ELECTROMYOGRAPHIC STUDIES ON THE OVINE UTERUS

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ABSTRACT

There are many reports in the literature, using a variety of techniques, indicating that hormonally induced movements of the uterus play a major role in the reproduction process. The purpose of this study was to explore some of these phenomena by measuring electro-myographical responses from the uterus of intact and ovariectomized ewes both before and during their normal breeding season.

Six healthy non-pregnant Romney ewes of about 50 kg body weight were selected for the investigations which were carried out between September 1987 and February 1988.

Electrodes, usually in groups of three, were surgically implanted at pre-selected sites (cervix, uterine body, uterine horn, oviduct) on the reproductive tract of the ewes, leads plugged into a universal AC amplifier, and the output recorded on a four channel ink writing chart recorder.

Four of the six ewes were ovariectomized at the time of implantation of the electrodes. Indwelling silastic catheters were inserted into the external jugular veins of these ewes to enable oxytocin to be injected without disturbing the animal. All ewes were housed individually indoors in pens that allowed them to move freely during recording sessions. Recordings began about 10 days after surgery had been carried out.

The electromyographs (emgs) were described using generally accepted terms such as the presence of spikes, their amplitude, the presence of bursts and of episodes of activity, whether electrical activity was co-ordinated or uncoordinated, and the direction of propagation of the activity. A motility index (MI) was derived as a method of measuring electrical activity of the uterus over prolonged periods of time.

During the anoestrous period in both intact and ovariectomized ewes an emg pattern of myoelectrical complexes characterised by alternating phases of co-ordinated bursts of electrical activity and quiescent periods were observed. This basic pattern of activity, and responsiveness to uterine stimulants such as oxytocin and Glandin N (a PGF2α analogue), was greater in the intact

ewes as compared to the ovariectomized ewes at this time. Although such a difference could be anticipated, a clear answer as to whether it is a general phenomenon, or whether seasonal differences exist, could not be determined because of the limitations in design of this study.

A distinct pattern of emg activity was also identified during different phases of the oestrous cycle in the ewe during the natural breeding season. This pattern could be mimicked by administration of the exogenous steroid hormones oestrogen and progesterone in the ovariectomized ewes. Four days after daily oestradiol-17b injections (50μg s.c.) the emgs of the ovariectomized ewes showed a marked increase in amplitude and in burst frequency and a considerably increased response to oxytocin and Glandin N. When this was followed by progesterone administration (50mg. s.c.), even with oestrogen injections continuing, inhibition of activity and reduced responsiveness to oxytocin and Glandin N was equally marked.

Withdrawal of progesterone, but with continuing oestrogen administration, resulted in a recovery from the inhibition and a response that was even greater than before the progesterone had been given. This suggests both an inhibiting and a potentiating action of progesterone on uterine electrical activity, a finding which adds some support to Csapo's classical withdrawal of the 'progesterone block' as one of the pre-requisites for initiation of normal parturition in sheep.

While oestrogen has a clear role to play as a stimulator of electrical activity it also seems capable of exhibiting a biphasic response with a period of depressed activity occurring before the positive stimulus occurs. Whether this is a function of dose or some other factor could not be established in this study. Its potentiating effect on the action of both oxytocin and Glandin N in these experiments adds further significance to the attention that should be paid to the reproductive status of the animal when clinical use is made of these substances.

The direction of propagation of action potentials recorded in these studies depended on the general level of emg activity. When this was low the direction of propagation was cervico-tubal, when high, the proportion of action potentials is in both directions from the tubal end of the uterus. A

clear understanding of such a mechanism, and its significance, must await further study.

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ELECTROMYOGRAPHIC STUDIES ON THE OVINE UTERUS

I. INTRODUCTION

It has long been recognised that the movement of sperm from the site of ejaculation to the oviduct is due not only to the motility of sperm cells but also to contractions of the female reproductive tract; in all species in which it has been timed it has been found that only a few minutes are required from deposition of sperm in the vagina/cervix until they reach the fallopian tube. Furthermore there seems to be no relationship between the rate at which semen is propelled through the uterus and the distance separating the cervix and the vagina from the oviducts (Nalbandov, 1976).

The probable importance of hormones in this process was indicated by Nalbandov (1976) who found, in cows, that mating caused a rise in intra-mammary pressure and that strong uterine contractions were induced by the stimulus of natural mating, artificial insemination, manipulation of the vulva, or even by the sight of a bull. This same author reported that increased activity was noted in isolated uteri perfused with oxytocin and, even in such preparations, semen deposited in the cervix was transported to the oviducts in five minutes. All these observations taken together were interpreted to mean that coitus induces nervous impulses that reach the posterior lobe of the pituitary via the hypothalamus activating the release of oxytocin; this then causes the uterine and oviduct contractions that are responsible for the rapid movement of semen from the site of ejaculation to the oviduct (Nalbandov, 1976).

Pusey *et al.* (1980) demonstrated in the rat that myometrial activity also plays a role in the distribution of blastocycts throughout the uterus. This process had earlier been suggested by Hafez (1964) to play a role in the process of spacing and trans-uterine migration of blastocysts in the ewe.

The control of myometrial activity around oestrus is very complex. In the ewe, Naaktgeboren and Van Der Weyden (1973) first demonstrated,

by recording the electrical activity of the myometrium, that the changes in the contraction pattern of the uterus are caused by the changing progesterone : oestrogen ratio. Quinlivan and Robinson (1969) reported reduced fertility in animals treated with progesterone impregnated intravaginal sponges to synchronize oestrus. This reduction in fertility can be attributed, at least in part, to decreased sperm transport to the oviduct resulting from reduced uterine motility.

In many species progesterone is considered essential for the maintenance of uterine quiescence during pregnancy and progesterone withdrawal is considered essential for the normal evolution of uterine contractility associated with both natural (Thorburn *et al.*, 1977) and induced parturition (Currie *et al.*, 1973).

It thus seems clear that hormonally induced movements of the uterus play a major role in the reproduction process. The purpose of this study therefore was to explore some of these phenomena by measuring electro-myographical responses from the uterus of both intact and ovariectomized ewes. Electro-myograms (emgs) were recorded from an intact ewe before, during and after a period of natural oestrus. Studies were also made of the emg of the uterus of an intact ewe in the anoestrous state and of ovariectomized ewes: in both types of experimental preparations commonly used exogenous hormones were administered to the ewe being investigated and the responses recorded. The results of these studies form the basis of this thesis.

II. LITERATURE REVIEW

II.1. The Reproductive Cycle of Sheep.

II.1.A. The breeding season

Ewes are seasonally poly-oestrous animals. The annual reproductive cycle consists of a breeding (oestrus) season and a non-breeding (anoestrus) season. The breeding season in New Zealand usually begins in March and is characterized by successive 16-17 day oestrous cycles. The duration of oestr us is about 36 h with ovulation occurring 24 h after its onset. In June/July, the ovarian cycles cease and the anoestrous season begins (Ward, 1986).

This seasonality ensures the young are born at a time of year when they have the best chance of growing to maturity but it does place restraints on modern farmers who wish to maximize farm output. With a cycle of 5 months pregnancy and 3 months lactation, it is theoretically possible to produce 3 sets of lambs every 2 years. However, the very strong seasonal photo-periodic influence upon fertility makes this difficult to achieve. Whether the objective is to maximize the efficiency of labour-intensive management systems as in Europe, or to maximise the efficiency of labour-extensive farming systems as in Australia and New Zealand, mating management strategies require better control of the onset of breeding activity (Kennaway *et al.*, 1987).

Numerous procedures have been used to induce out of season breeding (McDonald, 1986), but all have failed to account for the persisting inhibitory photo-periodic signals the sheep receive during anoestrus.

The first attempts to influence sheep breeding by manipulating day length appear to be those performed by Yeates (1949), who demonstrated that decreasing periods of light at a time of year when sheep were normally anoestrus induced oestrous cycles. This remains a method of producing more than one crop of lambs a year in housed sheep. Yeates also found that the actual length of the daily light period was not important, thus showing that breeding was not dependent upon a critical

total amount of light.

More recently it was reported that blinded sheep were able to respond to artificial changes in light period only when housed with a sighted ram (Legan and Karsch, 1980). Further advances in our understanding of seasonality have come with the recognition of the vital role that the pineal gland and its hormone melatonin plays in relaying photo-periodic information to the endocrine system; treatment with melatonin can be used to bring forward the beginning of the breeding season (Arendt *et al.*, 1983).

During seasonal anoestrus the pituitary contains large amounts of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and the ovaries contain small numbers of large follicles. Corpora lutea (indicating ovulation) are found only at the end of the anoestrous period (Ward, 1986). This increase in gonadotrophins in the pituitary gland may be due to a higher sensitivity of the pituitary to the negative feedback of oestradiol during anoestrus. Changes in annual photo-period modulates the negative feedback of oestradiol-17b on the tonic LH secretion in the ewe (Legan and Karsch, 1980). The duration of secretion of the pineal hormone melatonin is responsible for the changes in oestradiol feedback (Bittman *et al.*, 1983). The breeding season of ewes is therefore characterized by lower oestradiol negative sensitivity at the hypothalamic/pituitary level and this permits the LH pulse frequency to increase during the peri-ovulatory period, resulting in an LH surge and ovulation (Legan *et al.*, 1977).

The development of biologically active, biodegradable, continuous release formulations of melatonin should allow sheep producers to gain almost complete control of the time their animals can conceive and thus maximize their lamb production. However, there are still many questions to be answered concerning how melatonin acts at the neuroendocrine level to promote cyclic ovarian activity and maximize the number of ova shed by the ewes (Kennaway *et al.*, 1987).

II.1.B. The oestrous cycle in ewes.

The principal steroids secreted in the cyclic ewe are progesterone and oestradiol-17b (Pant *et al.*, 1977). On the day before oestrus one or more follicles grow rapidly and the concentration of oestradiol-17b in the blood increases from about 10 to 20 pg/ml. The oestrogen causes behavioural oestrus. A positive feedback from oestradiol, along with hypothalamic GnRH, stimulates release of LH and FSH from the pituitary. The concentration of LH in the blood rises to a peak of about 80 ng/ml 10 h after the beginning of oestrus, and then both LH and estradiol concentrations fall rapidly. LH stimulates ovulation, which occurs about 14 h after the LH peak, i.e. about 24 h after the beginning of oestrus. Throughout the rest of the oestrous cycle, the LH concentration remains very low (2 to 3 ng/ml). At the same time as the LH peaks, FSH reaches a maximum of about 170 ng/ ml and then falls rapidly. Unlike LH, it rises to a second peak 24 h after the first. After oestrus, FSH concentration is elevated at day 3 and from day 8 to 12 it rises to about 80 ng/ml and then declines to about 40 ng/ml before the next oestrus. At ovulation, stimulated by LH and prolactin, the granulosa cells form the corpus luteum (CL), which secretes progesterone. Maximum concentrations of progesterone are reached in the blood at days 8-9 of the cycle and remain high until days 13-14 when the corpus luteum starts to regress (Ward, 1986).

On the twel th day in the nonpregnant (and pregnant) ewe, prostaglandin F2a (PGF2a) increases in concentration and reaches a peak on the fourteenth day at a concentration in the uterine vein of about 10ng/ml. PGF2a, which is established as the luteolysin in the sheep, reaches the corpus luteum by means of the close apposition of the uterine vein and ovarian artery (Ward, 1986).

The release by the ovary of oxytocin, in amounts that are greatest at the time of luteal regression, suggests a role in luteolysis; however hysterectomy depletes the ovary of oxytocin without preventing luteolysis by the prostaglandin analogue, cloprostenol (Sheldrick and Flint, 1983). Wathes *et al.*, (1986) suggest that ovarian oxytocin stimulates the release of endometrial PGF2a, which in turn decreases

luteal progesterone production and also causes the release of further oxytocin.

It is not yet clear whether the appearance of endometrial oxytocin receptors triggers this chain of events or whether the fall in progesterone secretion is induced by some other factor. In the ewe the concentration of endometrial oxytocin receptors changes from a maximum at oestrus to an almost undetectable level during the mid-luteal phase before increasing again on day 15 of the cycle (Roberts and McCracken, 1976). In both the ewe and the cow, PGF2a itself is not luteolytic during the first few days of the cycle (Rowson *et al.*, 1972; Haresign, 1978). The period between days 3 and 6 when oxytocin does induce luteolysis in cows and goats is therefore presumed to reflect a time when sufficient receptors of the appropriate type are present for oxytocin to act on the endometrium and for PGF2a to act on the corpus luteum; such a situation may not occur in the ewe (Wathes *et al.*, 1986).

The corpus luteum shows histological evidence of regression on the 15th day and by day 16 the concentration of progesterone is basal (less than 0.2ng/ml). FSH released from the pituitary because of a reduction in negative feedback from progesterone stimulates oestradiol production from growing follicles. The increased oestradiol in turn acts as a positive feedback on the hypothalamic/pituitary axis leading to a peak of LH. This LH peak stimulates ovulation (Hafez, 1980).

Progesterone is believed to be a necessary precursor for the production of oestrous behaviour by the oestradiol (Hafez, 1980).

Synthetic GnRH stimulates release of physiological amounts of LH and FSH (Ward, 1986). The separate release of FSH without LH at the end of oestrus may be explained by the effect of the previous high concentrations of oestradiol depleting LH in the pituitary by the positive feedback mechanism resulting in ovulation. This treatment can also cause release of FSH (and not LH) in anoestrous ewes because of the higher sensitivity of these ewes to the negative feedback of oestradiol on the release of LH when they are in the state of anoestrus (Wheaton *et al.*, 1982).

II.1.C. Pregnancy and parturition in ewes

If pregnancy follows fertilization in the ewe, ova remain in the fallopian tubes for about 4 days and enter the uterus at the 8 to 16 cell stage. Attachment occurs between 14 and 18 days, which means that the blastocyst is able to prevent the cyclical regression of the corpus luteum before being attached. This may be brought about by inhibition of the release of the uterine luteolysin (PGF2a) (Fitzpatrick, 1986).

Following conception the corpus luteum persists and peak di-oestrous values of progesterone are maintained and gradually increase to about 60 days of gestation when there is a further increase. This later rise is due to the placental contribution to progesterone production (the placenta takes over the role of progesterone production in the ewe by about day 50 of gestation). Levels remain high until the last week of pregnancy when they decline rapidly (Arthur *et al.*, 1982).

The concentration of progesterone is significantly higher in multiple pregnancies (Basset *et al.*, 1969). Maximum progesterone concentration in the peripheral blood of ewes with a single lamb was 3.78 ng/ml between days 105-110, and 5.09 ng/ml between days 125-130 in ewes with twins (Emady *et al.*, 1974).

Oestrogen concentration in the peripheral circulation remains low until a few days before parturition when it starts to rise and then suddenly increases to about 400 pg/ml at the time of lambing. This is followed by a rapid fall (Challis, 1974).

During pregnancy prolactin concentrations fluctuate between about 20 and 80 ng/ml; however, they start to increase and reach a peak (between 400 and 700 ng/ml) on the day of lambing (Kann and Denamur, 1974).

It is now well established that the foetus influences the time of its own birth (Liggins, 1977). The sequence of events starts with activation of the hypothalamus and pituitary of the foetus by a mechanism that is, as yet, unknown. It is however certain that about 5 days before birth adrenocorticotrophic hormone (ACTH) is secreted and stimulates the foetal adrenal glands to liberate cortisol. Cortisol acts on the steroid-secreting cells of the foetal cotyledon, which for

most of pregnancy have been secreting the progesterone essential for the maintenance of pregnancy. Cortisol increases activity of the enzyme 17a-hydroxylase, and thereby causes a decrease in progesterone secretion. Similarly, cortisol acts on the maternal sheep placenta to cause an increase in steroid-17, 20-lyase, which, in the face of increased steroid 17a-hydroxylase activity results in an increase in the production of androstenedione, the precursor of oestrogen (Casey and MacDonald, 1986). Foetal oestrogen crosses the placenta, where it becomes unconjugated oestradiol. At the same time the clearance rate of progesterone increases (Flint *et al.*, 1975).

The change to oestrogen dominance over progesterone is important in several respects but particularly in the production of prostaglandins in the cells of the myometrium and maternal placenta. Unconjugated oestrogen is most closely related to the increased synthesis of prostaglandins, since there is a marked increase in the concentration of prostaglandins in uterine venous blood after the administration of oestradiol to the pregnant ewe (Liggins *et al.*, 1972). Both PGF2a and PGE2 are powerful stimulants of uterine muscle. Uterine contractions themselves facilitate the further release of intra-cellular lysosomal enzymes that synthesize prostaglandins and the whole process becomes self-perpetuating.

The ultimate effect of the uterine contractions, in combination with cervical relaxation and other changes, is to advance the foetus into the cervix and anterior vagina, where it stimulates sensory receptors and initiates Ferguson's reflex, with the release of large amounts of oxytocin from the posterior pituitary. This augments the myometrial contractions, and results in the liberation of even more prostaglandins so that the whole sequence becomes a cascade with a positive feedback. The significance of second stage contractions with reflexly synchronized abdominal effort is to increase the efficiency and decrease the duration of second stage labour, when the risks of anoxia and other hazards are maximal. Each uterine contraction outlasts the accompanying abdominal spasm and is important in maintaining uterine "tone" (Fitzpatrick, 1986).

Parturition involves more than mere contraction of the uterus however. The increase in expulsive force must be coincident with a decrease in resistance which for practical purposes, means softening of the collagen of the cervix, and relaxation of the uterine , vaginal and pelvic ligaments. Studies on the sheep cervix by Fitzpatrick (1979) have revealed that the dense connective tissue of the wall softens dramatically at the end of pregnancy and ultimately becomes gel-like, so that the cervix is held together during the passage of the foetus by the external muscle layer and the mucosa alone. Both the total amount of collagen and its concentration fall and there is a marked increase in the water content. Histologically, the collagen bundles become de-segregated and the fibrils dispersed. This is associated with detectable increases in the proteoglycan constituents. Together these changes, which are influenced by the decline in progesterone secretion and increase in oestrogens, prostaglandins and relaxin, increase the distensibility of the cervix over a period of 12-18 hours.

II.1.D. The post-partum period in ewes

Circulating concentrations of LH are low during the early post-partum period in ewes (Restall and Starr, 1977). The following explanation for this was offered by Nett (1987). During pregnancy the hypothalamo-hypophysial axis is suppressed by the high concentrations of progesterone and oestradiol in the circulation. The high concentration of these steroids inhibits secretion of GnRH from the hypothalamus, resulting in inadequate stimulation of the pituitary gonadotrophs to maintain synthesis of LH. The depleted stores of LH in the anterior pituitary gland must be restored after parturition before normal oestrous cycles can begin. Concentrations of FSH are not suppressed however (Moss *et al.*, 1985), a phenomenon that may be due to the relative lack of follicular development during late gestation and the absence of negative feedback from folliculostatin from the follicles (Miller *et al.*, 1982).

The importance of suckling on the duration of post-partum anoestrus is demonstrated in sheep by the response to experimentally

induced pregnancy during seasonal anoestrus so that lambing occurs during the breeding season. Dried-off ewes usually return to oestrus after about one month, while suckled ewes present the first oestrus some weeks later (Hafez, 1980).

Expulsion of the foetal-placental unit at parturition is accompanied by a dramatic decrease in the concentration of oestradiol and progesterone in the circulation (Burd *et al.*, 1976). This leads to the removal of their negative feedback actions on the hypothalamo-hypophysial axis. From an endocrinological viewpoint, once this phase is complete, the female should be ready to resume normal oestrous cycles. However, even though the function of the hypothalamo-hypophysial axis returns to normal within a relatively short period of time after parturition (about 35 days in the ewe--Wise *et al.*, 1986), during this period three changes occur permitting a gradual recovery in gonadotroph function:

(a) an increase in the frequency of LH pulses induced by an increase in the concentration of mRNAs for the subunits of LH in the axis;
(b) an increase the concentration of receptors for oestradiol in the hypothalamus and pituitary gland which become sensitive to the positive feedback effects of oestradiol to release LH; and
(c) the morphology of the gonadotrophs also returns to a state similar to that observed in cycling ewes.

Even after this recovery of function the fact that frequent suckling, and other environmental factors, will suppress pulsatile secretion of LH suggests that during the post-partum period, a two-phase recovery of the hypothalamo-hypophysial-gonadal axis occurs (Nett, 1987). The first phase, which lasts from 2 to 5 weeks after parturition, is characterized by infrequent discharges (e.g. one pulse every 4-8 h) of GnRH into the hypothalamo-hypophysial portal circulation. This mode of GnRH secretion stimulates the biosynthetic machinery in the gonadotroph and the rate of synthesis of LH increases. However, the pulses of GnRH are sufficiently spaced that only a small portion of the newly synthesised LH is secreted into the circulation.

The increased rate of synthesis of LH coupled with its relatively slow rate of release creates a situation in which pituitary stores of LH are replenished. Since the magnitude of the pulse is dependent on the quantity of LH stored in the anterior pituitary the early part of this phase of recovery fails to induce follicular maturation. Only after pituitary stores of LH have returned to their normal level are the pulses of LH that are released into the circulation of sufficient amplitude to stimulate follicular growth. This marks the beginning of the second phase of the recovery process. During this phase the increased circulating concentrations of LH stimulate growth of ovarian follicles and the secretion of oestradiol.

An important effect of oestradiol is to stimulate production of its own receptors in the hypothalamus and anterior pituitary gland thus increasing the sensitivity of these tissues to the positive feedback effects of itself. This positive feedback effect of oestradiol is the result of small increases in circulating concentrations for short periods of time in contrast to the prolonged periods of very high concentrations during late gestation that produce a powerful negative feedback effect. At this point, the frequency of discharges of GnRH increases, in turn producing more frequent pulses of LH. These events lead to the final stages of follicular development and culminate in ovulation (Nett, 1987). This author also proposed that the first stage of this recovery process (i.e. events leading to increased pituitary stores of LH) is relatively independent of the suckling stimulus and environmental stressors. The second phase of the recovery (i.e. events leading to an increased frequency of discharges of LH), however, appears to be tightly coupled to the suckling stimulus and environmental stressors.

Assessment of the literature suggests that the environmental stressors in ewes may be the persisting inhibitory photo-periodic signals they received during anoestrus. These signals affect the pineal gland and influence melatonin production which in turn affects the endocrine system. This may be achieved by a mechanism similar to that which operates in the second phase of Nett's "two phase recovery"

theory. If this is correct, Legan and Karsch's (1980) suggestion that the pituitary has a higher sensitivity to negative feedback of oestradiol during the anoestrous phase may be incorrect.

II.2. Myometrial Activity

Myometrium contractility is affected by the underlying endocrine events of the oestrous cycle, pregnancy and parturition. During pregnancy, the myometrium relaxes to accommodate the developing foetus and products of conception while at the end of pregnancy, it provides the rhythmic tonic contractions of parturition which facilitate the expulsion of the uterine contents. These uterine events occur in response to the release of oxytocin, PG, and other hormones, and reflect changes in the cellular components of the myometrium such as the formation of gap junctions, synthesis of receptors and contractile protein, and the generation of enhanced electrical activity.

II.2.A. Structure and organization of the myometrial contractile unit

In most species the uterine wall is composed of three distinct layers. An inner endometrium lines the lumen of the organ, while the myometrium itself comprises two layers, an outer longitudinal layer overlying an inner circular layer. The longitudinal layer consists of bundles of smooth muscle cells that are generally oriented along the long axis of the uterus. The bundles interconnect to form a network on the surface of the uterus (Csapo, 1962). Contraction of the longitudinal muscle tends to shorten the uterus and constrict its lumen.

Muscle cells of the circular muscle layer are arranged concentrically around the longitudinal axis of the uterus. These muscle cells are arranged more diffusely and the bundle arrangement is not as apparent as with the longitudinal layers. Contraction of the circular muscle layer constricts the uterine lumen. Muscle cells of both muscle layers occupy a major part of the uterine wall, but the myometrium is not an homogeneous muscle tissue. It is composed of muscle cells that are embedded in connective tissue as a matrix which consists of collagen, gap junctions and other cells such as fibroblasts, blood and

lymphatic vessels, and nerves. Every myometrial muscle cell, together with its matrix of connective tissue elements, is the functional unit for uterine contractility (Verhoeff and Garfield, 1986).

II.2.B. Special features of the ultrastructure of the functional units of the myometrium

Myometrial cells like other smooth muscle cells are spindle shaped cells 2 to 5 um in diameter and 50-100 um in length, with a single nucleus situated in the widest portion of the cell body (Bergman, 1969). The cellular components responsible for the dynamic contraction are contractile proteins, plasma membrane, sarcoplasmic reticulum (SR) and gap junctions (Verhoeff and Garfield, 1986).

(a) **The contractile apparatus:** The protein components of the cell that respond to calcium (Ca) fluctuations and utilize the chemical energy of ATP to result in either shortening or development of tension are termed collectively the contractile apparatus. In smooth muscle, the major contractile proteins are myosin, actin and tropomyosin. The minor components of the contractile apparatus include proteins that are involved in the Ca dependent regulatory mechanism (Wallenburg, 1983). Electron microscopic studies have identified at least three different types of myo-filaments in uterine smooth muscle cells, namely thick (myosin), thin (actin) and intermediate filaments as well as microtubules (Verhoeff and Garfield, 1986).

(b) Plasma membrane: The plasma membrane (sarcolemma) of uterine smooth muscle cells is a trilaminar structure thought to be composed of phospholipids and proteins. Intra-membranous protein particles are seen in freeze - fracture replicates of the plasma membrane (Garfield, *et al.*, 1978). These particles are more numerous on the protoplasmic than on the external face of the membrane. The volume of a myometrial cell is about 140,000 times less than that of a skeletal myocyte. The small cell size correlates with a large surface to volume ratio. On the surface of the smooth muscle cells many flask-

shaped invaginations of the plasma membrane are open to the exterior and are closely associated internally with mitochondria and the sarcoplasmic reticulum. They contribute substantially to the cell surface area. The relatively enormous area of plasma membrane in conjunction with a small intra-cellular volume tends to facilitate both diffusion of ions and conduction of excitation between the cells. This also favours rapid exchange of materials between the cells and their external environment (Riemer and Roberts, 1986). The myometrial plasma membrane plays an important role in the contraction - relaxation cycle of the cell during uterine contractions as

(i) it acts as the anchoring point for the thick filaments;
(ii) it has a role in ion movement causing depolarization and repolarization of the myometrium;
(iii) it contains receptors for hormone and neurotransmitter binding;
(iv) it contains gap junctions and
(v) it has a key role in Ca mobilization.

(c) The sarcoplasmic reticulum (SR): Uterine smooth muscle cells have an extensive system of SR consisting of a network of tubules and sacs within the cytoplasm whose volume has been estimated to be 2-7 % of the cell volume. The granular reticulum (GR) and agranular reticulum (AR) are continuous and the AR makes close contact with surface vesicles, plasma membrane and gap junctions.

Functionally the GR is probably involved in synthetic processes within the cell while the AR appears to be involved in calcium storage and release and therefore control of muscle contractility (Riemer and Roberts, 1986).

(d) The gap junctions: Gap junctions consist of symmetrical portions of the plasma membrane from two opposing cells (Peracchia, 1980). Intramembranous proteins protrude through the membranes spanning the gap between. Gap junction proteins within opposed cell membranes are thought to align themselves and create channels from the cytoplasm

of one cell to another. These channels are supposed to be the sites of electrical and metabolic coupling between cells by providing for the passage of current and direct exchange of metabolites between cells (Hooper and Subau-Sharpe, 1981). The channels may not always be open and are believed to be dynamic structures. Degradation is thought to occur by either dispersal of gap junction particles from within the plasma membrane or internalization of the entire gap junction within one of the cells by endocytosis and subsequent degradation by lysosomes (Verhoeff and Garfield, 1986).

Garfield *et al*. (1979) studies of the development of gap junctions in the myometrium during parturition demonstrated that:

(i) myometrial gap junctions are absent, or present in low frequency throughout pregnancy;

(ii) at the end of term, gap junction areas increase;

(iii) the junctions are present in high frequency and the size increases during delivery of the foetus and

(iv) the gap junctions begin to disappear within 24 h after delivery.

II.2.C. Myometrial contraction

Myosin, the principal element of muscle contraction, optimises the interaction with the other major contractile protein actin. The enzyme, myosin ATPase facilitates conversion of the chemical energy of ATP into motion/force during contraction. At the molecular level the mechanism of contraction is identical in all muscles and the common link between the contractile regulation of skeletal and smooth muscles is calcium. However, calcium regulation is organized differently in the two types of muscle (Huszar, 1986).

II.2.D. Electrophysiology of myometrial contractility

The uterine muscle cell is bounded by a semipermeable membrane and the distribution of ions on either side of the membrane determines an electrical charge on it. The membrane potential of the resting

myometrial cell (RMP) is normally charged eletro-negatively on the inside so that a potential difference of between 20-81 mv exists across the membrane. In common with other contractile cells, the uterine smooth muscle cell undergoes a cycle of electrical events which involves depolarization of the cell membrane followed by repolarisation, which precedes mechanical activity. This electrical activity is known as the action potential (AP). In skeletal muscle and in nerves, the action potential results from a momentary increase in the permeability of the cell membrane to sodium ions. As a result positively charged ions enter the cell and depolarise the membrane (Finn and Porter, 1975).

Action potentials (APs) can be recorded from the myometrium of an oestrogen-treated non-pregnant animal when a single muscle cell is impaled with a micro-electrode (Marshall, 1962). The activity of the muscle is characterised by alternating phases of APs and quiescent periods, the AP trains coinciding with the contraction phase of the mechanical activity. The action potential is recorded as a signal which is positive in direction and has an amplitude which varies depending upon the resting membrane potential (Kao, 1967) but which lies usually between -30 and -70 mv. The large APs are accompanied by an "overshoot" i.e. the signal becomes positive for a period. The overall duration of the AP is usually of the order of 100ms, i.e. 10-20ms positive sweep (when the signal is moving from the RMP to the AP peak) and 70-80ms negative sweep (in which the signal returns to the RMP). Since the APs in uterine muscle cannot be prevented by ganglionic and nerve blocking agents their generation is believed to be myogenic. Thus uterine smooth muscle is classified as being of the "single - unit" type i.e. muscles which are characterized by spontaneous activity which is initiated in pacemaker areas within the tissue that then spreads throughout the whole muscle. This type of muscle is able to develop active tension as it stretches (Bozler, 1948).

The spontaneous generation of action potentials in most smooth muscle cells results from at least two types of fluctuations of their electrical activity: slow, rhythmic oscillations of the membrane

potential and more rapid, localized depolarization of the membrane. The slow oscillations, variously called "basic electrical rhythm", "pacemaker potential," or simply "slow waves," are especially prominent in the longitudinal muscle layer of the myometrium during parturition. Their duration may be only seconds, or minutes, depending on the particular tissue and its environment. In these muscles the rapid, localized depolarization appears at the crest of the slow wave and generates action potentials that are called pacemaker potentials or prepotentials. The prepotentials determine the action potential frequency, the duration of the burst of action potentials, and hence the force and duration of each contraction. The slow waves set the intervals between contractions and hence the contraction frequency (Marshall, 1980).

It has been suggested that slow waves spread across the longitudinal muscle layer and into the underlying circular muscle layer and thus serve to synchronize spike discharge and contractions over a large area of muscle. In some situations, for example, the mid or non-pregnant uterus prepotentials often arise spontaneously in the absence of well-organized slow waves and generate action potentials that are conducted to neighbouring cells. Under such circumstances, spike discharge is frequently synchronous in various areas of the muscle because of the multiple foci of pacemaker activity. These contractions are irregular in frequency, amplitude and duration (Marshall, 1980).

The electrical activity leads to an increase in intra-cellular Ca and produces Ca release from the ER. When the amount of Ca needed for maximum activation of the contractile proteins exceeds a threshold limit, the myometrial cell starts to contract. This whole process is termed excitation-contraction coupling (Marshall 1980).

After contraction, the electrogenic pump activity increases and the membrane repolarizes bringing the potential below threshold for spike generation. Ca is removed from the cytoplasm by extrusion to the extra-cellular fluid, and by uptake into the ER and rebinding to the binding sites in the plasma membrane. The myometrial cell is then

relaxed and the cycle of contraction phase and quiescent period is completed (Marshall, 1980).

Action potentials and slow waves are conducted throughout the muscle through low-resistance pathways formed by the gap junctions. During the spread of conduction the tension development is dependent on the co-ordinated spread of excitation throughout neighbouring regions.

II.3. Effects of Hormones on Emg Activity

II.3.A. Oestrogen

Oestrogen increases myometrial responsiveness by several known mechanisms:

(a) The increased responsiveness of the myometrium to stretch and increased spontaneous contractile activity characteristic of the oestrogen-dominated uterus probably arises, at least in part, from an oestrogen-induced change in membrane potential. This facilitates spontaneous depolarization of pacemaker cells (Marshall, 1980).

The resting membrane potential of the myometrial cells in immature or ovariectomized animals is about 35 mv and at this potential the membrane is relatively inexcitable, supposedly because the membrane is in a state of a prolonged steady depolarization. This state is intrinsically less excitable presumably because of the high degree of inactivation of Na conductance. A stronger stimulus is required to increase Na conductance sufficiently to produce an action potential. Oestrogen treatment brings the membrane potential into the range where spontaneous discharge of action potentials occurs (about 50 mv) and the muscle becomes rhythmically active (Marshall, 1980). The mechanism by which the RMP is elevated is not clear, although it is possible that oestrogen stimulates the enzymatic apparatus necessary for active ion transport (Porter, 1975).

