SOME EFFECTS OF OXYTOCIN ON

IN VITRO PREPARATIONS

OF UTERINE MUSCLE

BY

HUMPHREY GETHIN REES   D.V.M. N.D.A.

OF

Department of Animal Health

Massey University

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Signed . . . .

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INTRODUCTION

Diverse but related factors influence the initiation of parturition, Ganong (1959). Nervous, endocrine, and physical elements all converge synchronously to bring about the final act of labour, but the triggering mechanisms of parturition remain undefined.

This study was undertaken with a view to defining experimentally some of the factors that influence the onset of parturition. Initially, existing in vivo methods for recording uterine activity were considered as a means of demonstrating the onset of first stage labour in ruminants. If the time sequence of this phenomenon could be established, factors contributing to its onset would be examined in further studies.

External methods of measuring intra-uterine pressure in man such as the guard ring tokodynamometer described by Smyth (1957) are unsuitable for domestic animals due to anatomical differences. Internal methods using electrodes and microballoons, described by Csapo (1963), would be difficult to keep in place in animals and would require special recording equipment. Telemetric methods of recording myometrial electrical potential and intra-uterine pressure changes, as described by Dracy A.E. (1965 - personal communication) appeared most satisfactory for in vivo uterine recording, as the animal is not connected physically to stationary recording equipment.

However, all these methods of in vivo uterine recording required equipment and animals beyond the scope of resources available. This investigation has been limited therefore to an in vitro study on

uterine preparations isolated from laboratory animals. It has been designed to examine the effects of hormonal pretreatment and stage of pregnancy on isotonic response of the isolated uterine strip to synthetic oxytocin.

The objective has been an evaluation of factors that influence isolated rat uterus activity under standard conditions. The effects of various regimes of hormonal pretreatment on the in vitro response of uterine preparations to oxytocin are described. Initially these observations were made on preparations from ovariectomized guinea pigs, and later on preparations from ovariectomized rats. Subsequently observations were made on non-gravid horn preparations from hemi pregnant rats.

CONTROL OF UTERINE ACTIVITY AND ITS MEASUREMENT.

REVIEW OF THE LITERATURE.

A. UTERINE PHYSIOLOGY.

INTRODUCTION.

The fundamental mechanisms associated with physiological control of uterine action are not well understood.   There is good evidence that both nervous and endocrine systems are intimately concerned with uterine activity and that these are interwoven through the neuro-endocrine complex in the hypothalamus.   Many natural substances such as oxytocin, vasopressin, adrenaline and histamine are known to exert an effect on uterine muscle, most often stimulating, sometimes inhibiting e.g. adrenaline on the non-pregnant cat uterus (Reynolds, 1965).   It is well established that oxytocin is concerned in the birth process, and steroid hormones especially oestrogens and progestogens have been shown to influence uterine response to oxytocin. The effects of nervous factors, steroid hormones, and oxytocin that are associated with uterine action are discussed in detail.

I.   Influences of the nervous system on the uterus.

Although the uterus is innervated by sympathetic and parasympathetic fibres the effects of stimulating these nerves differs both between species and between individuals of the same species (Reynolds, 1965).   Even the various muscle layers of an individual uterus have been shown to respond differently (Gruber, 1933).

There are varying views as to the manner in which these effects are brought about.   Dale for example (cited by Gruber, 1933) thought that during pregnancy there was a change in the relationship of the nerve fibres so that motor fibres became dominant over the normally powerful inhibitory ones whereas Gruber considered that the muscle cells themselves became more responsive to the impulses from the motor fibres. Schofield (1952), working with the rabbit, found that stimulation of the hypogastric nerves caused first excitation of the uterus followed by inhibition, effects that could be imitated by adrenaline or nor-adrenaline. The nerve fibres concerned were thought to be adrenergic and as the effects of their stimulation could not be abolished by C5 or nicotine, probably post ganglionic.   Stimulation of the nervus erigens produced responses in only 25% of the rabbits examined.   Schofield suggested that the role of the uterine nerves was to control blood supply and glandular secretion in this organ.

In the rabbit, Cross (1958a) found that mechanical dilatation of the vagina induced sharp contractions of the uterus soon after parturition indicating a mechanism of motor innervation of the uterus triggered by pressure receptors situated away from the organ itself. He was able to demonstrate this mechanism in the non pregnant rabbit pretreated with oestrogen.   The responses resembled those produced by small doses of adrenaline or nor-adrenaline and survived thoracic spinal

transection yet were abolished by spinal anesthesia. Cross (1958b) concluded from this work that dilatation of the vagina in parturient rabbits activated sympathetico-adrenal and neurohypophyseal pathways capable of initiating uterine contractions. Cross also observed an absence of all somatic and autonomic reflexes with a reduction in foetal movements in pregnant rabbits to which either general or spinal anaesthesia was administered, yet delivery of the young in these cases, following physiological doses of oxytocin, compared favourably with oxytocin induced parturition in non anaesthetised does. This evidence suggests that oxytocin induced uterine response in this species is independent of nervous connections.

Csapo, Takeda and Wood (1963) and Csapo and Jacob (1963) also suggest that motor nerve innervation to the uterus can be triggered from pressure receptors within the uterus itself and they regard distension of the uterus as a vital factor controlling electrical and mechanical activity of uterine smooth muscle in both rabbits and man.

Summary of nervous effects.

It seems clear that the uterus can contract independently of the central nervous system and that parturition can proceed to completion in the absence of CNS control (Kurdinowski, 1904; Flemming, 1932). It appears equally clear that nervous connections can influence muscular activity at least in the rabbit uterus (Cross, 1958a), and that oxytocin has the ability to override this form of control (Cross, 1958b).

II.   Hormonal influences on the uterus.

1.   Oestrogens and progestogens.

Both classes of hormone are principally produced by the ovary

although other tissues appear capable of taking over this role, particularly during pregnancy, and both are intimately concerned with the whole reproductive complex. The three most common natural oestrogens are oestrone, oestriol and oestradiol - 17 beta and of these the latter is considered the most important (Gorbman and Bern, 1962). In addition there are many other intermediary steroids with oestrogenic properties. There are a number of progestogens, of which progesterone is the final oxidised product, with pregenalone and 17 alpha-hydroxyprogesterone as important precursors of other steroid compounds. It is often stated that oestrogen enhances uterine contractile power and progesterone has the reverse effect though this is probably an over-simplication (Schofield, 1952). Frank and Gustavson (1925) first demonstrated a stimulating effect of oestrogen on the myometrium in vitro though it is now realised that it is necessary to pretreat the uterus with the oestrogen, and a stimulatory effect is not seen when the oestrogen is administered to the uterus in vitro. The stimulatory effect is not an invariable one, however, and the response varies with the stage of the oestrous cycle, pregnancy, or pseudopregnancy. There may also be differences between species.

(i) The biochemical actions of oestrogens on uterine tissue.

These are not fully understood but the following biochemical effects have been recorded:

(a) Water content.

Oestrogens cause uterine tissue to increase its water uptake. This is said to occur as a result of enzymatic and electrolytic changes within the uterus itself (Velle, 1963; Csapo, 1956a).

(b) Ionic exchange.

Intracellular K^+ increases in uterine smooth muscle under the

influence of oestrogen, and this phenomenon may be responsible for increased water uptake (Csapo, 1956a).

In the rat single injections of oestrogen bring about an immediate increase in cell K^+ which reaches maximum 8 hours after injection. This increase is accompanied by a decrease in cell Na^+, which returns to a higher level 3 - 4 days after the oestrogen administration.

The Cl^- content of the uterus is proportional to the K^+ level that is present, but conversely the Cl^- and the Na^+ levels bear an inverse relationship (Burnstock et al, 1963).

(c)   Protein synthesis.

Amino acids are assimilated into the uterus under the influence of oestrogen, and thereby protein synthesis is increased (Velle, 1963; Schofield, 1954).

(d)   Muscle tonus.

"Plasma spaces" in the uterus contain actomyosin filaments which increase when oestrogen is added and which decrease on its withdrawal. Oestrogen has been regarded as the limiting factor in the synthesis and maintenance of this contractile substance (Csapo, 1962).

In the rat the effect of oestrogen on uterine smooth muscle is complex according to Gennel (1940). During the initial phase after ovariectomy uterine tonus is high and oestrogen at this stage reduces the tone and uterine contractions follow.

In the rabbit the uterus is unresponsive to oestrogen at the height of psuedopregnancy but becomes highly responsive one day after hypophysectomy or ovariectomy in the pseudopregnant animal (Velle, 1963).

(ii)   Effects of progesterone.

(a)   General effects.

Progesterone increases endometrial cellular response, e.g.

deciduomata formation in the event of mechanical stimuli being applied, but decreases the sensitivity of the myometrium to oxytocin. Thus local proliferation of the endometrium occurs under natural conditions when a blastocyst contacts the uterine lining at implantation (Boyd, 1959). The inhibitory effects of progesterone on uterine activity have been demonstrated in the pregnant rabbit (Csapo, 1963), and pregnant rat (Daykin P.W., 1966 - personal communication).

