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THE EFFECT OF CLOPROSTENOL ON HUMAN LUTEAL STEROID AND PROSTAGLANDIN SECRETION *in vitro*

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1 Human luteal tissue slices from days 18, 21 and 25 of the menstrual cycle were superfused *in vitro* with Medium 199 alone or containing cloprostenol (1 μg/ml). Concentrations of progesterone, oestradiol-17β and prostaglandins $F_{2\alpha}$ and E_2 were determined in the superfusate samples.

2 Secretion of steroids and prostaglandins was maintained at an approximately constant level throughout the experiments (21 h in one case) when the tissue was perfused with M199 alone.

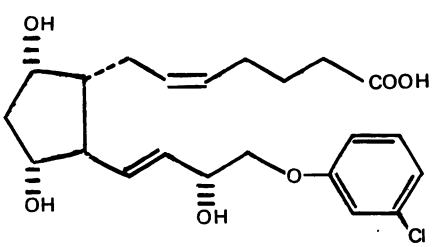
3 Superfusion with cloprostenol (1 μg/ml) resulted in an initial depression of progesterone and oestradiol-17β but this was not maintained, levels returning to control values or showing an increase, while superfusion with cloprostenol continued. Cloprostenol is not therefore considered to be luteolytic at this dose and under these conditions for human luteal tissue *in vitro*.

4 Superfusion with cloprostenol (1 μg/ml) also resulted in a large stimulation of secretion of endogenous prostaglandin $F_{2\alpha}$ following a short lag phase. This stimulation was possibly due to the initial depression of progesterone secretion. A short-lived stimulation of prostaglandin E_2 secretion was also observed.

5 The significance of the increase in prostaglandin E_2 secretion and the interrelationships between the various changes observed with cloprostenol are difficult to interpret.

Introduction

Luteal function in many mammalian species appears to be controlled by the secretion of a uterine luteolysin which, in most cases, is almost certainly prostaglandin $F_{2\alpha}$ (Poyser, 1973). However, human luteal function seems to be independent of uterine control since neither hysterectomy (Beavis, Brown & Smith, 1969; Beling, Marcus & Markham, 1970) nor congenital absence of Fallopian tubes, uterus and vagina (Brown, Kellar & Matthew, 1959; Fraser, Baird, Hobson, Michie & Hunter, 1973) disrupt cyclical ovarian function. Attempts to induce luteal regression in women by intravenous infusions of prostaglandin $F_{2\alpha}$ have been unsuccessful (Jewelewicz, Cantor, Dyrenfurth, Warren & Vande Wiele, 1972) but direct injection of prostaglandin $F_{2\alpha}$ into the human corpus luteum does induce luteolysis (Korda, Shutt, Smith, Shearman & Lyneham, 1975) and specific prostaglandin $F_{2\alpha}$ receptors have been found in the human corpus luteum (Powell, Hammarstrom, Samuelsson & Sjoberg, 1974). Consequently an intra-ovarian luteolytic role for prostaglandin $F_{2\alpha}$ has been proposed for the human corpus luteum. The observations that human luteal tissue produces prostaglandins *in vitro* (Challis, Calder, Dilley, Forster, Hillier, Hunter, Mackenzie & Thorburn, 1976a) and that infusions of prostaglandin $F_{2\alpha}$ reduce human luteal progesterone secretion *in vitro* (McNatty, Henderson & Sawyers, 1975) support this hypothesis.



Cloprostenol (ICI 80,996), a derivative of prostaglandin $F_{2\alpha}$, has been shown to be a potent luteolytic agent in several species including sheep, rats and mares (Dukes, Russell & Walpole, 1974). In this paper we present the preliminary results of an in-

vestigation into the action of this compound on human luteal steroid and prostaglandin secretion *in vitro*.

Methods

Source of tissue

Ovarian tissue samples were obtained at laparotomy on days 18, 21 and 25 in 28 day menstrual cycles, from 3 women undergoing hysterectomy for non-malignant conditions (fibroids or endometriosis). The stage of the menstrual cycle was determined from the menstrual history and supported by ovarian and endometrial histology. The project was accepted by the Regional Ethical Committee.