(b) Oestrogen participates in the formation of gap junctions between adjoining myometrial cells (Garfield *et al.*, 1980). In the rat and guinea pig uterus (Puri and Garfield, 1982), there is a good correlation between gap junction formation and an increase in

circulating oestrogens. During pro-oestrus, the electrical and mechanical activities of the rat uterus *in vivo* are well synchronized, but the synchrony during di-oestrus is much less (Ishikawa and Fuchs, 1978). During pregnancy, irregular synchronous activity prevails in all animal species studied whereas a good synchronization and rapid propagation of contraction waves are observed during the parturient stage (Fuchs and Poblete, 1970).

(c) Oestrogen induces hypertrophy of the myometrial cells and stimulates their synthesis of the contractile proteins, myosin ATPase and ATP, through the induction of specific RNA and protein synthesis. Thus the influence of oestrogen on the myometrium includes membrane, metabolic and structural changes which promote excitation and contraction (Mckerns, 1977).

(d) According to Reynolds (1935), prolonged treatment with high doses of oestrogen renders the uterus immotile. This is contrary to the contemporary belief of the function of oestrogen. He demonstrated that uterine muscle is quiescent when the blood level of oestrogen is very high. Under physiological conditions, uterine quiescence is observed during the pro-oestrus surge of oestrogens in cyclic rats and during the pre-parturient surge of oestrogen in pregnant rats. The withdrawal effect of oestrogen levels in blood is observed in late oestrus and met-oestrus and during the early post-partum period, when strong spontaneous contractions accompany the fall in ovarian oestrogen secretion (Fuchs, 1978).

(e) A further oestrogen effect described is the formation of membrane receptors for certain uterotonic agents. The most important of these are oxytocin receptors (Fuchs *et al.*, 1983) and a-adrenergic receptors (Roberts *et al.*, 1981). Increased receptor numbers lower the threshold for stimulation and increase the response to a given dose of oxytocin by recruiting more units to contract. The effect of oestrogens is selective; thus the receptors for prostaglandin E2 and F2a do not appear to be increased by oestrogens (Wakeling and Wyngarden, 1974) and the number of b-adrenergic receptors is decreased (Roberts *et al.*,

1981). Oestrogens also appear to stimulate prostaglandin synthesis in a rather complex fashion that requires interaction with progesterone (Castracane and Jordan, 1975).

Oestrogens increase the number of specific binding sites for oxytocin in rat (Soloff and Sweet, 1982) and rabbit (Nissenson *et al.*, 1978) myometrium without having any effect on the affinity of the receptor. In the rat the increase in receptor number occurs abruptly several hours before labour in parallel with an increased sensitivity to oxytocin. The increase in sensitivity to oxytocin has been attributed to the increased number of oxytocin-receptors.

(f) High levels of oestrogen correlate with increased uterine nor-epinephrine concentration indicating that the sympathetic nerves are also a specific target tissue for oestrogen action (Marshall, 1981). Oestrogen also has the potential to alter the local concentration of adrenergic agonists at several other levels. It exerts a direct action on catecholamine metabolism in sympathetic neurons by the inhibition of extraneuronal catecholamine uptake (Iverson, 1973). Also, the oxidation products of oestrogen, primarily 2- and 3- hydroxy derivatives (known collectively as catechol-oestrogens due to their structural similarity to catechol), have been demonstrated to inhibit the enzymes tyrosine hydroxylase and catechol methyl transferase (COMT) *in vitro* (Lipsett *et al.*, 1982). Both enzymes are involved in smooth muscle contraction. However the occurrence of this phenomenon *in vivo* and its possible significance to neuronal function in the myometrium has not been demonstrated.

In summary the main influences of oestrogen on myometrial function are:

(i) they increase the capacity of the uterus to contract;

(ii) they promote the synchronization of the contractile units by suppressing locally generated spontaneous activity while increasing the excitability to humoral agents and nerve transmitters; and

(iii) they render the control of myometrial contractions more precise.

II.3.B. Progesterone

Progesterone appears to have little influence on myometrial responses unless the uterus has been exposed to oestrogen. Oestrogen induces the synthesis of myometrial progesterone receptors which are normally low in concentration (Sakamoto *et al.*, 1986).

The mechanism by which progesterone diminishes myometrial excitability is not completely clear, although the following observations have been made:

(a) Progesterone produces an increase in resting membrane potential in the myometrium from about 50 mv to 65 mv (Marshall, 1980). This hyper-polarizing effect would be expected to reduce excitability and the conduction of electrical impulses and impair the propagation of contraction waves along the uterus (Marshall, 1962). The contractions elicited at various parts of the uterus therefore remain synchronous and localized (Fuchs, 1978).

In the rat and rabbit myometrium, the membrane potential of the muscle cells gradually becomes more negative reaching a maximum (about 60 mv) around mid-pregnancy and remaining at this level until the end of term. During this period the uterus may show some spontaneous contractions but these are localized, irregular, and weak. About 24 h before parturition the membrane begins to depolarize and the uterus becomes progressively more active. At parturition the membrane potential is about 50 mv and uterine contractions spread uniformly through the muscle (Casteels and Kuriyama, 196›).

To investigate these observed changes, Casteels and Kuriyama (1965) measured the distribution of ions and their permeability in the myometrium of the rat at various stages of pregnancy. They found no significant differences in the Na, K or Cl concentration gradients during pregnancy. On the other hand the K permeability gradually increased until mid-pregnancy and then remained high until near the end of gestation. Na permeability was relatively low throughout pregnancy but increased several days before delivery. Carsten (1979) demonstrated

that progesterone promoted calcium uptake by the SR and diminished the intra-cellular calcium. The electro-physiological changes in the myometrium that accompany pregnancy might therefore be explained on the basis of these permeability variations.

(b) Progesterone inhibits the formation of gap junctions (Garfield *et al.*, 1980). Changes in the levels of steroid hormones may initiate synthesis of proteins associated with gap junctions. In the myometrium, oestrogen stimulation is required both to achieve full development of gap junctions and to permit progesterone to inhibit their formation. This may be the result of oestrogen stimulating the formation of receptors for progesterone which enter the cell and express an inhibitory effect on protein synthesis. The inhibition of gap junction formation by progesterone is probably the main reason why progesterone withdrawal is essential for the delivery of live young in sheep and many other species (Garfield *et al.*, 1979).

(c) Contractile capacity is maintained under the influence of progesterone. This is indicated by the development of tension in the electrically stimulated uterus of progesterone-treated rabbits and rats (Csapo, 1956). The concentration of high energy phosphate compounds and actomyosin within the myometrial cells also increases throughout pregnancy, reaching a maximum several days before parturition (Csapo, 1969). Thus uterine protein synthesis is maintained under the influence of progesterone and the accumulation of total protein is increased, probably because the synthesis of oestrogen-induced proteolytic enzymes is suppressed by this hormone (Fuchs, 1984).

(d) Progesterone suppresses the action of oestrogens by inhibiting the replenishment of oestrogen receptors. Thus a potentially important effect of progesterone is to modulate the effects of oestrogen by decreasing the concentration of oestrogen receptors and in turn, diminish the response to oestrogen (Riemer and Roberts, 1986).

(e) At least in the uterus of rats and rabbits progesterone inhibits the formation of oxytocin receptors (Nissenson *et al.*, 1978; Fuchs *et al.*, 1983). Progesterone probably exerts this action through

its inhibitory effect on the replenishment of nuclear oestrogen receptors (Fuchs, 1986). There appear to be species differences, however, as progesterone does not inhibit the formation of oxytocin receptors in the human and guinea pig uterus (Alexandrova and Soloff, 1980).

(f) Progesterone increases the formation of b-adrenoceptors (Roberts *et al.*, 1981), and, as with vascular muscle, activation of these receptors leads to relaxation. This is the basis for tocolytic therapy with b - agonists in human pre-term labour.

(g) Prostaglandin F2a and E2 receptors, by contrast, are not inhibited by progesterone and appear to be increased, at least in the rat, hamster and rhesus monkey (Wakeling & Wyngarden, 1974). The finding that progesterone enhances the myometrial responses to PGE1, PGE2 and PGF2a in rats (Fuchs, 1974) corroborates these findings.

(h) Progesterone modifies the oestrogen-induced changes in myometrial sympathetic nerves. It causes a reduction in nor-epinephrine content. Further, high levels of progesterone during pregnancy are associated with an almost complete loss of nor-epinephrine from the uterine sympathetic nerves (Marshall, 1981). Progesterone pellet implantation in one uterine horn of the guinea pig was found to mimic the functional adrenergic denervation changes characteristic of pregnancy (Bell and Malcolm, 1978).

II.3.C. Oxytocin

Oxytocin (OT) is the most potent known endogenous uterotonic agent. The threshold concentration for *in vitro* stimulation of the oestrogen-primed rat uterus is 5 to 30 μ.u/ml (Fitzpatrick and Bently, 1968). The *in vivo* potency of oxytocin is especially striking when compared with other uterotonic agents. PGF2a has about 1/150 to 1/500 the potency of OT on the rat uterus *in vitro* (Moslev *et al.*, 1972) and only about 1/2500 of the potency of OT *in vivo* (Fuchs, 1974). This great potency makes the measurement of circulating OT levels very difficult, and it is only recently that evidence for a physiological

role for oxytocin in the mechanism of parturition has become clear.

(a) The electrophysiological actions of oxytocin on the uterus differ in the reports of different research workers. One view of the electrophysiological action of oxytocin is that it can produce spike discharges only when the myometrial membrane is ready for such stimulation. Marshall (1963) stated that the "prerequisite for the action of oxytocin on the excitable membrane is that the membrane potential be near threshold for the discharge of propagated action potentials". This view is intimately tied in with the progesterone block hypothesis (Csapo, 1956) and it has been suggested that in the presence of a high resting potential, oxytocin cannot elicit a response (Csapo, 1961). In his view, oxytocin is seen as having a double role; thus -

- if the resting potential is high, but near threshold for spike generation, oxytocin depolarizes and causes spike production;
- if the resting potential is low oxytocin causes repolarization and then spike production.

The evidence for these conclusions was obtained from the pregnant rat and rabbit myometrium, which appear to respond in similar ways (Csapo, 1961).

Kleinhaus and Kao (1966) investigated oxytocin effects on the uterus by measuring emg activity and concluded that:

- oxytocin initiates spike production in quiescent preparations;
- increases the frequency of burst discharges;
- increases the number of individual spikes in each burst;
- increases the amplitude of the spikes.

The studies of Marshall (1962) have shown that oxytocin exerts its action on the cell membrane, where the slight depolarization occurs at the same time as the slight reduction in the spike amplitude and increased uterine tone; it is dependent upon adequate extra-cellular Na and Ca.

Kleinhaus and Kao (1969) have suggested that oxytocin acts by

increasing the number of entry 'gates' for Na into the muscle cell. The plasma membrane of uterine smooth muscle contains a calcium-magnesium stimulated ATPase system which is implicated in the transport of calcium from the intra-cellular to the extra-cellular compartment. OT has been shown to inhibit the calcium-magnesium ATPase (Soloff and Sweet 1982).

The mechanism of action of oxytocin may not be limited to the cell membrane alone. Carsten (1974) showed that OT inhibits calcium uptake by the SR, therefore causing higher free calcium levels in the cytoplasm.

(b) The stimulation of uterine contractions during spontaneous labour results from a combined action of OT and prostaglandins. Recent evidence suggests that OT is more important for the initial phase of labour in women whereas prostaglandin F2a seems essential for the progression of labour and cervical dilatation (Fuchs, 1986). Several pieces of evidence support this idea:

(i) A marked rise in OT receptor concentrations occurs at term and maximal values are reached in early labour when plasma PGFM levels do not rise significantly. This rise in OT receptors has been shown to render the uterus responsive to levels of plasma OT which do not differ significantly from those found in pregnant women before the onset of labour. The rise in OT receptor concentration may, therefore, be the event that initiates labour (Fuchs *et al.*, 1982).

(ii) OT may be the stimulus that initiates prostaglandin production in uterine tissues during labour. Support for a receptor-mediated stimulation of PGF2a by OT derives from experiments in sheep in which OT-induced PGF2a release from the endometrium was shown to be proportional to the concentration of OT receptors in this tissue. Oxytocin stimulated prostaglandin production by this apparently receptor-mediated process (Roberts and McCracken, 1976). Furthermore in the pregnant rat uterus, OT was found to increase both prostacyclin and PGF2a release (Chan, 1980).

Several reports indicate that the increase in PGF2a is caused directly by OT action and not by uterine contractions (Chan, 1980; Roberts and McCracken, 1976) .

(iii) Ethanol is the only substance known to block the release of OT from the neurohypophysis *in vivo*, and administration of ethanol will stop early but not advanced labour (Fuchs *et al.*, 1967). In humans the rise in circulating PGFM level is not significant until cervical dilatation is more than 4-5 cm after which a rapid increase is observed (Fuchs *et al.*, 1983).

All these results suggest that throughout active labour PGF2a production is a major factor in myometrial activity whereas oxytocin may be more important in the initial stages of the parturition process.

(c) A rise in the concentration of OT receptors primarily caused by ovarian hormones decreases the threshold for uterine stimulation by OT. Oestrogen increases the number but not the affinity of the OT receptors (Fuchs, 1986). Nissenson *et al.*, (1978) also suggested that the effect of oestrogen involves the synthesis of new OT receptor proteins; progesterone is suggested by the same author to prevent this increase in receptor number. Judging from the decline in OT receptor numbers following parturition or hormone withdrawal, the turnover of receptors is relatively rapid (Fuchs, 1986).

Stretch and distension of the uterine wall induces uterine growth in intact as well as in ovariectomized animals and improves cell to cell communi:ation; it also acts synergistically with oestrogen in promoting myometrial OT receptor concentrations. In the presence of a rather constant endocrine milieu in the days preceding delivery, it is an attractive hypothesis that the rapidly increasing distention of the human uterus contributes to the rise in OT receptor levels at term (Fuchs, 1986).

II.3.D Prostaglandins

The ability of prostaglandins to stimulate the uterus to contract at any time of gestation, and interrupt pregnancy at all stages, has led to a number of studies of their possible role in the physiological activation of the uterus during parturition. A current question is whether PGs are involved in the initiation and maintenance of uterine contractions or whether they are released as a consequence of parturition and participate in other cellular functions during this period.

(a) The effect of PGs on luteolysis has been well defined in the ewe where a close association between increased uterine venous concentrations of PGF2a and decreased progesterone levels were recorded at the end of the luteal phase (Thorburn *et al.*, 1973). This luteolytic effect of PGF2a in ewes has been shown to be mediated by a reduction in total blood flow to the luteal ovary, and a very marked decrease in blood flow to the luteal tissue itself (Nett and Niswender, 1981). In late pregnancy the ewe, unlike the goat and sow, does not depend on the CL as a source of progesterone and a different mechanism for progesterone withdrawal is necessary. Since the placenta is the source of steroids in late pregnancy in the ewe, increased foetal cortisol exerts its effects by acting on the placental enzyme systems to increase the production of oestrogen rather than progesterone; this, in turn,

causes increased prostaglandin synthesis.

(b) The effects of prostaglandins and oxytocin on smooth muscle cell contraction at the molecular level have been summarized by Krall and Korenman (1977). Their theories are based on studies which have tested the effects of myometrial agonists and suppressors on molecular parameters i.e. cAMP and adenyl cyclase activity, protein kinase activity, and Ca++ binding and transport. They state that hormones, and other compounds that inhibit myometrial activity, attach to specific binding sites on receptors with the result that cellular cAMP levels are increased. Increased cAMP activates protein kinase and these enzymes translocate to the membranous components of the cell (e.g. the

sarcolemma, and the sarcoplasmic reticulum) where phosphorylation of specific membrane proteins results in increased uptake and sequestration of Ca++. The free Ca++ inside the cell is therefore decreased resulting in the inactivation of actomyosin adenosine triphosphatase (ATPase) and relaxation of the muscle cell. Activation of the myometrium (e.g. by b-agonists) can reverse these effects so Ca++ is free to enter the smooth muscle cells and elicit a contraction (Haluska, 1985).

(c) Oestrogen increases myometrial PGF2a concentrations (Thorburn *et al.*, 1977). Experiments in sheep (Liggins *et al.*, 1973) revealed that progesterone can prevent glucocorticoid-induced elevation of PGF2a concentration in uterine venous blood but not in the myometrium. This observation led the authors to suggest that progesterone might inhibit the release of PGF2a even though both it and oestrogen induce PGF2a synthesis. Treatment of sheep with diethylstilboesterol increases myometrial PGF2a and administration of progesterone in doses high enough to block labour also blocked the oestrogen-stimulated increase in myometrial PGF2a. Thus, the increase in PGF2a just prior to parturition is probably triggered by a coincident increase in oestrogen.

(d) Fuchs (1978) suggested that foetal or maternal neurohypophyseal hormones (e.g. OT) may provide the stimulus for increasing uterine PGF2a production during labour. Fuchs (1981) demonstrated that OT stimulates PGE2 and PGF2a production in *decidua parietalis* but not in *decidua vera* scraped from the membranes or the myometrium. The stimulation of prostanoid production by OT may be receptor-mediated, since *decidua parietalis* has high and *decidua vera* low OT receptor concentrations (Fuchs *et al.*, 1984). Support for a receptor-mediated stimulation of PGF2a by OT also derives from experiments in sheep, in which OT-induced PGF2a release from the endometrium was shown to be proportional to the concentration of OT receptors in this tissue (Roberts *et al.*, 1976). In the pregnant rat uterus, OT was found to increase both prostacyclin and PGF2a release, and the increase in PGF2a is caused directly by OT action and not by uterine concentrations (Chan, 1980).

II.3.E Relaxin

Relaxin is another hormone which has a profound effect on myometrial activity. It's main function was thought to be the relaxation of the pubic ligaments in preparation for delivery of the foetus at the end of pregnancy (Hisaw, 1926). However, recent evidence has shown that this hormone has other important functions. Relaxin's structural similarity to insulin is manifested in its effects on glycogen storage capabilities (Vasilenko *et al.*, 1980) and uterine growth (Vasilenko *et al.*, 1984). Relaxin not only uncouples excitation - contraction of myometrial SMCs (Schwabe *et al.*, 1978), but also coordinates myometrial activity at parturition (Downing *et al.*, 1980). Finally, relaxin has been shown to increase the distensibility of the uterus (Currie, 1979) presumably by affecting its connective tissue components. The inhibitory effect of relaxin on myometrial activity is produced by the stimulation of cAMP (Judson *et al.*, 1980; Cheah and Sherwood, 1980) and by a decrease of myosin light-chain kinase (MLCK) activity.

II.3.F. Vasoactive intestinal peptide (VIP)

Peptide neurotransmitters, like the VIP, have a powerful relaxing effect on the myometrium (Ottesen *et al.*, 1981) and it has been demonstrated, by immunocytochemical methods, that nerves supplying both the blood vessels and smooth muscles in the uterus contain VIP (Ottesen *et al.*, 1981). The contractility of uterine muscle strips is inhibited by VIP in a concentration-dependent manner.

Closer to *in vivo* conditions, both the electrical and mechanical activity of myometrial explants were inhibited by systemically infused VIP, even if the explants were stimulated by PGF2a or by OT (Ottesen, 1983). This myometrial modulatory response is a part of gestational physiology, because synthesis of VIP is modulated by steroid hormones.

The inhibitory action of VIP on myometrial smooth muscle is not affected by a- and b-adrenergic blockers, by atropine or by blockers of

nerve transmission such as tetrodotoxin (Ottesen, 1983). This suggests that VIP acts directly on the smooth muscle cells. The relaxing effect of VIP is not due to competition with OT or PGF2a, because where there is a large excess of these agents, the contractions are still inhibited. Thus, it is most likely that there are specific VIP receptors on uterine smooth muscle cells (Ottesen, 1983).

II.4. Uterine Motility Studies with Particular Reference to the Ewe

II.4.A Methods of studying uterine motility

Uterine motility has been investigated in a variety of ways. At the cellular level isolated tissues have been used to study ion fluxes across membranes, or the electrical activity of individual cells. At the whole animal level, endocrinological principles have been employed to examine the level of hormones in body fluids and to study the effects of exogenous hormones. In domestic animals numerous techniques have been explored:

(a) Visual inspection of uterine contraction during laparotomy or endoscopy under general or local anaesthesia (Brinsfield and Hawk, 1969);

(b) Intra-uterine or intra-myometrial pressure recording (Gillette and Holm, 1963);

(c) Tension recording of the myometrium using strain gauges fixed on the uterine wall (Bass and Gallantine, 1964);

(d) Cineradiography of the nonpregnant uterus utilising radiopaque materials deposited in the uterine lumen (Fischel *et al.*, 1978);

(e) Extra-cellular electromyography (emg) of the myometrium using various electrodes (Kao, 1959; Csapo *et al.*, 1963; Naaktgeboren and Van Der Weyden, 1973; Ruckebusch and Bueno, 1976).

An emg is a recording of electrical activity from a portion of a muscle (Theodore *et al.*, 1960). This activity can arise from direct electrical or hormonal stimulation or it can occur spontaneously. The potentials from the surface of several neighbouring cells are recorded.

At the same time, inactive cells, depolarized cells and repolarizing cells can make contact with the electrode and they can therefore summate each other's effect or abolish it so the potential of this electrode is the sum of the action potentials of the surrounding muscle fibres. A reliable reflection of muscle activity is obtained. The measured potentials have quite small amplitudes and, in most cases, do not represent the activity of one cell. Thus many of the recorded spikes have several peaks due to the combined activity of neighbouring cells. Larger spikes are recorded when there is good co-ordination of the activity as is found at parturition when gap junctions increase in number (Naaktgeboren and Van Der Weyden, 1973).

The electromyographic technique described by Naaktgeboren *et al.* (1973) has been widely used to register myometrial activity. This depends on the fact that electrical activity in muscle cells is generally closely related to the initiation and propagation of their mechanical muscle activity (Finn and Porter, 1975). The level of synchrony between the electrical activity of the individual myometrial cells influences the characteristics of emgs (Marshall, 1959). The recorded local electrical activity of the myometrium and the local rise and fall of the intra-uterine pressure generally correspond extremely well (Csapo *et al.*, 1963).

It appears very important in studies of uterine motility that recording devices remain outside the uterine lumen. The presence of open-ended or balloon tipped catheters, or other types of sensors in the uterus of the non-pregnant ewe, is likely to distort uterine motility as well as fertility, no matter whether these devices are present for some hours or several months (Hawk, 1970). In addition, the cycle length may be shortened by the presence of intrauterine instruments (Hawk, 1965). More recently, it has been found in post-partum rats, that an intrauterine pressure recording balloon caused new gap junctions (low resistance paths between adjacent cells) to form (Wathes and Porter, 1982). Thus, for accurate recording of normal uterine activity, the omission of any intrauterine monitoring device seems to be an absolute prerequisite.

Sensors such as small pressure transducers implanted in the uterine wall do not appear to interfere with normal uterine activity but in chronic preparations these sensors may migrate into the uterine lumen (Lehrer and Schindler, 1971). This is not a feature of surface electrodes. Normal uterine physiology is unaltered, or at the most only slightly disturbed by surface electrodes. This is demonstrated by the fact that after electrodes had been implanted ewes may become pregnant and deliver healthy offspring after a gestation of normal duration (Van Der Weyden *et al.*, 1981).

II.4.B Uterine motility observations recorded from the uterus of the intact ewe

The uterine activity in the intact cycling ewe has been investigated *in vivo* by several techniques:

(a) Recording myometrial emg activity (Naaktgeboren *et al.*, 1973; Prud'home, 1976; Ruckebusch and Bueno, 1976; Shipilov *et al.*, 1977; Harding *et al.*, 1982; Sigger *et al.*, 1984; Garcia-Villar *et al.*, 1982; Toutain *et al.*, 1985);

(b) Measuring intra-myometrial pressure (Spilman *et al.*, 1972);

(c) Measuring Intra-luminal pressure changes (Mann, 1969); and

(d) Direct observation of uterine contractions (Brinsfield and Hawk, 1969; Croker and Shelton, 1973; Lehrer and Schindler, 1974; Hawk, 1975; Rexroad, 1980).

It is well known that uterine motility varies with the endocrine function of the ovaries (Finn and Porter, 1975), and with seasonal and other environmental factors e.g. confronting the ewe with rams (Van Der Weyden, 1983). The myometrial activity of the ewe varies throughout the entire oestrous cycle. The uterus shows an increased activity during oestrus

as well as a change in the predominant direction of propagation of contractions during this period. However, the results of different studies vary or even totally conflict.

There is a close relationship between the electrical and mechanical activity of the myometrium in the cycling ewe (Garcia-Villar *et al.*, 1982). During the luteal phase of the cycle the ovine uterus is virtually inactive (Naaktgeboren *et al.*, 1973). Lehrer and Schindler (1974) in their studies of uterine contractions using laparoscopy and endoscopy during the natural cycle of ewes did not observe any contractions of the uterine horns during di-oestrus and reported that the horns were in a coiled position; during oestrus the horns uncoiled during contraction. Van Der Weyden *et al.* (1981) were unable to detect any distinct patterns of uterine activity and indicated that there is little if any difference between the electrical activity recorded from the uterus during di-oestrus and that during the early stage of gestation. The fact that the ovine uterus is quiescent at this time may well be important for implantation of the blastocyst which takes place about the 15th day of pregnancy (Boshier, 1969).

Trans-uterine migration has been reported to occur in only 4-10% of ewes with one corpus luteum, whereas a figure of 87.5% was found in ewes with two corpora lutea in one ovary (Casida *et al.*, 1966; Scanlon, 1972; Reimers *et al.*, 1973). In the latter case it was never found that both embryos migrated to the horn contra-lateral to the ovary with the two corpora lutea. Although the ova reach the uterus 3-4 days post oestrus (Edgar and Asdell, 1960), Abenes and Woody (1971) argued that transuterine migration takes place between the 10th and 14th day after oestrus. These facts suggest that uterine motility does not play a major role in the delicate process of transuterine migration in this species.

Uterine quiescence during the luteal phase of the cycle has been attributed to the inhibitory action of progesterone (Lye and Porter, 1978).

The electrical activity of the oviduct has also been reported to be minimal or absent in the di-oestrous ewe (Larks *et al.*, 1971; Ruckebusch and Bueno, 1976). However, unlike the uterine and tubal activity, the electrical and mechanical activity of the cervix persists throughout the oestrous cycle, and for up to five months after ovariectomy (Garcia-Villar *et al.*, 1982). An important finding in these latter experiments was that the cervix displayed its own spontaneous motility, which could not be considered as the transmission of the upper uterine activity to a passive cervix. This was demonstrated by recording emgs from the whole genital tract during the luteal phase and after ovariectomy when the horns are quiescent. The cervix remained active. Its emgs mainly consisted of short spike bursts of low amplitude. This pattern differs markedly from that of the uterine horns which display spike bursts of high amplitude and long duration only during active periods (i.e. oestrus). Furthermore after hysterectomy, the cervix continued to display the same activity as before.

All these observations suggest that hormonal status does not have the same influence on the motility of the different parts of the genital tract, and that the motility of the cervix and horns differ in their requirement for oestrogen stimulation. Moreover endogenous progesterone levels were not sufficient to inhibit the activity of the cervix. Both hormones, however, administered at pharmacological levels, were able to modify the activity of the cervix e.g. exogenous oestradiol benzoate markedly increased the motility of both horns and cervix while large amounts of fluorogestone acetate given by intra-vaginal sponge completely inhibited the motility of the whole tract (Garcia-Villar *et al.*, 1982). This may be one of the reasons why the complete inhibition of normal cervical activity is implicated in the lower fertility observed in ewes after oestrus is synchronized by progesterone (Quinlivan and Robinson, 1969).

During oestrus, the electrical activity of the myometrium is not

only characterized by single, well coordinated and well propagated bursts, but also by the so-called "episodes of activity" which recur at increasing frequency and also became more pronounced. Single bursts between the "episodes of activity" gradually disappear (Van Der Weyden, 1983). To some extent, these episodes were similar to the episodes of emg activity of 5-10 min. duration seen in the ovine uterus after the 4th week of pregnancy (Naaktgeboren *et al.*, 1973). However, in the pregnant uterus the "episodes of activity" usually appeared simultaneously in both horns and have been shown to accompany a slow, tonic increase of intra-uterine and intra-myometrial pressure (Garcia-Villar *et al.*, 1982; Harding *et al.*, 1982). Uterine electrical activity during ovine oestrus appears to be comparable with that described in the oviduct (Larks *et al.*, 1971; Ruckebusch and Bueno, 1976). In the cervix "episodes of activity" seem to be the predominate pattern of electrical activity throughout the entire oestrous cycle. Only at the end of oestrus is the activity of the cervix found to be synchronous with that of the uterine horn.

Van Der Weyden (1983) demonstrated that a good relationship existed between uterine electrical activity around oestrus and peripheral plasma concentrations of both progesterone and oestrogen. Electrical activity recorded approximately one day before the onset of oestrus illustrates that the subsequent increments in activity coincide with decreasing or basal plasma progesterone and increasing oestrogen levels. Oestrogen levels started to decline around the time of the LH surge and was followed, with some delay, by a decline in uterine activity. Therefore, maximal uterine activity, as indicated by maximal amplitudes, burst frequency and the total duration of electrical activity, was invariably found around the LH peak and during some 10 hours after that. At this time, when conduction is enhanced because of gap junction development, a high proportion of bursts clearly involved tubo-cervical or cervico-tubal directed contractions. This occurs during the period of high sexual receptivity in the ewe.

The predominant direction of propagation of contractions during oestrus is still not very clear, and the results of the various studies are conflicting. According to several investigators most contractions are propagated in a cervico-tubal direction during the early part of oestrus, but during late oestrus the majority occur in a tubo-cervical direction (Croker and Shelton, 1973; Hawk, 1975). However, Prud'homme (1976) investigated the uterine activity during the first 15 h of oestrus in sheep, and showed that 60% of the contractions occurred in a tubo-cervical direction.

An interesting observation was made by Ruckebusch and Bueno (1976) when they noted that tubo-cervically directed contractions continued as cervico-tubally directed contractions in the contra-lateral horn. The same phenomenon has also been reported to occur in the post-partum rabbit (Carter *et al.*, 1971) and it has been suggested to exist in the parturient sow (Taverne *et al.*, 1979). Since it has been shown by Garcia-Villar *et al.* (1982) that the ovine cervix has intrinsic spontaneous activity, the cervico-tubally directed activity might well have been triggered by cervical activity. Ruckebusch and Bueno (1976) argued that cervico-tubally directed electrical activity may spread along the oviduct, whilst on the other hand, tubo-cervically directed activity sometimes appeared to originate in the oviduct.

A recent report by Toutain *et al.* (1985) has shown that tubo-cervically directed activity is the most frequent form of uterine contraction throughout oestrus in the ewe. The percentage of descending propagations followed a typical time-dependent development during oestrus, with maximal frequency of contractions at the peak of uterine myo-electrical activity. These results agree with those reported by Van Der Weyden (1983); Prud'home (1976); Garcia-Villar (1982) but they sharply disagree with those of Croker and Shelton (1973), Hawk (1975) and Rexroad (1978; 1980). These latter three groups of authors reported a predominance of contractions that moved towards the oviduct during early oestrus and in the opposite direction during late oestrus.

Their investigations of uterine motility however, were conducted as acute experiments under anaesthesia and it is known that anaesthesia can affect uterine contractility (Friedman, 1965). Furthermore, during these experiments the uterus was spread over the surgical drape, a situation that can hardly be regarded as physiological.

The existence of a pacemaker area in the uterus remains controversial. The view that myometrial cells can act as 'pacemakers' is based on the observation that some action potentials are preceded by a period of gradual depolarization called the pre-potential or slow wave similar to that observed in cardiac pacemaker cells (Marshall, 1959). Kao (1967) suggested that any myometrial cell can, for reasons unknown, become a 'pacemaker' and initiate APs. The 'pacemaker' cells do not form a discrete anatomical structure and may be located singly or in groups throughout the myometrium (Finn and Porter, 1975). Their activity apparently can be induced by stretch (Bozler, 1947). This concept is somewhat tenuous however, since it is based on an analogy with the shape of pacemaker potentials recorded elsewhere (principally in the heart), and on the assumption that APs recorded, which lack a pre-potential, have been triggered by conduction from 'pacemaker' cells. It is difficult to understand how apparently randomly occurring pacemaker activity is responsible for initiating the coordinated contractions which can be recorded from uteri under certain conditions (Finn and Porter, 1975). Although Sigger *et al*. (1984) reported that pacemakers are not restricted to the tips of the uterine horns, Toutain *et al*. (1985) showed that about 75% of APs propagated during oestrus originate at the tubal end of the horns - this therefore may be considered as the most active pacemaker site.

Several possible explanations for the different patterns of motility exist. Hawk (1975) and Rexroad (1978) observed by direct visual inspection that exogenous oestradiol significantly increased the number of contractions moving toward the oviducts. Walsh *et al*. (1979) demonstrated a counter-current exchange mechanism for progesterone

between the utero-ovarian vein and the ipsilateral ovarian artery in non-pregnant and pregnant ewes. This counter-current exchange mechanism may also be applicable to other steroids e.g. oestradiol (Van Der Weyden, 1983). Thus, tissues vascularized by the ovarian artery (i.e. the oviduct and cranial portion of the uterine horn) might be perfused with blood containing increased levels of oestradiol. In this way, the myometrial cells of the cranial part of the uterus might become more excitable than those of the caudal part (Marshall, 1980). Rexroad (1980) on the other hand reported that during oestradiol-induced oestrus in ovariectomized ewes, the anterior part of the uterus contained a higher concentration of PGF2a than the posterior part. Prostaglandins may well be important in stimulating uterine motility during oestrus (Lye and Porter, 1978).

