **24**, 136-137.
- BEHRMAN H. R., McDONALD G. T., GREEP R. O., 1971. Regulation of ovarian cholesterol esters : evidence for the enzymatic sites of prostaglandin-induced loss of luteal function. *Lipids*, **6**, 791-796.
- BINDER D., BOWLER J., BROWN E. D., CROSSLEY N. S., HUTTON J., SENIOR M., SLATER L., WILKINSON P., WRIGHT N. C. A., 1974. 16-aryloxyprostaglandins : A new class of potent luteolytic agent. *Prostaglandins*, **6**, 87-90.
- BLAND K. P., HORTON E. W., POYSER N. L., 1971. Levels of prostaglandin F_{2x} in the uterine venous blood of sheep during the oestrous cycle. *Life Sci.*, **10**, 509-517.
- BRUCE N. W., HILLIER K., 1974. The effect of prostaglandin F_{2x} on ovarian blood flow and corpora lutea regression in the rabbit. *Nature (New Biology)*, **249**, 176-177.
- BRUCE N. W., MOOR R. M., 1975. Ovarian follicular blood flow in sheep. *J. Reprod. Fert.* (In Press.)
- CALDWELL B. V., 1969. Uterine factors influencing corpus luteum function. In : RASPE G., *Advances in Bioscience*, **4**, 399-415. Pergamon Press, Vieweg.
- CALDWELL B. V., MOOR R. M., 1971. Further studies on the role of the uterus in the regulation of corpus luteum function in sheep. *J. Reprod. Fert.*, **26**, 133-135.
- CERINI M. E. D., CHAMLEY W. A., FINDLAY J. K., GODING J. R., 1972. Luteolysis in sheep with ovarian autotransplants following concurrent infusions of luteinizing hormone and prostaglandin F_{2x} into the ovarian artery. *Prostaglandins*, **2**, 465-470.
- CHAMLEY W. A., BUCKMASTER J. M., CAIN M. D., CERINI J., CERINI M. E., CUMMING I. A., GODING J. R., 1972. The effect of prostaglandin F_{2x} on progesterone, oestradiol and luteinizing hormone secretion in sheep with ovarian transplants. *J. Endocr.*, **55**, 253-263.
- CHAMLEY W. A., CEVINI J. C., GODING J. R., 1973. Luteal function in sheep with ovarian autotransplants given concurrent infusions of prolactin and prostaglandin F_{2x} into the ovarian artery. *Prostaglandins*, **4**, 711-716.
- COLLETT R. A., LAND R. B., BAIRD D. T., 1973. The pattern of progesterone secretion by the autotransplanted ovary of the ewe in response to ovine luteinizing hormone. *J. Endocr.*, **56**, 403-411.
- COUDERT S. P., PHILLIPS G. D., PALMER M., FAIMAN C., 1972. Prostaglandin F concentration in the peripheral blood of the ewe during the estrous cycle. *Prostaglandins*, **2**, 501-509.
- COUDERT S. P., PHILLIPS G. D., FAIMAN C., CHERNICKI W., PALMER M., 1974 *a*. A study of the utero-ovarian circulation in sheep with reference to local transfer between venous and arterial blood. *J. Reprod. Fert.*, **36**, 319-331.
- COUDERT S. P., PHILLIPS G. D., FAIMAN C., CHERNICKI W., PALMER M., 1974 *b*. Infusion of triitated prostaglandin F_{2x} into the anterior uterine vein of the ewe : absence of local venous-arterial transfer. *J. Reprod. Fert.*, **36**, 333-343.
- DEANE H. W., HAY M. F., MOOR R. M., ROWSON L. E. A., SHORT R. V., 1966. The corpus luteum of the sheep : relationships between morphology and function during the oestrous cycle. *Acta Endocrin.* (Kbh), **51**, 245-263.
- DUKES M., RUSSELL N., WALPOLE A. L., 1974. Potent luteolytic agents related to prostaglandin F_{2x} . *Nature*. (In Press.)
- GINTHER O. J., DEL CAMPO C. H., RAWLINGS C. A., 1973. Vascular anatomy of the uterus and ovaries and the unilateral luteolytic effect of the uterus : A local veno-arterial pathway between uterus and ovaries in sheep. *Amer. J. Vet. Res.*, **34**, 723-728.
- GODING J. R., McCRACKEN J. A., BAIRD D. T., 1967 *a*. The study of ovarian function in the ewe by means of a vascular autotransplantation technique. *J. Endocr.*, **39**, 37-52.
- GODING J. R., HARRISON F. A., HEAP R. B., LINZELL J. L., 1967 *b*. Ovarian activity in the ewe after autotransplantation of the ovary or uterus to the neck. *J. Physiol. Lond.*, **191**, 129-130.
- GODING J. R., BAIRD D. T., CUMMING I. A., McCRACKEN J. A., 1971. Functional assessment of autotransplanted endocrine organs. *Acta Endocrin.* (Kbh), Suppl. 158, 169-191.