Treatment of tissue

Ovarian tissue was transported to our laboratories in a sterile container immediately on removal from the patient. On arrival in the laboratory the corpora lutea were placed in sterile medium (M199 Wellcome Reagents Ltd.) at pH 7.4. The medium contained no antibiotics or serum at this or any other stage in the experiment. The corpora lutea were then trimmed free of connective tissue and 0.4 mm slices were obtained with a hand microtome. These tissue slices were each then halved and each half slice (50 mg) was accurately weighed and placed in a sterile superfusion chamber. The superfusion system used was as described before (Rodway, Dodson & Watson, 1976) except that the Millipore filters were replaced by nylon gauze. The whole system was set up in a cold room at 4°C, in a heated water bath maintaining the coils, superfusion chambers and contents at 37°C. The apparatus was sterilized 12 h before use by pumping through a 5% (v/v) solution of sodium hypochlorite. Two hours before use the apparatus was rinsed through with distilled water for 1 h and with M199 for the remaining time. Retention of radioactive steroids and prostaglandins by the perspex chambers and the polyethylene tubing was found to be negligible.

In every experiment two blank chambers were set up containing no tissue. Two control chambers containing human luteal tissue were also set up and these and the blank chambers were superfused with M199 alone throughout the experiment. Two experimental chambers containing human luteal tissue were also set up. Initially these chambers were superfused with M199 to allow collection of control samples. Subsequently superfusion was continued with M199 containing cloprostenol (1 μg/ml). After this experimental period, which varied in duration and frequency of sample collection for different experiments, superfusion was again carried out with M199 alone. The duration of sample collection was the same for blank, control and experimental chambers for any given experiment. Also in every experiment a preliminary 90 min superfusion of blank, control and experimental chambers was carried out with M199 and the samples collected during this time were discarded. A flow rate of 20 ml M199/h was used in all experiments for all chambers. All samples were stored at −20°C until extracted and assayed.

Analysis of samples

All samples were analysed for progesterone, oestradiol-17β, prostaglandin $F_{2\alpha}$ and prostaglandin E_2 by radioimmunoassay in each case. All samples were checked for parallelism with the standard curve in every assay and at least three aliquot volumes were assayed in duplicate for every sample.

Tritiated steroid and prostaglandins (Radiochemical Centre, Amersham) had the following specific activities: [^{3}H]-progesterone 255 mCi/mg, [^{3}H]-oestradiol-17β 344 mCi/mg, [^{3}H]-prostaglandin $F_{2\alpha}$ 452 mCi/mg and [^{3}H]-prostaglandin B_2 288 mCi/mg.

Quality controls were made up in each case (at a concentration of 5 ng/ml) on the day of the experiment, so that the quality control had been stored for the same length of time as the experimental samples.

The cross-reactivity of cloprostenol was checked in all assays for progesterone, oestradiol-17β, prostaglandin $F_{2\alpha}$, and prostaglandin E_2 and was in all cases less than 0.001% at 50% binding of the antibody. No displacement of the standard curves for any of the above compounds was noted in the presence of cloprostenol at a concentration of 1 μg/ml.

Progesterone assay. Samples were extracted and assayed for progesterone by methods previously described (Patek & Watson, 1976), within three weeks of the experiment, where possible. Blanks gave increasing values and quality controls gave variable values if samples were stored for longer than eight weeks. Where analysis was carried out within this period the interassay coefficient of variation, calculated from the quality controls was 6.2% the mean quality control value being 4.72 ng/ml. The intraassay coefficient of variation was 4.6%. Blank sample values were always <5 pg progesterone/ml M199 and it was not considered necessary therefore to adjust the values obtained for control and experimental samples. All samples showed parallelism with the progesterone standard curve.

Oestradiol-17β assay. Sample aliquots of various volumes were extracted twice with three volumes of redistilled diethyl ether. Recovery of tritiated oestradiol-17β using this extraction method was 86–88%. The dried extracts were assayed by radioimmunoassay using a slight modification of the method of Wu & Lundy (1971). Samples could not be stored for longer than eight weeks without disruption of blank and quality control values. Where analysis