(b) Effects associated with oestrogen.

Progesterone with oestrogen is responsible for cyclic changes in the female reproductive tract of most mammals, and is concerned with uterine development during pregnancy. The rat, however, does not experience a functional luteal phase in its oestrous cycle, and progesterone may not be as important in the cyclic changes of this species (Eckstein and Zuckerman, 1956).

Progesterone following oestrogen priming in rabbits causes marked uterine hypertrophy, whereas oestrogen alone produces only limited uterine development. The proliferative effects of progesterone are antagonised however if oestrogen is given simultaneously. The sequential action of these hormones appears essential for their synergistic effect (Schofield, 1964).

(c) Influence on parturition.

Progesterone injections delay parturition in both the rabbit and the rat, when given close to term, Csapo (1956a) and Daykin P.W., (1966 - personal communication). An increase in Na^+ and a decrease in K^+ occurs within the myometrium as a result of these injections. This counteracts the depolarizing action of endogenous oxytocin on the uterus, and certainly under these circumstances the uterine muscle of the pregnant rabbit is

unable to contract (Schofield, 1964; Csapo and Corner, 1952). This is a total inhibition capable of preventing both spontaneous and induced labour and some change other than simple cessation of production is thought to be responsible for the removal of the progesterone block to parturition in this species (Schofield, 1964).

In the bovine on the other hand McDonald and Hays (1958) showed that progesterone injections given during the last month of the gestation period had no effect on gestation length, and in man, Pose and Caldeyro Barcia (1958) state that progesterone has no depressing effect on the myometrium, and that injections of this hormone do not prevent either premature labour, or parturition at term. The results of these workers show that production of progesterone is maximal at the time when normal spontaneous labour occurs in women, and indicate that a distinct species difference exists between women and rodents as far as the effects of progesterone administration are concerned.

2. The Polypeptide hormones.

Oxytocin.

(a) Posterior lobe function.

The posterior lobe of the pituitary, referred to as the neuro-phypophysis, is composed of the pars nervosa and the pars intermedia. In the absence of neural stimuli no hormones are released from either of these areas. Hormones extracted from the posterior lobe are octa-peptides produced in the supra optical and para-ventricular nuclei of the hypothalamus and they migrate along the supra-optico pituitary tract to the pars nervosa where they are stored. Afferent nerve endings in the hypothalamus are thought to secrete a substance, not yet identified, that can cause the release from protein bonding of these octa-peptides and their subsequent

release from storage in the posterior lobe (Nalbandov, 1964).

The mammalian neurohypophysis secretes two octa-peptides, oxytocin and arginine vasopressin (or in pigs lysine vasopressin), (Ferguson and Heller, 1965). Both oxytocin and vasopressins have several actions that may be pressor with rise in blood pressure, oxytocic with uterine contraction and ejection of milk, or antidiuretic resulting in the control of water balance in the body fluids. For each hormone, however, one action predominates and in the case of oxytocin the greatest effect is on contraction of the uterus. In its pure form the pressor and anti-diuretic effects are slight.

(b) Oxytocin action at parturition.

Experimental evidence from several species indicates that oxytocin is closely associated with the birth process. In rabbits, Cross (1958b) considered milk ejection by normal parturient does to be indicative of spontaneous labour. In these experiments Cross found that doses of oxytocin, which effected a prompt delivery of living young in parturient does under general and spinal anaesthesia, also produced the degree of milk ejection that was observed in the normal rabbits during labour. In addition he was able to show that the neurohypophyses of the normal does were depleted of oxytocin and vasopressin following parturition. This latter phenomenon has also been demonstrated in both rats and bitches (Dicker and Tyler, 1953a, b). In the goat Folley and Knaggs (1965) have also been able to demonstrate a rise in blood oxytocin at the time of parturition while van Dongen and Hays (1966) report detectable levels of oxytocin in the blood plasma within a few minutes of delivery in cattle. They suggested that initiation of calving was not dependant on oxytocic activity in the blood but that it did play a physiological role in expulsion of the calf.

In man, infusion of oxytocin at 1-8 mU/min. produces 1st stage

labour in near term subjects (Berde, 1959). But Hawker and Robertson (1957) failed to detect any increase in concentration of oxytocin in the blood of women in labour compared to the early stages of pregnancy. These workers point out however, that blood assays of oxytocin reflect the balance of secretion to destruction, and do not give an accurate check on the secretory output of the neurohypophysis in labour.

(c) Uterine response to oxytocin.

Characteristic uterine responses to oxytocin are long sustained slowly declining contractions, and at about the time of parturition an intravenous injection of oxytocin gives exactly the same kind of response as that seen during delivery (Berde, 1959). There is good evidence from assay work by many authors that within limits, the degree of contraction exhibited by pregnant and non-pregnant uterine strips in vitro, is in proportion to the dose of oxytocin administered (Cupps and Asdell, 1944: Csapo, 1956a; Holton, 1948; Stewart, 1949; Gaddum, 1938; Jung, 1961a).

Little is yet known about the precise biochemical mechanism of the uterine effect of oxytocin. The electrophysiological studies of Jung (1961b) indicate that the primary action of oxytocin on uterine muscle is the lowering of membrane potential. This is followed by a series of tetanic action potentials which are attended by mechanical contraction. With increasing doses of oxytocin the amplitude of the action potentials as well as the time that elapses before the peak potential is reached both decrease, but the muscle remains contracted long after the action potentials have died away. Evans et al (1958) suggest that the action of oxytocin on uterine muscle is not exclusively mediated via changes in membrane potential, for some uteri will respond to oxytocin even though their membrane potential has been subdued by immersion in potassium Ringer solution.

The pharmacological action of oxytocin can be summarised by

stating that its stimulating action on the uterus whether isolated or in situ is influenced by the species, the stage of the sexual cycle, the stage of gestation if pregnant, and other factors. The response of the isolated organ is markedly influenced by the concentration of Ca ions and to a lesser extent Mg ions.

(d) Species.

Several species have been used to study the effects of oxytocin on uterine smooth muscle, but much of the critical work on intact animals concerns the rabbit (Knaus, 1929, 1930; Assali et al, 1958; and Cross, 1958a, b).

Oestrogen dominance of the myometrium is essential for a satisfactory oxytocic response in rabbits. Progesterone on the other hand reduces the sensitivity of the myometrium to oxytocin administration (Knaus, 1929, 1930). Cross, (1958b) also working with rabbits, produced graded uterine contractions that coincided with increased infusion rates of an extract containing oxytocin.

Other species that have been studied include the guinea pig (Bell, 1941), the rat (Brunner et al, 1956, 1957; Evans et al, 1958). the cow (Fitzpatrick, 1957; Van Demark and Hays, 1952), and man (Cockrill et al, 1934; Chalmers et al, 1951; Berde and Cerletti, 1956; Caldeyro Barcia, 1958; Csapo, 1963; and Berde, 1959).

(e) Stage of cycle and stage of pregnancy.

Knaus (1929) found that with the entire rabbit after injections of luteal extract, the uterus failed to contract in the usual manner when posterior pituitary extract was administered. This worker then demonstrated that excised strips of rabbits uterus were insensitive to posterior pituitary extract if the animals had previously received injections of corpus luteum

extract or possessed functional corpora lutea in the ovaries (Knaus, 1930).

Bell (1941) found that a 50 mU dose of oxytocin was required to elicit one contraction in the guinea pig at the beginning of pregnancy but 1 mU was sufficient during the terminal states while Fitzpatrick (1957) found that the cow in mid-pregnancy required approximately ten times more oxytocin than a cow just before labour to produce equivalent contractions of the corpus uteri. The pregnant rabbit, however, behaves differently, since from the 2nd to 29th day of pregnancy it does not respond to oxytocin even if the hormone is administered in very large doses (Fitzpatrick, 1957).

Berde (1959) states that in pregnant women there is a marked increase in uterine sensitivity to oxytocin as term approaches and Fuchs and Fuchs (1963) found that both spontaneous contractability and in vitro response of human uterine strips to oxytocic stimuli vary with the phases of the menstrual cycle and during the period of gestation.

(f) Side effects.

The synthetic oxytocin, Syntocin, is pure oxytocin and consequently has less of the pressor and antidiuretic effects of posterior pituitary lobe extracts which contain varying proportions of vasopressin and other pharma-cologically active materials that can be extracted from the pars nervosa (Berde and Cerletti, 1956).