The variation of cervical motility (Garcia-Villar *et al.*, 1982) and the propagation of uterine contractions during the peri-ovulatory period (Toutain *et al.*, 1985) deserve attention in regard to the transport of spermatozoa in the genital tract. In ewes, spermatozoa are deposited into the vagina and have to be carried through the cervix before reaching the utero-tubal junction. The mode of transport remains obscure and large discrepancies exist in the values given for the rate of passage (Mattner and Braden, 1963). Spermatozoa seem to pass the ovine cervix mainly through active sperm-tail flagellation, but uterine and/or cervical activity might also play a role (Lightfoot and Restall, 1971; Hafez, 1973). It has been clearly established that the efficacy of uterine and cervical motility in assisting sperm passage depends on the direction of contractions (Blandau 1973; Fischel *et al.*, 1978). Hawk (1975) reported that at 5h after the start of oestrus, 67% of the uterine concentrations originated at the cervix near the uterine body and moved anteriorly; in contrast, by 48h after the end of oestrus 75% of the contractions originated at the utero-tubal junction and moved toward the cervix. Unfortunately, these results were obtained in anaesthetized ewes during short periods (5-10 min.) of visual inspection; they do not take account of the alteration in the direction

of propagation during the myo-electrical cycle as suggested by Prud'home (1976) who used electromyographic techniques and observed a relationship between the direction of propagation and the frequency of bursts. He reported that during low frequency spiking (irregular spiking activity), the propagation was directed mainly from the utero-tubal junction to the cervix, while during high frequency spiking (regular spiking activity), the propagation was directed mainly from the cervix toward the utero-tubal junction. Garcia-Villar *et al.* (1982), comparing this with their own results, suggested that during oestrus, the uterus and the cervix displayed a synchronized pattern of myo-electrical complexes such that every 40 min. a series of cervico-tubal contractions (lasting for 5-7 min.) pick up spermatozoa from the reservoir at the cervix and move them to the uterus. This could explain the slow but continuing release of spermatozoa into the horns over a period of hours after insemination.

Toutain *et al.*, (1985) argued that, during regular cervical spiking, mechanical activity of the cervix is maximal, closing the cervical ring and impairing flow from the uterus into the vagina. In contrast, during cervical irregular spiking activity, mechanical activity of the cervix is reduced or absent, and cervical compliance greater, so that tubo-cervical uterine propagation would lead to a flow of spermatozoa and uterine secretions through the cervix. Due to the high prevalence of tubo-cervical propagation during oestrus, he considered that one of the main roles of uterine motility during irregular spiking activity is to flush spermatozoa from the uterus into the cervix and vagina. Such an hypothesis is supported by the fact that after intra-uterine insemination, large numbers of motile and immotile spermatozoa enter and traverse the cervix in the caudal direction, and of the total spermatozoa recovered from the tract, about 80% pass caudally to the vagina (Lighfoot and Restall, 1971).

This back and forth motion of sperm cells between the uterus and cervix could be important in reducing the number of spermatozoa reaching the oviducts by progressively selecting the more vigorous of them and

eliminating those that are immotile. When the cervix is closed, not only cervico-tubal propagation, but also opposing contractions may be necessary to achieve sperm transport toward the oviducts. This is because the anatomy of the horn places more uterine fluid at the tubal end of the uterus where it is more ventral than at the cervix. Consequently, tubo-cervical propagation is necessary for the movement of luminal fluid toward the cervical end to achieve its close contact with the spermatozoa that have recently arrived at the internal cervical os. A back and forth motion of luminal fluid within the uterus may be responsible for the actual transport of sperm to the uterus. In this respect the reciprocal propagation (descending in one horn and immediately ascending in the other horn), which is the most frequent pattern of propagation at the end of oestrus, seems well designed to achieve rapid sperm transport towards the oviducts near the time of ovulation.

II.4.C. Uterine motility observations recorded from the uterus of the ovariectomized ewe

Two weeks after ovariectomy , ewes show no spontaneous uterine activity unless they receive steroid treatment (Van Der Weyden, 1983). Rawlings *et al.* (1977), Pant *et al.* (1977), and Karsch *et al.* (1980) treated ovariectomized ewes with a progesterone-oestradiol regimen which aimed to simulate patterns and levels of these hormones measured during the natural cycle; this treatment regimen induced a cycle in which the serum LH and the incidence of oestrous behaviour closely resembled that of the natural cycle . The steroid treatment regimen was started between two and four weeks after ovariectomy.

Van Der Weyden (1983) concurrently measured emg activity in his ewes and demonstrated that the emg activity measured throughout the artificial cycle was within the range of those recorded for the natural cycle i.e. a marked predominance of tubo-cervically directed burst activity during the major part of the artificial oestrus.

Prud'homme (1976) administered oestradiol benzoate to ovariectomized ewes and investigated the uterine electrical activity during the first 15h of the induced oestrus. A predominance of tubo-cervically directed uterine activity was observed in these chronic preparations. It thus seems likely that the predominance of tubo-cervically directed uterine activity seen during oestrus is induced by oestradiol.

Lye and Porter (1978) recorded intra-uterine pressure by means of a water-filled balloon placed in the uterine lumen and demonstrated that progesterone exerts an inhibitory action on uterine activity in the ewe. In their experiments, ovariectomized ewes were treated with oestradiol-17b (50 ug s.c. daily) for three days, then the oestradiol treatment was combined with progesterone (50mg in oil) for 3 days, before the animals were again injected with oestradiol alone. They noted that spontaneous uterine activity declined to very low levels unless oestrogen treatment was given from the day surgery was performed to introduce the recorded device. Under oestrogen treatment, spontaneous activity as shown by pressure changes occurred regularly with a frequency of approximately 20 cycles /10 min and a maximum amplitude of 20 to 50 mm Hg.

In the experiments conducted by Lye and Porter (1978) oxytocin (500mu iv) and prostaglandin F2a (10 ug/min into the uterine lumen) markedly stimulated uterine activity and particularly the frequency of the pressure cycles. When progesterone was administered, the effect was quite profound. Intra-uterine pressure cycles declined steadily in both frequency and amplitude with the inhibition being maximal at 72h. Furthermore, the progesterone dominated uterus was virtually unresponsive to oxytocin and PGF2a. Another group of oestrogen-treated ewes were given one injection of a high dose (100mg) of progesterone in oil; this caused a steady decline in the amplitude and frequency of contraction which reached a minimum at 48 h and was maintained for a further 36 h. Recovery was complete by 115 h. They concluded that progesterone has a marked , reversible , inhibitory action upon myometrial activity in the oestrogen-treated ovariectomized ewe, a finding that is consistent with the earlier reports of Csapo (1956).

Lye and Porter (1978) also consider that the nature of the inhibition is similar to that observed in the progesterone-treated rabbit thus confirming the 'classical progesterone block' theory described by Csapo (1956).

Van Der Weyden (1983) showed that the first progesterone administration after artificially induced oestrus invariably resulted in total inhibition of uterine electrical activity within 12h. This was markedly shorter than the lag-time of 48h. or more reported by Lye and Porter (1978) but could be due to the completely different administration schedules of the steroids in the two sets of experiments. In Van Der Weyden's animals, the oestradiol benzoate injection always resulted in increased uterine electrical activity within some 10h after injection. An alternation of quiescent and active periods during the artificial oestrous periods in ewes, as in the work of Lye *et al.*, (1983), was never observed.

Lye *et al.*, (1983) recorded continuous series of low-amplitude, low-frequency intra-uterine pressure cycles in their ewes. However, subcutaneous administration of oestradiol at doses of 25 or 50 ug oestradiol-17b, or 50 ug oestradiol benzoate, resulted in uterine quiescence for several hours (5-7h) followed by a prolonged period of increased activity with intermittent quiescent periods. These periods of quiescence were related to the time of hormone administration, and the alternating pattern could be sustained for several months if the ewes were treated with oestradiol daily. It is worth noting that Continho and De Mattos (1968) have described inhibitory effects of oestrogen on the uterus in rabbits and Downing *et al.* (1978) have published similar findings in rats. In all of these reports the response to oxytocin appears to remain even though the spontaneous activity is abolished or markedly reduced by the oestrogen. Myometrial activity also appears to exhibit an alternation of activity and quiescence in the intact pig during the period of rising oestrogen levels in early oestrus (M.A.M. Taverne, personal communication cited by Lye *et al.*, 1983).

The mechanism by which oestradiol exerts its biphasic action is unknown. It is possible to postulate mechanisms to account separately

for the inhibition (e.g. synthesis of an inhibitory substance in response to oestrogen), and for the periods of enhanced activity (e.g. prostaglandin secretion, or increased populations of oxytocin receptors- Alexandrova and Soloff, 1980), but it is very difficult to postulate a mechanism which would account for the alternation of these two states. Lye and Chillis, (1982) in reporting their experiments in which oestrogen-treated ewes were infused with the PG synthetase inhibitor meclofenanic acid, suggested that this oestrogen-induced pattern of activity was prostaglandin-dependent since both the high-activity and quiescent periods were abolished by this drug. The stimulatory effects of prostaglandins in sheep are well known (Lye and Porter, 1978), and Lye and Challis (1982) have shown that prostacylin (PGI-2) can inhibit spontaneous uterine activity in sheep . Other possible mechanisms could take account of catecholamines (Downing and Porter, 1980).

The suggestion has been made (Porter, 1979) that oestradiol-induced inhibition of myometrial activity may be mediated by relaxin synthesized in the uterus . There is little evidence for or against the existence of such a mechanism in the ewe although relaxin inhibits the spontaneous activity of the ovine myometrium *in vivo* (Porter , 1979) yet leaves the response to oxytocin unimpaired. Further, uterine synthesis of relaxin has been reported for the guinea-pig (Pardo and Larkin, 1982).

Windmoller *et al.* (1983) found that in some animals uterine responsiveness to exogenous oxytocin increased within 5-6h of starting oestrogen treatment. This finding is consistent with other observations in which 6h oestradiol treatment was required to induce oxytocin-stimulated PGF2a release from the ovine uterus (McCracken *et al.*, 1981). Oestradiol increases PGF2a synthesis in the ovine uterus (Horton and Poyser, 1976). These workers also administered MFA (meclofenamic acid) to examine whether the increase in oxytocin response might result from an increase in prostaglandin biosynthetic capacity. The failure of MFA to influence the effect of exogenous oxytocin suggests that prostaglandin production is not a critical factor in the enhanced oxytocin response (Thorburn and Challis, 1979). The uterine response to

PGF2a also rose in some animals within 5-6h after the start of oestrogen administration, and was maximal by 30h .It is possible that oestrogen enhances PGF2a responsiveness by increasing the PG receptor population (Windmoller *et al.*, 1983).

Porter and Lye (1983) demonstrated that the progesterone 'block' of the ovine myometrium can be partly but significantly overcome by oestradiol-17b .Thus in ewes receiving intra-luminal injections of 250 or 500 ug oestradiol-17b there was a significant recovery of spontaneous intra-uterine pressure cycles as well as an increase in the resting pressure, the frequency, and the mean maximum amplitude of the responses to oxytocin (500 mu iv) by 24h, and in many cases by 12h after treatment compared with control ewes treated with the vehicle only. This recovery was observed despite the continued administration of progesterone (50 mg daily s.c.). The changes in myometrial activity occurred despite the fact that the plasma progesterone levels did not alter significantly throughout the experiments. Accordingly it was argued that the changes in myometrial behaviour following the administration of oestradiol-17b cannot be attributed to a withdrawal of progesterone. A similar increase in myometrial activity and responsiveness without a fall in plasma progesterone levels was reported in pregnant ewes given stilboestrol (Liggins *et al.*, 1972). Other workers have reported a stimulatory action of stilboestrol during late pregnancy in ewes (Cahill *et al.*, 1976). However, because of the complex nature of the hormonal environment in late pregnancy, it is not certain from these reports that oestradiol acted directly on the myometrium. None the less Porter and Lye's (1983) results do support such an action.

How oestradiol functions to antagonize the action of progesterone is uncertain. The claim by Liggins *et al.* (1972) that the myometrial threshold for oxytocin in pregnant ewes was significantly reduced by the intra-aortic infusion of PGF2a suggests that oestradiol may exert its anti-progesterone effects by causing the synthesis and release of this substance. Porter and Lye (1983) could not demonstrate a significant change in oxytocin responsiveness when their progesterone-treated ewes were infused with PGF2a for up to 8h. The results of partial reversal of

the myometrial progesterone 'block' in the non-pregnant ewe *in vivo* by oestradiol (a hormone that is markedly elevated in the plasma pre-partum) suggests this participates in the initiation of normal parturition by accelerating the recovery of the uterus from the effects of progesterone. The finding by Rawlings *et al.* (1978) that ewes passively immunized against oestrogen were still able to deliver normally , does not conclusively eliminate the participation of oestrogen in normal ovine parturition because, as the authors themselves point out,a locally operating mechanism involving oestrogen would not have been excluded.

Porter (1979) reported that myometrial activity was abolished abruptly but reversibly in 4 out of 5 ewes by the intravenous injection of porcine relaxin. Recovery began only after about 90 min. and was not complete until 3-4h after the injection. During the relaxin-induced inhibition the myometrium responded to oxytocin administered intravenously in doses of 250 mu. One ewe in this study received intra-uterine infusions of 2.5 and 5.0 ug PGF2a per min. during the period of relaxin inhibition ; the former dose evoked a slight and the latter a marked response from the myometrium. The rate of rise of intra-uterine pressure and the mean amplitude of pressure cycles were significantly decreased at 1, 1.5 and 2h after the relaxin injection.

Oestradiol was reported by Lye *et al.* (1983) to inhibit spontaneous uterine activity in ewes. The oxytocin and PGF2a responses however, were preserved, and this revealed a close similarity between oestrogen and relaxin inhibition (Porter, 1979). The suggestion was made that the action of oestrogen may be mediated through the secretion of relaxin. However , the physiological role for relaxin in ewe myometrial activity is still not clear. Porter (1979) suggested that a possible role for relaxin near term, is that it permits a co-ordinated evolution of myometrial activity following the decline in progesterone and the dramatic rise in oestradiol levels. This enables the conductivity and oxytocin sensitivity of the uterus to increase to a peak that is synchronized with the release of a centrally originating signal capable of overriding relaxin inhibition thus culminating in labour (Porter,

1979). This hypothesis concerning the function of relaxin is supported by the observation of Toutain *et al.* (1983) of a distinct inhibitory phase of the gravid horn starting 43.3 ± 11.27 hours before parturition which is characterized by a dramatic decrease in the duration of emg bursts. After the inhibitory period, which lasted about 10 hours, both the gravid and the non-gravid horns showed regular bursts of a duration which progressively increased and led to almost continuous electrical and mechanical activity (Lye *et al.*, 1983).

III. GENERAL MATERIALS AND METHODS

III.1. Animals

The experiments were carried out on 6 pluriparous Romney ewes between September 1987 and February 1988 when they were in their anoestrous season. Healthy nonpregnant ewes of about 50 kg body weight, were selected. The ewes were housed individually indoors in pens about 2.0mx2.0m that allowed them to move freely during recording sessions. Hay and water were made available *ad libitum* and supplemented with fresh grass and nuts. The pens were exposed to natural light and ambient temperature. At night artificial light was only on when hormone injections were given. Experiments began about 10 days after surgical implantation of the electrodes.

III.2. Electrodes

Electrodes were made from 1 metre lengths of Teflon coated multistrand stainless steel wire (Cooner Wire AS 633, Cooner Wire Company, Chatsworth Callfornia). A knot was tied 150 mm from one end and the insulation stripped from a 2mm length immediately distal to the knot. The shaft of a 26g hypodermic needle was crimped onto the nearby free end to assist during fixation of the electrade to the uterus.

III.3. Surgery and anaesthesia

After 24h without food, the ewes were premedicated by intramuscular administration of acetyl promazine (0.25mg/Kg ACP C-VET Limited, Bury St.Edmunds, UK) and anaesthesia was induced by administration of thiopentone sodium (12mg/kg) into a jugular vein. An endotracheal tube was inserted and anaesthesia was maintained by inhalation of halothane and oxygen using a semi-closed circle system. The wool was clipped from a generous area near the surgical site and the skin-prepared with alcohol 70% and hibitane tincture.
The area was draped and an abdominal incision was made obliquely in the groin to allow access to the uterine horns. These were gently

exteriorized . In four of six animals the ovaries were removed. Electrodes were implanted in groups of three usually in a triangular arrangement with about 5mm separating each member of a group. These groups of electrodes were located at preselected sites (Fig. 1) on the surface of the uterus. The hypodermic needles with attached wire were passed into the myometrium and tied so that the section stripped of insulation was embedded in the muscle. The surplus wire with its attached needle was trimmed. The electrode wires were led through a stab wound on the flank, passed subcutaneously and exteriorized just below the transverse processes of the lumbar vertebrae. The abdominal incisions were closed with Dexon (3 metric) and the skin with metal clips.

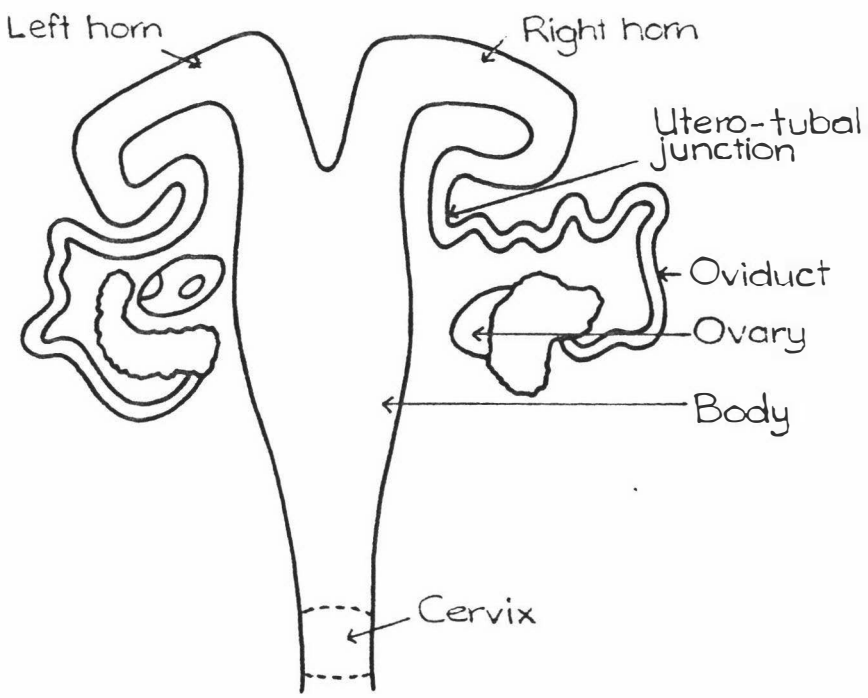
Minor modifications to the surgical approach were made as experience was gained in exteriorization of the uterus and to facilitate placement of the electrodes.

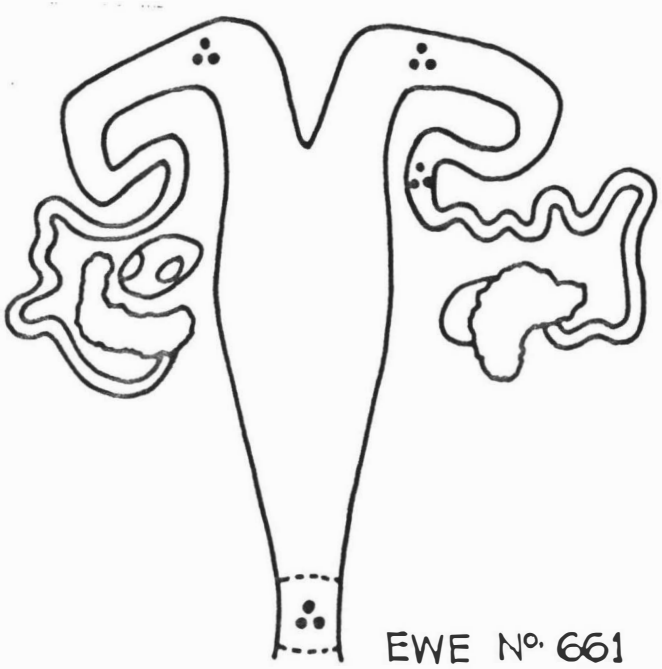
In the four ovariectomized ewes an indwelling silastic catheter was inserted into the external jugular vein, to enable injection of oxytocin (50ml x 10iu/ml, Ethical Agents Ltd. 16 Garfield St. Parnell, Auckland) without disturbing the animal. The catheter was flushed at least once daily with a sterile heparinized physiological saline solution.

Antibiotic (Streptopen, Glaxo Animal Health (N.Z.) Limited, 1025 Tremaine Avenue, Palmerston North, N.Z.) was administered for five days post-operatively and the skin clips were removed after seven days.

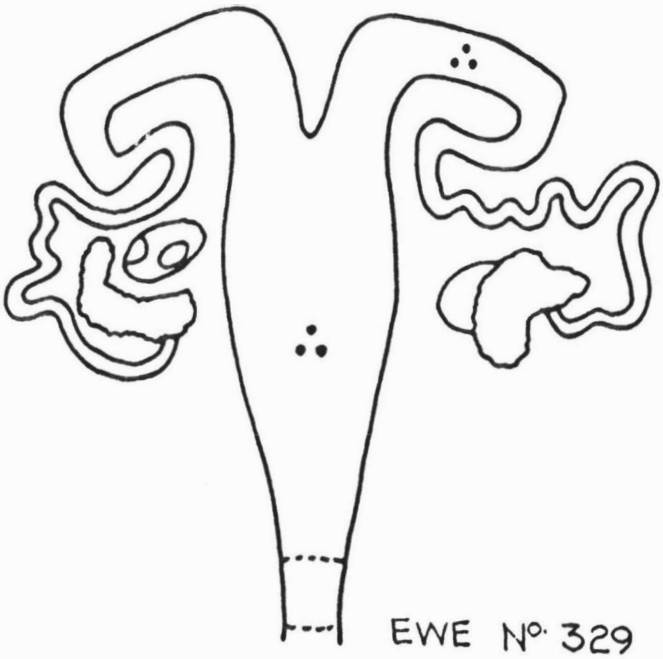
Fig. 1: **The positions of electrodes sutured at preselected sites on the uterus among 6 ewes.**

(the sites selected for individual ewes are shown by dots in the following diagrams to this figure on pages 51, 52 and 53).

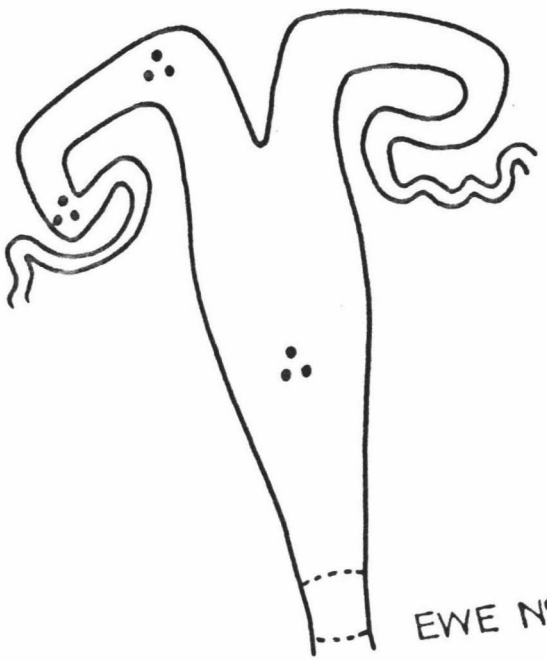




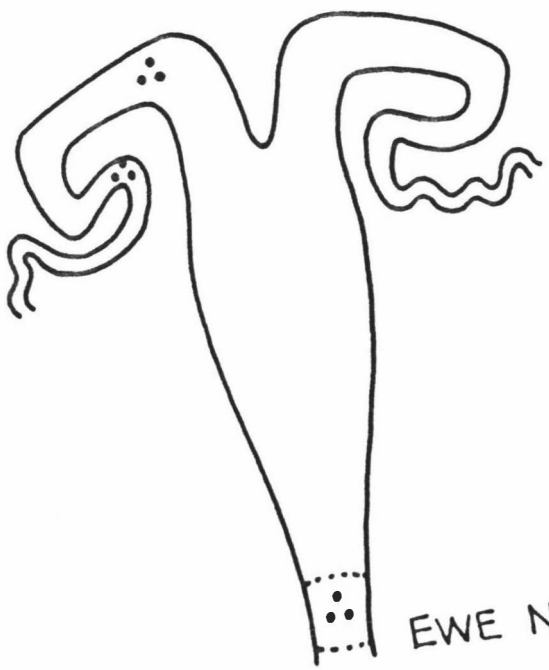
EWE Nº. 661



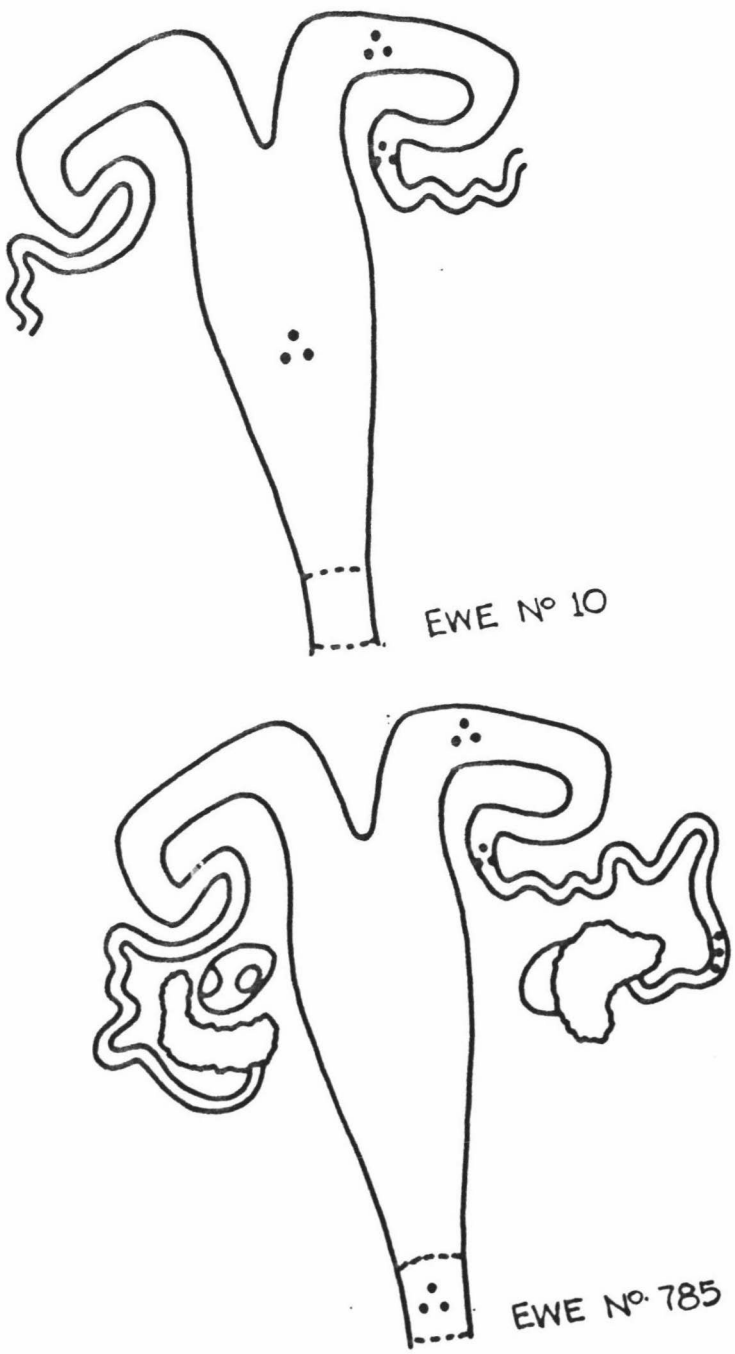
EWE Nº. 329



EWE No. 476



EWE No. 248


EWE No 10
EWE No. 785

III.4. Recordings

For recordings the wires were soldered to an edge connector, which was held against the body of the ewe by a body stocking (Presnet Medic Corp NZ). The shielded extension leads from the edge connector were plugged into the input of a universal AC amplifier (Tektronix, Inc., Portland, Oregon, U.S.A.) and the output was recorded on a four channel ink writing chart recorder (Gould Inc., Instruments Division, Cleveland, Ohio, U.S.A.).

III.5. The Electromyograms

The following terms have been used to described the emg data:

(i) spikes: short lasting (200msec) fluctuations in potential. As the electrodes are in contact with many muscle cells, these spikes represent the activity of a discrete population of the cells

(ii) bursts: periods of continuous spiking activity usually of about 5-25 sec duration. There are no periods of rest between spikes

(iii) episodes of activity: these represent prolonged periods (up to 10 min) of electrical activity consisting of grouped bursts with short periods of rest (<15sec) in between.

(iv) co-ordinated or uncoordinated electrical activity: The activity was considered to be co-ordinated when the emgs showed bursts with intermittent periods of rest at relatively regular intervals. This kind of electrical activity enables the number of bursts per unit time to be counted. Unco-ordinated activity is characterized by single and grouped spikes and/or bursts which are not well separated by definite periods of quiescence.

(v) propagation of electrical activity and propagation time: The interval (sec) before the appearance of electrical activity at the neighbouring electrode groups implanted along the uterine horn. Propagation can be in a tubo-cervical direction or in a cervico-tubal direction. Sometimes the activity does not first appear at the electrodes near the tubal or the cervical end ; also the activity may simultaneously appear at various positions on the uterus. In these cases

a mixed pattern of propagation can be seen in one horn. Sometimes the electrical activity is propagated along the entire horn.

(vi) amplitude: The height of the spikes, expressed in microvolts.

The various patterns of emgs that were recorded are shown in Figs 2-5.

Fig. 2: **Active and quiescent periods of emg during anoestrus**

(The record is from sheep number 329 showing spontaneous myometrial electrical activity from the uterine body (upper) and horn (lower) during anoestrus. This record was made at a paper speed of 0.25mm/sec. The vertical bar at the beginning of the trace represents a calibration of 200uv).

(i) The active periods lasted 4-5 mins. and the quiescent periods about 16-17 mins.

(ii) The propagation of the electrical activity along the uterus was from body to horn, i.e. cervico-tubally.

Bar a: the episodes consisted of regular co-ordinated bursts.

Bar b: the intervals between episodes contained several unco-ordinated bursts or spikes.

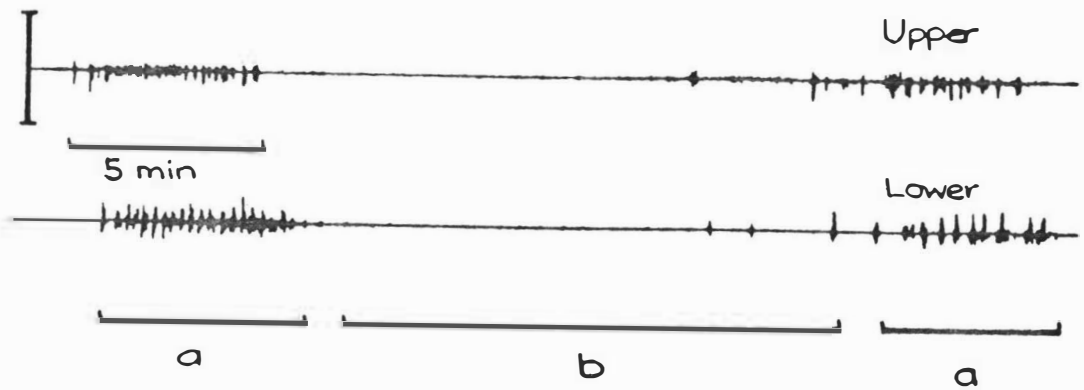


Fig. 3: An example of the method of measuring the duration of bursts, the interval between bursts, the burst frequency, spike frequency and amplitude of the spike.

(The record is from ewe 329, and shows the pattern of emg induced by oestrogen and PGF2a during the period of anoestrus. Two paper speeds were = 1mm and 5mm/sec. Calibrations: Both horizontal bars represent 10sec. The upper vertical bar represents 2500uv).

(i) a1: beginning of a burst.
a2: end of a burst.
a1 to a2: duration of a burst.
a2 to a3: interval between bursts.
a1 to a4: The total time for calculating frequency of the bursts. On this record the time was 180sec. and there were 9 bursts. That is 3 bursts per min.

ii) The number of action potentials (spikes) in a burst were counted and divided by the duration of the burst to obtain the action potential frequency.

A. shows the maximal amplitude (peak to peak) which is of a spike in a burst. In this example the measurement for the lower trace is 1750uv.