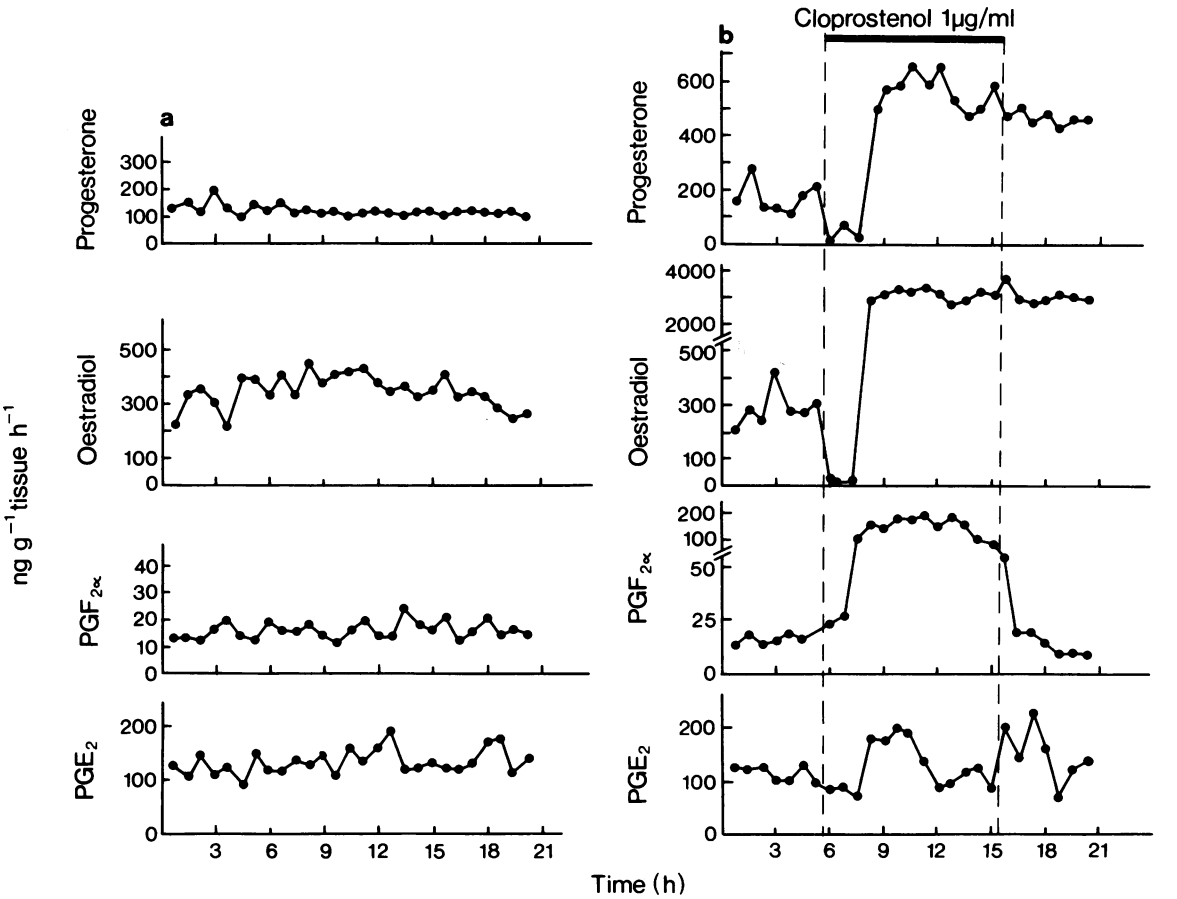


Figure 1 Day 18 corpus luteum. (a) Mean amounts of steroids and prostaglandins in consecutive samples of superfusate from two control chambers containing tissue superfused with M199 *in vitro*. (b) Mean amounts of steroids and prostaglandins in consecutive samples of superfusate from two chambers containing tissue superfused with M199 or cloprostenol in M199 (horizontal bar) *in vitro*.

was carried out within this time the interassay coefficient of variation was 7.9%, the mean quality control value being 5.53 ng/ml. The intra-assay coefficient of variation was 5.5%. Blank sample values were always between 30 and 50 pg oestradiol-17β/ml M199 and quality controls, control and experimental samples were adjusted appropriately. All samples showed parallelism to the oestradiol-17β standard curve.