Oxytocin even in the synthetic form however, is not entirely without side effects in vivo, for Berde and Cerletti (1956) demonstrated its antidiuretic effect when used in high doses in man. In rats it causes increased excretion of water, sodium, potassium and chloride (Brunner et al, 1956, 1957), and the diuretic effect is brought out more clearly in thirsting rats and in rats loaded with physiological saline. Oxytocin has no diuretic effect in hypophysectomized animals, but if such animals are given NaCl and desoxycorticosterone they will exhibit a diuretic response. Brooks and

Pickford (1957) demonstrated the diuretic effects of oxytocin in the dog. Chalmers et al (1951) and Caldeyro Barcia (1958) have shown that rapid intravenous injections of high doses of oxytocin cause a transient and occasionally steep fall in blood pressure in man; and Ahlquist and Woodbury (1947) found that small doses of oxytocin cause vasodilation in the pregnant canine uterus.

Uterine spasm in conscious sheep and dogs has been reported following large doses of crude pituitary extracts with a high vasopressin content. The spasm is due to impaired circulation within the uterus resulting from intense myometrial contraction. Uterine ischaemia occurs when high doses of these extracts are used and compression of the blood vessels by the contracting myometrium is the suggested cause (Assali et al, 1958).

(g) Ionic influence.

Depolarisation of the myometrial cell membrane occurs when oxytocin is administered (Caldeyro Barcia 1960; Burnstock et al, 1963). Oxytocin increases the permeability of K^+ through the cell membrane. This lowers the membrane potential, and thus reduces the excitability threshhold of the cell (Caldeyro Barcia, 1960). The presence of oxytocin in late pregnancy, however, lowers slightly the resting potential of all myometrial cells and renders the uterus more susceptible to shock (Burnstock et al, 1963).

Repolarisation of the membrane occurs under the influence of progesterone which tends to reverse the K^+ gradient. This may be responsible for the antagonistic effect of progesterone pretreatment on oxytocin action (Csapo, 1956a).

Summary of hormonal effects.

The evidence indicates that both steroid and polypeptide hormones exert marked effects on uterine contractability. Pretreatment with

combinations of ovarian steroid hormones such as oestradiol and progesterone alters the degree of sensitivity of the myometrium apparently by altering the threshold at which depolarisation of the cell membrane occurs, and, depending on the particular steroid combination that is used, the effects of electrical, mechanical or polypeptide stimuli may be either enhanced or depressed (Csapo, 1956b).

Oestrogenic substances produce enzymatic and electrolytic changes in uterine tissue which alter ionic gradients and increase protein synthesis in myometrial cells. These changes tend to increase the susceptibility of the uterus to stimuli (Burnstock et al, 1963; Velle, 1963; Schofield, 1961).

Progestogens on the other hand influence similar processes but in some way not understood cause different effects. Under progesterone domination the uterus hypertrophies but contractability of the myometrium is suppressed, these affects varying in degree according to the amount of oestrogen that is present - in general terms both hormones acting synergistically promote both uterine development and activity. The inhibitory effect of progesterone alone on spontaneous activity of the pregnant uterus is quite conclusive for the rabbit but appears to vary in degree between the other species (McDonald et al, 1954; Schofield, 1961; Pose and Caldeyro Barcia, 1958; Csapo, 1963; Csapo, de Mattos and de Sousa Filho, 1963).

Both oxytocin and the vasopressins that can be isolated from the posterior pituitary gland have an oxytocic-like action on uterine smooth muscle but of these oxytocin has the greatest effect. Naturally occurring oxytocin, which contains elements of other pharmacologically active substances, possesses a number of side effects attributable in part to these substances while synthetic oxytocin possesses side effects only to a slight degree (Berde and Certletti, 1956; Gorbman and Bern, 1962).

The uteri of several species in both the pregnant and non pregnant

state show a graded response to oxytocin which is proportional to the amount of hormone administered (Bell, 1941; Evans et al, 1958; Van Demark and Hays, 1952; Caldeyro Barcia, 1958). This effect may be modified by the stage of the cycle as demonstrated by Knaus (1929, 1930) in the rabbit and Fuchs and Fuchs (1963) in the human or by the stage of pregnancy as demonstrated in several species. The threshold for oxytocin action on the uterus diminishes with advancing pregnancy and the hormone appears to play a vital role at the time of parturition (Berde and Cerletti, 1956; Cross, 1958b). It appears to exert its influence on the uterus by depolarising the electrical potential of the myometrial cell and synergistic action on the uterus by combinations of oestrogens and progestogens in suitable ratio can facilitate this effect (Caldeyro Barcia, 1960; Burnstock et al, 1963).

B. RECORDING UTERINE ACTIVITY IN VIVO.

I. External methods.

These have been described by Reynolds (1965) under the headings of Electrical and Mechanical Recording, but their use has been confined mainly to man.

These external methods do not appear to have a place in recording uterine activity in animals. Low electrical potentials of the uterus would be difficult to sense through the thick musculature of the ruminant abdomen, and might be confused with potentials emanating from the rumen (Reid et al, 1960). Equipment for recording mechanical changes is applied to the ventral abdomen in man with the patient in dorsal recumbency; this would be impractical in ruminant animals.

1. Recording of electrical potential.

Diphasic electrical currents appear all over the uterus during labour and synchronise with contractions. These electrical changes can

be detected by needle electrodes placed in the abdominal wall, or by an electrode implanted *per* *vaginum* high in the fornix.

2. Recording of mechanical changes.

This is carried out by means of a Tokodynamometer in man and the common type in use has been described by Smyth (1957). Basically these consist of flat plates which for a given force make a variable area of contact with the abdominal wall. Plethysmographs (Kornmesser and Nyboer, 1962) and a Tokometer applied to the cervix (Kelly and Schleifer, 1962) will also record mechanical changes.

II. Intra-abdominal methods.

These methods have been studied by Csapo (1963) in rabbits and in man, and in cattle by Gillette and Holm (1963). They involve the implantation of electrodes into the myometrium, and the introduction of pressure sensing balloons between the foetal placenta and the endometrium; both procedures require a laparotomy.

1. Electrical potential.

Silver electrodes are inserted tangentially into parturient uteri at various sites; for example, ovarian, fundic, and cervical regions of the myometrium. The free ends are passed to the outside and connected to a recorder.

2. Pressure changes.

Microballoons are inserted through a slit in the myometrium so that they rest between the placental membranes and the myometrium in the case of the Csapo method, but remain imbedded in the uterine musculature in the method used by Gillette. In both cases the free ends are connected to a transducer outside the animal and joined to a recorder. These methods

have produced data from which these authors can predict, to some extent, the time sequences of parturition.

Caldeyro Barcia (1958) has recorded amniotic pressure changes by introducing open ended catheters through the abdominal wall and myometrium into the amniotic sac in man.

All these intra-abdominal methods, however, have the disadvantage of connections between the subject and the recording equipment, which would make uterine recording in a natural environment impractical.

3. Telemetric methods.

These have the advantage of being able to sense and transmit to a receiver recorder, changes in electrical potential and pressure without connecting leads between the subject and the recording equipment. Prensky (1963) describes active and passive methods of telemetric recording. In the passive method "radar like" pulses are transmitted to an implanted oscillator in the uterus, and are recorded on their return by a receiver. Frequency changes in the message received is taken as an indication of uterine activity. With the active method voltage from the uterus is conveyed via electrodes to an oscillator secured on the animal's back which is capable of transmitting information that can be received by antennae surrounding the animal's pen.

Summary of in vivo methods.

All the methods of in vivo uterine recording that have been described involve both surgical interferences and extensive recording equipment. These two factors would have made it extremely difficult to obtain adequate results from large numbers of domestic animals.

It was decided therefore to confine this experimental investigation

to an *in vitro* study using preparations from larger numbers of laboratory animals. It was considered that knowledge gained from this type of study would be helpful in interpreting results from *in vivo* experiments to be carried out on a smaller number of farm animals at a later date.

C. RECORDING UTERINE ACTIVITY IN VITRO.

Basically two experimental approaches are concerned with *in vitro* studies on mammalian uterine preparations.

I. Partial severence of uterine connections.

(a) The tubal end of the uterine horn is detached from its connections at laparotomy, and the cervical end is anchored to an immobile rod inserted in the abdomen. The free end of the uterus is then attached to a kymograph lever by a piece of cotton thread (Gaddum, 1959).

(b) A platinum hook with cotton thread attached, is passed around the central point of a uterine horn. A plexiglass basin-like cover with an aperture in the centre of its base is inserted bottom upwards into the abdominal cavity via the laparotomy incision. The ovarian and cervical ends of the uterine horn are anchored at opposite points on the rim of the plexiglass cover. The cotton thread from the free end of the platinum hook is passed through the aperture in the base of the cover for attachment to a kymograph lever (Schofield, 1954).

In both methods the abdomen of the anaesthetised subject is filled with physiological fluid.

II. Total excision of the uterus.

(a) Portions of the excised uterus are assembled in a superfusion apparatus (Gaddum, 1953), in which the perfusing fluid flows over the uterine preparation continuously.

(b) The excised strip is immersed totally in a Magnus type organ bath containing physiological fluid (Gaddum, 1959).   This method with certain modifications was selected for this study and is described further under the chapters on experiments, materials, and methods.