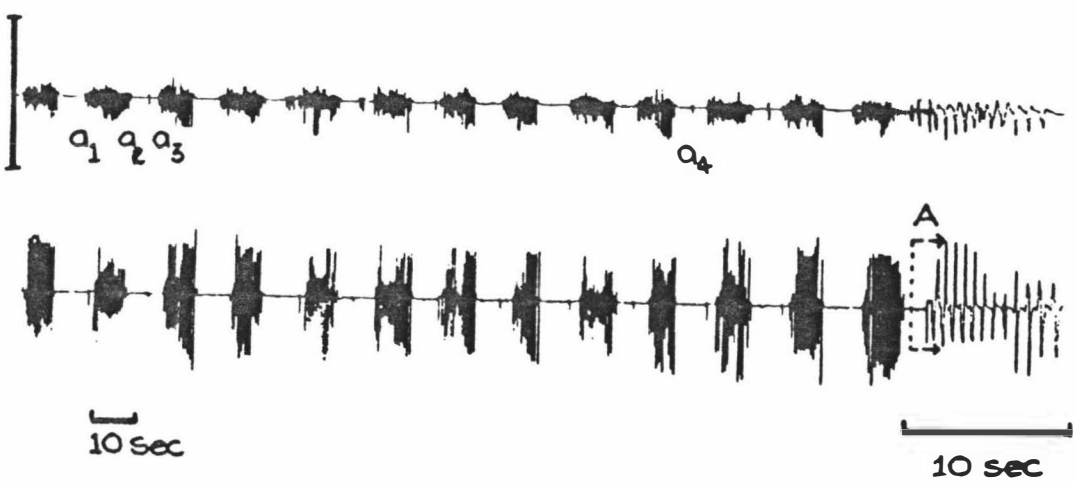


Fig.4 (a & b) An example showing the propagative direction of bursts and episodes (see pp 58a & 58b).

(These records are from ovariectomized ewe 785. Both slow (0.05mm per second) and fast (0.25mm per second) paper speed records of emg were recorded on the fourth day of oestrogen treatment (50ug s.c. daily). A = oviduct; B = utero-tubal junction; C = uterine horn; D = cervix.

4 a: The horizontal bars represent episodes of emg activity. Both bar 1 and bar 2 in four positions are synchronized in emg activity.

(Calibration: The vertical bar at the beginning of the trace represents 400uv).

i) The amplitude in trace C (horn) is much higher than that recorded from the other positions of the electrodes.

ii) Bursts frequencies in trace B (tubal end) are higher than that of the other positions.

4 b: a: Burst a1-a9 are propagated tubo-cervically, b is cervico-tubal in direction along the uterus.

(Calibration: The vertical bar at the beginning of the trace represents 400uv).

(i) The amplitude in trace C (horn) is much higher than that of the other three positions.

(ii) Bursts frequencies in trace B (tubal end) are higher than that of the other positions.

(iii) most bursts in trace A (oviduct) are propagated from tubal end of uterus to the oviduct.

Fig. 4a

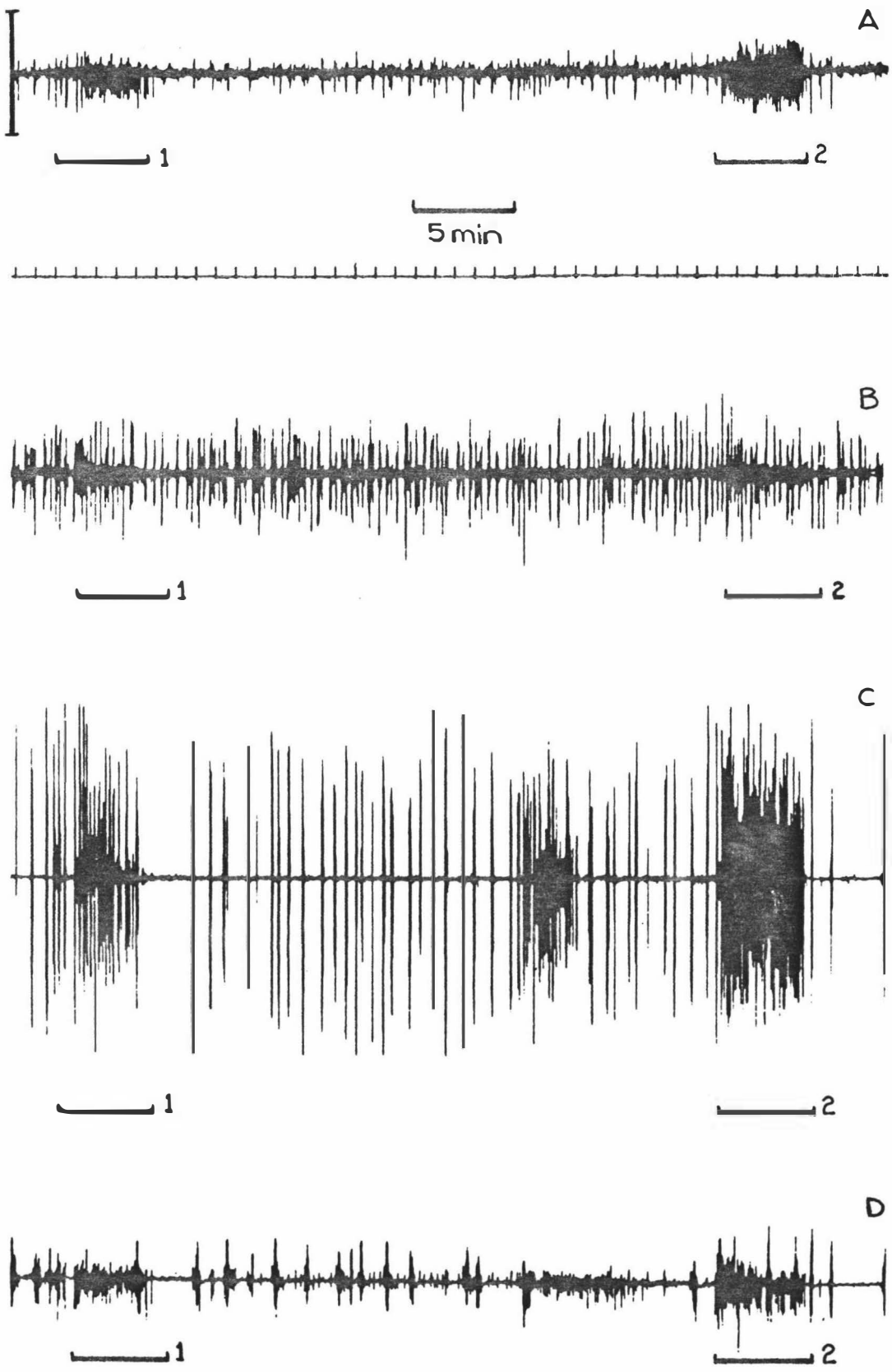


Fig. 4b

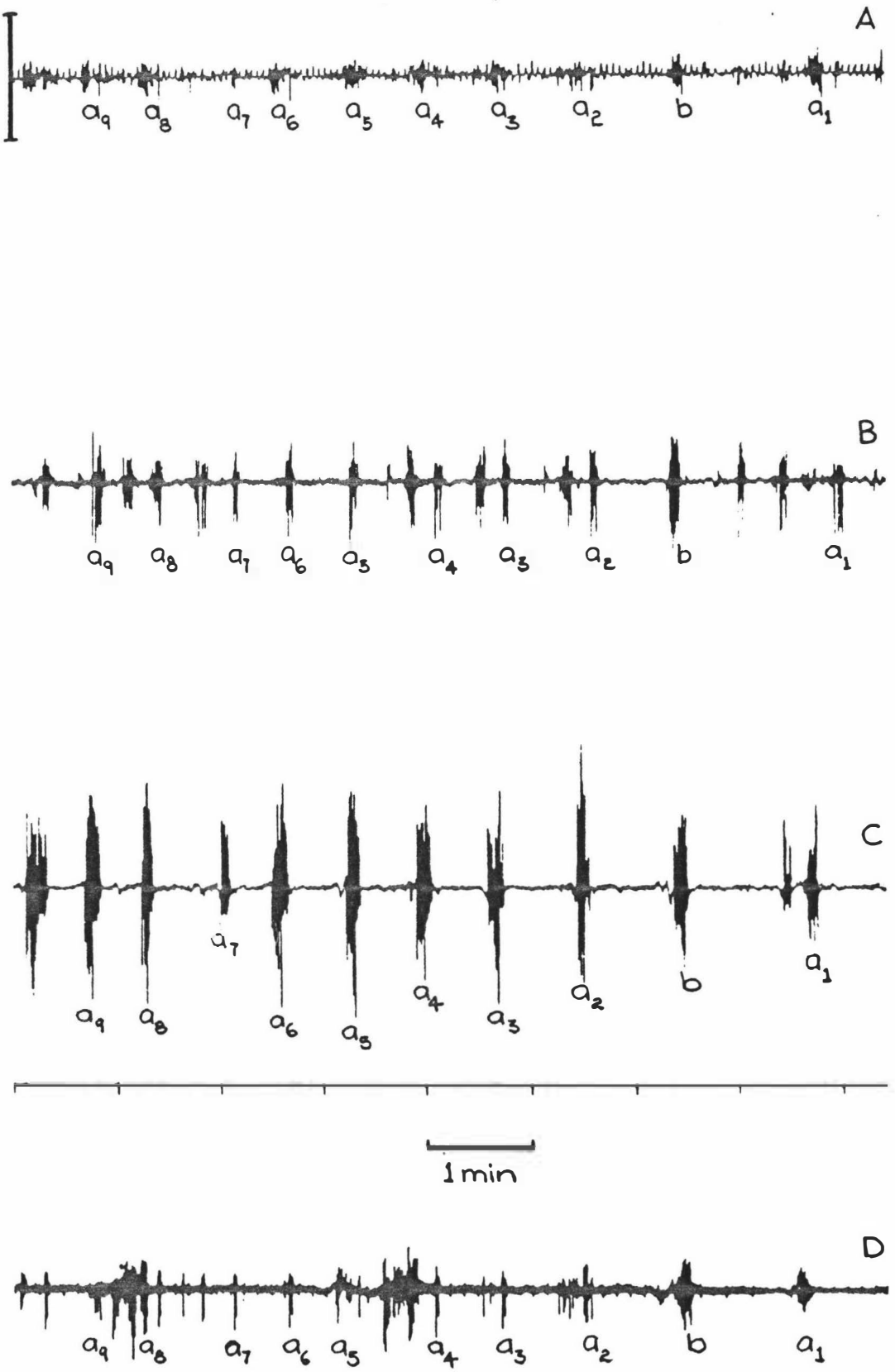

A
a9 a8 a7 a6 a5 a4 a3 a2 b a1
B
a9 a8 a7 a6 a5 a4 a3 a2 b a1
C
a9 a8 a7 a6 a5 a4 a3 a2 b a1
1 min
D
a9 a8 a7 a6 a5 a4 a3 a2 b a1

Fig. 5: An example of co-ordinated and uncoordinated patterns of electrical activity (see p 59a).

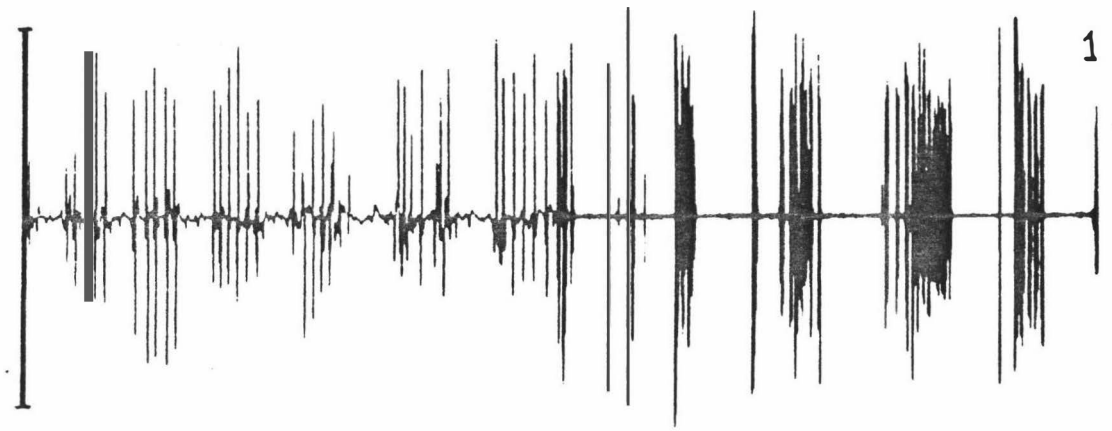
(These records are from ovariectomized ewe 10 showing progesterone inhibition of emg activity which was initially stimulated by oestrogen. Two paper speeds were used: 0.05mm per second and 1mm per second. This recording is from the right horn of the uterus.

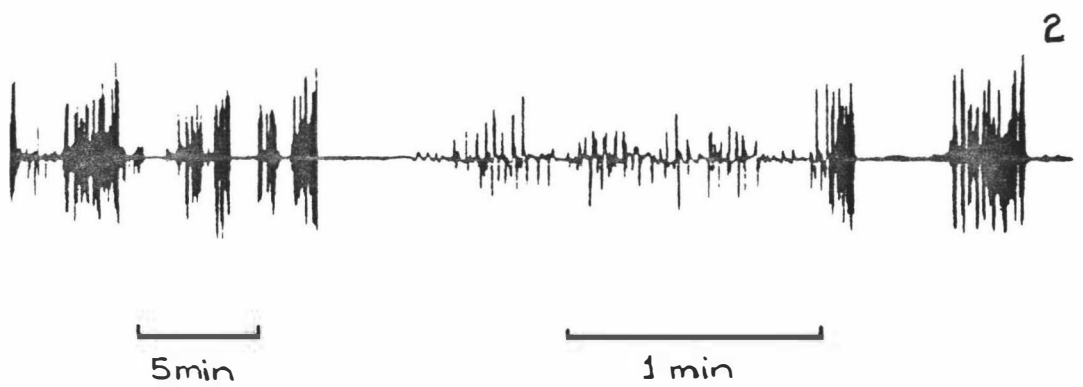
(Calibration: The vertical bar is 1000uv for all three traces.)

(1) Upper trace: The oestrogen induced emg activity before injection of progesterone: note the electrical activity is well coordinated.

(2) Middle trace: 6 h after first injection of progesterone (50mg s.c) with oestrogen injection (50ug/d s.c.) continuing. Note the electrical activity is still coordinated and spike amplitudes reduced.

(3) Lower trace: 52 h after starting progesterone injection (50mg s.c./d) with oestrogen injection (50ug s.c. daily) con tinuing: note the electrical activity is now uncoordinated and the spike amplitudes reduced.


1


2
5min
1 min


3

III.6. Quantitative analysis of the Electromyograms

A motility index (MI) was derived as a method of measuring electrical activity of the uterus over prolonged periods of time. The formula was

$$MI = \frac{Ns \times Ts \times As}{3600 \times 1000} \times 100$$

where

MI = motility index

Ns = the numbers of spikes in one hour (measured by counting the numbers of spikes over 1 minute and multiplying x 60)

Ts = 0.2 second (200 msecs), (a constant time for each individual spike - see literature review)

As = the average amplitude of all spikes (derived by measuring an example of spikes in uv during the time period under consideration, the measurement being made on traces at high paper speeds).

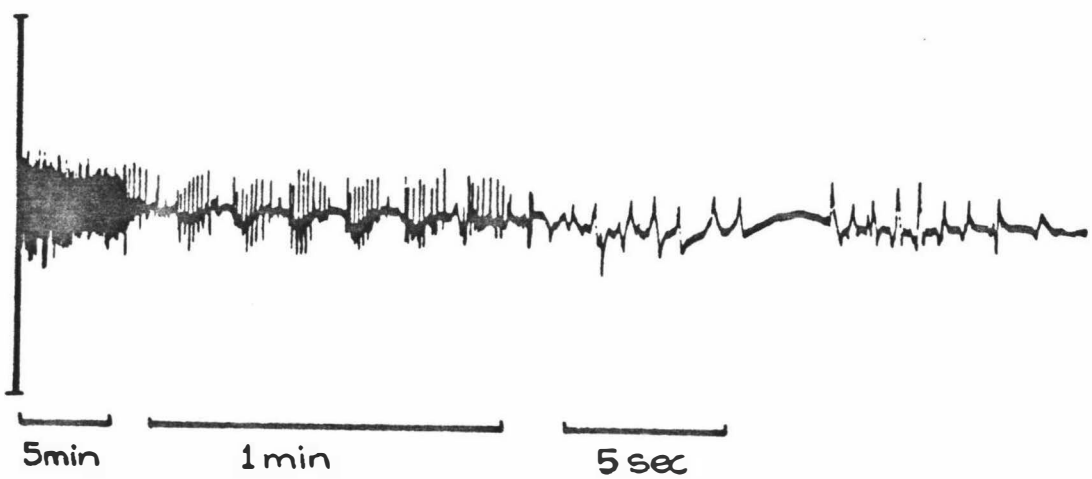
The product of Ns x Ts x As was then represented as a percentage of the total amount of possible activity over a one hour period (3600 records) if a unit of amplitude of 100 uv was taken as the constant.

Fig. 6: An example of calculating the motility index (Ewe 329)
The emg shows the effects of oxytocin during the stages of recovery from progesterone inhibition.

(Calibration: The vertical bar is 2500 uv, the horizontal bars represent the paper speed as shown in the figure.)
There are about 9 spikes/burst and 6 bursts/minute = 3240 spikes/hour = Ns. Ts = 0.2sec. As is estimated to be 500uv

$$\text{Thus MI} = \frac{3240 \times 0.2 \times 500}{3600 \times 1000} \times 100 = 7.33\ \%$$

The character of this particular emg pattern can be described as one of high frequency, high amplitude and co-odinated emg activity. The MI = 7.33%.



IV. RESULTS

IV.1 Myometrial Emg Activity in the Intact Ewe during Anoestrus

The following results were obtained from ewe 329 during the period from September to November in 1987.

IV.1.A. The resting Emg activity of the uterus (Fig. 2)

The electrical activity was organized into myoelectrical complexes that were propagated from the body of the uterus to the horn. A regular basal emg pattern was observed which comprised 2-3 episodes of regular high frequency bursts per hour. The duration of each episode was 4-5 mins. and the interval between successive episodes was about 16-17 min. Every episode contained 15-20 co-ordinated bursts each consisting of 5-7 spikes. During the interval between the high frequency episodes there were irregular spikes or uncoordinated bursts.

IV.1.B. The effect of exogenous oxytocin on the resting emg activity of the uterus during anoestrus (Fig. 7).

The sensitivity of the uterus to oxytocin persisted during anoestrus. Intramuscular injection of oxytocin (20 i.u.) was followed within 30 mins. by increased emg activity which lasted up to 5 hours. During this time there was a loss of the regular complexes of activity but by 7h post injection, the normal anoestrus pattern of complexes had returned.

IV.1.C. The effect of exogenous oestrogen on the resting emg activity of the uterus during anoestrus (Fig. 8).

Administration of oestradiol benzoate 2mg i.m. (5mg/ml, Intervet, International B.V. Boxmeer-Holland) reduced the regular resting emg activity of the uterus after a latency of about 2h. Individual bursts then disappeared and the emg became quiescent for about 3h before activity resumed and gradually increased in both amplitude and frequency. Fifteen hours after oestrogen administration the uterus had reached its highest level of emg activity, and emg complexes consisting of alternating phases of co-ordinated activity and intervals of inactivity typical of the untreated anoestrous ewe resumed.

IV.1.D. The effect of exogenous oxytocin on oestrogen induced emg activity during anoestrus (Fig. 9).

Fig. 7: **Oxytocin effect on spontaneous emg activity during anoestrus**

(This record is from ewe No 329. The paper speed was made at 0.05mm per sec. Upper trace = uterine body, lower trace = uterine horn).

A. Record obtained before the administration of oxytocin 20iu. i.m.

B. During the action of oxytocin (30min after oxytocin injection).

C. Emg recorded 7h after oxytocin injection.

Calibrations: The vertical bar represents 300μv for all three records.

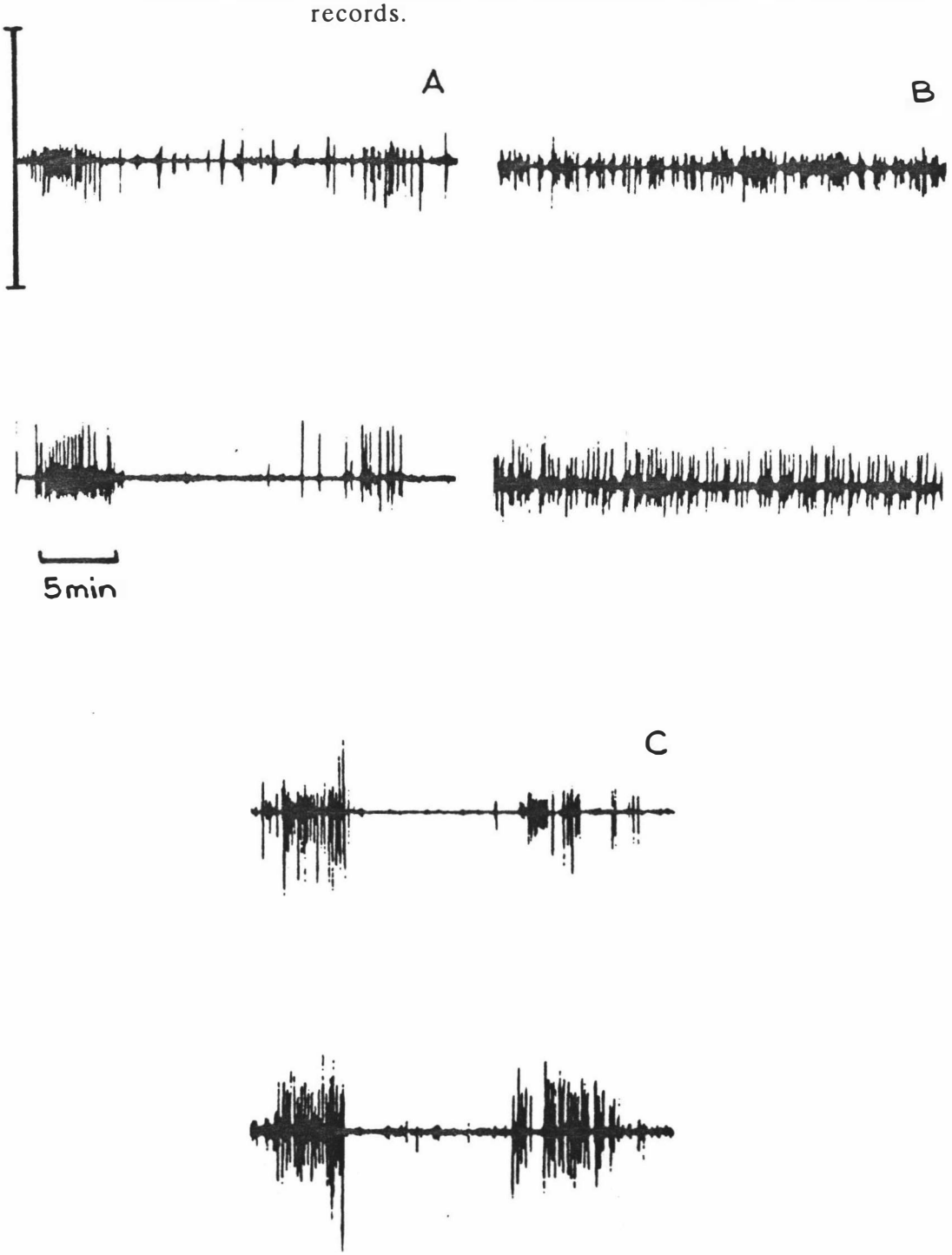


Fig. 8: **Oestradiol benzoate both inhibits and stimulates myometrial emg activity in ewe 329 during anoestrus:**

A. 1hr. after administration of oestradiol benzoate.

B. 4hrs. after administration of oestradiol benzoate.

C. 15hrs. after administration of oestradiol benzoate.

Calibrations: Vertical bar for A and B = 500μv, for C = 1000μv.

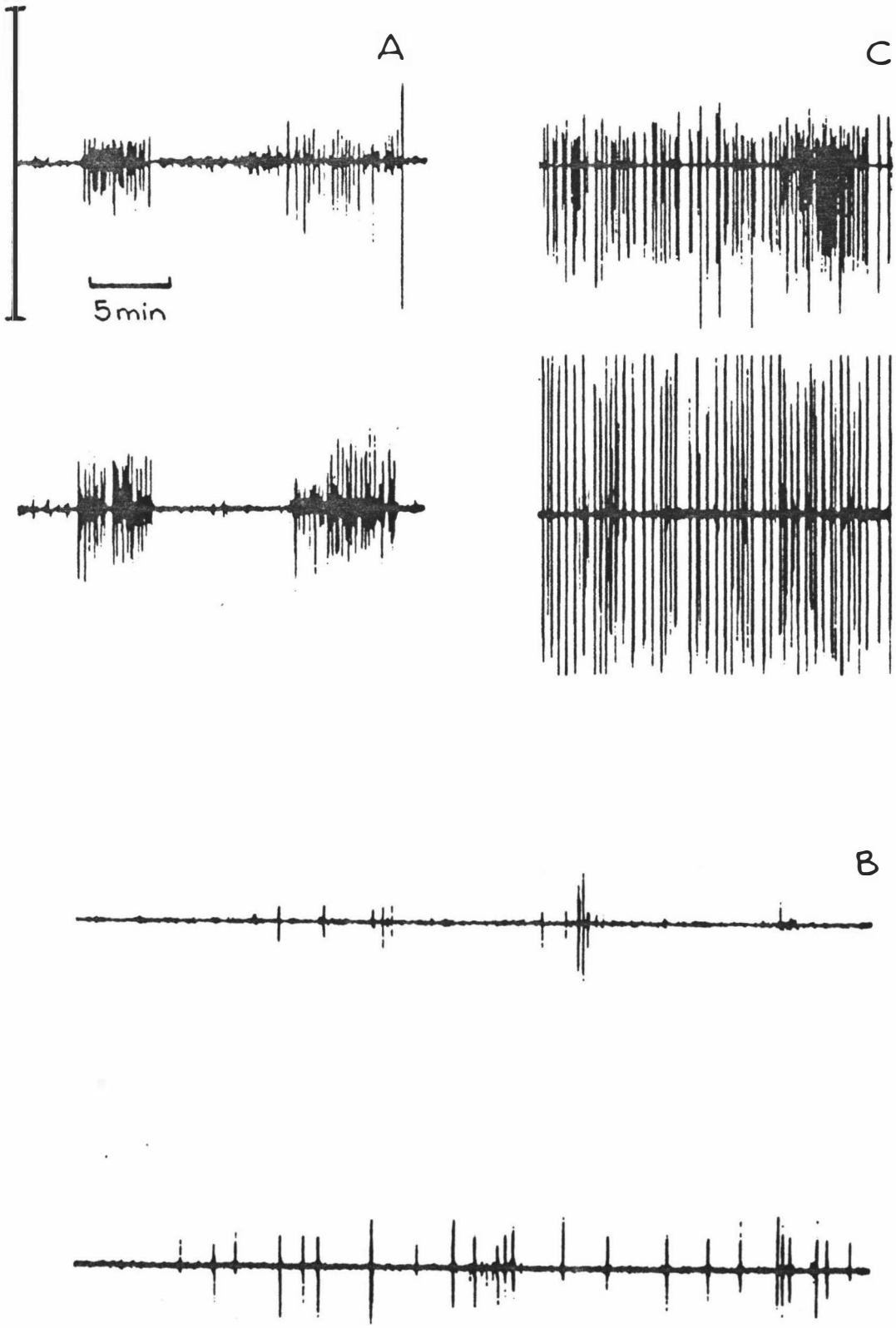
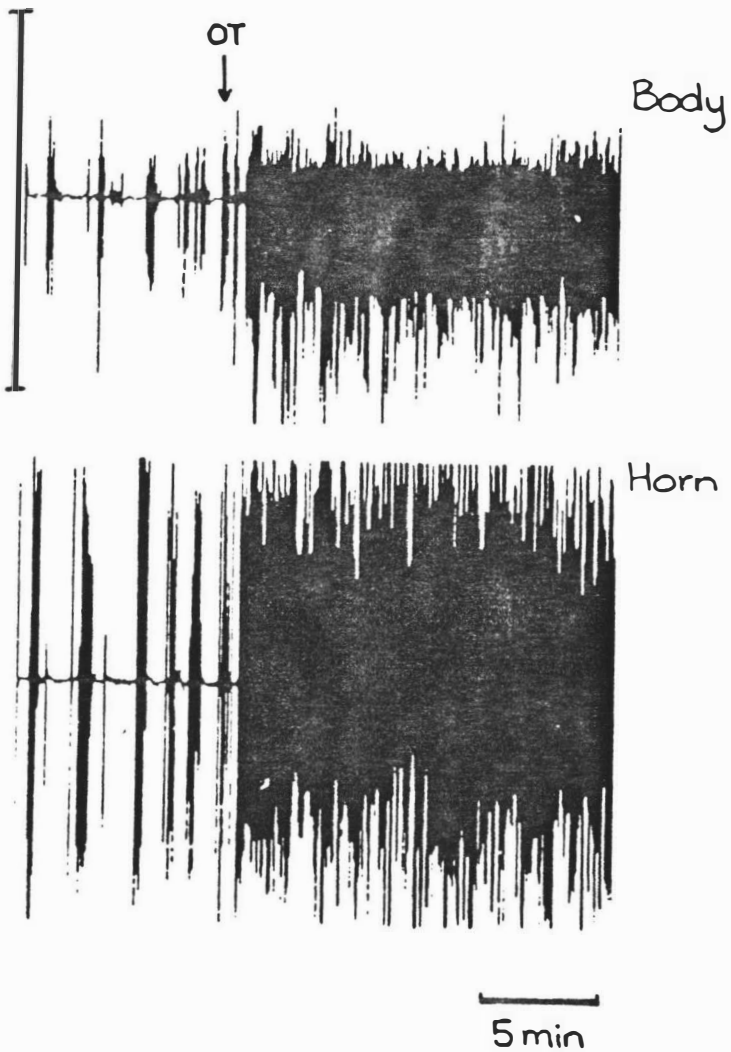


Fig. 9: **The effect of oxytocin on oestradiol induced emg activity.**

(18hrs. after administration of 2mg oestradiol to ewe 329. The emg activity increased within seconds of oxytocin injection shown at arrow -20iu i.m.).

Calibration: Vertical bar represents 1000μv.



Under the influence of oestrogen, the sensitivity of the uterus to oxytocin was greatly increased compared with the oestrogen deprived uterus. Table I summarises the changes that were observed.

IV.1.E. The effect of PGF2a on oestrogen induced emg activity during anoestrus (Fig. 10).

When PGF2a (Glandin N, Ethical Agents Ltd., P.O. Box 37-043, 19 Garfield Str., Parnell, Auckland, New Zealand) was injected 26h after oestradiol, the uterus responded within 8 mins and the response lasted about 4h. The emg activity following intramuscular PGF2a injection i.m. was similar to that induced by oxytocin (M.I. = 30%).

IV.1.F. The changes in myometrial activity when stimulated by a second injection of oestrogen.

Thirteen days after the initial oestrogen injection, the pattern of emgs had returned to the anoestrous pattern of activity so that regular complexes of emgs alternated with episodes of inactivity. The effect of oxytocin (20iu. i.m.) on emg activity at this time was to increase the frequency of spikes in the active periods although this increase was less than was obtained after the initial treatment with oestradiol. However, when a second injection of oestrogen (2mg i.m.) was given, the emg activity increased both in frequency and in amplitude. This became evident about 15h after the injection. The direction of propagation of myoelectrical activity was the same as that seen during spontaneous activity i.e. from body to horn of the uterus. An injection of PGF2a (0.5ml. i.m.) also stimulated uterine activity after the uterus was influenced by oestradiol.

All the responses following the second injection of oestrogen were qualitatively similar to those obtained after the first injection but they differed quantitatively. Spike amplitudes, frequency and the responses to injection of oxytocin, PGF2a and oestrogen were all reduced (Table II, Figs. 11 & 12).