Prostaglandin $F_{2\alpha}$ assay. Sample aliquots of various volumes (20–200 μl) were acidified with equal volumes of pH 4.0 citrate buffer and extracted twice with two volumes or redistilled diethyl ether. Recovery of tritiated prostaglandin $F_{2\alpha}$ with this extraction method was greater than 95%. The dried extracts were assayed by solid-phased radioimmunoassay by methods previously described (Dighe, Emslie, Henderson, Rutherford & Simon, 1975). Blanks and quality controls were found to store well at −20°C in terms of prostaglandin $F_{2\alpha}$ but all samples were extracted and analysed as quickly as possible, none the less. The interassay coefficient of variation was 5.7%, the mean quality control value being 4.81 ng/ml. The intraassay coefficient of variation was 4.1%. Blank sample values were always less than 1 pg prostaglandin $F_{2\alpha}$/ml M199 and were therefore disregarded. All samples showed parallelism with the prostaglandin $F_{2\alpha}$ standard curve.

Prostaglandin E_2 assay. All sample aliquots were extracted as soon as possible by the method described for prostaglandin $F_{2\alpha}$. Recovery of tritiated prostaglandin E_2 by this extraction method was 95–98%.

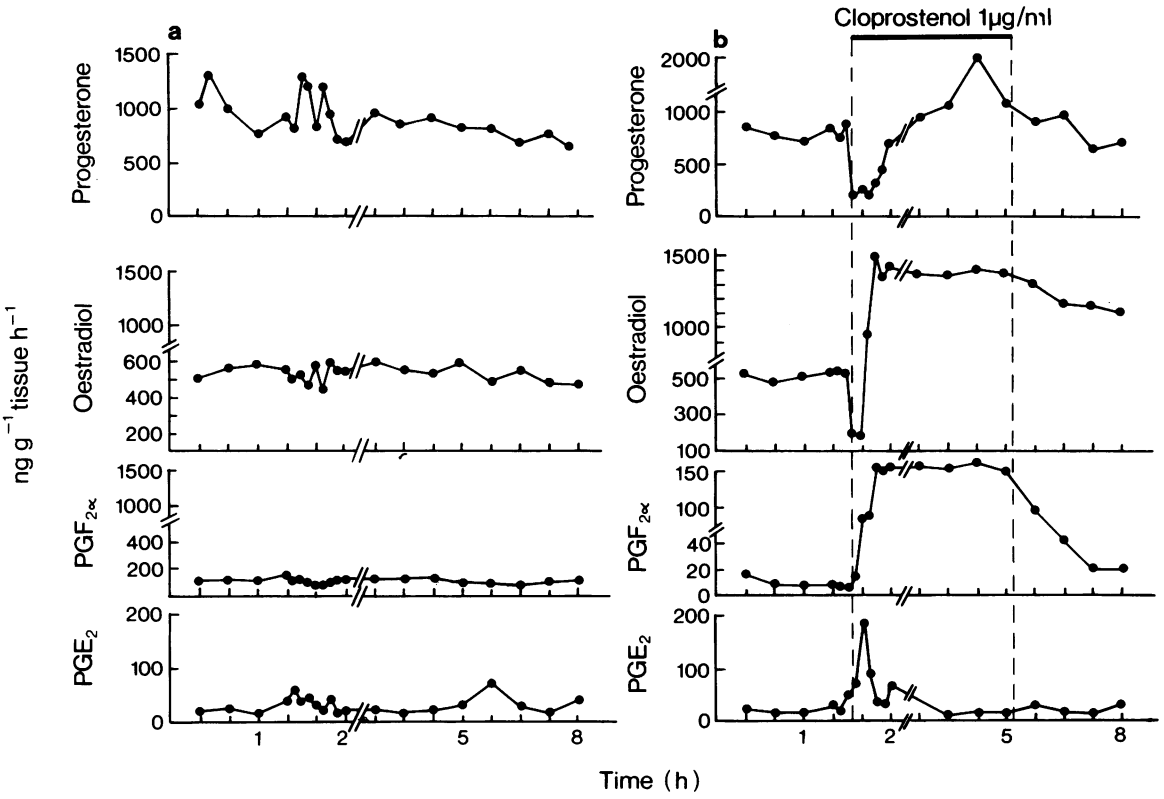


Figure 2 Day 21 corpus luteum. (a) Mean amounts of steroids and prostaglandins in consecutive samples of superfusate from two control chambers containing tissue superfused with M199 *in vitro*. Samples were collected at 20 min intervals for 80 min, 5 min intervals for the next 40 min and 45 min intervals thereafter. (b) Mean amounts of steroids and prostaglandins in consecutive samples of superfusate from two chambers containing tissue superfused with M199 or cloprostenol in M199 (horizontal bar) *in vitro*. Samples were collected at 20 min intervals for 80 min, 5 min intervals for the next 40 min and 45 min intervals thereafter.