In both variants of the total excision technique, one end of the uterine strip, the cervical end in the experiments carried out in this study, is attached to a stationary rod while the opposite end is attached by cotton thread to a kymograph lever.

Conclusion on in vitro methods.

As the experiments to be carried out required that a relatively large number of preparations should be examined within a short space of time to obtain the necessary between animal group comparisons, "Total Excision of the Uterus Method (b)" was considered to be the most suitable technique and this was the approach adopted in this study.

D.   PHYSICAL FACTORS INFLUENCING THE ACTIVITY OF UTERINE PREPARATIONS IN VITRO.

Robson (1933) stated that isolated muscle strips have the dis- advantages of an artificial environment, without the advantages of natural anabolic and katabolic connections, neither are there ovarian nor neuro- hypophysial connections.   Gaddum (1959) claims the Magnus method to be the simplest means of measuring the action of drugs on plain muscle.   The bath is kept at body temperature and oxygen or air is continuously bubbled through the bath.   The response depends on many factors, such as temperature, tension in the muscle, and ionic status.   Magnification due to the lever, frequency and magnitude of stimulation, oxygen supply, and source of energy are also factors that influence the extent of the response.

1. <u>Temperature.</u>

Harris (1947) has shown that within certain limits, increased temperature increases uterine activity while Burnstock et al (1963) also state that uterine activity is temperature dependent. Increased intracellular Na^+ is associated with loss of intracellular K^+ during dissection at room temperatures. Extrusion of Na^+ is accompanied by an uptake of K^+ when warmed again in a tissue bath. Raising the temperature by 10^oC polarises the membrane and abolishes spike discharge.

Fuchs (1966) suggests that lowering of the temperature decreases spontaneous uterine activity in the rabbit due to reduction in the influx of Ca^{++} while Holton (1948) showed that spontaneous activity of the rat uterus could sometimes be overcome by lowering the temperature of the bath. Csapo (1954) found the duration of isotonic contraction cycles of the rabbit uterus decreased as the temperature was lowered, but found isotonic shortening of the unloaded uterus to be insensitive to temperature when tension was zero.

Isometric tension of isolated uteri is practically zero at 10^oC, but increases in a straight line relationship with temperature up to 30^oC. Between 30^oC and 40^oC the tension temperature curve becomes asymptotic with maximum effect at 40^oC. In Csapo's experiments however, isotonic shortening of the unloaded uterus had no temperature dependance between 15^oC and 40^oC; at 15^oC isotonic shortening could be maximal but tension minimal.

2. <u>Tension, isometric and isotonic contraction.</u>

The tension, or resistance to stretch, of individual uterine preparations varies with many circumstances, especially hormonal and ionic status. Csapo (1954) states that isotonic shortening of the loaded uterus

refers to all the intermediate states between shortening of unloaded muscle (no resistance) and isometric tension (maximum resistance), the variable parameter being the load.

Burnstock et al (1963) state that spontaneously active smooth muscle is sensitive to stretch and responds to stretching by depolarisation the membrane response being roughly parallel to that occurring during the onset of spontaneous contraction. In both cases there is a fall in membrane potential. Csapo (1954) working with rabbits, found that if slight stretch was applied, the uterus would adapt itself to the new length up to a certain point, first by a small increase and later by a decrease in the resting tension. If this limit of adjustment was over-stepped, the resting tension, indicated by a slight elevation of the tracing above the base line, would become significant and would increase by subsequent stretch.

Isotonic shortening, unlike isometric tension, can be maximal in a case of partial activation of the preparation and does not therefore reveal the proportion of activated and non-activated units and Csapo concludes that isotonic shortening of the unloaded uterus gives no quantitative information about the functional state of the system. Similarly Schofield (1954), working with intact rabbits, concluded that the isotonic method may record maximum activity when a few muscle cells are contracting, but the isometric method records a maximum effect only when all contractile units are involved and is sufficiently sensitive to distinguish between contractile capacities of individual units under different conditions. She claims that isotonic methods previously used to record uterine contractions are grossly inaccurate (Schofield, 1961).

Csapo and Corner (1952) showed that the "Staircase Phenomenon", which is a gradual increase in tension after stimulation has begun, can be measured by isometric but not with the isotonic methods. The "Staircase

Phenomenon" is of particular value in measuring hormonal effects on uterine tension. Oestrogen increases tension or gives a positive staircase effect that can be reversed by progesterone.

An isotonic apparatus can be modified for isometric recording by the incorporation of a strain gauge. One end of the instrument is attached to the muscle strip so that the preparation is assembled under a known degree of tension. The strain gauge is then attached to either a kymograph lever, an electric recorder, or to an oscilloscope. Fluctuations in isometric tension can thus be identified for each stimulus that is applied. Such a modification was not available for this work.

3. Ionic status.

From what has previously been described, it is clear that most factors influencing uterine activity exert their influence through changes in ionic status of the smooth muscle. It is well established that contraction of both smooth and striated muscle is associated with changes in ionic balance between the muscle cell and its environment. It would be expected, therefore, that the ionic balance of the medium used in in vitro studies on the uterus would markedly influence the behaviour of the preparation.

(i) Effects of different ions.

(a) Potassium ions.

Jung (1961) showed in rats that in vitro uterine contractions bear a direct relationship to the concentrations of extracellular potassium. Burnstock et al (1963) state that an inverse relationship exists in most species between resting potential and external K^+ concentration. Thus an increase in external K^+ would be expected to cause a depolarization and a decrease in external K^+ a hyperpolarization. Schild (1966) states that

smooth muscle can be completely depolarized by immersion in "high potassium Ringer solution" and such a preparation is incapable of generating or conducting electrical impulses but retains its ability to respond to stimulant and relaxant drugs.

(b) Sodium ions.

Evans et al (1958) showed that the in vitro electrical potentials of rat uteri were considerably subdued when the Na^+ content of the Ringer solution was removed and replaced with K^+. Uteri treated in this way, however, were still sensitive to stimuli.

On the other hand, Csapo (1956a), working with rabbits, reports that increases in Na^+ content of Ringer solution reduces the sensitivity of in vitro myometrial strips.

(c) Calcium ions.

Low concentrations of Ca^{++} produce a contractile response in uterine muscle that increases as concentrations of the ion are raised (Schild, 1966). If, however, Ca^{++} is removed from a preparation, myometrial tension drastically declines (Csapo, 1956a). Spontaneous activity of isolated rat uterus in standard Locke solution declined when the Ca^{++} level was reduced according to de Jalon, Bayo and de Jalon, (1945 cited by Burn et al, 1950).

(d) Magnesium ions.

Schild (1966) found that the Mg^{++} can have either an antagonistic or a synergistic influence on the effects of certain other ions. The positive or negative relationship of Mg^{++} in these instances depended upon the relative proportions in which the other ions were present.

(ii) Ionic inter-relationship.

The contractile response of the uterus to Ca^{++} was antagonised by

Mg^{++} when the Ca^{++} levels were low.   This response was potentiated by Mg^{++} when Ca^{++} levels were high (Schild, 1966).

Increases in intracellular K^+ are accompanied by a fall in Na^+. An inverse relationship also exists between Na^+ and the Cl^- according to Burnstock et al (1963).   These authors state that the Na^+ K^+ Cl^- relationship of in vitro uterine preparations is dependent upon the hormone levels to which they have been subjected.

(iii) Ion-hormone inter-relationship.

(a) Oestrogens.

Burnstock et al (1963) states that in work with rats, intracellular K^+ increases in the uterine smooth muscle under the influence of oestrogen and that this is accompanied by a fall in Na^+.   In this species, single injections of oestrogen bring about an immediate increase in cell K^+ and an increase in cell Cl^- which reaches a maximum 8 hours after injection.   Four days after the single injection the Cl^- concentration is low and the Na^+ content high.

Csapo (1956b) states that in rabbits when Ca^{++} is removed the myometrial tension drastically declines if the uterus is dominated by oestrogen - but very little change occurs if dominated by progesterone.

Best and Pickles (1963) state that Mg^{++} content of the human uterus is increased by oestradiol.

(b) Progestogens.

Csapo (1956b) states that in the rabbit, the progesterone dominated myometrium has a membrane potential close to critical value in normal Krebs solution.   If its membrane is further depolarized by increasing the K^+ outside only contracture increases, and active tension is not developed. Under progesterone domination a strong Ca^{++} complex is formed which cannot

be washed out of the muscle - and the muscle remains unchanged if Ca^{++} is removed from the environment.

Burnstock et al (1963) state that in rabbits and in man the progesterone dominated uterus either binds Ca^{++} more firmly or more Ca^{++} has to be displaced before excitability is lost while Csapo (1956b) states that all the effects of progesterone can be explained by increases in Na^{+} and decreased K^{+} content of the myometrial cells and probably by a more stable Ca^{++} complex in the membrane or in the myoplasm; but which of these three alterations is the limiting and first reaction, and how it is influenced by progesterone is not known.

(c) Oxytocin.