- GODING J. R., CUMMING I. A., CHAMLEY W. A., BROWN J. M., CAIN M. D., CERINI J. C., CERINI M. E. D., FINDLAY J. K., O'SHEA J. D., PEMBERTON D. H., 1972. Prostaglandin F_{2x} , « the » luteolysin in the mammal? *Gynec. Invest.*, **2**, 73-97.
- GODING J. R., 1973. The demonstration that PGF $_{2x}$ is the uterine luteolysin in the ewe. In : DENAMUR R. and NETTER A. *Le Corps Jaune*, 311-323. Masson et C^e, Paris.
- HANSEL W., CONCANNON P. W., LUKASZEWSKA J. H., 1973. Corpora lutea of the large domestic animals. *Biol. Reprod.*, **8**, 222-245.
- HARRISON F. A., HEAP R. B., LINZELL J. L., 1968. Ovarian function in the sheep after autotransplantation of the ovary and uterus to the neck. *J. Endocr.*, **40**, xiii.
- HEARNSHAW H., RESTALL B. J., GLEESON A. R., 1973. Observations on the luteolytic effects of prostaglandin F_{2x} during the oestrous cycle and early pregnancy in the ewe. *J. Reprod. Fert.* **32**, 322-323.

- HORTON F. W., POYSER N. L., 1974. Elongation of oestrous cycle in the guinea pig following active immunization against prostaglandin F_{2α}. *Prostaglandins*, **5**, 349-354.
- INSKEEP E. K., BUTCHER R. L., 1966. The local component of utero-ovarian relationships in the ewe. *J. Anim. Sci.*, **25**, 1164-1171.
- JANSON P. O., ALBRECHT I., AHREN K., 1974. Effects of prostaglandin F_{2α} on ovarian blood flow and vascular resistance in the pseudopregnant rabbit. *Acta Endocrin.* (Kbh). (In Press.)
- KIRACOFÉ G. H., MENZIES C. S., GIER H. T., SPIERS H. G., 1966. Effects of uterine extracts and uterine or ovarian blood vessel ligation on ovarian function of ewes. *J. Anim. Sci.*, **25**, 1159-1163.
- LAND R. B., PELLETIER J., THIMONIER J., MAULÉON P., 1973. A quantitative study of genetic differences in the incidence of oestrus, ovulation and plasma luteinizing hormone concentration in the sheep. *J. Endocr.*, **58**, 305-317.
- LAND R. B., SCARAMUZZI R. J., WHEELER A. G., BAIRD D. T., 1974. Unpublished observations.
- LIGGINS G. C., GRIEVE S. A., KENDALL J. Z., KNOX B. S., 1972. The physiological roles of progesterone, oestradiol 17 β and prostaglandin F_{2α} in the control of ovine parturition. *J. Reprod. Fert., Suppl.* **16**, 85-103.
- MCCRACKEN J. A., GLEW M. E., SCARAMUZZI R. J., 1970. Corpus luteum regression induced by prostaglandin F_{2α}. *J. Clin. Endocr.*, **30**, 544-546.
- MCCRACKEN J. A., BAIRD D. T., GODING J. R., 1971. Factors affecting the secretion of steroids from the transplanted ovary in the sheep. *Recent Progr. Horm. Res.*, **27**, 537-582.
- MCCRACKEN J. A., CARLSON J. C., GLEW M. E., GODING J. R., BAIRD D. T., GRÉEN K., SAMUELSSON B., 1972 a. Prostaglandin F_{2α} identified as a luteolytic hormone in the sheep. *Nature* (New Biology), **238**, 129-134.
- MCCRACKEN J. A., BARCIKOWSKI B., CARLSON J. C., GRÉEN K., SAMUELSSON B., 1972 b. The physiological role of prostaglandin F_{2α} in corpus luteum regression. In : BERGSTRÖM S., *Advances in the Biosciences*, **9**, 599-624. Pergamon Press, Vieweg.
- MCCRACKEN J. A., BAIRD D. T., CARLSON J. C., GODING J. R., BARCIKOWSKI B., 1973. The role of prostaglandins in luteal regression. *J. Reprod. Fert., Suppl.* **18**, 133-142.
- MATTNER P. E., THORNBURN G. D., 1969. Ovarian blood flow in sheep during the oestrus cycle. *J. Reprod. Fert.*, **19**, 547-549.
- MOOR R. M., ROWSON L. E. A., 1966 a. Local uterine mechanisms affecting luteal function in the sheep. *J. Reprod. Fert.*, **11**, 307-310.
- MOOR R. M., ROWSON L. E. A., 1966 b. Relationship between embryo and corpus luteum in sheep. *J. Endocr.*, **34**, 233-239.
- MOOR R. M., 1968. Effect of embryo on corpus luteum function. *J. Anim. Sci.*, **27**, Suppl. 1, 97-118.
- MOOR R. M., HAY M. F., SHORT R. V., ROWSON L. E. A., 1970. Corpus luteum of the sheep : effect of uterine removal during luteal regression. *J. Reprod. Fert.*, **21**, 319-326.