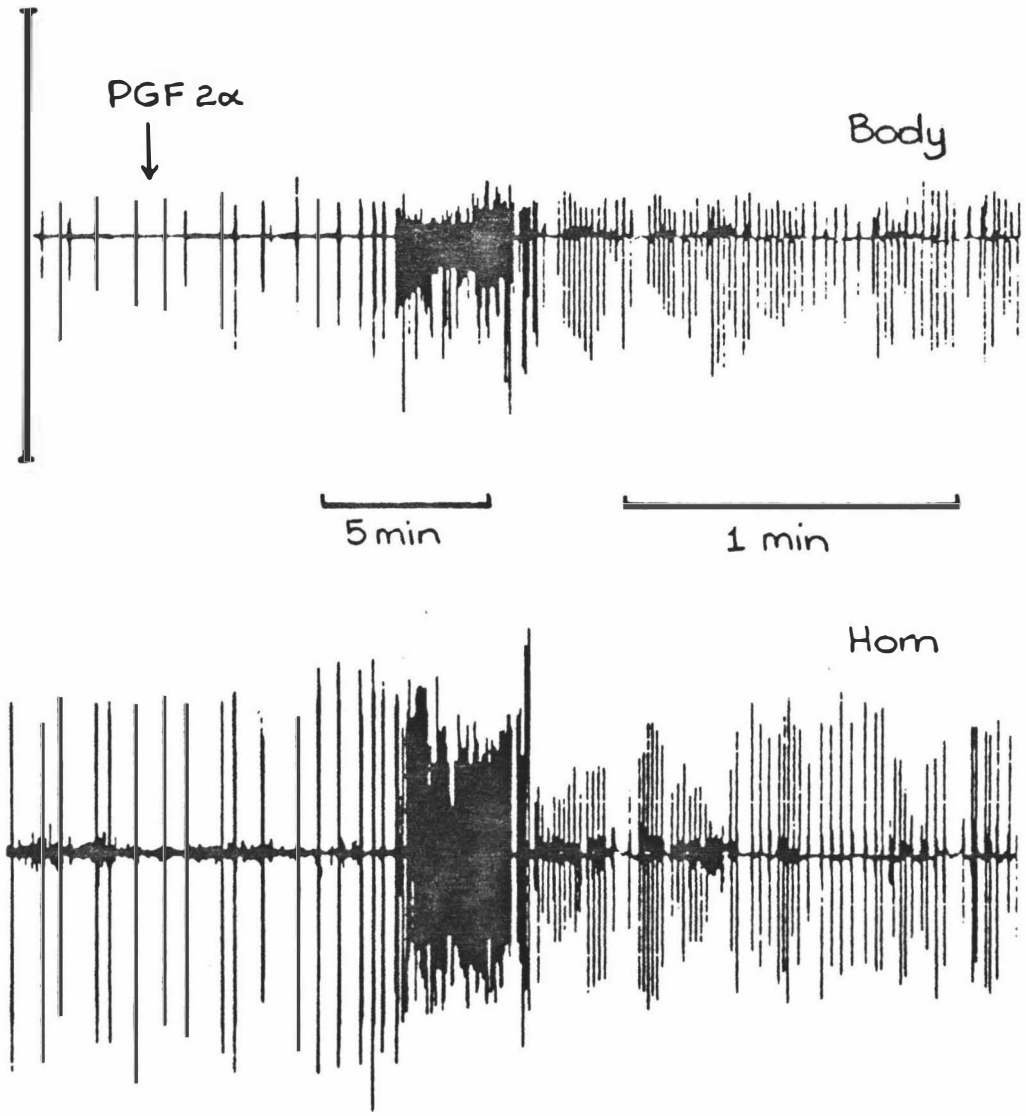
20 u. i.m Oxytocin / Physiological Status	Time between OT injection and e.m.g response	Duration of action	Bursts/h	Amplitude μV	Spikes per burst	Motility Index
			Before OT / After OT	Before OT / After OT		Before OT / After OT
No oestrogen treatment	30 min	4 h	72 / 180	200 / 200	10	0·80% / 2·00%
18 hours after oestrogen treatment	Seconds	6 h	180 / 400	1000 / 1000	15	15·00% / 33·33%

Table I: **The effect of oxytocin both on spontaneous and oestradiol induced emg activity (Ewe 329)**

Fig 10: **The effect of PGF2a on oestradiol induced emg activity during anoestrus.**

(Record from ewe 329).

Calibrations: The vertical bar represents 2500 μv. Chart speed 0.05mm/sec and 0.55mm/sec as shown.



EWE Nº 329

Status of uterus	The time between injection and response	Duration	Burst/h frequencies	Amplitude	Spikes per Burst	Motility Index
			Before OT i.m / After OT i.m	Before OT i.m / After OT i.m		Before OT i.m / After OT i.m
Resting e.m.g	30m	4h	46/h / 100/h	100 µv / 100 µv	10	0·26 % / 0·55 %
Oestradiol induced e.m.g	5 m	6h	105/h / 170/h	300 µv / 300 µv	18	3·3 % / 5·1 %

Table II: **The effect of oxytocin on spontaneous and oestradiol induced emg activity 13 days after last oestradiol injection of ewe 329 (compare with Table I).**

Fig 11: **Characteristics of the emg recorded 13 days after the initial injection of oestrogen.**

(All records are from the horn of the uterus).

Upper trace: spontaneous emg activity during anoestrus.

Middle trace: the effect of oxytocin on spontaneous emg activity during anoestrus (compare with Fig. 9).

Lower trace: the effect of 20 iu oxytocin 15h. after the second injection (2mg i.m.) of oestradiol (compare to Fig. 9).

Calibrations: The vertical bar represents 400μv. Chart speed is 0.05sec in all records.

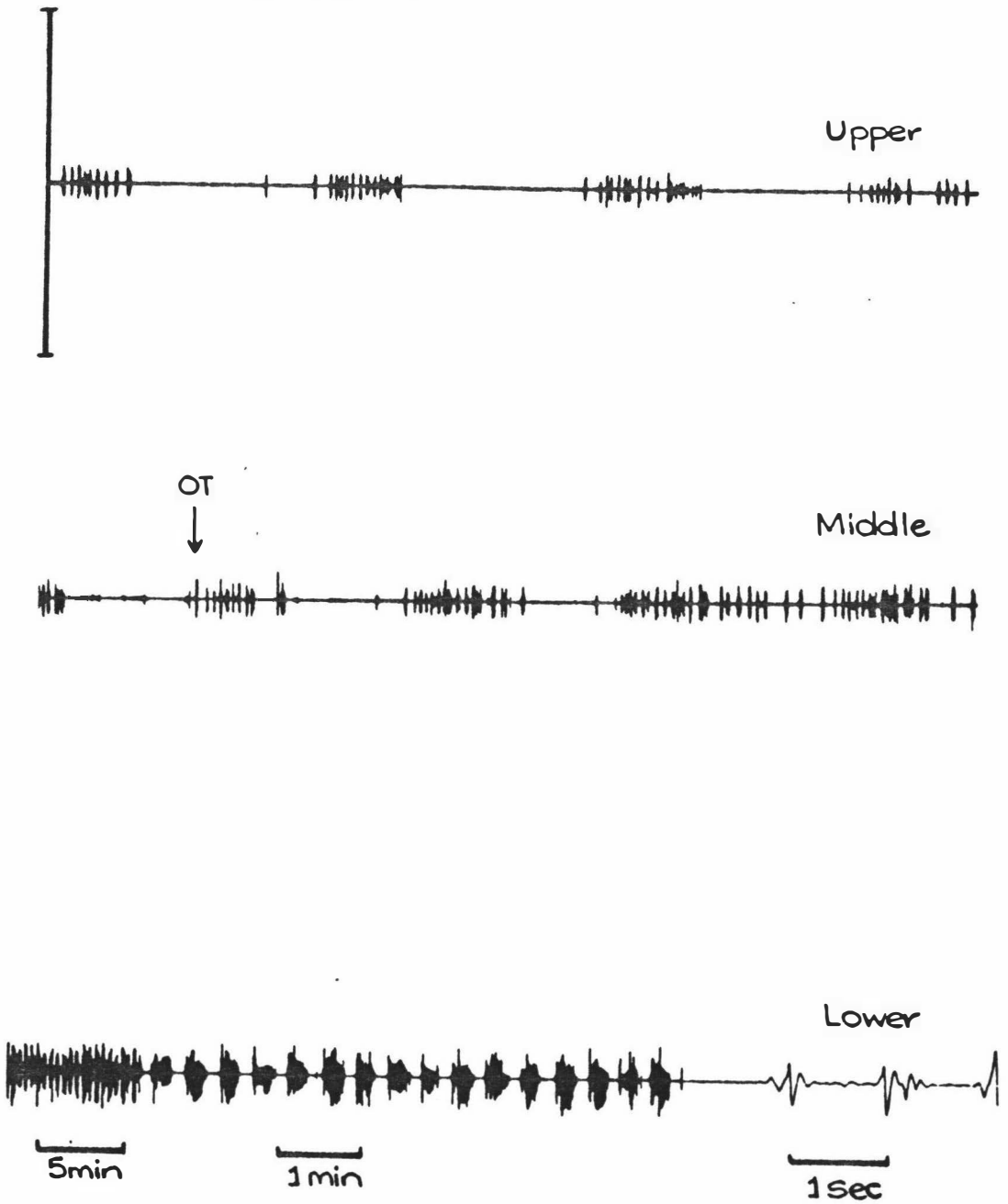
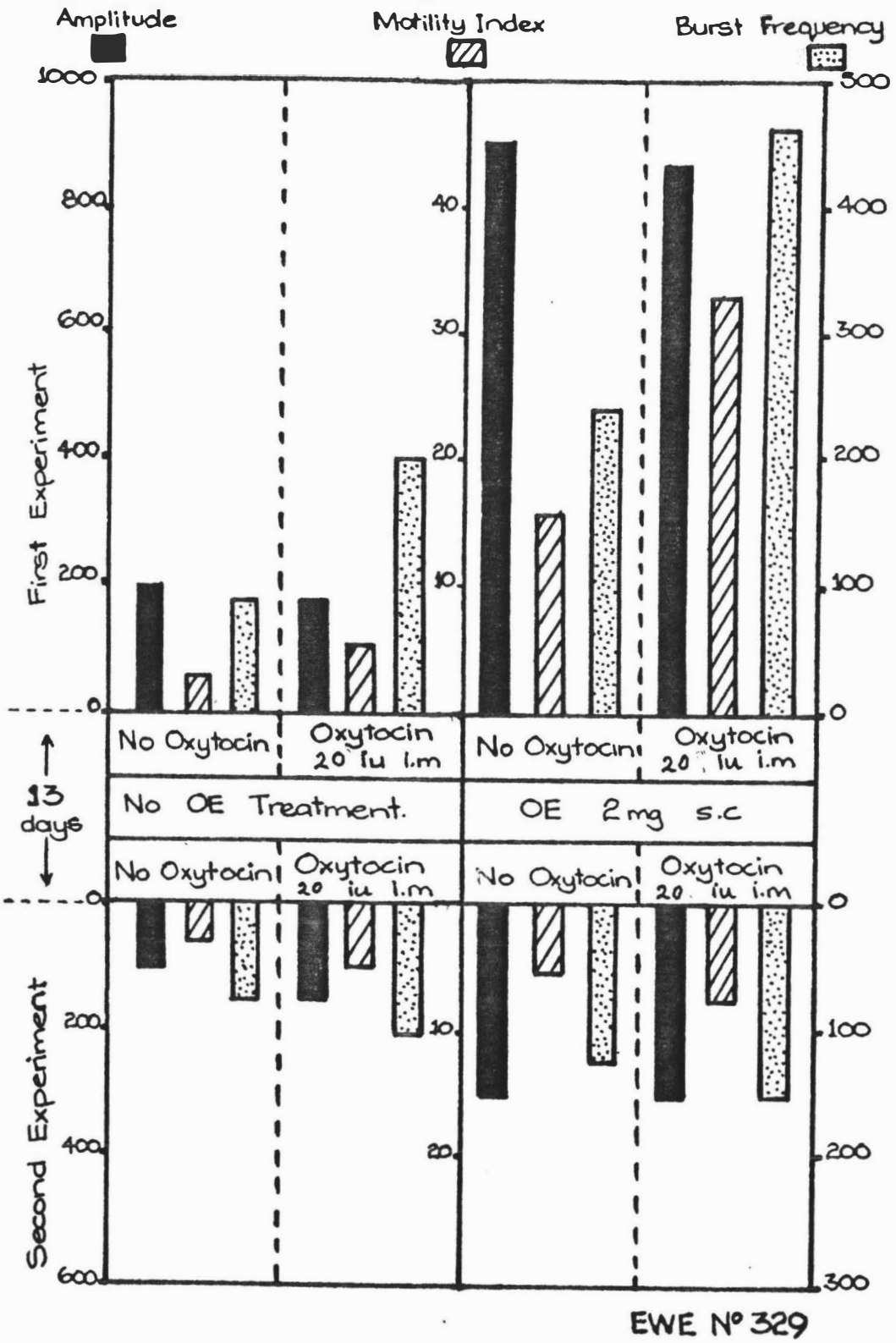


Fig. 12: The effects of oestrogen and oxytocin on amplitude, burst frequency and motility index during two experiments on intact anoestrous ewe 329.



IV.2 Myometrial Activity in the Ovariectomized Ewe during the Anoestrous Season.

IV.2.A. Specific features of the design of this experiment.

Four ewes (numbers 476, 10, 248, 785) were used for the experiment (Appendix I). The investigations were carried out from November 1987 through to February 1988 while the ewes were in their anoestrous season. Treatments began 10 days after ovariectomy. Each animal was subjected to two regimes which lasted 30 days and 40 days respectively. Thirty days were allowed between regimes - during the 30 day period no hormones were administered.

The responses were examined during five stages within each regime. These stages were:

(i) The pattern of emg in ovariectomized ewes observed 10 days after surgery in regime one and after 30 days without treatment in regime two.

(ii) The pattern of emg activity observed after administration of oestradiol-17b (50μg/day s.c.) (Estradiol Cypionate, 2mg/ml, The Upjohn Company, Kalamazoo, USA).

(iii) The effect of 50mg s.c. of progesterone (Progestin, Chemavet Distributors Ltd., Auckland) for 3 days (regime one) or 4 days (regime two) on the pattern of emg activity. During this period the administration of oestrogen was continued.

(iv) The pattern of emg activity after progesterone treatment was discontinued but while oestrogen was being continued.

(v) The pattern of emg activity after oestrogen treatment ceased.

Experimental groups: ewes in these groups (regimes one and two) were followed for the five stages of the protocol as outlined above.

Control groups: ewes acting as 'controls' did not receive progesterone treatment during that particular regime.

During these two regimes each ewe was used both as a control animal and as an experimental animal.

Throughout each of the five stages of the trial the sensitivity of the uterus of each ewe was tested by injections of both oxytocin and PGF2a.

IV.2.B. The general patterns of electrical activity

(a) Resting emg activity and the effects of exogenous oxytocin and PGF2a on it (Fig. 13).

Very low levels of emg activity were observed in the ovariectomized ewes 10 days after surgery (regime 1) and 30 days after stopping the administration of the hormones (regime 2). Although weak, the emg activity still showed the basic resting pattern of regular complexes of high frequency spikes alternating with quiescent periods that had been seen during the anoestrous period in intact ewes.

During this stage the uterus was only slightly sensitive to oxytocin. Oxytocin increased the frequency of single spikes within 30 mins of administration but had no effect on their amplitude.

(b) Oestrogen induced emg activity in the ovariectomized ewe and the effects of exogenous oxytocin and PGF2a on it (Figs. 14 & 15).

Four days after daily oestradiol-17b injections had been administered, the emgs had increased amplitudes markedly and the burst-frequency was 300-500 per hour. This pattern was stable in individual animals from that day on, but the differences between animals were large. Generally, at this stage, the patterns of emg activity became well co-ordinated and regular and single or grouped spikes were seldom observed. Every burst consisted of about 10 spikes of nearly equal amplitude and each burst lasted about 10 seconds. The periods of rest between bursts lasted about 20 seconds. There were 1-2 episodes of activity in each hour.

Fig. 13: **The pattern of emg activity in ovariectomized ewe 247 during the anoestrous season.**

Upper trace: regime 1: 10 days after surgery.

Middle trace: regime 2: 30 days without hormone treatment.

Lower trace: the action of oxytocin on the pattern of regime 1.

Calibrations: The vertical bar on the left of upper trace represents 500μv for all three records. Chart speed is 0.05mm/sec.

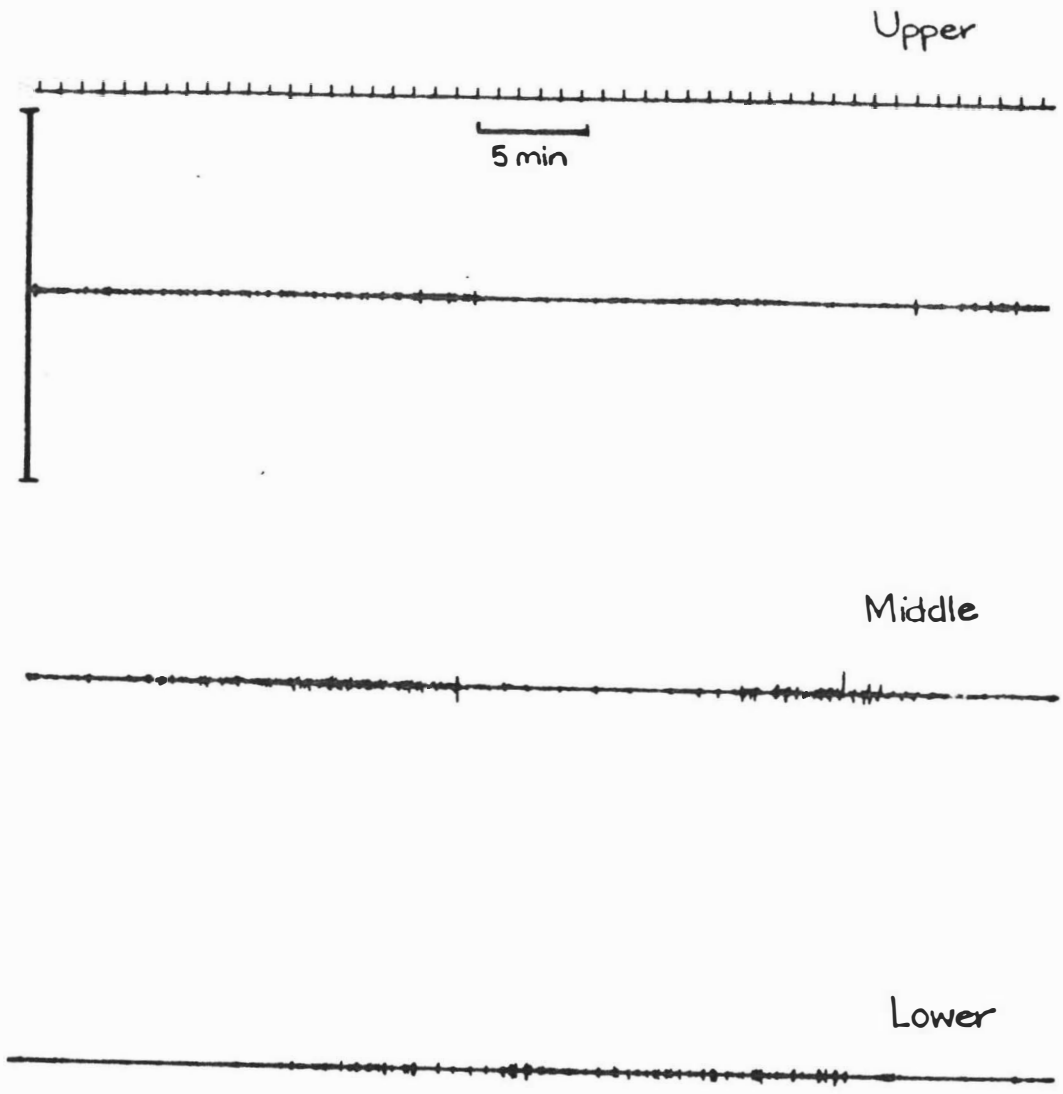


Fig. 14: **The pattern of emg activity induced by oestradiol-17b (50μg s.c. daily) on ovariectomized ewes during the anoestrous season.**

(All these records are from the uterine horns of ewes 476, 248 and 785 made on the 4th day of administration of oestradiol).

Calibrations: Vertical bar is 2500μv for all four records. Chart speed is 0.05mm/sec.

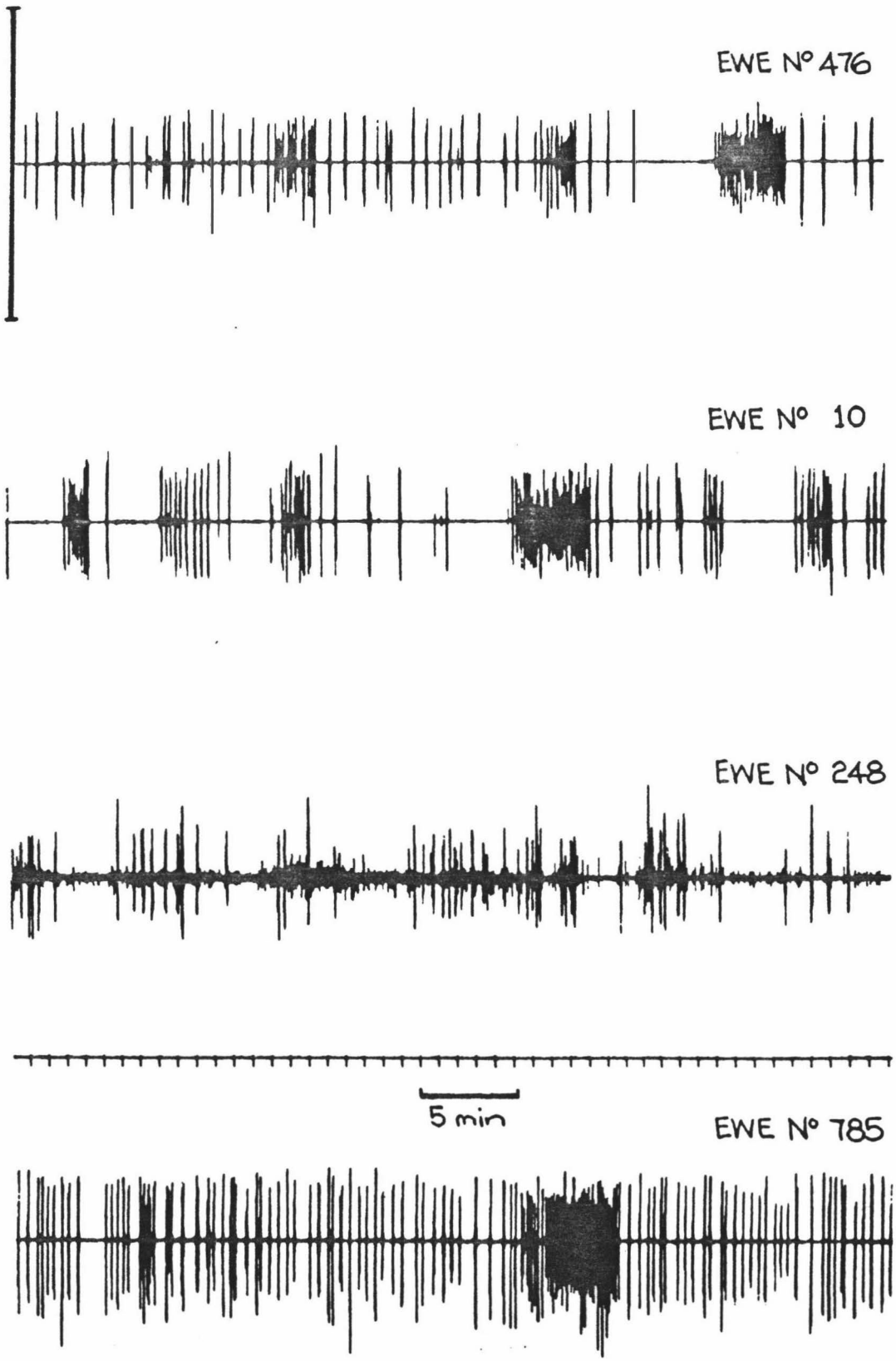
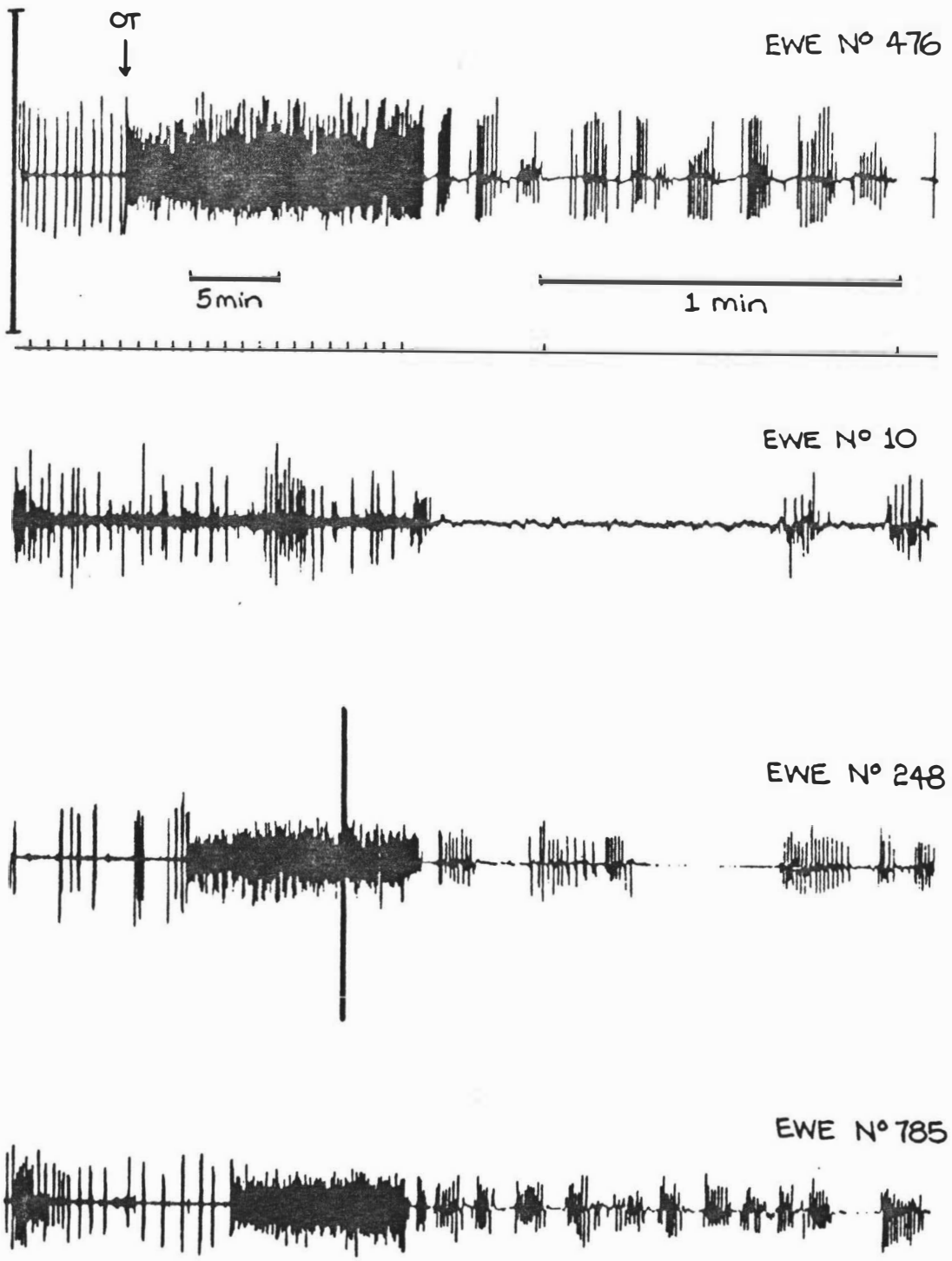


Fig. 15: **The effects of oxytocin 500mu/i.v) on the oestrogen induced emg activity of ovariectomized ewes during the anoestrous season.**

(These records are from ewes 476, 248, 10 and 785 made on the 4th day of administration of oestradiol).

Calibrations: The vertical bar represents 1500μv. Two chart speeds were used as indicated in the figure.



It was noted that during stage 2, the uterus was very sensitive to the test with oxytocin and PGF2a. Both of these stimulants increased the spike frequencies greatly and the latency before a response was obtained was reduced.

(c) **The effects of progesterone on oestrogen-induced emg activity.**

The pattern of emgs induced by oestrogen during stage 2 was changed in three respects after progesterone injection:

(i) There was a reduction in the amplitude of spikes by almost 50% within 10h and an almost complete suppression within 30h after the injections of progesterone had begun (Fig. 16a & trace C1 of Fig 16b).

(ii) There was a change in the frequency of bursts. During the first 5h after progesterone injection several bursts were concentrated to form a complex of activity; this was followed by an interval of inactivity with single bursts appearing during this interval. Initially, the duration of episodes and the following intervals were similar but subsequently the intervals became progressively longer and the number of the bursts in the episodes decreased.

(iii) The third change concerned the amplitude of spikes within a burst: before progesterone injection, all spikes in a burst were of similar amplitude but about 7h after administration of progesterone some of the spikes became much smaller than others and some seemed to disappear. This trend continued so that after 30h most bursts were suppressed and a few were left with only one or two spikes. The emg activity became uncoordinated before it was markedly inhibited by progesterone some 30h after the injection. During this inhibited period, exogenous oxytocin and PGF2a were still able to increase the frequencies of single and grouped spikes but they did not increase their amplitudes (Fig. 16b).

Once administration of progesterone was stopped the myometrial activity began to recover in the presence of continuing oestrogen

Fig 16a: The development of inhibition after progesterone administration

Trace B1: 5h after progesterone administration began. Bursts are grouped, there are about 25 bursts in a group and very few single bursts occur in the intervals between episodes.

Trace B2: 10h after progesterone administration began. The frequency of bursts within episodes is reduced and the amplitude of spikes is reduced.

Trace B3: 22h after progesterone administration began. Both the number of bursts in a group and the amplitude is reduced but the frequency of bursts remains about the same.

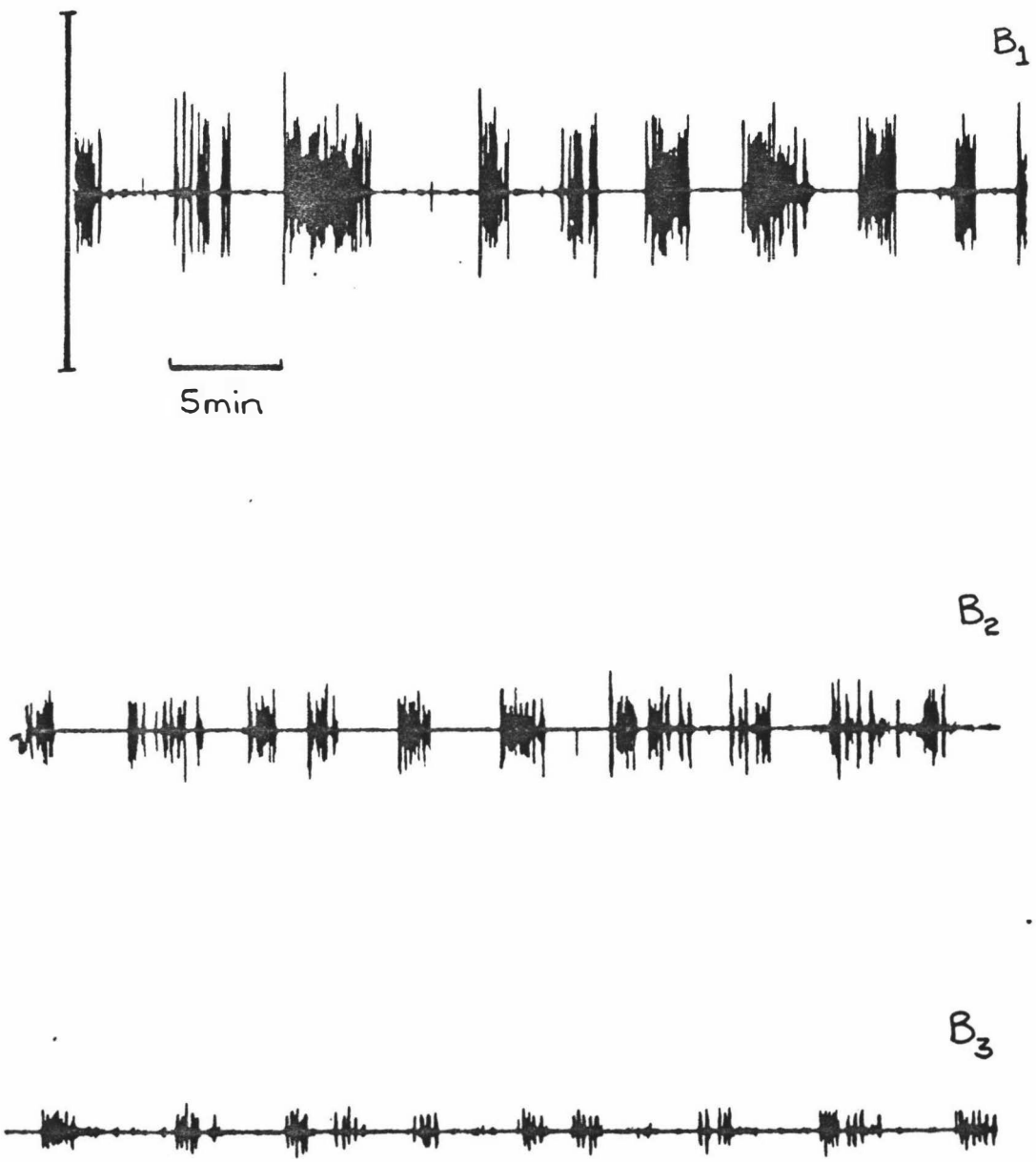
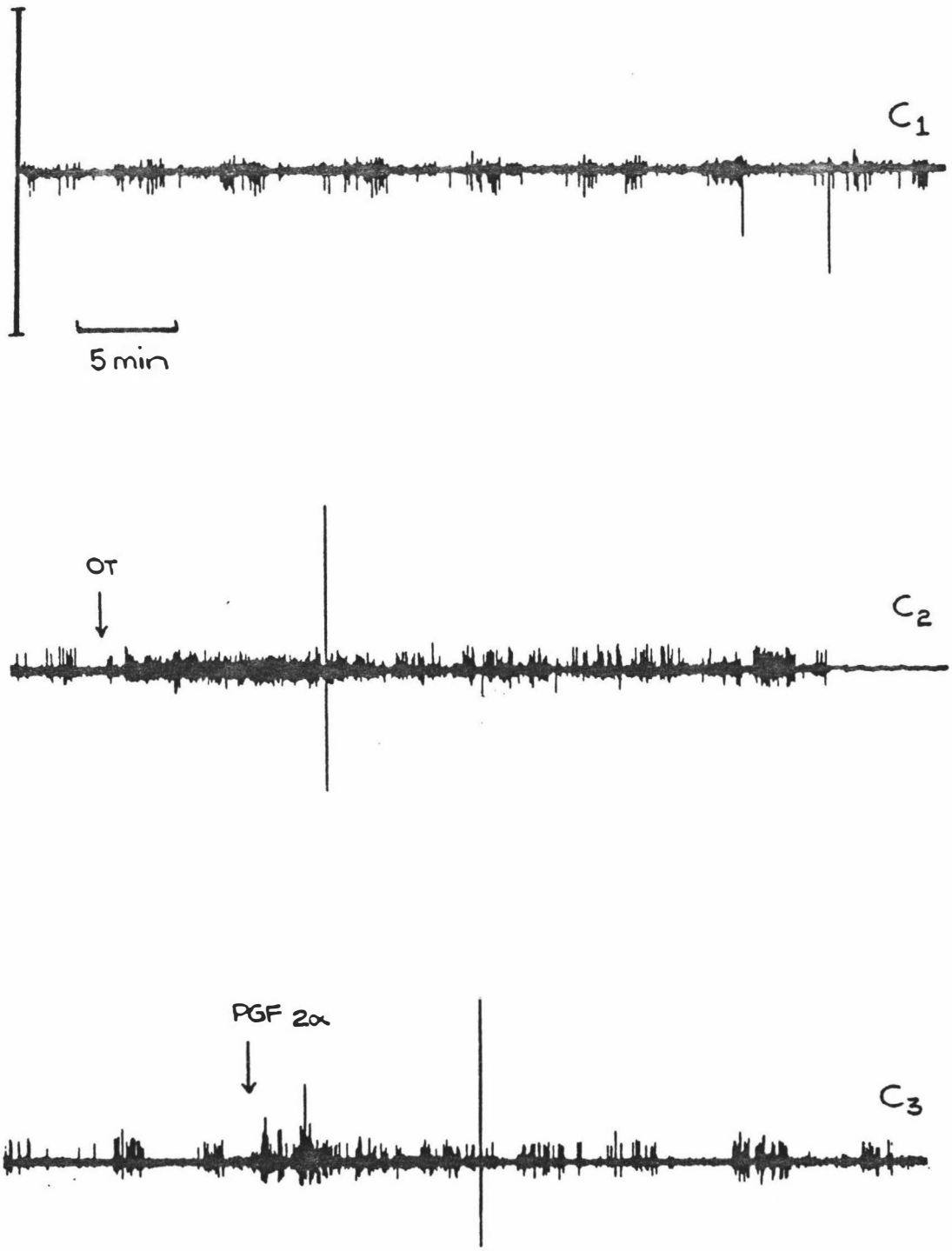


Fig. 16b: **The effect of oxytocin and PGF2∝ when the uterus is inhibited by progesterone.**

Trace C1: 32h after beginning progesterone administration.