Stewart (1949) has shown that uterine sensitivity to oxytocin in the guinea pig can be increased by extra Mg ions. Evans et al (1958) showed that the rat uterus will still respond to oxytocin even though all the Na^{+} has been replaced by K^{+} and the membrane potential consequently subdued.

4. Technique.

(a) Magnification of the lever.

Some magnification of response is essential to the accuracy of measurements made from the records of isotonic uterine contractions. The extent of this magnification may vary on occasions between experiments. However, a four-fold magnification of the lever has been adopted commonly for the recording of isotonic uterine contractions (Holton, 1948; Gaddum, 1953).

(b) Frequency and magnitude of stimulations.

Because of fatigue, in vitro uterine preparations tend to contract

to a lesser extent following an increase in the frequency of stimulation. Contractions following responses to maximum stimulants are reduced for the same reason. In oxytocin assays the interval between administrations has been five minutes in the rat method, and ten minutes for that of the guinea pig (Holton, 1948; Gaddum, 1953; Stewart, 1949).

(c) Oxygen supply.

An inadequate oxygen supply curtails markedly the activity of in vitro uterine strips (Gaddum, 1953). An oxygen supply above apparent optimum does not increase uterine activity unless by rate of flow it is allowed to stimulate the preparation by mechanical means (Harris, 1947).

(d) Source of energy.

Ringer and Dale, (cited by Harris, 1947), found in vitro uterine preparations to be dependent upon an available source of carbohydrate for maximum activity and more recently de Jalon, Bayo and de Jalon (1945 - cited by Burn et al, 1950) showed that the spontaneous activity of the isolated rat uterus in standard Locke's solution could be markedly reduced by reductions in the glucose content of the standard solution.

Summary of the physical factors affecting in vitro preparations.

It is apparent that numerous inter-related factors influence the activity of in vitro uterine preparations. These factors have been mentioned under the headings of temperature, tension, ionic status and technique.

The effects of all these factors apart from those discussed under technique can be explained according to Csapo (1956b) by an increase or decrease of Na^+ and K^+ within the myometrial cell, with or without the presence of Ca^{++} complex at the cell membrane capable of exerting a stabilizing effect

upon this exchange. An alteration in the concentration of any of these three ions can be the limiting and first reaction. The way in which such an alteration is influenced by temperature, tension, or hormonal action is not fully understood (Burnstock et al, 1963).

In experiments with in vitro uterine preparations therefore, it is essential to keep temperature, tension, and hormonal levels constant. The Ringer solutions used must be of an identical formula with all ingredients made up daily in constant proportions (Harris, 1947). It is equally important that the other aspects of technique mentioned must be kept as constant as possible if any conclusions are to be drawn from in vitro preparations either within or between groups of experimental subjects.

EXPERIMENTS WITH GUINEA PIGS

A. Introduction

The complexity of myometrial function was outlined in the review of literature, and this indicated certain gaps in our knowledge of the physiological events leading up to parturition. Reports of foetal expulsion from perfused gravid uteri in the absence of all nervous and vascular connections, suggest that intrinsic mechanisms are responsible for increased myometrial activity in the terminal stages of pregnancy.

Oxytocin however, is known to cause uterine smooth muscle to contract in most species; and in the non-pregnant mammal its effect is accentuated greatly if administered during oestrus. Clearly certain hormonal influences associated with the oestrous cycle affect the degree of oxytocin response. It became important therefore to examine the effect of hormonal interactions on the contractability of uterine smooth muscle before interpretations could be made from parameters recorded at specified stages of pregnancy.

A supply of female guinea pigs was available, and *in vitro* experiments to examine the effects of oxytocin on uterine preparations from specimens of this species were performed.

B. The Techniques Used in Experiments on Guinea Pigs

A total of 35 mature female albino guinea pigs bred in the Massey "floor colony", were isolated from males from birth onwards. When sufficient numbers over 400 g in weight became available for experiment, bilateral ovariectomy was carried out under "nembutal anaesthesia". Aseptic techniques were adopted and approach to the ovaries was made by incision through both flanks. Each guinea pig was identified numerically with a metal ear tag when under anaesthesia.

1. Housing and Feeding:

The animals were kept on wire floor cages in a room maintained at 65°F. They were fed a diet of commercial pellets supplemented with carrots and cabbage.

2. Treatment:

Subcutaneous injections of oestradiol monobenzoate in oil *(B.D.H.). progesterone in oil *(Organon) and sterilised arachis oil were given for assigned pretreatment periods.

3. Examination of the Specimen:

Fifteen minutes prior to examination the guinea pigs were stunned, decapitated and the abdomen opened along the linea alba. The uterus was dissected out and cut free at the cervix. The right horn was identified with a metal clip placed on its ligamentous attachments at the anterior end, and the entire excised uterus placed in room temperature Ringer solution where it was trimmed free of fatty material. The Ringer solution was prepared in accordance with British Pharmacopeia (1948) directions for oxytocin assay as recommended by Stewart (1949), and this same formula was used in all the guinea pig experiments described.

The selected uterine horn was cut at its junction with the cervix and a platinum hook attached to the cervical end. This was then attached to the oxygen inflow tube of a Magnus type organ bath containing Ringer solution at 31°C. The tubal end was attached by cotton thread to a kymograph lever. Oxygen, under 90 mm pressure, was allowed to bubble into the organ bath at a slow but constant rate that did not disturb the assembled tissue.

* B.D.H. = British Drug Houses.
* Organon = Organon Laboratories.

Weights of the animals were measured on an Avery scale which was sensitive to 0.2 g. Uterine strip and adrenal weights were measured on a torsion balance sensitive to 0.001 g.

(i) Apparatus:

A 50 ml Magnus type organ bath, with a two-way tube at its base, was connected at the inflow to a coil of glass tubing containing three times the Ringer solution volume necessary to fill the organ bath. The outflow was connected to a rubber tube leading to the sink. This Magnus bath and its glass coil, whose opposite end was connected to an external reservoir of Ringer solution, was immersed in a water jacket maintained at $31^{\circ}C$ by a thermostatically controlled immersion heater.

A kymograph lever was mounted on a retort stand so that its fulcrum was situated anterior to the organ bath with the frontal writing point touching lightly on the smoked kymograph paper.

(ii) Attachment of Specimen:

The lever was balanced to give a four-fold magnification. A cotton thread joined to the tubal end of the uterine horn was attached to a predetermined point on the lever posterior to the fulcrum by means of a 1 g piece of plasticine so that the specimen was stretched by a force of this amount. One hour after assembly the temperature of the bath was raised to $37^{\circ}C$, the lever returned to the horizontal position, and its cotton attachment to the uterine strip tightened accordingly.

(iii) Administration of Oxytocin:

"Syntocinon", a proprietary preparation of synthetic oxytocin (Sandoz Ltd., Switzerland), was diluted with distilled water to concentrations of 0.0001 mille-units (mU) to 50 mille-units (mU) in 1 ml.

The oxytocin doses were placed in test-tube racks immersed in the water jacket and held there for at least 15 minutes prior to administration. These doses were administered for 1 and 2 minute periods prior to washing, by injection of the prewarmed solution into the organ bath taking care not to disturb the preparation.

The organ bath was washed twice with fresh Ringer solution at $37^{\circ}C$ at the end of each administration period, and again before the beginning of the next, 10 and 15 minute periods separating the administrations.

4. Assessment of Response:

The kymograph papers were varnished and dried. The amplitude of the contractions recorded on the drum were measured in centimeters. The greatest amplitude recorded for each dose of oxytocin was taken as the index of response to the particular dose.

5. Statistical Analysis:

Analyses of variance (Snedecor, 1956) were used to compare the within animal and between animal variation, and to estimate the possible significance of treatment effect. Statistical analysis was not carried out on the first preliminary experiment. This was run to determine the oxytocin dose range most suitable for experiments that followed.

C. Experiment I.

"To establish a range of oxytocin doses that could be selected to stimulate ovariectomised guinea pig uteri after exogenous hormonal treatment."

1. Method

      Twelve guinea pigs ranging from 428 - 780 g weight were ovariectomised a minimum of 4 days prior to treatment. Four pigs were allotted randomly to each of the following treatments.

Progesterone	5 mg and Oestradiol 10 μg daily for 7 days
Progesterone	5 mg daily for 7 days
Oestradiol	10 μg daily for 7 days
Arachis oil	(Control) daily for 7 days

      Treatments were staggered so that one pig became available for examination daily on four consecutive days in each of three consecutive weeks.

      Oxytocin doses, ranging from 0.0001 mU to 50 mU were used on the first representative of each pretreatment, 0.0001 mU to 100 mU on the second, and 0.0025 mU to 50 mU on the third. Oxytocin doses were applied for two minutes in each case before the bath was washed, and fifteen minutes was allowed to elapse between each administration.