Prostaglandin E_2 was assayed as prostaglandin B_2 equivalents, the conversion of prostaglandin E_2 to prostaglandin B_2 being carried out by the method of Dozois & Thompson (1974). Efficiency of conversion was found to average 86% ± 4.6% (mean ± s.e. mean). Conversion was carried out no later than the day following the experiment. Quality controls were treated exactly as the samples. After conversion, prostaglandin B_2 was estimated by radioimmunoassay by the method described for prostaglandin $F_{2\alpha}$ by Patek & Watson (1976) except that the antibody used had been raised against prostaglandin B_2-bovine serum albumin. All converted quality controls and samples showed parallelism with the prostaglandin B_2 standard curve. The prostaglandin B_2 content of the quality controls and samples without conversion was also determined and was always less than 5 pg prostaglandin B_2/ml M199. The interassay coefficient of variation for quality controls after conversion was 8.2%, the mean quality control value being 4.22 ng/ml the intraassay coefficient of variation for samples after conversion was 7.6%.

Results

All results quoted are the mean values for samples from two superfusion chambers each containing luteal tissue and each maintained under the same regime.

The patterns of secretion of steroids and prostaglandins for human luteal tissue superfused *in vitro* with M199 alone throughout the experiment are shown in Figures 1a, 2a and 3a for days 18, 21 and 25 of the menstrual cycle, respectively. Figures 1b, 2b and 3b show the patterns of secretion of steroids and prostaglandins for the same tissues, for days 18, 21 and 25 of the menstrual cycle respectively, when superfused with cloprostenol (1 μg/ml) in M199.