Trace C2: the effect of oxytocin (500mu given i.v) 35h after beginning progesterone administration.

Trace C3: the effect of PGF2∝(1.5mg given i.m) 37h after beginning progesterone administration.



treatment. During recovery the first change to the emgs was an increase in the frequency of single or grouped spikes. This occurred within about 5h of the last administration of progesterone (Fig. 16c, trace D1) so that the pattern of amplitude and low-frequency spikes characteristic of progesterone inhibition changed to one of low-amplitude and high frequency spikes which were poorly co-ordinated. Then, after about 30h (Fig. 16c, trace D2), the spikes began to regroup into bursts that contained inconsistent numbers of spikes of irregular amplitude. As the time after progesterone administration increased to about 55h (Fig. 16c, trace D4), some of these inconsistent bursts tended to become uniform in the number of spikes they contained and later, in their amplitudes as well. The emgs also became more co-ordinated. The progressive increase in the amplitudes of the spikes from the beginning of recovery until the myoelectrical complexes reappeared is shown in Fig. 16c.

The amplitudes of the spikes continued to increase so that they became greater than those recorded before progesterone administration. The main differences between the emg activity recorded in the periods before and after the influence of progesterone were changes in the frequency and amplitude of spikes and the number of spikes within a burst. When the uterus had recovered from the period of progesterone inhibition all these properties of the emgs increased to levels greater than those induced by oestrogen alone.

The response to a single large dose of progesterone (100mg i.m.) given 4 days after a previous inhibition of the uterine emg using a lower dose of progesterone was to reduce the myometrial activity to its lowest level. This occurred at about 40h after the large injection. Following this the emg activity started to recover and took 48h to reach the level of activity present before this single injection. The amplitude of the emg kept increasing for another 24h to an amplitude higher than the previous levels that had been recorded (see Fig 16d, trace E).

Fig. 16c: **The emg pattern observed during the recovery from progesterone inhibition.**

Trace D1: 5h after the last progesterone injection. The single spike frequency has increased and the single spikes have disappeared with spikes now present in the intervals between the groups.

Trace D2: 35h after the last progesterone injection. Single spikes are grouping to form primary bursts - note also the very limited response to PGF2α

Trace D3: 44h after last progesterone injection. An increased response to oxytocin can be seen.

Trace D4: 55h after the last progesterone injection. The emg amplitude is clearly increasing.

Trace D5: 62h after last progesterone injection. The normal burst pattern has appeared.

Trace D6: 72h after last progesterone injection. The emg amplitude has increased markedly, bursts are regular and burst frequency high.

Trace D7: 76h after last progesterone injection. There is a rapid response to PGF2α administration.

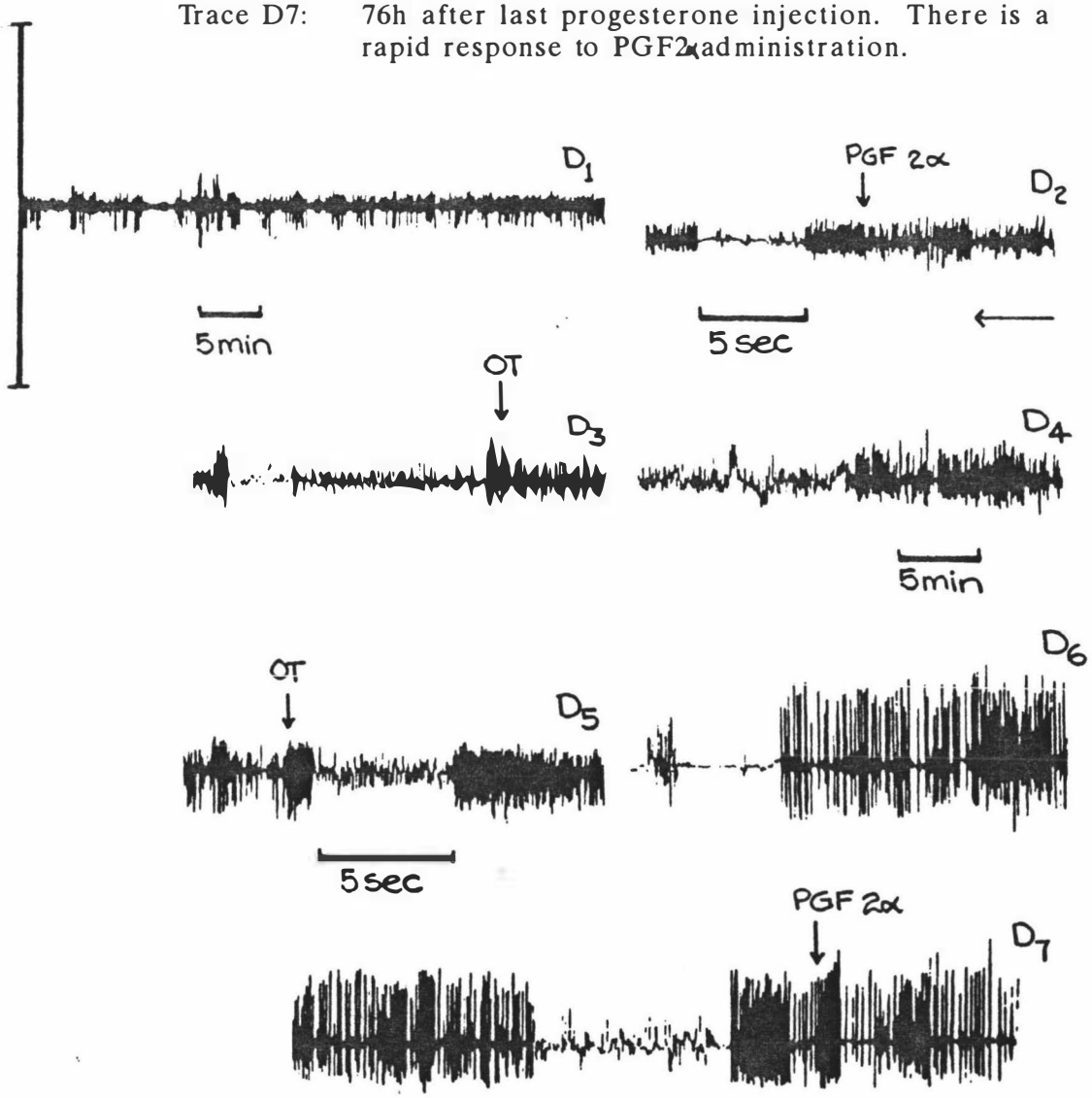
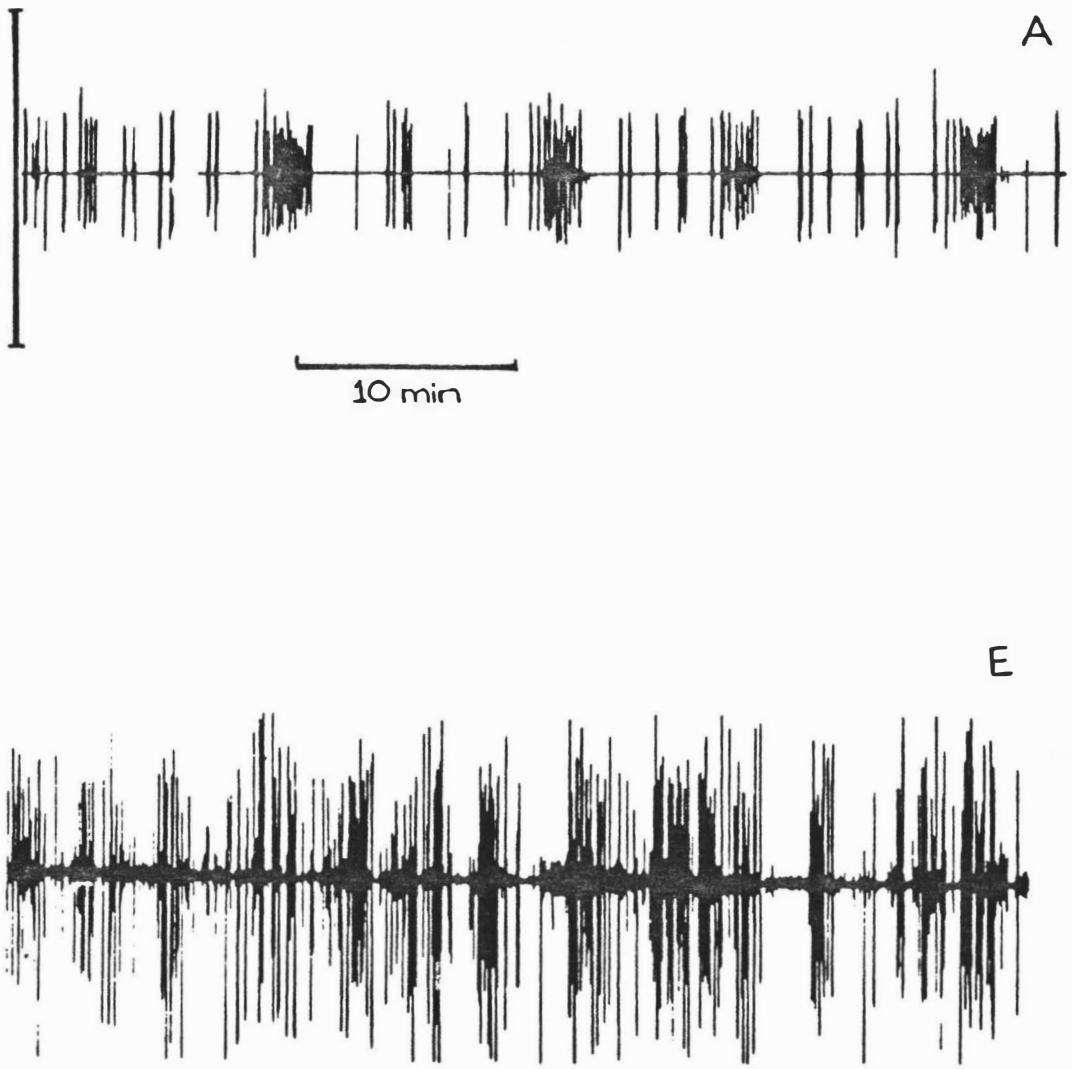


Fig. 16d: **The pattern of oestrogen induced emg in ewe 10 before progesterone administration (A) and in the same ewe 115h after a large dose of progesterone (100mg given i.m) given when the ewe had recovered from the progesterone treatment administered during regime one (E).**

(Note the high amplitude in E, vertical bar represents 2500μv).



The responses to oxytocin and PGF2a after progesterone administration depended on the level of emg activity prevailing at the time. In general both drugs could increase the frequency but not the amplitude of the emgs although this was not always the case (see Fig. 16b, traces C2 and C3 and Fig. 16c, traces D2, D3, D5, D7).

(d) The reduced responsiveness of the uterus in regime two of the experiment.

Thirty days after stopping the administration of all drugs the experiment was continued in the 4 ewes as "regime two". The changes observed during regime two were qualitatively similar to those of regime one but there were changes in sensitivity of the uterus:

(i) The emg activity of the uterus of the ovariectomized ewes fell to its lowest level 30 days after regime one had been completed; nevertheless it still showed the alternating complexes of emg activity and the subsequent quiescent periods which were present during regime one.

(ii) After daily administration of oestradiol-17b at the beginning of regime two the emg action potentials increased in both frequency and amplitude. The uterus of the oestrogen-treated ewe at this stage was more sensitive to stimulation by both oxytocin and PGF2a than the uterus of the ewes not under oestrogen treatment.

(iii) The changes of emg activity during both the phase of inhibition following progesterone administration and the phase of recovery from it were similar to that seen during regime one. An important difference was that after recovery of myometrial activity from the inhibition, the uterine emgs became much more pronounced than those which had been induced by oestrogen in regime two before progesterone treatment, i.e. the emg response was

enhanced and greater than at any earlier stage of the experiment.

At all stages of the experiment, the myometrial response in all ewes in regime two to the administration of exogenous oestrogen, progesterone, oxytocin and PGF2a was slower to manifest itself than that observed in regime one. Thus it needed 4 days of oestrogen administration to reach the highest level of emg activity in regime one but 8 days in regime two; it took 6 days to display the full changes that were seen with inhibition by progesterone and recovery from it in regime one but nearly 18 days were required in regime two.

The overall level of response in regime two was also reduced. Thus while oestradiol treatment itself induced high levels of emg activity in regime one, these values were reduced in regime two (in ewe 10, for example, there were 100 bursts/h and an amplitude of 1000μv in regime one compared with 90 bursts/h and an amplitude of 500μv in regime two). The highest levels of emg activity observed in the four ewes were burst frequencies of 200/h and amplitudes of 2250μv in regime one compared with 170/h and 500μv in regime two. The intensity of emg activity was clearly reduced during the second regime of the experiment.

(e) The direction of propagation of emg action potentials

The direction of propagation of action potentials appeared to depend on the level of myoelectrical activity present. At times of high emg activity, e.g. under oestrogen stimulation (Fig. 4), movement was in a tubocervical direction. At times when the emg activity was less, as in the anoestrous ewe, the dominant direction of propagation was from the cervix to the tubal end.

IV.2.C. Some quantitative aspects of the electromyograms recorded from the ovariectomized ewes.

(a) **Amplitude:** A difference in amplitude between animals during the same stage of oestrogen-induced emg activity was noted (see Fig. 15 where amplitude ranges from 1750μv in ewe 476 to 500μv in ewe 10). Different amplitudes were also recorded at different sites in the same ewe as demonstrated in Fig. 17. The highest amplitude was always recorded from the horn site and the lowest from the oviduct.

The effect on amplitude during the different stages during regime one is shown for ewe 248 in Fig. 18. Progesterone not only clearly inhibits oestrogen-induced activity but also appears to potentiate activity when the inhibitory phase passes.

(b) **Burst frequency:** A difference in burst frequency between animals during the same stage of oestrogen-induced emg activity was also observed (see Fig. 15) as were differences between different electrode sites in the same animal (Fig. 17). Highest frequencies were always recorded at the tubal end of the uterine horn, lowest frequencies at the oviduct. The burst frequency in both regimes one and two was always greatest during stage 2 of the respective regime (a phase where progesterone administration had ceased but oestrogen was continuing). Again a potentiating effect of progesterone is suggested (Fig. 19).

(c) **Spikes in burst:** Differences between animals are shown during the same stage of oestrogen-induced activity in Fig. 20 and differences between stage 2 and stage 4 of the experiment in the same ewe in Fig. 21. Again the stage 4 response is greater.

(d) **Motility index:** Differences between animals during the same stage of oestrogen-induced activity are shown in Table III. Table IV illustrates the very clear responses obtained with both oxytocin and PGF2a at the same stage. This is brought about mainly by an increase in bursts per hour (compare characteristics

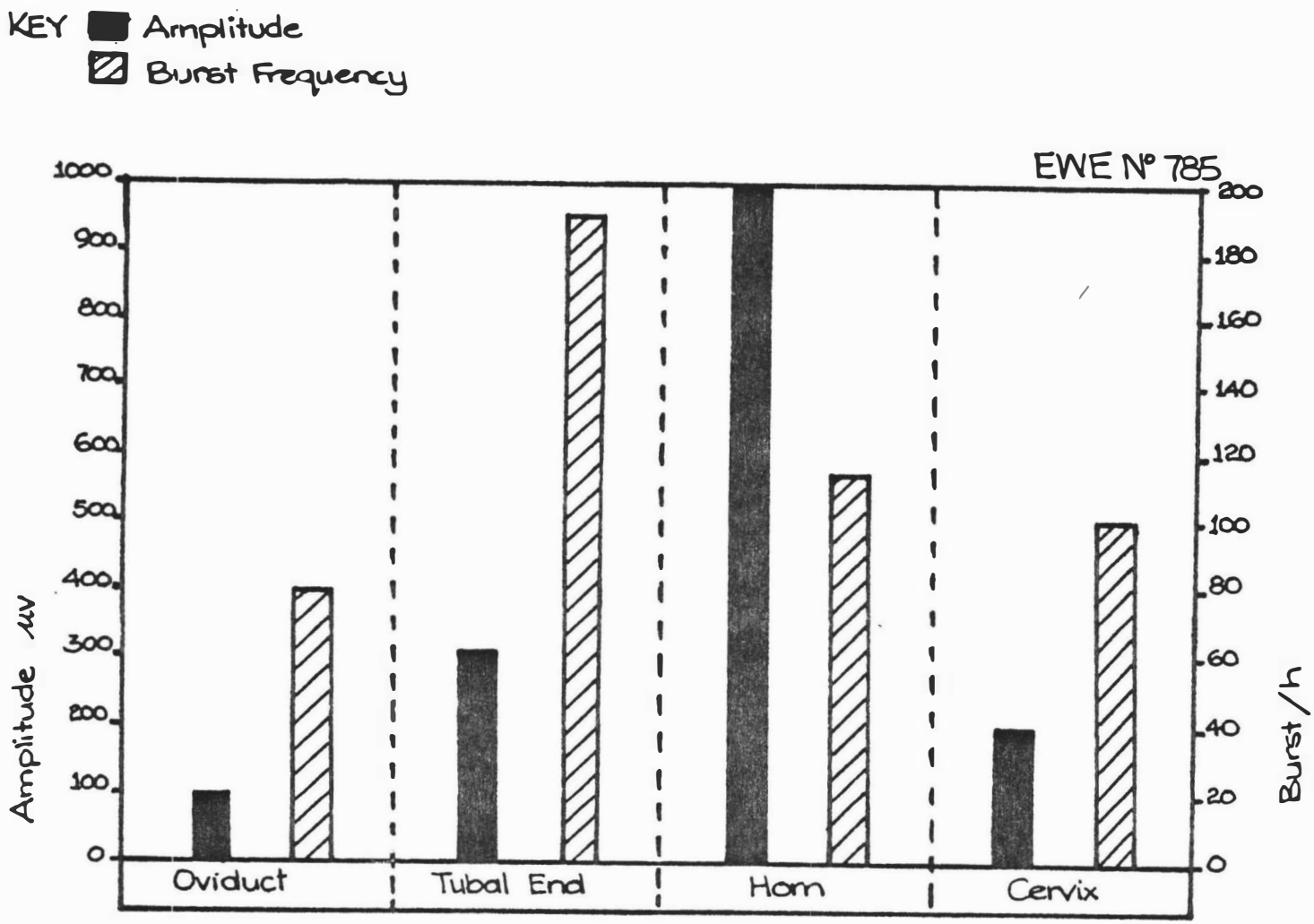


Fig. 17: **The difference in amplitude and burst frequency between 4 electrode positions on the reproductive tract.**

(Ewe 785, record taken during stage 2 of regime one).

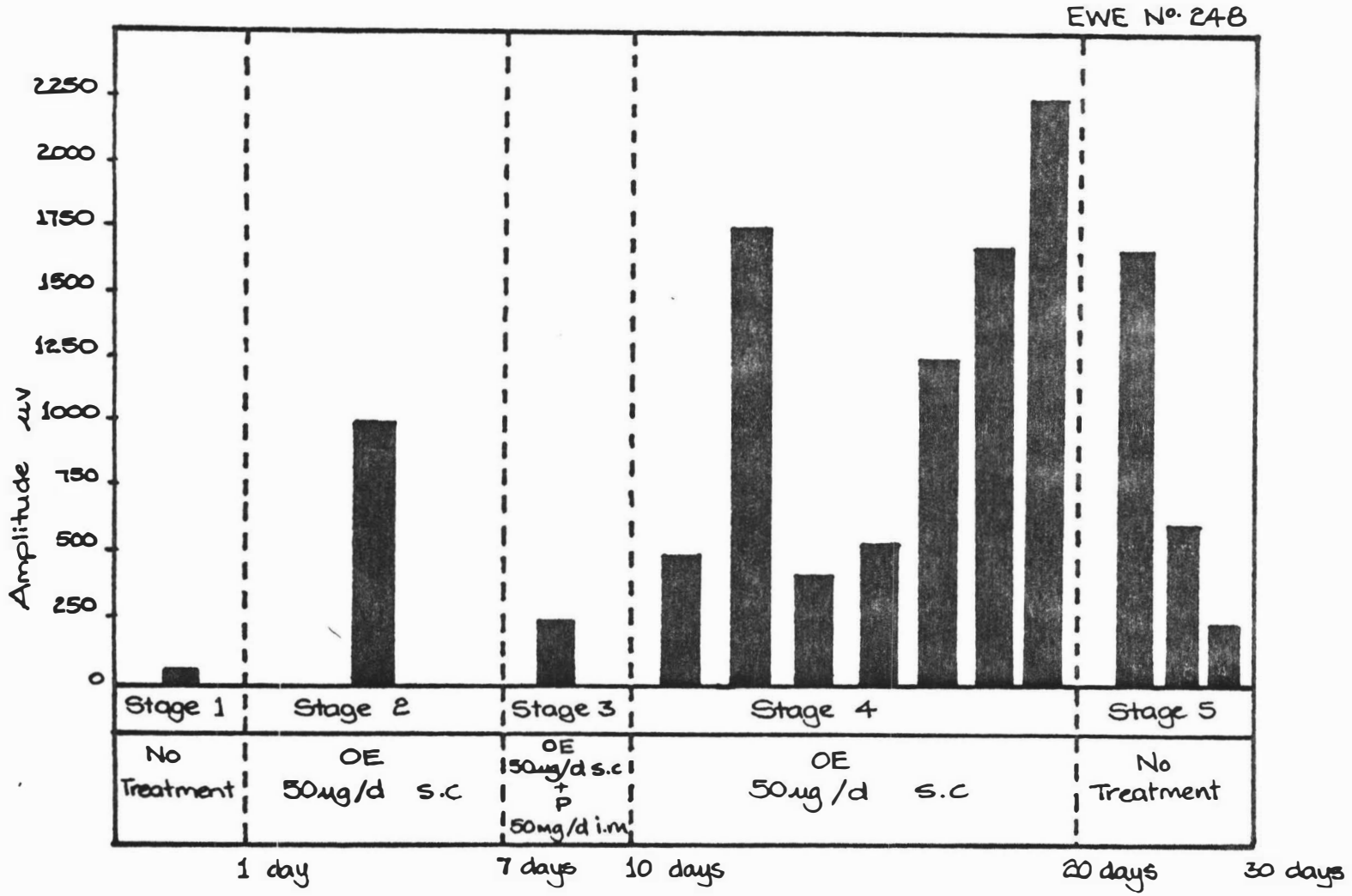


Fig. 18: **Emg amplitudes during different stages of treatment in regime one.**

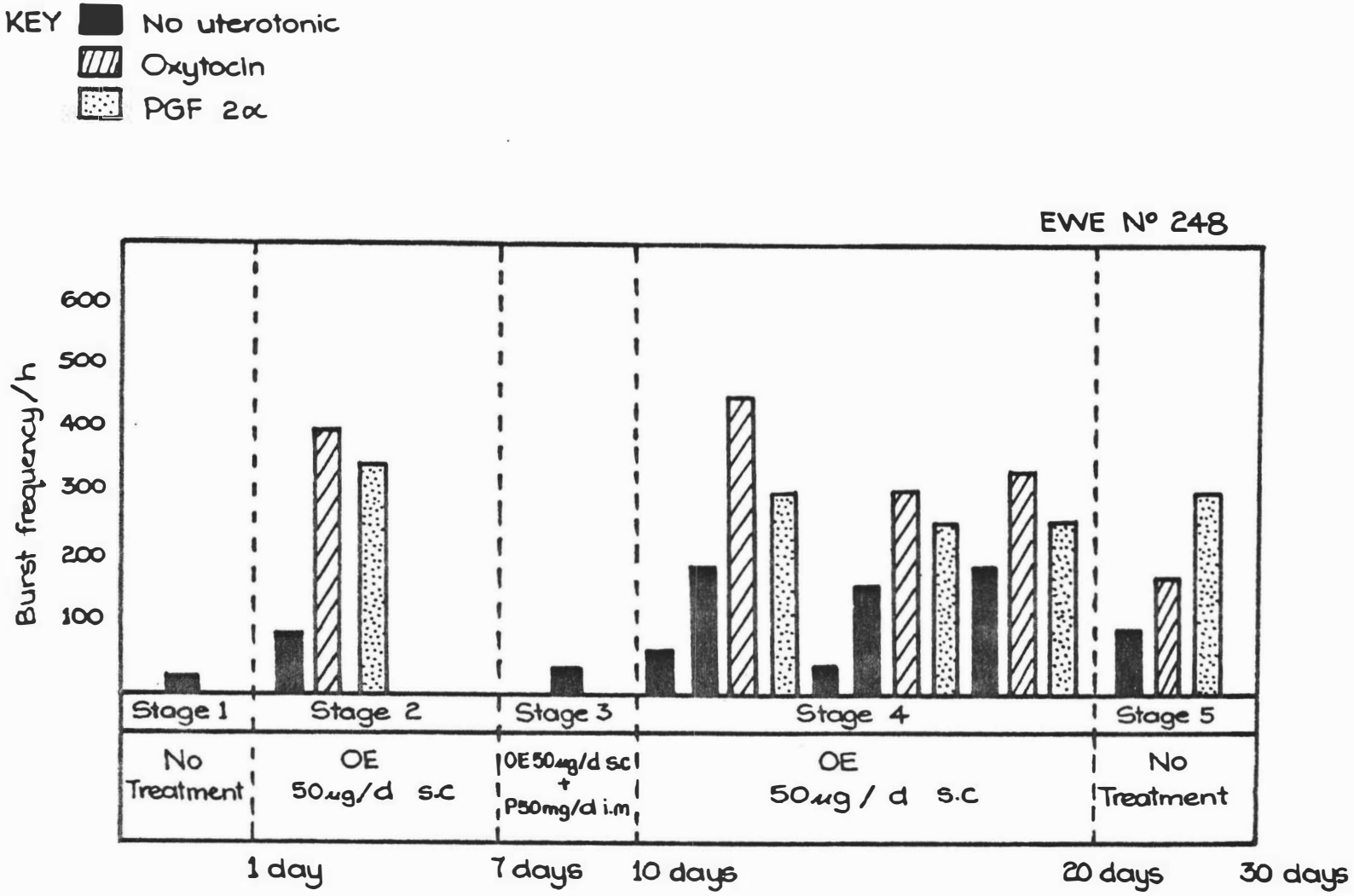


Fig. 19: **Differences in burst frequency at each treatment stage of regime one.**

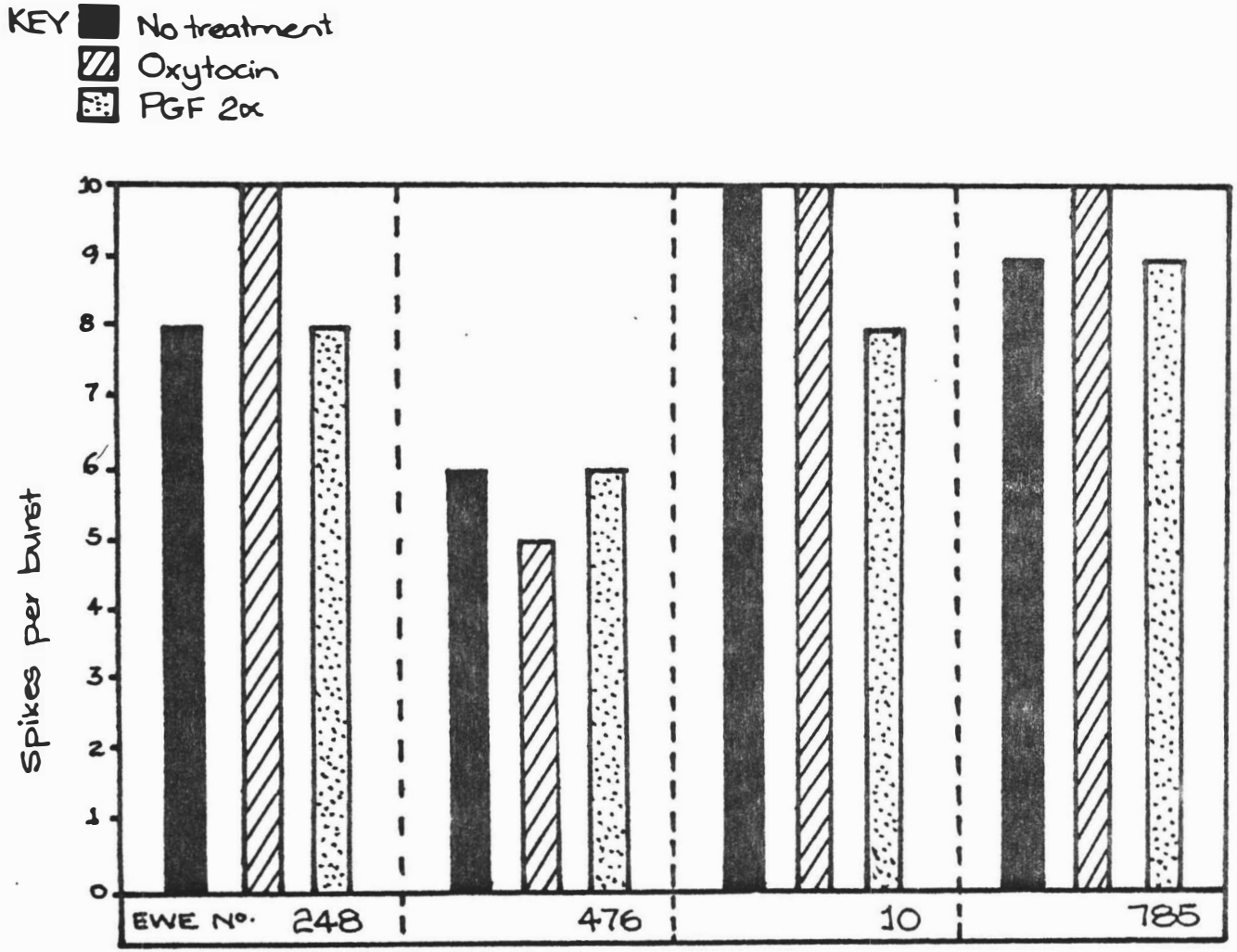


Fig. 20: **Number of spikes in a burst during the period of oestradiol induced emg activity in regime one.**

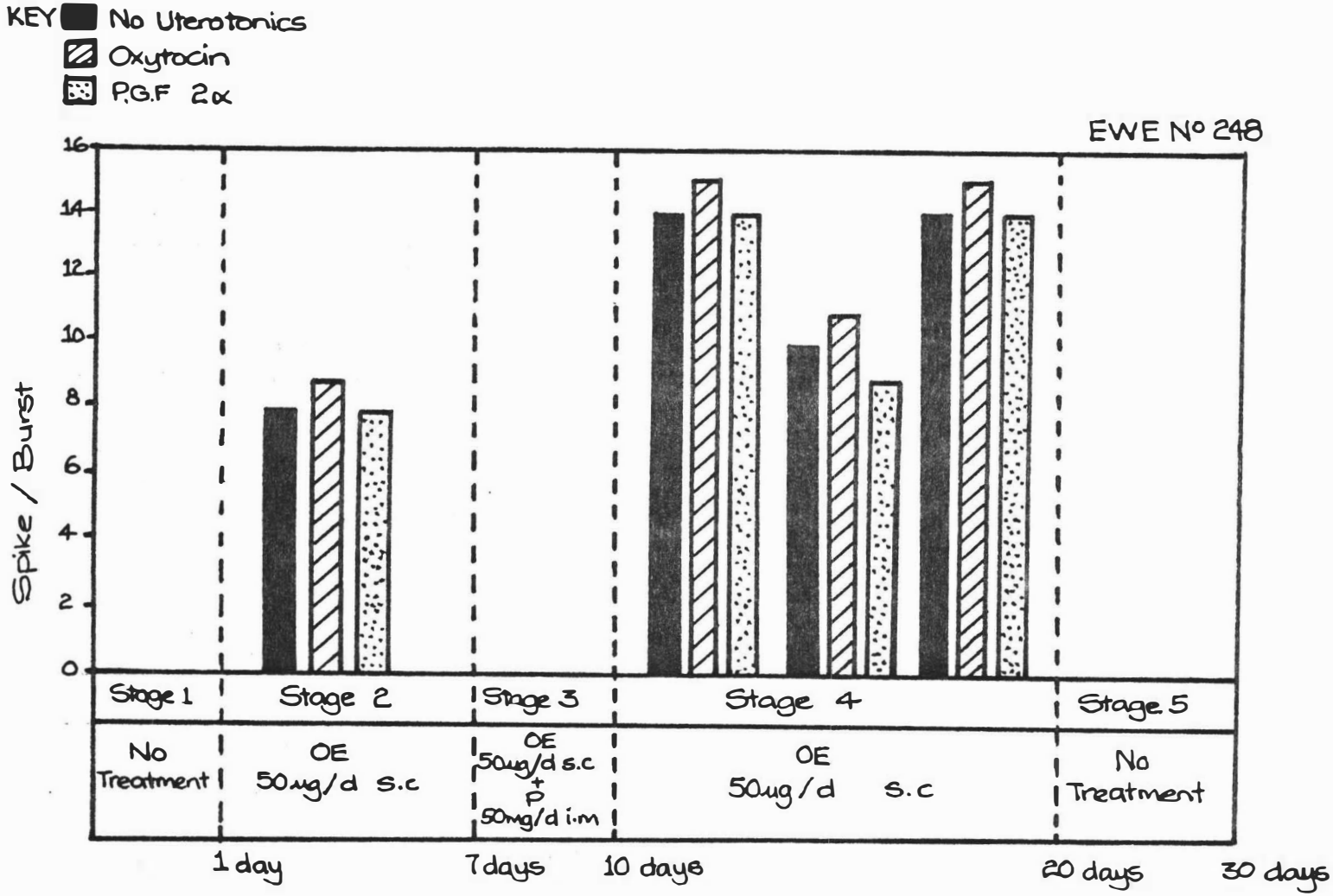


Fig. 21: **The number of spikes in a burst during each treatment stage of regime one.**

Ewe No	Amplitude uv	Burst per Hour	Spikes per Burst	Motility Index
476	1750	120	6	7·0%
10	500	80	10	2·2%
248	1000	90	8	4·0%
785	750	110	9	4·1%

Table III: **The differences in emg activity between animals during stage 2 of regime one.**

Ewe No.	Uterotonic	Amplitude μv	Burst per Hour	Spikes per Burst	Motility Index	
					With Uterotonic	Without Uterotonic
248	OT	1000	400	10	22·2 %	4·0%
	PGF 2α	1000	340	8	15·1 %	
10	OT	500	400	10	11·1 %	2·2 %
	PGF 2α	500	360	8	8·0 %	
785	OT	750	500	10	21·0%	4·1 %
	PGF 2α	750	370	9	14·0%	

Table IV: **The effect of oxytocin and PGF2a on the oestradiol induced electrical activity during stage 2 of regime one.**

of the same ewes in Tables III and IV). Fig. 22 illustrates the increased response seen during stage 4 where the inhibitory action of the progesterone given during stage 3 has waned. Fig. 23 again illustrates, using motility index as the ultimate criterion, the enhanced response observed in stage 4 of regime two and referred to earlier in the results under IV.2.B(d).