2. Results and Conclusions

      It was observed that the uteri from the control (Arachis oil) group showed spontaneous contractions and contracted markedly in response to Oxytocin; the effects of the arachis oil injections and the variability in the period between ovariectomy and experiment were considered possible reasons for this activity. It appeared that the threshhold dose of oxytocin was higher in the progesterone - oestrogen treated animals than in the others. It was concluded that:

(a) Administration of oxytocin for one minute was adequate to elicit a response.
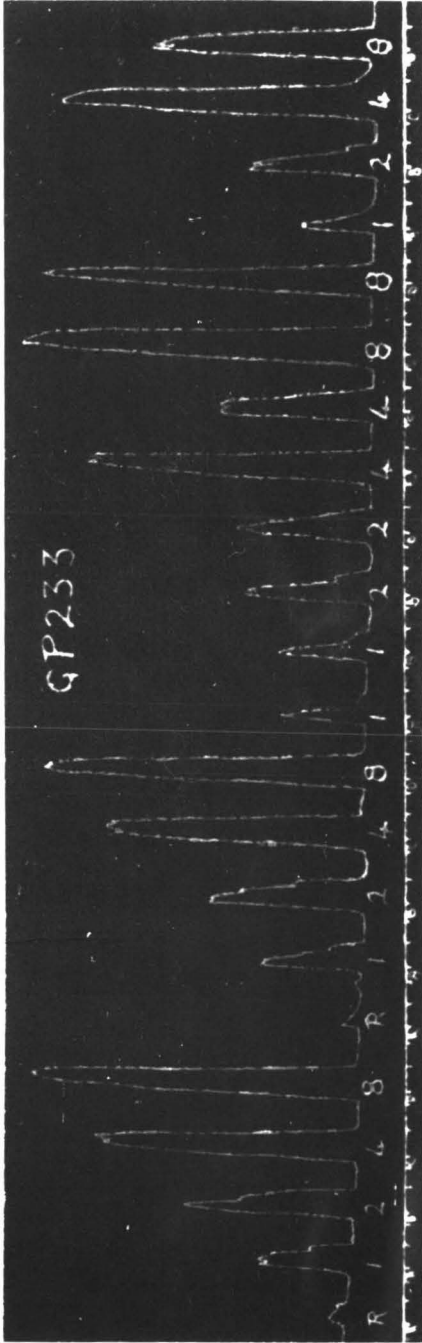
FIG. 1: RESPONSES OF GUINEA PIG ILEUM PREPARATION TO GRADED DOSES OF HISTAMINE

This tracing (retouched) indicates dose/response relations of the ileum of guinea pig 233. Ordinate: magnitude of response with Harvard solution. 1,2,4,8 = doses of histamine base.

(b) A dose range of 1 mU to 8 mU produced a satisfactory degree of

response.

D. Experiment II.

"To examine the effects of time since ovariectomy and arachis

oil pretreatment on oxytocin response."

1. Method

Four guinea pigs ovariectomized for 31 days, and 8 guinea pigs

ovariectomized for 21 days were randomly divided into three pretreatment

groups.   Group I received no treatment, Group II received 1 ml arachis

oil daily for six days, and Group III received no treatment but was

examined three weeks later than Groups I and II.   There were two guinea

pigs that had been ovariectomized 31 days in Group I and one in each of

Groups II and III.

Both uterine horns of each pig were assembled in quick

succession in two different organ baths.   One guinea pig was examined

per day, pigs from Groups I and II being examined on alternate days.

The treatment of each preparation was as follows:

R : R : 1 : 2 : 4 : 8 : R : 1 : 2 : 4 : 8 : 1 : 1 : 2 : 2 : 4 : 4 :

8 : 8 (15 minute intervals between all doses to this point) : 1 :

2 : 4 : 8 (10 minute intervals only between last 4 doses).

R = Ringer solution. 1 : 2 etc. = mU of oxytocin diluted in distilled water.

The doses were in contact with the uterine strip for one minute

before washing.   Ringer solution administration was regarded as a

treatment.   Thus each strip received 20 administrations of oxytocin and

3 administrations of Ringer solution in the treatment sequence.

The highest amplitude occurring within the one minute period of every administration, including the three separate applications of Ringer solution, was measured and recorded.

The guinea pigs were weighed at the time of ovariectomy and again prior to slaughter. The uterine strips were weighed immediately on removal from the organ bath at the conclusion of each test part.

3. Results and Discussion.

An example of the tracing obtained is shown in Fig. I facing page 34. The response to oxytocin was extremely variable - see Table I facing page 35. (For further details see Appendix I & II). Most preparations produced a contraction during each oxytocin administration. Some produced contractions during the Ringer solution administration, while others produced large contractions regardless of the dose administered. This spontaneous activity appeared to be inherent in some of these specimens.

Analysis of the variance between the groups was carried out for (a) the total activity (b) the total oxytocin response and (c) the total oxytocin response after the respective mean "Ringer response" had been subtracted from all oxytocin readings within each preparation. The differences between groups were not significant in any of these comparisons.

In this experiment the between animal variation of the animals used appeared to be high and the following analysis was therefore carried out in order to estimate the numbers of animals that would be required to improve experimental precision in future trials. Selected right horn readings from Groups I and II only were used. This was done because the results listed in Appendix I & II showed that readings from Guinea pig 202 in Group II contained evidence of excessive spontaneous activity. The

results from this animal were not considered worthy of analysis and in order
to avoid bias when making comparisons, these readings and those of the
corresponding animal in time in Group I, (pig 233), were excluded.   In
Group III the results from guinea pig 258 were again considered to be of
little use because of excessive spontaneous activity.   In this case readings
from corresponding guinea pigs in other groups could not be disregarded
without leaving insufficient data.   Therefore, no valid comparisons,
between Group III and the other two groups, could be made.

Right horn readings only were compared in Groups I and II as some
of the left horn readings in Group I were of a very small magnitude and
likely to produce invalid interpretations.   The discolouration of the
platinum attachment hook was the suspected cause of these low left horn
readings for a tissue hook in this condition may have lost its electrically
inert properties.   This would have interfered with the transmission of
galvanic current within the uterine strip and the contractability of the
myometrium would have been reduced.   The data used were the differences
between three responses for 1 mU of oxytocin and the three corresponding
8 mU responses.   These are summarised as follows: (see next page)

Treatment Group	G.P. No.	8mU-1mU Response (cm)	Total Response for Strip (cm)
I	257	2.1 4.6 5.8	12.5
I	251	7.2 5.9 8.2	21.3
I	203	3.0 3.2 2.8	9.0
II	246	11.1 12.2 9.7	33.0
II	250	3.1 4.8 3.3	11.2
II	246	1.1 2.5 4.9	8.5

The analysis of variance of this data is shown below, Table I(a).

TABLE I(a): *ANALYSIS OF VARIANCE GUINEA PIG EXPT. II.

Source of Variation	d.f.	M.S.	Component of Variance
Treatment	1	5.445	
Animals within Treatment	4	36.771	$s^2a = 11.642$
Readings within Animals	12	1.843	$s^2r = 1.843$

* Selected data only (see text).

This analysis did not indicate any significant treatment effect but did show that the variance between animals within a treatment (S^2a) was approximately ten times the variance within animals (S^2r). These estimates of the two components of the "error" were used to calculate the numbers of animals and readings that would give standard errors of various sizes. The indications were that for an S.E. less than 20% of the mean response 15 animals would be required in each treatment group. The effect of increasing the number of readings per animal from 1 to 4 was negligible.

In each group in the experiment the heaviest animal appeared to provide the preparation with the greatest activity and so a regression analysis was carried out to determine whether any relationship existed — no significant effects were found. The effect of weight however may have been confounded to some extent by variation in age of the animals concerned.

The possibility that the adrenal cortex of the ovariectomised guinea pig might be responsible for sensitising the uterus to oxytocin in the absence of ovarian influence was also considered and it was decided therefore to weigh the adrenal glands of the guinea pigs in future experiments.