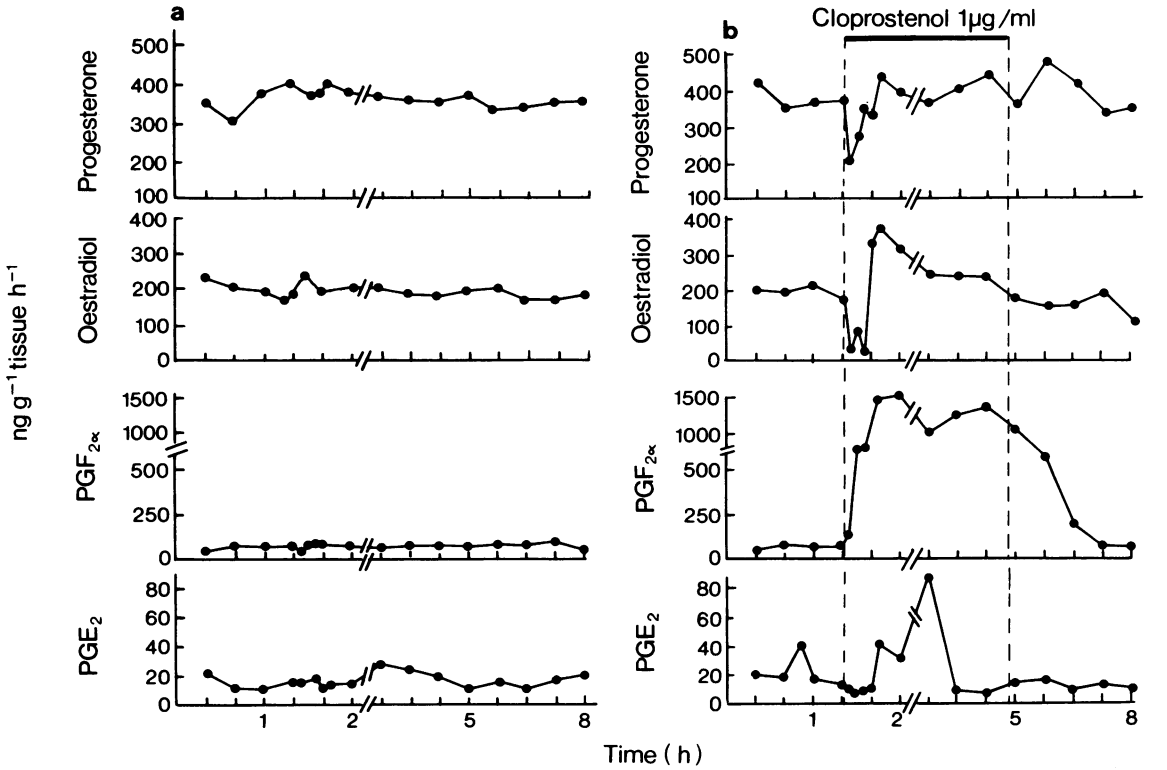


Figure 3 Day 25 corpus luteum. (a) Mean amounts of steroids and prostaglandins in consecutive samples of superfusate from two control chambers containing tissue superfused with M199 *in vitro*. Samples were collected at 20 min intervals for 80 min, 5 min intervals for the next 25 min and 45 min intervals thereafter. (b) Mean amounts of steroids and prostaglandins in consecutive samples of superfusate from two chambers containing tissue superfused with M199 or cloprostenol in M199 (horizontal bar) *in vitro*. Samples were collected at 20 min intervals for 80 min, 5 min intervals for the next 25 min and 45 min intervals thereafter.

Tissues superfused with M199 alone throughout the experiment maintained secretion of progesterone, oestradiol-17β, prostaglandin $F_{2\alpha}$ and prostaglandin E_2 at an approximately constant level. The day 18 human luteal tissue was superfused *in vitro* for 21 h, and even after this time the amounts of steroids and prostaglandins being secreted were still 95–98% of that found for the early samples. The other tissues were maintained only for 8 h and amounts of steroids and prostaglandins in the last few samples were equivalent to those in the early samples. It appears therefore that the tissue was still viable after either 8 or 21 h superfusion with M199 *in vitro*.

Superfusion with cloprostenol (1 μg/ml) caused a depression of progesterone secretion in every case, but this did not persist. The greatest effect in terms of duration of depression of progesterone secretion was seen for the day 18 human luteal tissue sample. The depression of progesterone secretion from this tissue was followed by 4-fold increase of progesterone secretion which persisted after superfusion with cloprostenol had ceased. The other tissue samples did not show such a marked increase in progesterone secretions following the depression in secretion. The day 25 luteal tissue sample showed the smallest overall response to cloprostenol.

The pattern of oestradiol-17β secretion following treatment with cloprostenol was similar for all tissues being an initial depression followed by a large potentiation. The greatest duration of depression of oestradiol-17β secretion and subsequent potentiation was seen for the day 18 luteal tissue sample. The day 25 luteal tissue sample gave the smallest response to this treatment.

Superfusion with cloprostenol resulted in an increase in endogenous prostaglandin $F_{2\alpha}$ secretion, following a short lag phase, in every case. The day 18 luteal tissue sample gave the smallest response in relation to prostaglandin $F_{2\alpha}$ secretion, the increase being approximately 10-fold, while the other tissue

samples (day 21 and day 25) showed a 15 to 20-fold increase in endogenous prostaglandin $F_{2\alpha}$ secretion.

The response of prostaglandin E_2 secretion to superfusion with cloprostenol was more variable but in every case an increase in secretion of endogenous prostaglandin E_2 was seen around the same time or just after the increase in prostaglandin $F_{2\alpha}$ secretion. However, the increase in prostaglandin E_2 secretion was not maintained, levels returning to control values before cessation of superfusion with cloprostenol.

Discussion

Superfusion with cloprostenol (1 μg/ml) *in vitro* resulted in a reduction in progesterone secretion for all the human luteal tissue samples studied (days 18, 21 and 25 of the menstrual cycle). However the reduction in progesterone secretion was not maintained, the amounts of progesterone secreted returning to control or even higher values while superfusion with cloprostenol continued. Cloprostenol could not therefore be considered to be luteolytic for human luteal tissue *in vitro* at this dose (1 μg/ml) and under the conditions used for these experiments. The speed with which progesterone secretion was depressed (within 5 min for day 21 and day 25 luteal samples) could indicate an inhibition of release of progesterone.