- NETT T. M., AKBAR M. A., NISWENDER G. D., 1974. Influence of prostaglandins PGF_{2α} on blood flow to the ovary and progesterone secretion in sheep. *J. Steroid Biochem.*, **5**, 400.
- PHARRISS B. B., WYNGARDEN L. J., GUTKNECHT D. G., 1968. In : ROSEMBERG E., *Gonadotropins*, 121-129. Geron-X, Los Altos, California.
- PHARRISS B. B., WYNGARDEN L. J., 1969. The effect of prostaglandin F_{2α} on the progestagen content of ovaries from pseudopregnant rats. *Proc. Soc. Exp. Biol. Med.*, **130**, 92-94.
- PHARRISS B. B., 1970. The possible vascular regulation of luteal function. *Perspectives in Biology and Medicine*, **13**, 434-444.
- PHARRISS B. B., CORNETTE J. C., GUTKNECHT G. D., 1970. Vascular control of luteal steroidogenesis. *J. Reprod. Fert., Suppl.* **10**, 97-103.
- PHARRISS B. B., TILLSON S. A., ERICKSON R. R., 1972. PGs in luteal function. *Recent Progr. Horm. Res.*, **28**, 51-89.
- PIPER P. J., VANE J. R., WYLLIE T. H., 1970. Inactivation of prostaglandins by the lungs. *Nature* (Lond.), **225**, 600-604.
- POWELL W. S., HAMMERSTRÖM S., SAMUELSSON B., SJÖBERG B., 1974. Prostaglandin F_{2α} receptor in human corpora lutea. *Lancet*, **1**, 1120.
- RESTALL B. J., HEARNshaw H. R., GLEESON A. R., THORNBURN G. D., 1973. Observations on the luteolytic action of prostaglandin F_{2α} in the ewe. *J. Reprod. Fert.*, **32**, 325-326.
- SALAMONSEN L. A., JONAS H. A., BURGER H. G., BUCKMASTER J. M., CHAMLEY W. A., CUMING I. A., FINDLAY J. K., GODING J. R., 1973. A heterologous radioimmunoassay for follicle-stimulating hormone : application to measurement of FSH in the ovine oestrous cycle and in several other species including man. *Endocrinology*, **93**, 610-618.
- SCARAMUZZI R. J., BAIRD D. T., WHEELER A. G., LAND R. B., 1973. The oestrous cycle of the ewe following active immunisation against prostaglandin F_{2α}. *Acta Endocrin.* (Kbh), *Suppl.* **177**, 318 (abstract).
- SCARAMUZZI R. J., BOYLE H. P., WHEELER A. G., LAND R. B., BAIRD D. T., 1974 a. Preliminary studies on the secretion of prostaglandin F from the autotransplanted uterus of the ewe after exogenous progesterone and oestradiol. *J. Endocr.*, **61**, xxxv.

- SCARAMUZZI R. J., BAIRD D. T., LAND R. B., WHEELER A. G., 1974 *b*. Steroid and prostaglandin levels in ewes bearing utero-ovarian autotransplants. *J. Steroid Biochem.*, **5**, 401.
- SCARAMUZZI R. J., CORKER C. S., YOUNG G., BAIRD D. T., 1975. Production of antisera to steroid hormones. *Proceedings of the Vth Tenovus Workshop, Cardiff*. Alpha Omega Alpha, Cardiff, 111-122.
- SHORT R. V., 1967. Reproduction. *Ann. Rev. Physiol.*, **29**, 373-400.
- SPEOFF L., RAMWELL P. W., 1970. Prostaglandin stimulation of in vitro progesterone synthesis. *J. clin. Endocr. Metab.*, **30**, 345-350.
- STORMSHAK F., KELLEY H. E., HAWK H. W., 1969. Suppression of ovine luteal function by 17 β -oestradiol. *J. Anim. Sci.*, **29**, 476-478.
- THORBURN G. D., HALES J. R. S., 1972. Selective reduction in blood flow to the ovine corpus luteum after infusion of prostaglandin F_{2 α} into a uterine vein. *Proc. Int. Union of Physiol. Sciences*, **3**, 145.
- THORBURN G. D., NICOL D. H., 1972. Regression of the ovine corpus luteum following infusion of prostaglandin F_{2 α} into the ovarian artery. *J. Endocr.*, **51**, 785-786.
- THORBURN G. D., COX R. I., CURRIE W. B., RESTALL B. J., SCHNEIDER W., 1972 *c*. Prostaglandin F concentration in the utero-ovarian venous plasma of the ewe during the oestrous cycle. *J. Endocr.*, **53**, 325-326.
- WILSON L. jr, CENEDELLA R. J., BUTCHER R. L., INSKEEP E. K., 1972. Levels of prostaglandins in the uterine endometrium during the ovine oestrous cycle. *J. Anim. Sci.*, **34**, 93-99.
- WILTBANK J. N., CASIDA L. E., 1956. Alteration of ovarian activity by hysterectomy. *J. Anim. Sci.*, **15**, 134-140.
- YANKEE E. W., BUNDY G. L., 1972. (15s)-15-methyl prostaglandins. *J. Amer. Chem. Soc.*, **94**, 3651-3652.