IV.3 The Emg Activity of the Uterus of the Cycling Ewe

Recordings from a cycling ewe showed a pattern of emg activity of regular waves over a 17 day period. Three phases were readily distinguished. Emg activity during the greater part of dioestrus was typified by alternating periods of activity and quiescence. Towards the end of dioestrus, the frequency of active periods increased and the spike or grouped spike frequency within the active episodes also increased (Fig. 24). At this time there is also an increase in spikes or grouped spike frequency within the quiescent period (Fig. 25, A3). Finally the basic pattern described above disappeared and the emg consisted of diffuse single or grouped spikes of low-amplitudes (Fig. 24, B1). As the pattern associated with dioestrus changes into that of pro-oestrus these single or grouped spikes develop into or mix with an increasing number of bursts with short intermittent periods of rest (Fig. 25, B1). At this stage regularity of the single and co-ordinated bursts is absent. However progressively bursts become more regular and single or grouped spikes become less frequent and finally disappear as oestrus occurs (Fig. 26, B3, B4).

The activity as oestrus begins is well co-ordinated and emg activity and amplitude increases from 250μv to 1000μv (Fig. 26, B3, B4, B5). The period of increased activity appears to include pro-oestrus (about 30h) as well as the early part of oestrus.

During the major part of oestrus, the uterus showed well co-ordinated and well propagated bursts of activity (Fig. 26, C1, C2). These episodes recurred at about 20 min. intervals and appeared simultaneously in the cervix, the tubal end and both horns of the uterus. Near the end of oestrus the burst frequency started to decrease.

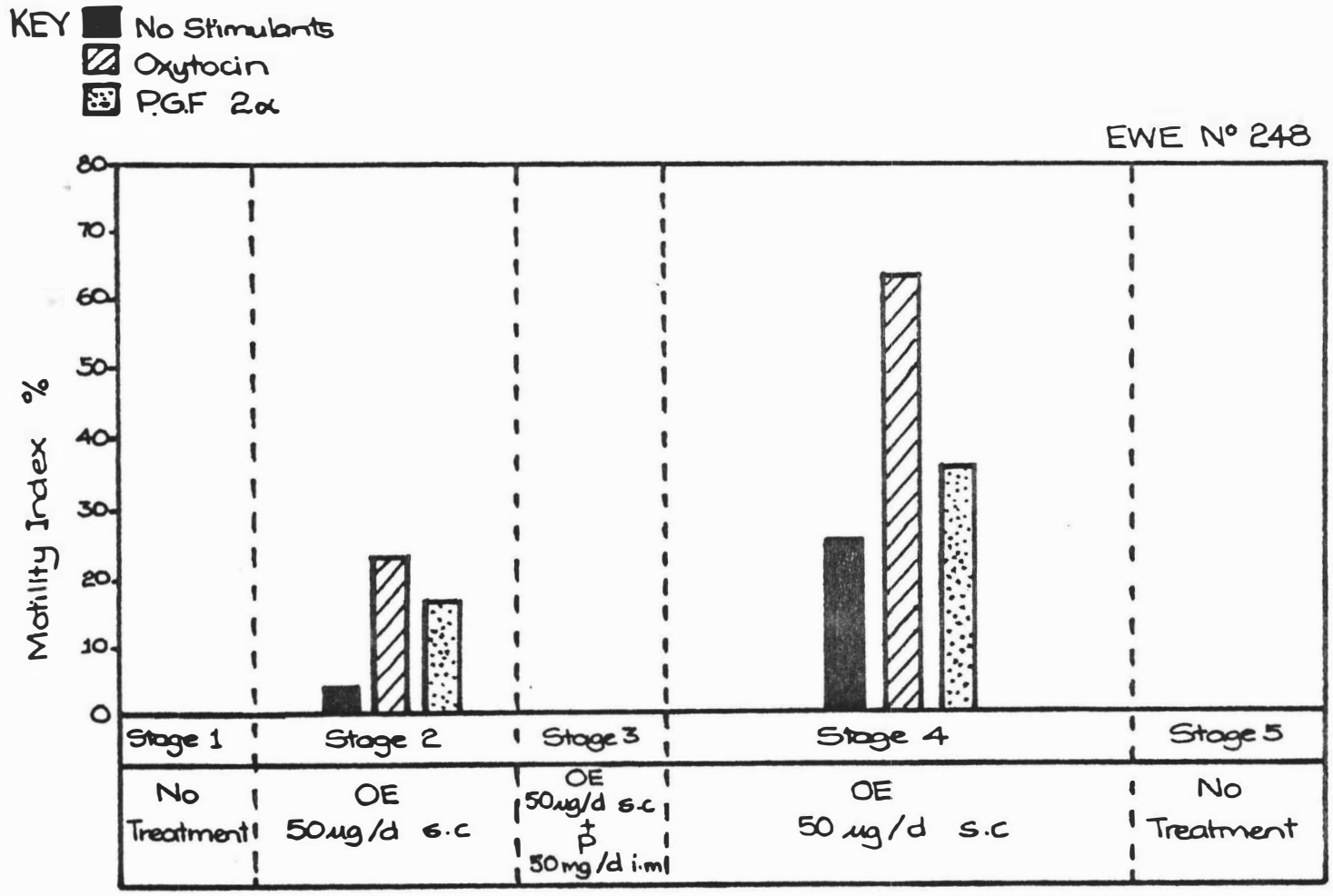


Fig. 22: **Motility Index during stages 2 and 4 of regime one.**

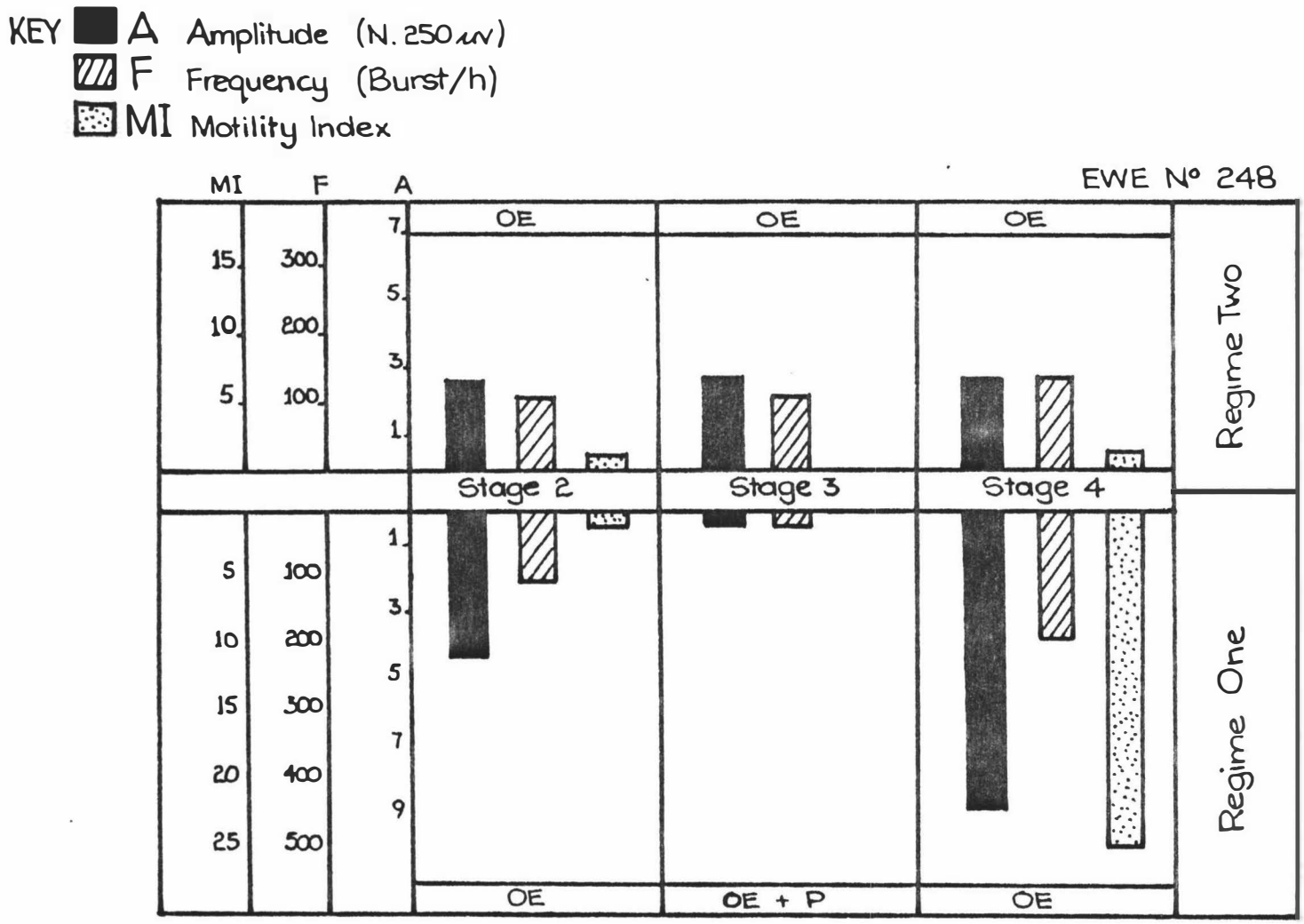


Fig. 23: **The characteristics of emg activity during stages 2, 3 and 4 of both regimes.**

Fig. 24: **Ewe 661 during dioestrus.**

(This ewe was marked by vasectomized ram on 23.3.88 and 8.4.88).

Trace A: recorded 10 days after being marked by ram

Trace B: " 11 " " " "

Trace C: " 12 " " " "

Bar a: active period (paper speed = 5mm per second and 0.05 per second).

Bar b: quiescent period.

Vertical bar is calibrated = 2500μv.

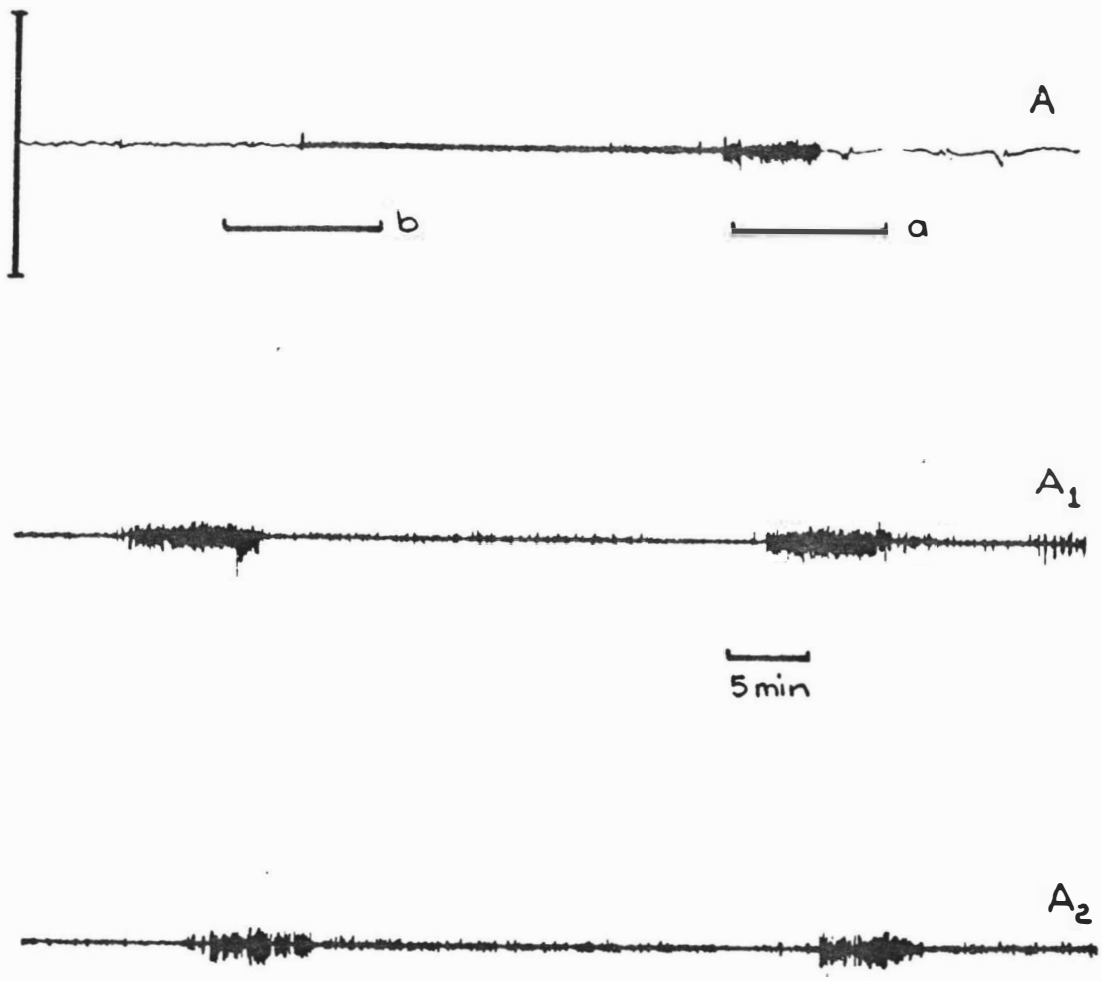


Fig. 25: **Ewe 661 during pro-oestrus.**

Trace A3: recorded 2000h 22.4.88 (marked by ram 8.4.88)

Trace B1: " 0800h 23.4.88

Trace B2: " 1000h 23.4.88

Chart speed = 0.05mm per second

Vertical bar = 2500μv

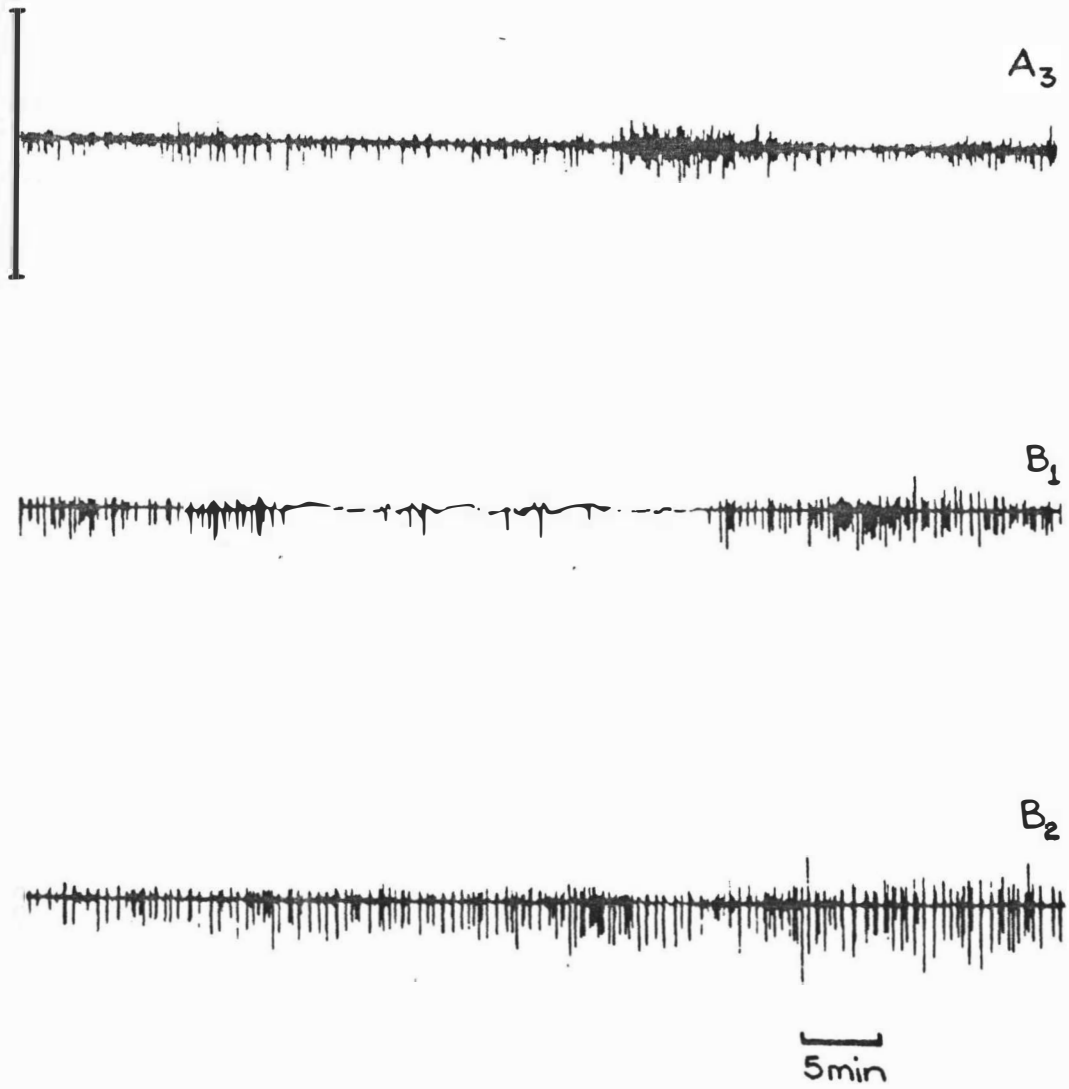


Fig. 26: **Ewe 661 during oestrus.**

Trace B3: recorded 1600h 23.4.88

Trace B4: " 0200h 24.4.88

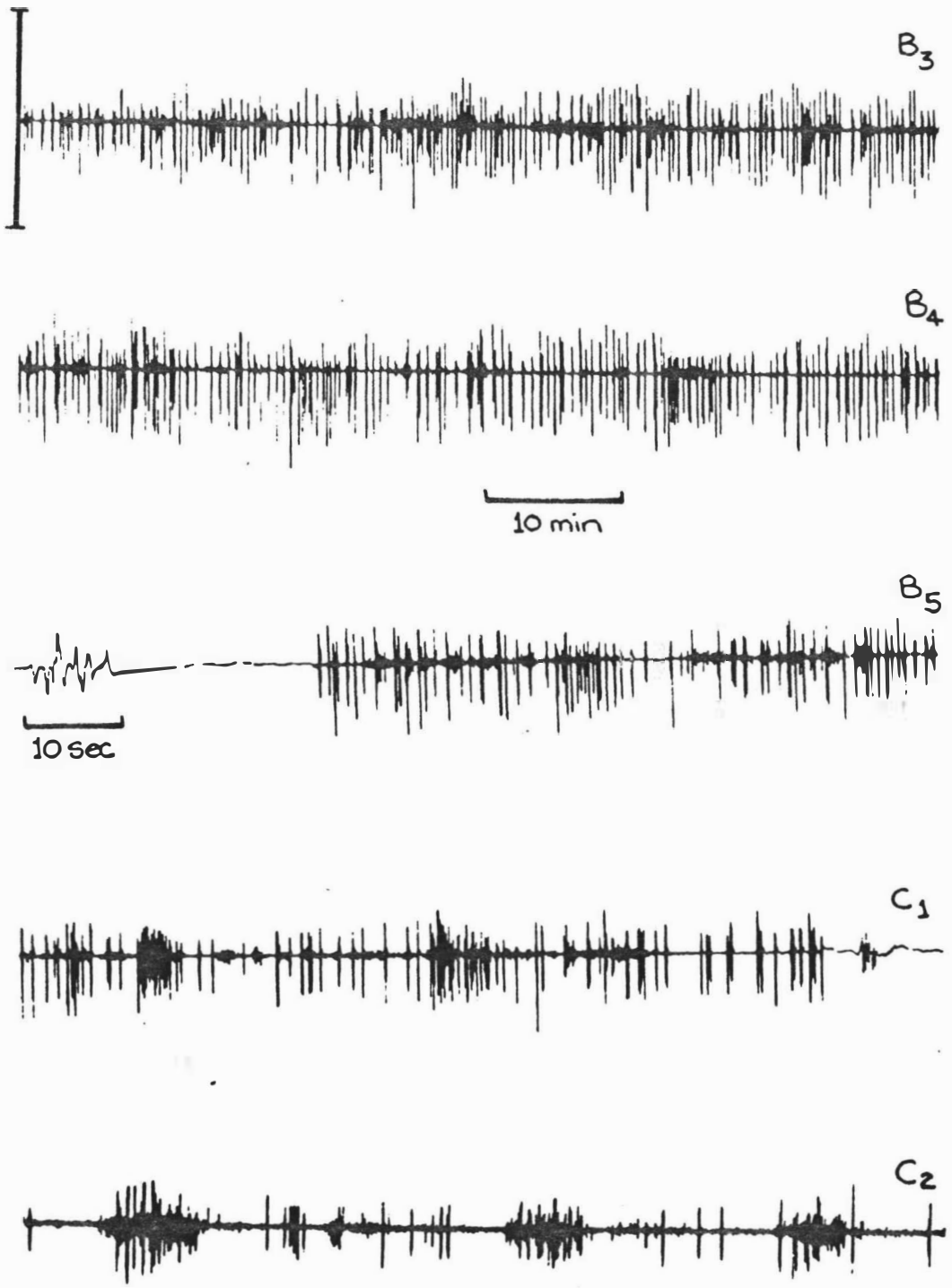
Trace B5: " 0800h 24.4.88

Trace C1: " 1400h 24.4.88

Trace C2: " 0300h 24.4.88

Chart speed = 0.05mm per second except where shown otherwise on the figure.

Vertical bar = 2500μv



As met-oestrus developed the bursts began to be grouped and these groups were further concentrated (Fig. 27, D1, D2, D3, D4). The met-oestral period occupied about 22h. Some single spikes appeared in between bursts and overall, the co-ordination of the activity diminished and the maximal amplitude recorded gradually decreased. This was followed over the next day by emergence of the typical pattern of alternating active and quiescent periods which lasted for about 2 days before the amplitude of bursts became undetectable and only single spikes were recorded during the short periods of activity (Fig. 28, E1, E2). The change in emg activity from the well co-ordinated oestrous type to the inhibited quiescent type that is characteristic of dioestrus occurred over about 40h.

The direction of propagation of action potentials during the 3-4 day active period in a natural cycle was mainly from the tubal end to the cervix. Activity may also be propagated in the reverse direction at this time however - particularly in the intervals between the highly active episodes (Figs. 29, 30). At the end of oestrus, emg activity recorded from the tubal end was much greater than that for the other three positions, as is evident in Fig. 30. Activity such as this may be associated with propagation both towards the oviduct and the cervix.

Fig. 27: **Ewe 661 during metoestrus/early dioestrus.**

Trace D1: recorded 0400h 25.4.88

Trace D2: " 0700h 25.4.88

Trace D3: " 1600h 25.4.88

Trace D4: " 0500h 26.4.88

Chart speed = 0.05mm per second

Vertical bar = 2500μv

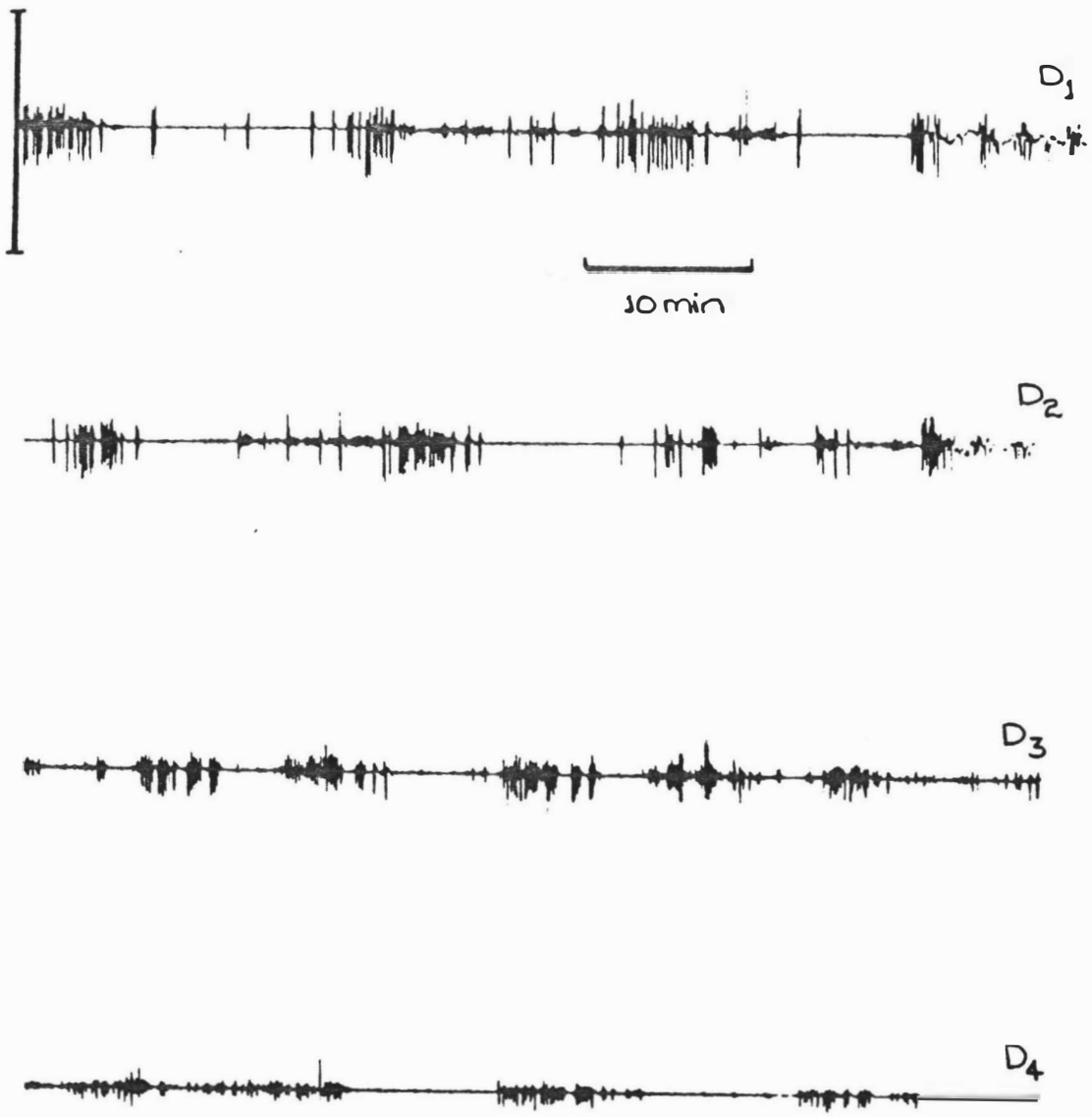


Fig. 28: **Ewe 661 during early dioestrus.**

Trace E1: Recorded 16700h 26.4.88

Trace E2: " 1700h 28.4.88

Chart speed = 0.05mm per second

Vertical bar = 2500μv

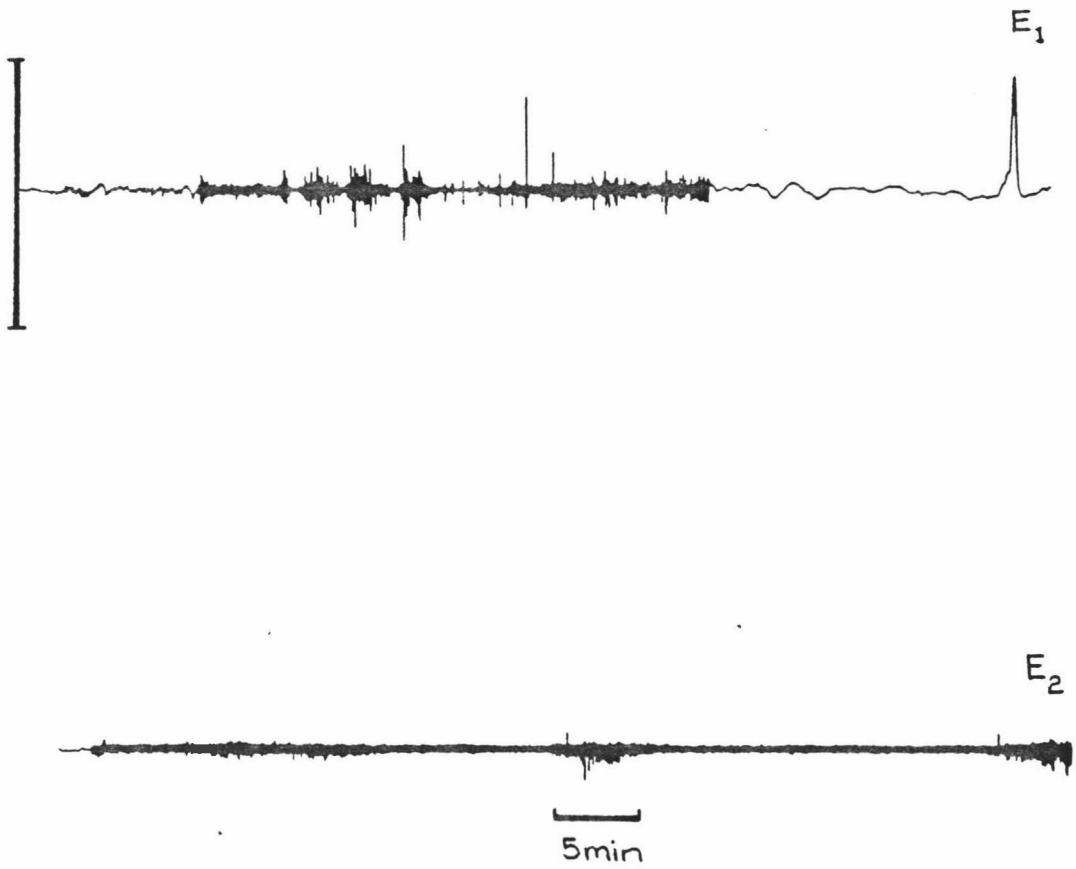


Fig. 29: **Ewe 661 recorded at mid-oestrus 8.4.88.**

Bar a: the active period where the propagated direction of emg activity is from tubal end of uterus to cervix.