Because of both inherent animal variation and variation due to other uncontrolled factors in this experiment no conclusions could be drawn as to the effects of either time of ovariectomy or of pretreatment with arachis oil. In spite of this a third experiment was carried out as it was considered that both slight modifications in technique together with pretreatment of the animals with steroid hormones might influence the uterine preparations to a degree that would exceed the effect of this variation.
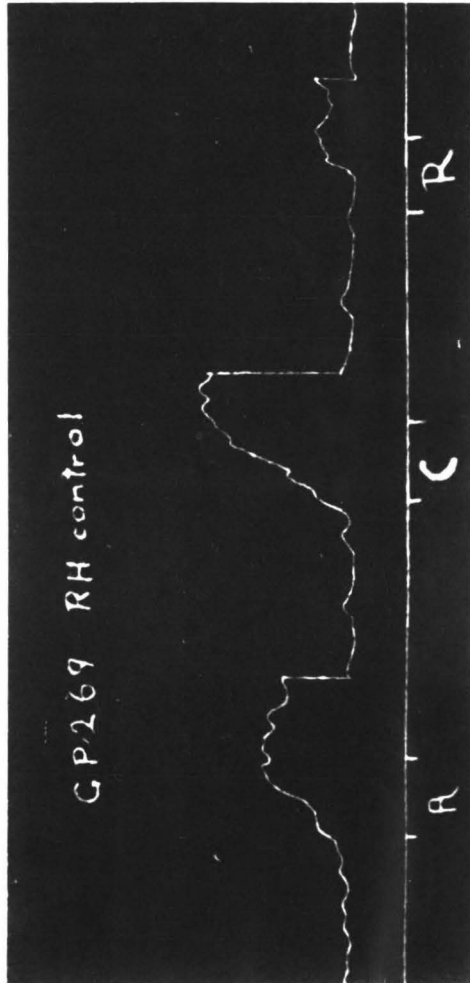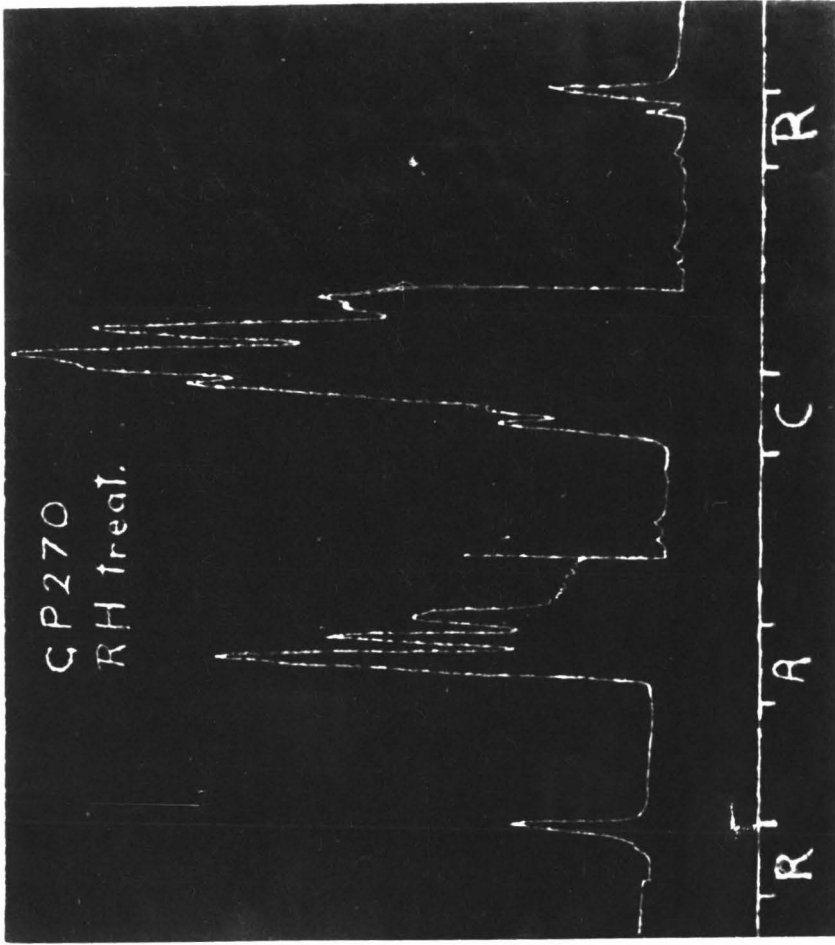
GP270
RH treat.

R   T   A   C   T   R

FIG. III

GP269 RH control

A   C   R

FIG. II

E. Experiment III.

"To determine whether treatment of ovariectomized guinea pigs with exogenous steroid hormones would alter the sensitivity of in vitro uterine strips to stimulus with oxytocin."

1. Method

As guinea pigs from closely related age groups were not available, similarity in body weight was used as a measure of uniformity. Guinea pigs weighing over 700 g were considered unsuitable and were not included.

Eleven guinea pigs that had been ovariectomized five weeks on the day of the experiment were weighed and grouped into pairs on a weight basis. The heaviest pig was discarded leaving 5 pairs.

One member of each pair was given 5 mg of progesterone subcutaneously on each of six consecutive days. 10 μg of oestradiol were given subcutaneously to this same pig on the fifth and sixth day. The other member of each pair acted as a control and received no treatment. This treatment regime was selected because it had been used successfully in implantation studies on ovariectomized guinea pigs (McDonald M.F., 1965 - personal communication).

Both horns were assembled as in the previous experiments except that monofilament nylon was used in place of platinum hooks for attaching the uterine strips. Syntocinon doses of 1 mU and 4 mU were made up with Ringer solution as the diluent.

Administration of the doses took the following order with a 15 minute interval between each dose.

Right Horn   Ringer; 1 mU; 4 mU; Ringer; 4 mU; 1 mU; Ringer.

Left Horn   Ringer; 4 mU; 1 mU; Ringer; 1 mU; 4 mU; Ringer.

The highest amplitude occurring within the one minute period of

TABLE II: SUMMARY OF RESULTS OF GUINEA PIG EXPERIMENT III (For full details see appendices III and IV).

GUINEA PIGS IN ORDER OF EXAMINATION	UTERINE HORN	GROUP I (PRETREATED)								GROUP II (CONTROLS)							
		UTERINE STRIP WT. (g)	TOTAL UTERINE ACTIVITY (cm)	TOTAL OXYTOCIN RESPONSE (cm)	TOTAL RINGER RESPONSES (cm)	MEAN RINGER RESPONSE (cm)	TOTAL CORRECTED* OXYTOCIN RESPONSE (cm)	WT. OF G.P. AT SLAUGHTER (g)	ADRENAL GLAND WT. (g)	UTERINE STRIP WT. (g)	TOTAL UTERINE ACTIVITY (cm)	TOTAL OXYTOCIN RESPONSE (cm)	TOTAL RINGER RESPONSES (cm)	MEAN RINGER RESPONSE (cm)	TOTAL CORRECTED* OXYTOCIN RESPONSE (cm)	WT. OF G.P. AT SLAUGHTER (g)	ADRENAL GLAND WT. (g)
1	L	0.38	39.3	33.9	5.4	1.8	13.4	440	0.25	0.14	9.4	6.4	3.0	1.0	1.3	520	0.19
	R	0.41	60.5	41.7	18.8	6.3	8.3		0.24	0.12	2.3	1.9	0.4	0.1	0.8		0.20
2	L	0.42	25.8	19.9	5.9	2.0	6.0	520	0.21	0.21	5.9	3.8	2.1	0.7	0.6	580	0.23
	R	0.43	49.8	34.3	15.5	5.2	6.8		0.21	0.20	2.1	1.4	0.7	0.2	0.3		0.24
3	L	0.50	52.7	39.8	12.9	4.3	11.3	535	0.22	0.10	18.0	11.6	6.4	2.1	1.6	560	0.22
	R	0.48	45.8	22.0	23.8	7.9	-4.7		0.23	0.12	7.9	4.7	3.2	1.1	0.2		0.26
4	L	0.46	61.5	46.6	19.6	6.5	8.0	530	0.21	0.16	8.0	6.2	1.8	0.6	2.0	615	0.19
	R	0.56	63.8	41.9	11.9	4.0	13.0		0.22	0.16	26.5	17.3	9.2	3.1	2.5		0.21
5	L	0.37	59.2	42.2	17.0	5.7	9.8	560	0.21	0.36	92.6	60.0	33.2	11.1	7.8	665	0.19
	R	0.41	67.8	53.8	14.0	4.7	17.6		0.18	0.41	95.3	62.9	31.7	10.6	10.3		0.24
	MEANS ± S.E.	0.442 ±0.027	52.6 ±8.6	37.6 ±5.7	14.5	4.84	8.95 ±1.14	517	0.22	0.198 ±0.027	26.8 ±8.6	17.6 ±5.7	9.17	3.06	2.74 ±1.14	588	0.22

* The total corrected oxytocin responses were calculated by subtracting the mean ringer solution response for each preparation from the mean response to 1 mU oxytocin and from the mean response to 4 mU, by the same preparation.   These corrected means were then totalled, to give the total corrected oxytocin response for the preparation.

every administration, including the three separate administrations of Ringer solution, was measured and recorded.

2.   Results

For examples of tracings obtained see Fig. IIA & IIB facing page 39.

A complete record of results from this experiment has been listed in appendices III and IV, and a summary of this data appears in Table II facing page 40.   The analyses of variance are summarised in Table's II(a)-(d) facing page 41.

Pretreatment with steroid hormones had clearly resulted in an increase in weight of the uterine strips and this result was highly significant ($P < 0.001$).   Total uterine activity had also been significantly increased by this treatment ($P < 0.05$) but the standard error of the experiment was high and so an analysis which compared the response totals recorded during the four oxytocin administrations only was carried out (Table II(c)).   Using this latter form of analysis there was again a significant effect of pretreatment on oxytocin response ($P < 0.025$) but the S.E. was proportionately the same as the S.E. for comparison of pretreatment effect on total uterine response.