A further, major, effect of the superfusion with cloprostenol was the large increase in secretion of endogenous prostaglandin $F_{2\alpha}$, which was seen in all samples, occurring after a lag phase of 15–45 minutes. A similar stimulation of endogenous prostaglandin $F_{2\alpha}$ secretion following treatment with cloprostenol has been observed in the sheep *in vivo* (Challis, Forster, Furr, Robinson & Thorburn, 1976b) but this followed a much longer lag phase (27–39 hours). The increased prostaglandin $F_{2\alpha}$ secretion seen for the human luteal tissue could be the result of progesterone withdrawal since inhibition of prostaglandin $F_{2\alpha}$ synthesis by progesterone has been demonstrated for human endometrial tissue *in vitro* (Cane & Villee, 1975). The appearance of endogenous prostaglandin $F_{2\alpha}$ in the uterine venous plasma of pregnant goats has also been observed following a decline in progesterone secretion resulting from treatment with exogenous prostaglandin $F_{2\alpha}$ *in vivo* (Currie & Thorburn, 1973) although the lag phase to increased endogenous prostaglandin $F_{2\alpha}$ secretion was again longer than observed in these experiments with human luteal tissue. In the guinea-pig (Poyser, 1976) and sheep (Barcikowski, Carlson, Wilson & McCracken 1974) oestradiol will initiate secretion of prostaglandin $F_{2\alpha}$ into the utero-ovarian vein and in woman intra-ovarian injections of oestradiol cause premature menstruation (Hoffman, 1960) suggesting a stimulation of luteolysis. However in these experiments with human luteal tissue *in vitro*, secretion of oestradiol-17β following cloprostenol treatment is depressed in every case until after the increase in secretion of endogenous prostaglandin $F_{2\alpha}$. It is hard to interpret the significance of either the initial depression or the subsequent stimulation of oestradiol-17β secretion seen for all the tissue samples in response to cloprostenol (1 μg/ml). Since the pattern of response for progesterone and oestradiol-17β are so similar it is tempting to consider that the changes in these steroids may be connected with each other. Although no direct comparison may be made between the *in vitro* and the *in vivo* situation, the possible effects of an increase in oestradiol-17β secretion at the level seen here could be an important consideration in relation to *in vivo* treatment with cloprostenol in humans.

The significance of the increase in prostaglandin E_2 secretion observed for all the human luteal tissue samples in response to cloprostenol is not clear.

The control (superfusion with M199 alone) levels of secretion of progesterone and oestradiol-17β agree broadly with those observed by Challis *et al.* (1976a) for static incubations. The levels of prostaglandins observed here for the control samples are, however, higher than those observed by Challis *et al.* (1976a). The prostaglandin $F_{2\alpha}$ levels were higher (100 ng g^{-1} h^{-1} and 70 ng g^{-1} h^{-1}) than the prostaglandin E_2 levels (30 ng g^{-1} h^{-1} and 15 ng g^{-1} h^{-1}) for the day 21 and day 25 human luteal tissue samples, respectively. The day 18 human luteal tissue sample, however, secreted more prostaglandin E_2 (125 ng g^{-1} h^{-1}) than prostaglandin $F_{2\alpha}$ (16 ng g^{-1} h^{-1}). These results contrast with those of Challis *et al.* (1976a) who found prostaglandin E_2 levels to be higher than prostaglandin $F_{2\alpha}$ levels in every case. However the conditions of our experiments differ extensively from those of Challis *et al.* (1976a) since we were using slices rather than homogenates and were also removing the secretory products of the tissue immediately, thus preventing any product-feedback which might otherwise occur. In this respect superfusion more closely resembles the physiological situation where many secretory products are continuously removed by the blood. Challis *et al.* (1976a) suggest that higher prostalandin levels may be indicative of the onset of cell autolysis. However,the maintenance of a constant pH (as gauged by medium colour) and maintenance of reasonable levels of steroid secretion throughout the experiments would tend to discount this possibility. In further experiments histological analysis of the tissue at the end of the experiments will answer this question.

The large increase in the secretion of endogenous prostaglandin $F_{2\alpha}$ by the human luteal tissue *in vitro* seen here in response to cloprostenol especially in day 21 and day 25 samples, indicates that the human corpus luteum has a very large potential for the secretion of prostaglandins. This observation supports the hypothesis of an intra-ovarian physiological rôle for prostaglandin $F_{2\alpha}$ in the human.

The authors recognize the limitations resulting from the small number of observations presented in this study but believe that the similarity of response obtained for each sample makes these observations worthwhile. Further work is in progress relating to cyclical variations in steroids and prostaglandins and the effects of prostaglandins on steroid and prostaglandin secretion by human luteal tissue superfused *in vitro*.

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