Bar b: the quiescent period.

Chart speed = 0.25mm per second

Vertical bar = 2500μv

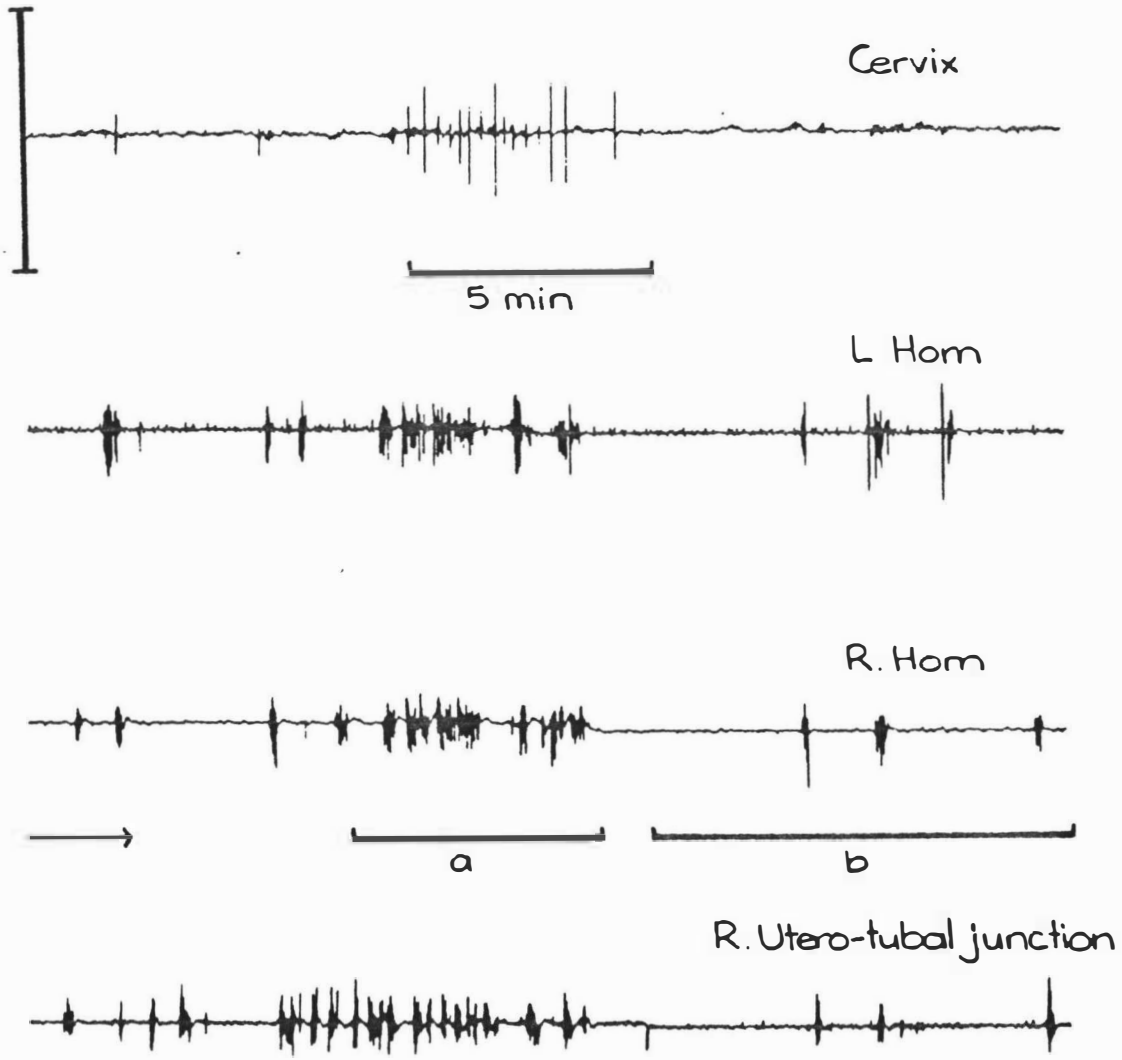


Fig. 30: **Ewe 661 recorded at late oestrus 9.4.88.**

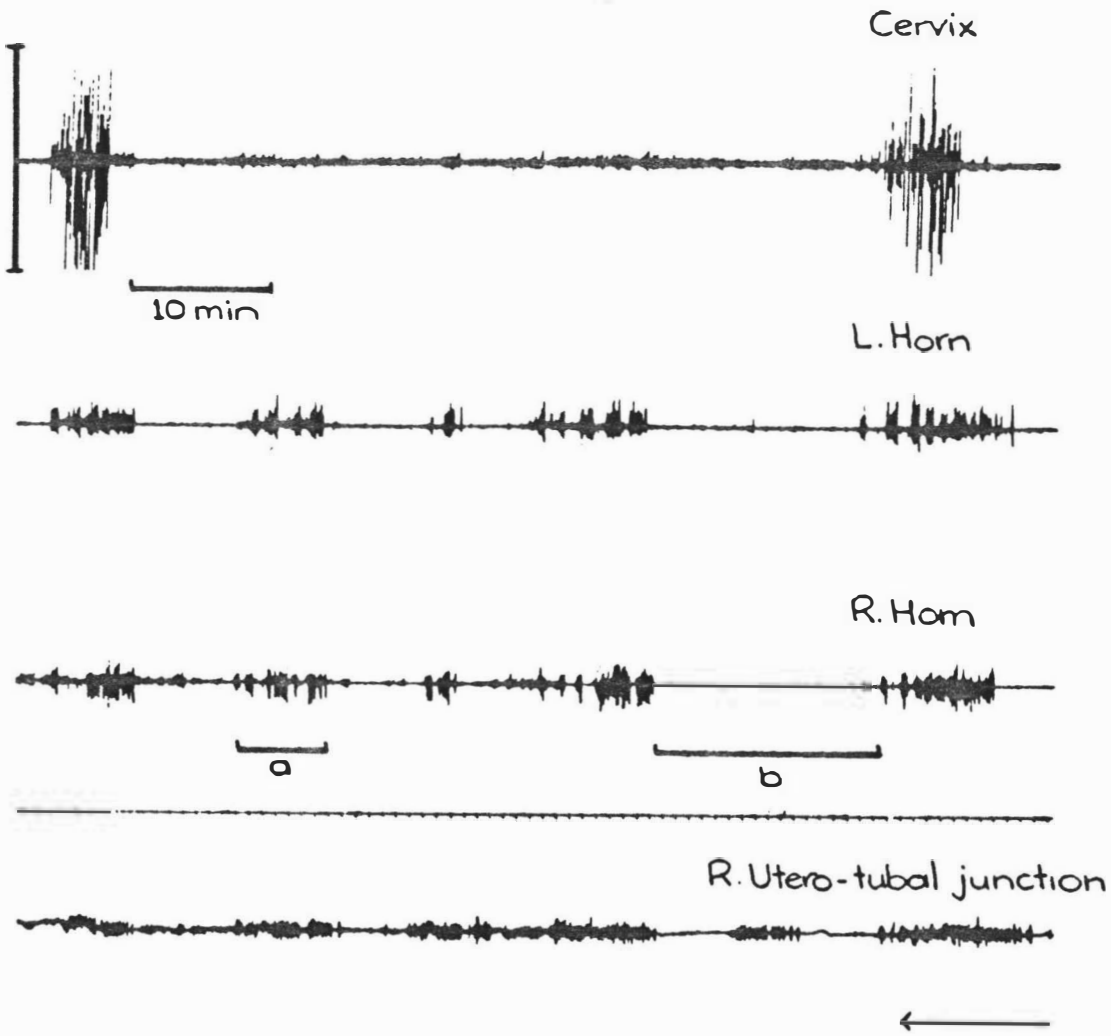
Note the burst frequency of tubal end is higher than other positions, more bursts are grouped and the intervals between episodes become longer than previously, and the direction of propagation of action potential is mainly from tubal end to cervix.

Bar a: active period.

Bar b: quiescent period.

Chart speed = 0.05mm per second

Vertical bar = 2500μv.



V. DISCUSSION

V.I. Emg Activity during Anoestrus and in the Ovariectomized Ewe

In the present study, a regular resting emg was recorded during anoestrus in both the intact (Fig. 2) and the ovariectomized ewe (Fig. 13). This resting emg was organized into myoelectrical complexes characterised by alternating phases of co-ordinated bursts and periods that were followed by a period of quiescence; during some quiescent periods diffuse single spikes or irregular uncoordinated bursts were observed from time to time.

The uterine emg activity recorded during anoestrus in the intact ewe was much greater than the basic pattern recorded from the ovariectomized animal as indicated in Figs. 2 and 13. This may be attributable to circulating endogenous oestrogen. Although the plasma oestrogen level during anoestrus is nearly the same as that during the luteal phase of the natural cycle (Rawlings et al., 1977) it may cause some uterine activity in the absence of any inhibitory influence of progesterone since corpora lutea which secrete progesterone are not formed until the end of the seasonal anoestrous period.

Although depressed compared with the intact ewe a regular pattern of myoelectrical activity appeared in the ovariectomized ewes (Fig. 13), even in the absence of any ovarian source of sex steroid hormones. This raises the possibility that such activity may be regulated by the autonomic nervous system. Owman et al. (1986) in various types of denervation experiments established that the uterus is innervated by fibres from adrenergic ganglion formations located in the vaginal wall or in the parametrial tissue outside the utero-vaginal junction.

The action of adrenaline and nor-adrenaline can be either excitatory or inhibitory depending on the dominant adrenoreceptor type present in the muscle cell. In myometrial smooth muscle, activation of a-receptors usually results in excitation whereas b-receptors mediate inhibition.

The stimulatory actions of epinephrine and norepinephrine are associated with depolarization, an increase in membrane conductance and accelerated spike discharge resulting in increased muscle tension. However, these stimulatory effects depend on the presence of Ca in the external medium. The inhibitory

actions of the adrenergic amine are characterized by a cessation of action potential discharge and a hyperpolarization of the cell membrane. B-receptor activation both produces membrane hyperpolarization and suppresses spontaneous pacemaker discharge. These effects arise from an increase in membrane conductance to K^+ or an increase in the potential gradient for K+ across the cell membrane and an increase in the K+ selectivity of the membrane. Another prominent action of b-adrenergic amine is stimulation of cellular metabolism. This stimulation is related to an increase in the level of cAMP within the muscle cell, which in turn activates a protein kinase that causes the binding of Ca to the cell membrane and to intra-cellular sites. There is also an increased outward extrusion of Ca across the cell membrane. In this way the amount of "activator" Ca available to the contractile elements is reduced and the muscle relaxes. The increased binding of Ca to the cell membrane is also believed to suppress the pacemaker electrical activity (Marshall, 1980).

Owman and Sjoberg (1977) showed that the adrenergic nerve supply to the genital tract in the female has functional properties that differ from those of the rest of the peripheral sympathetic system. A major difference in comparison with other sympathetically innervated regions is the remarkable sensitivity of these nerves to sex steroids. Oestrogen, for example, markedly increases the norepinephrine content in the individual adrenergic fibres, whereas progesterone causes a reduction towards normal levels (Falck et al., 1975). Experiments involving ovariectomy and reimplantation of ovarian tissue (Falck et al., 1974) have suggested that this control of adrenergic nerve function, as reflected in the transmitter levels is governed by the ovaries. Owman et al., (1986) showed that profound changes occur in the uterine adrenergic innervation in the course of pregnancy. The neuroeffector relationship becomes remodelled reflecting the unique sensitivity of the nerve supply to the action of sex steroid and there is progressive decay in the tissue norepinephrine content as pregnancy proceeds. This is first due to functional inactivation through reduced formation, uptake and storage of the transmitter substance. In the guinea pig myometrium the inactivation finally leads to structural degeneration and disappearance of the adrenergic fibres around the growing conceptus. Accordingly the myometrium enters a state of 'denervation supersensitivity' as parturition is approached (Owman et al., 1986). Marshall (1981) showed high levels of oestrogen correlate with

increased uterine norepinephrine concentration suggesting that the sympathetic nerves are also a specific target tissue for oestrogen action.

Roberts et al. (1981) demonstrated that progesterone increases the formation of b-receptors and Marshall (1981) demonstrated that it modifies the oestrogen-induced changes in myometrial sympathetic nerves specifically causing a reduction in norepinephrine content. Furthermore high levels of progesterone during pregnancy are associated with an almost complete loss of norepinephrine from the uterine sympathetic nerves.

It is thus clear from these reports that the uterus receives an extensive autonomic nerve supply which is very sensitive to ovarian sex steroids. The present experiments support the notion that the autonomic nerve supply may contribute to the basic pattern of uterine emg activity observed in the ovariectomized ewe. Although oestrogen enhances this pattern of activity in both frequency and amplitude, the basic pattern remains unchanged (Fig. 14).

Furthermore the experiments reported in this thesis highlight the inhibitory influence of progesterone on oestrogen-induced emg activity (Fig. 16a) and demonstrate that additional oestrogen administration results in an enhancement of activity to above the levels observed before progesterone was given (Fig. 16c). This phenomenon possibly reflects the potential of progesterone to stimulate uterine receptors as described by Wakeling and Wyngarden (1974) who showed that prostaglandin F2a and E2 receptors rather than being inhibited by progesterone appear to be increased in numbers at least in rats, hamsters and rhesus monkeys. Fuchs (1974) also found that progesterone enhanced the myometrial responsiveness to PGE1, PGE2 and PGF2a in rats.

V.2 The Direction of Propagation of the Action Potentials

The direction of propagation of the action potentials in the present experiments appeared to vary with the level of emg activity. There was a greater tendency for action potentials to conduct from the tubal end of the uterus to the cervix when the uterine emgs were very pronounced, e.g. when induced by oestrogen (Fig 4a, b) and during the periods of increased activity observed during oestrus in the cycling ewe (Figs. 29, 30). At other times, particularly when there is reduced activity, the dominant direction of

propagation of action potentials was cervico-tubal (Fig. 2). These results can be reconciled with those of Croker and Shelton (1973), Hawk (1975) and Rexroad (1978, 1980), who reported a predominance of contractions that moved towards the oviduct during early oestrus and in the opposite direction during late oestrus (this despite their experiments being conducted acutely with the animals under anaesthesia). Their so-called early oestrus may be the same as the period termed pro-oestrus in this thesis when the emg was not very active and before the episodes of increased emg had appeared. However, the results differ from those of Van Der Weyden (1983) and Prud'homme (1976), who reported that during the first part of oestrus most contractions were propagated towards the cervix whereas during the remainder of oestrus there was usually no predominance of activity in either direction. Van Der Weyden (1983) and Prud'homme (1976) also observed tubo-cervical propagation of oestradiol-induced emg activity in ovariectomized ewes as was the case in the present experiments.

Another feature of the current work was the finding that both the frequency and the duration of episodes with increased bursts were greater at the tubal end of the uterine horn (Figs. 4, 29, 30). The increased frequency and total duration of bursts at the tubal end probably represent activity that is not propagated along the entire horn and oviduct and may represent pacemaker activity that may be conducted in either a tubo-cervical or tubo-oviductal direction.

The observation that the direction of propagation of action potentials depends on the general level of emg activity suggests that the control mechanisms are necessarily complex. The following factors may influence the direction of propagation of the action potentials:

1. Propagation of emgs from the cervix to the tubal end at times of low levels of resting emg activity could be governed by the sympathetic nerve system.

   The sympathetic nerve supply to the female reproductive tract is complex (Owman et al., 1966, 1986) in that the ovary is supplied separately, whereas the adrenergic innervation of the oviduct, uterus and vagina is mixed to varying degrees in different species and

originates not only from the paracervical ganglia but also from the prevertebral inferior mesenteric ganglia. Furthermore Garcia-Villar et al. (1982) established that the ovine cervix has intrinsic spontaneous activity and suggested that cervico-tubally directed activity may well be triggered by directed activity from the cervix. Ruckebusch and Bueno (1976), however, argued that cervico-tubally directed electrical activity may spread along the oviduct, whilst on the other hand tubo-cervically directed activity sometimes appeared to originate in the oviduct.

In the present study both the frequency and the total duration of the bursts were invariably greater at the tubal end of uterine horn and the action potentials from this point spread in both directions, a finding which supports Ruckebusch and Bueno's argument.

2. The emg action potentials propagated in a tubo-cervical direction during oestrus and oestrogen-induced activity may be regulated by the levels of electrical activity from action potentials generated by many muscle cells. That means when the tubal end has higher emg activity with more muscle cells in a unit firing, the voltage at this point will be higher than at other positions. This higher voltage will force the electrical current to flow from the tubal end of the uterine horn in both directions and overcome the opposing spontaneously directed cervico-tubal activity governed by the sympathetic nervous system. Why higher levels of electrical voltage exist at the tubal end of the uterine horn but not at other positions where the emg activity is high, is not clear. It may be influenced by the following:

- A greater supply of blood vessels at the tubal end region than at other parts of the uterus, and therefore the area of tubal perfusion by steroids is greater.

- The blood perfused to the tubal end might contain higher concentrations of oestradiol. This has been suggested by Walsh et al. (1979), who demonstrated a counter-current exchange mechanism for progesterone between the utero-ovarian vein and the ipsilateral ovarian artery in non-pregnant and pregnant ewes. Such a mechanism may also be applicable to other

steroids, eg. oestradiol. Thus, tissue vascularized by the ovarian artery (i.e. the oviduct and cranial portions of the uterine horn) might be perfused with blood containing higher levels of oestradiol. In this way, the myometrial cells of the cranial part of the uterus may become more excitable (Marshall, 1980).

- The concentration of oxytocin and PG receptors induced by oestrogen or progesterone may be higher in this region than at other positions. Rexroad (1980) noticed that during oestradiol induced oestrus in ovariectomized ewes, the anterior part of the uterus contained a higher concentration of PGF2a than the posterior part. PGF2a may stimulate uterine activity during oestrus (Lye and Porter, 1978).

- The number of sex steroid hormone receptors may be higher at the tubal end than at other positions.

- The actions of the adrenergic nervous system may be more amenable to change by progesterone or other factors at the tubal end of the uterine horn than at other positions.

It seems that in late pregnancy, the tubal end is the only part of the uterus where functional adrenergic transmission may take place since adrenergic innervation has been eliminated through structural degeneration or neuronal inactivation in other regions (Owman et al., 1986). If this also happens in cycling animals, the nerve system of the tubal end may contain more receptors for oestrogen stimulation.

In summary, when the emg activity is low, the direction of propagation of action potentials is cervico-tubal and may originate near the cervix under sympathetic nervous stimulation. When the emg activity is very high, the propagation of action potentials is in both directions from the tubal end of the uterus. This is pushed by the higher bio-electrical potential which arises because of the greater number of muscle cells firing as a unit at this site when either plasma oestrogen is higher or progesterone is withdrawn.

V.3. The Influence of Oestrogen

In the present experiments, the stimulating action of oestrogen on myometrial responsiveness and emg activity was clearly shown during the anoestrous period in both intact and ovariectomized ewes. This effect probably arises from an oestrogen-induced increase in membrane potential which facilitates spontaneous depolarization by pacemaker cells (Marshall, 1980); an additional possibility is the stimulation of gap junction formation which could enhance conduction and synchrony between cells (Sims et al., 1982). Furthermore Mckerns (1977) reported that oestrogen stimulates the induction of specific RNA and protein synthesis in myometrial cells. This causes them to hypertrophy, with an increased synthesis of contractile protein, metabolic enzymes and ATP. Thus the influence of oestrogen on the myometrium includes membrane, metabolic and structural changes which promote excitation and contraction.

Fuchs et al. (1983) reported that a further oestrogen effect is the formation of membrane receptors for utero-tonic agents, principally oxytocin. This increase in receptor number lowers the threshold for stimulation and increases the response to a given dose by recruiting more units to contract. Thus the pharmacological excitability of the myometrium is increased.

During the development of oestrogen-induced emg activity, and the development of inhibition by progesterone and subsequent recovery from it, the emg response to exogenous oxytocin and PGF2a seems to be related to the emg activity prevailing at the time. When the emg activity is very high there is a greater sensitivity of the uterus to oxytocin and PGF2a. The emgs are of greater amplitude and spike frequency and the responses last longer. When the uterus is totally inhibited by progesterone, the action of oxytocin and PGF2a is short and only increases in the number of single spikes were recorded. Looked at from the point of view of clinical use of these stimulants, attention needs to be paid to the reproductive status of the animal at the time, or alternately, to pre-stimulation of the uterus using oestrogen to increase its sensitivity before either oxytocin or PGF2a is used.

There is some evidence that oestrogen is not always stimulatory in its response. Wakeling and Wyngarden (1974), for example, reported that oestrogen does not increase the receptors for PGF2a while Roberts et al.

(1981) observed that oestrogen actually decreased the receptors to PGF2a. The results described in this thesis do not support the findings of this group of workers as the ability of PGF2a to stimulate emg activity was increased greatly by single courses of oestrogen treatment in both intact and ovariectomized anoestrous ewes (Figs. 10, 15). Possibly mechanisms other than an increase in receptors are more important in achieving such an effect.

Reynolds as long ago as 1935 reported that prolonged treatment with high doses of oestrogen renders the uterus immotile and Fuchs (1978) described the same result with the surge of oestrogen during pro-oestrus in pregnant rats. They associated the phenomenon with high levels of oestrogen at a time when the uterus was quiescent. Downing et al. (1978) demonstrated the same inhibitory function of oestrogen in ovariectomized rats, while Lye et al. (1983) reported that spontaneous intrauterine pressure cycles were abolished for periods of several hours in ovariectomized non-pregnant ewes within 8h of an injection of 50μg oestradiol-17b. Following the quiescent period, a phase of intense uterine activity ensued and persisted for several hours before again being replaced by a period of quiescence. During the quiescent periods the uterus retained its responsiveness to oxytocin and PGF2a. Increasing the dose of oestradiol failed to prolong the period of quiescence. In the experiments reported in this thesis this inhibitory action of oestrogen on uterine emgs was also observed in the intact anoestrous ewe after a single injection of 3mg oestrogen.

The mechanism by which oestradiol exerts its biphasic action is unknown. The inhibition is possibly due to synthesis of an inhibitory substance in response to the oestrogen and the period of enhanced activity may arise from either prostaglandin secretion or an increased population of oxytocin receptors. It is very difficult, however, to postulate a mechanism which would account for the alternate occurrence of these two states. Lye (1983), from experiments in which oestrogen treated ewes were infused with the PG synthetase inhibitor meclofenamic acid, suggested that oestrogen induced patterns of activity were prostaglandin-dependent since both the high activity and quiescent periods were abolished by this drug. Lye and Challis (1982) have also shown that prostacyclin (PG12) can inhibit spontaneous uterine activity in sheep.

There are other mechanisms that could explain the responses seen. Catecholamines, for example, offer one such possibility although Downing and Porter (1980) found that neither depletion of uterine catecholamine stores with reserpine nor blockade of [alpha] or ß adrenoreceptors altered the extent or the time course of oestradiol inhibition in rats.

A further suggestion made by Porter (1979) was that oestradiol-induced inhibition of myometrial activity may be mediated by relaxin synthesized in the uterus. There is no evidence for or against the existence of such a mechanism in the ewe although relaxin inhibits the spontaneous activity of the ovine myometrium in vivo (Porter, 1979) and leaves the responses to oxytocin unimpaired.

V.4: The Influence of Progesterone

In the present experiments progesterone also exhibited a biphasic action - it not only inhibits emg activity by reducing amplitudes and eliminating burst appearance as is commonly accepted, but also has the potential action of increasing emg activity by enhancing the amplitude and the frequency of bursts after the emg activity recovers from its inhibition (Fig. 16c and 16d). In particular it shows an increase in the frequency of bursts during the recovery period compared with the period of inhibition. This was observed with both the induced emgs of the ovariectomized ewe and with the spontaneous oestrous emg of the normal cycling ewe (Fig. 8). This 'stimulatory' action of progesterone does not seem to have been described before in any species. The many experiments that have been carried out in this area appear to have been directed mainly at discovery and explanation of the phenomenon of inhibition and the effect of withdrawal of progesterone from blood plasma on the initiation of parturition.

The early studies of Csapo (1956) on the rabbit uterus demonstrated that the progesterone-dominated uterus is capable of contracting under (electrical) field stimulation, but that conduction between and recruitment of myometrial cells is functionally blocked. More recent reports have suggested other possible mechanisms for a progesterone-induced decrease in uterine excitability. For example, it has been demonstrated that oestrogen-induced gap junctions are inhibited in pregnant sheep until progesterone levels decline near term (Garfield et al. 1979).

Lye and Porter (1978) have rekindled the Csapo hypothesis that progesterone blocks myometrial activity during pregnancy and that withdrawal of this "block" is a prerequisite for parturition in sheep. In their experiments ovariectomized ewes were treated with oestradiol-17b for 3 days and then the oestradiol treatment was combined with progesterone for 3 days before finally the animals were again injected with oestradiol alone. Intra-uterine pressure records obtained from a water-filled balloon in the uterine lumen showed that spontaneous uterine activity declined to very low levels unless oestrogen treatment was given from the day of surgical intervention to implant the balloon. Under oestrogen treatment, spontaneous activity was marked, pressure cycles occurred regularly with a frequency of approximately 20 cycles each 10min. and reached a maximum amplitude of 20 to 50mmHg. In their animals oxytocin (500mu i.v.) and PGF2a (10μg/min into the uterine lumen) markedly stimulated uterine activity, particularly the frequency of the pressure cycles.

The effect of progesterone in the above experiments was quite profound. Intrauterine pressure cycles declined steadily in both frequency and amplitude (less than 10mmHg) the inhibition being maximal at 72 hours. Furthermore, the progesterone dominated uterus was virtually unresponsive to oxytocin and PGF2a. The authors concluded that "progesterone has a marked reversible, inhibitory action upon myometrial activity in the oestrogen-treated ovariectomized ewe and is consistent therefore, with the earlier reports that progesterone reduces myometrial activity". They also considered that the nature of the inhibition is very similar to that observed in the progesterone-treated rabbit and thus the response conforms to the 'classical' progesterone 'block' as first described by Csapo (1956).

The experiments reported in this thesis concerned with emg activity in the ovariectomized ewe provide further support to this classical progesterone block theory. Furthermore they contribute additional information by outlining the ability of progesterone to increase myometrial activity when it is presented with a declining background of oestrogen.

A number of reports have indicated that spontaneous activity is inhibited during the luteal phase of the cycle and suggest that progesterone produced by the corpus luteum exerts an inhibitory action on the ovine myometrium at

this time (Naaktgeboren and Van Der Weyden, 1973; Croker and Shelton, 1973; Ruckebush and Bueno, 1976). The investigation recording emg activity in a normal cycling ewe in this thesis confirmed these earlier findings and furthermore, revealed a much higher emg activity (specially in burst frequency) to be present during the period of recovery from progesterone inhibition, i.e. during pro-oestrus compared with met-oestrus (in the latter case the newly formed corpus luteum starts to produce progesterone).

The mechanism for an increase in emg activity when progesterone is given in the presence of a declining background of oestrogen in ovariectomized ewes is unknown. It might relate to the regulation of PG synthesis. Thorburn and Challis (1979) commented that oestrogen alone administered to the ovariectomized ewes for ten days failed to stimulate endometrial PGF production as judged by the concentration of PGF in the endometrium and the secretion of PGF into the utero-ovarian vein. In contrast treatment with progesterone for 10 days caused a significant increase in the endometrial PGF content and PG synthetase activity. In their studies, the greatest PGF production was achieved by combined progesterone (10 day) plus oestrogen (last 3 days) treatment. Following earlier experiments in sheep Liggins et al. (1973) reported that progesterone can prevent glucocorticoid-induced elevations of PGF concentration in uterine venous blood but not in the myometrium, an observation that suggests that progesterone might inhibit PGF release while both progesterone and oestrogen promote PGF synthesis.

Further work (Louis et al., 1977) has demonstrated that oestradiol treatment alone has no effect on the prostaglandin F concentration in uterine caruncles or inter-caruncular tissue in non-pregnant bilaterally ovariectomized sheep; this was also the case with the concentrations of PGF in the utero-ovarian vein or PGFM in the jugular vein. Oestradiol, however, did accumulate in the uterine tissue. Progesterone treatment, on the other hand, provoked a significant increase in the concentration of PGF in the caruncles, a significant increase in the release of PGF from the caruncles during incubation with arachidonic acid, and an increase in the mean concentration of PGFM in the jugular vein. When oestradiol was superimposed on a progesterone-primed system there was a further marked increase in the PGF content of the caruncles, release of PGF into the utero-ovarian vein, and increase in the concentration of PGFM in the jugular vein.

All these data are consistent with a requirement for progesterone in activating 'prostaglandin synthetase' activity, and promoting PGF production largely from the caruncles. After progesterone priming both the synthesis of PGF by the caruncles and PG release into the vascular system is increased whereas oestradiol treatment alone is without effect.

The mechanism by which the steroid hormones regulate endometrial PGF2a synthesis is of some interest. In rats (Boshier and Holloway, 1973) and in sheep (Louis et al., 1977) treated with exogenous progesterone, the endometrium contains large lipid droplets which may act as stores of precursors such as arachidonic acid for PG synthesis (Thorburn, 1977; Thorburn and Challis, 1979). The nature of this lipid is uncertain although preliminary observations suggest that it may be triglyceride. Thorburn and Challis (1979) suggest that progesterone may induce the enzyme required for the synthesis of this triglyceride containing arachidonic acid, while oestrogen may induce the acylating enzyme necessary for hydrolysis. The priming effect of progesterone may be not only on the 'prostaglandin synthetase' enzymes, but may be also related to the availability of fatty acid precursors in the uterus (Louis et al., 1977). Further than this, concurrent administration of oestrogen may lead to the labialization of lysosomes thus releasing phospholipase A2 which in turn may release arachidonic acid as a precursor for the 'prostaglandin synthetase' complex. This would explain the additive effect of progesterone plus oestradiol treatment. The proposition is supported by observations of lipid droplets in the endometrium following progesterone administration and by the decrease in the concentration of these droplets following the addition of oestradiol.

Another possibility that could explain why myometrial activity appears to be enhanced after recovery from progesterone inhibition, but with continuing exposure to oestrogen, comes from work by Louis et al., (1977) and Challis and Thorburn (1979). They showed that the ovine endometrium has the ability to concentrate oestradiol-17B presumably because of high levels of oestrogen receptors in progesterone treated animals - tissue levels may be many times plasma levels. Higher emg activity was observed in oestrogen plus progesterone treated animals than where oestrogen treatment alone was administered in the experiments reported in this thesis. The increased response could be explained as a consequence of the higher levels of

oestrogen in the endometrium that could have resulted from the administration of progesterone.

V.5: Uterine refractoriness

The decreased responsiveness of the uterus of the ovariectomized ewes to the treatments in regime two (see section p.83 of this thesis) is deserving of comment. Relatively high myometrial activity occurred during regime one of the experiment and the uterus appeared to take a considerable time to recover from this. Increased refractoriness of the cells of the myometrium through a reduction in receptor sites for hormones could be responsible for the change in sensitivity between the two regimes. Whether a similar response would have been observed in the intact ewe in either the anoestrous season or the breeding season was beyond the scope of investigations planned in this project. If such a phenomenon is widespread further experiments would need to be designed to determine the nature of such a response.

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APPENDIX I

EWE 329, 50 kg, surgery on 17.9.1987. A low mid-line incision 15cm long was made in the ventral abdomen. Electrodes were sutured into the myometrium at 2 sites (see p 51). One was on the dorsal body of the uterus about 3cm from the cervix and the other was on the great curvature of the right uterine horn about 80mm distal from the first point and 70mm distal to the utero-tubal junction. This ewe was not ovariectomized and recording began on 23.9.1987.

EWE 476, 45 kg, operated on 24.9.1987. Low-ventral midline incision. Electrodes were sutured on the uterine body, left horn, and the junction of the left horn with the oviduct (see p 52). This ewe was ovariectomized. Recording began on 10.12.1987.

EWE 248, 50 kg, surgery on 25.11.1987. Right side groin area incision 10cm length. This approach made it easy to find the uterus and exterminates. Three positions are ventral side of cervix, left horn and the junction of the left horn with oviduct (see p. 52). Recording began on 10.12.1987. This ewe was ovariectomized.

EWE 10, 55 kg, surgery on 26.11.1987. Right side groin area, incision 10cm length. Three positions are body, horn and the junction of the right left horn with oviduct (see p. 53). This ewe was ovariectomized. Recording began on 10.12.1987.

EWE 785 was operated on on 9.12.1987. 60 kg. Right side groin area incision 15cm long. Four positions were made. They were ventral cervix, right horn, right junction and right oviduct (see p. 53). She was ovariectomized. Recording began on 12.1.1987.

EWE 661, surgery on 11.2. 1988. 50 kg. Right side groin area incision 15cm long. Four positions were made. These were cervix, left horn, right horn and the right junction of the horn (see p. 51). Recording began on 23.3.1988 when the first oestrus occurred and the ewe was marked by ram. Recording ceased on 8.5.1988. During this period, the length of normal cycling in this ewe was about 17 days.