The total "ringer responses" and the mean "ringer responses" were then analysed in a similar manner but no significant differences were shown between the two experimental groups using either set of figures.

Finally the mean "ringer responses" were subtracted from the mean 1 mU oxytocin response and from the mean 4 mU response for the preparation concerned.   These corrected means were totalled for each preparation and subjected to analysis of variance (Table II(d)).   This analysis demonstrated that the significance of the pretreatment effect

TABLE II(a):   ANALYSIS OF VARIANCE OF UTERINE STRIP WEIGHTS

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	0.2977	41.3	P 0.001
Error	18	0.0072		

TABLE II(b):   ANALYSIS OF VARIANCE OF TOTAL UTERINE RESPONSE

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	3333.36	4.53	P 0.05
Error	18	736.16		

TABLE II(c):   ANALYSIS OF VARIANCE OF TOTAL OXYTOCIN RESPONSE

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	1998.01	6.0	P 0.025
Error	18	332.95		

TABLE II(d):   ANALYSIS OF VARIANCE OF CORRECTED OXYTOCIN RESPONSE

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	192.82	14.9	P 0.001
Error	18	12.94		

was improved when allowance for spontaneous activity had been made. Using this correction technique the S.E. of the overall group means was reduced relative to the mean response.

At least in the control group there appears to be a relationship between body weight and uterine activity. Correlation coefficients between body weight and overall uterine activity were estimated for each group. In the pretreated group the correlation ($r = 0.147$) was not significant ($P > 0.1$) but in the control group the correlation ($r = 0.816$) was highly significant ($P < 0.001$). This result appears to be due almost entirely to the very active responses of the uterus of the heaviest animal in the group.

A similar situation appears to exist with respect to the relationship between body weight and uterine strip weight. The estimated correlation coefficients were 0.307 ($P > 0.1$) and 0.859 ($P < 0.001$) for the pretreated and control groups respectively.

The total adrenal weights for each group were almost identical.

3. Comment.

The results of this experiment appear to demonstrate quite clearly that the ovariectomised guinea pig uterus, when "sensitised" with a suitable combination of steroid hormones, will increase its _in vitro_ response to an oxytocin stimulus. These findings were of course not unexpected in view of the published works referable to this type of effect discussed earlier.

The finding that significant positive correlations between body weight and uterine weight, and body weight and uterine activity, were found for the control but not the treated groups is of some interest and suggests that either the control animals used may not have been true controls in that one member of the group, the heaviest, represented a different

population, or that pretreatment may have masked the effect of body weight in the other group. The true effect of body weight in this type of experiment could only be assessed by further work with more uniform animal groups.

There was no indication that adrenal weight had an association with uterine response.

F. General Discussion on Guinea Pig Experiments

Doses of oxytocin from 1 mU to 8 mU had proved satisfactory for eliciting responses in this type of experimental preparation. These doses produced significantly greater effects in preparations from animals that had been pretreated with oestradiol and progesterone. However, during these experiments two major problems emerged. The first was the large degree of variation in response between animals on the same pretreatment. There was some indication that weight or weight as a function of age was involved, but clearly there were many other ill-defined factors. Some preparations showed continuing spontaneous activity whilst others would scarcely respond to doses of oxytocin which produced good responses in other members of the same group.

The second problem was the apparent sensitivity of some preparations to the emptying and filling of the organ-bath. When the Magnus-type bath was emptied, the uterus was left suspended free of physiological fluid for a few seconds. Under these circumstances, the preparation tends to sag under its own weight and this occurred especially with the large specimens. In addition, the temperature insulating effect of the Ringer solution was removed and it was noticed that when the air-temperature was suddenly lowered by opening a door, a contraction usually followed.

Although it would appear that these problems could be reduced by better selection of animals and changes in technique, many other workers have recorded difficulties in the use of the guinea pig in experiments of this type.   Dale and Laidlaw (1912) recommended that guinea pigs weighing between 200 and 250 g be used and Kochmann (1921) finding it difficult to obtain animals of this weight, investigated methods suitable for larger animals, but these were only partially successful.   Similarly, Holton (1948) found it difficult to obtain suitable guinea pig material.   Stewart (1949), Thorp (1950) and Lewis (1964) all found the guinea pig uterus to be unsatisfactory for work with oxytocin substances since the preparations failed to give reproducible results.   Further, Thorp (1950) stated that the guinea pig uterus was incapable of giving a sufficient number of responses for satisfactory statistical analysis.   Gaddum (1938) quoted the limits of error for oxytocin assay on guinea pig material as $\pm$ 20 per cent, which would seem to be a conservative estimate.

The guinea pig as a species has certain reproductive patterns that are a disadvantage as far as studies on uterine smooth muscle are concerned.   Firstly, it has an oestrous cycle of $16\frac{1}{2}$ days, a gestation period of 68 days, and only rarely does the gravid female produce more than 3 to 4 young per litter.   These factors make it difficult to accumulate uniform specimens in sufficient numbers for this type of uterine work.   Secondly, certain anatomical features of the reproductive tract make this an unsuitable species for future experiments.   The cervix for instance has two internal openings but only one common external os, and the uterine horns join distally to form what appears externally as a single uterine body.   In point of fact however, the horns are separated by a thin median septum as far as the lower cervical segment where they

fuse together. For this reason, hemiovariectomy could not cause the establishment of unilateral pregnancies for use in future *in vitro* experiments. There would also be considerable difficulty in inserting microballoons transcervically into either uterine horn for *in vivo* experiments.

G. Conclusion

Although it was shown *in vitro* in guinea pigs that the overall uterine response to two levels of oxytocin was markedly increased when these animals were pretreated with a combination of oestradiol and pro-gesterone, greater uniformity of experimental material and certain adjustments in technique seemed necessary if a precise definition of the pretreatment levels likely to produce optimum effect was to be determined.

Moreover evidence from several authors indicating the unsuitability of the guinea pig as a source of uterine material for individual oxytocin assays was not disproven. Indeed it became apparent that the disadvantages recorded by these authors were accentuated in the between animal group comparisons that were carried out. Certain physiological and anatomical features in the guinea pig raised further doubt as to the suitability of this species for the uterine studies that were planned.

For these reasons, and because the output of the guinea pig colony accessable to the author was not large enough to allow adequate selection, a decision to continue the investigation using the white rat as an experimental animal was made. Large numbers of a closely inbred strain of Sprague-Dawley rats were readily available.

PRELIMINARY EXPERIMENTS WITH THE RAT.

A. INTRODUCTION.

The rat was considered to be a suitable species for a further study of the effects of hormonal interaction on the mechanical activity of uterine smooth muscle for two main reasons. Firstly, large numbers of an inbred strain of Sprague-Dawley rats were readily available. Secondly, because of certain physiological and anatomical features the rat is an ideal source of uterine material.

Gestation in the rat lasts 21.5 - 22 days. There are approximately 12 pups per litter in most instances with the sexes evenly represented. Ovariectomy can be carried out on prepubertal females as early as three weeks of age (Munford R.E., 1966 - personal communication). Thus for the purpose of this study a pregnant female rat would provide suitable experimental animals within six weeks from the time of being mated.

In the normal unmated rat, oestrus recurs approximately every 4 - 5 days, and the rat will breed at all times of the year if conditions are favourable. The age at which rats become sexually mature depends on the strain and diet, but usually puberty occurs at approximately 50 days of age (Blandau and Money, 1943). In the prepubertal rat the vagina is not canalised and in most cases the complete lumen does not appear until the first heat period. Very clear cut cyclic changes occur in the vaginal contents of the rat from puberty onwards. Thus the stage at which the uterus of the intact animal is affected by ovarian hormones can be detected readily (Long and Evans, 1922).

Ovulation occurs spontaneously but in the unmated rat the corpora lutea of the normal cycle are only slightly functional and the uterus is

probably not influenced by its secretions (Long and Evans, 1922).
According to these authors, if sterile mating occurs, the corpora lutea
do become functional and the endometrium is sensitised so that pseudo-
pregnancy occurs lasting for approximately 14 days.

Following normal copulation a vaginal plug is formed and its
effect is not only to keep sperm within the female reproductive tract,
but also according to Long and Evans (1922) to stimulate the vagina and
cervix so that the corpora lutea become functional and pseudopregnancy
or pregnancy results. The conditions are the same if fertilisation
occurs for the changes of pseudopregnancy are identical with the first
part of pregnancy. The time of mating for pregnant and pseudopregnant
animals can therefore be recorded with reasonable accuracy.

Surgical opening of the ovarian bursa is said to reduce the
fecundity on the operated side (Kelly, 1939). The uterus is of the
bicornuate type, which consists of two horns and a short unpaired segment,
which comprises the corpus and cervix. In spite of their apparent
external fusion the two horns remain separate as far as the paired
external orifices. The latter are difficult to detect among the folds
of the vaginal part of the cervix (Long and Evans, 1922). These features
were considered important for the establishment of hemipregnant prepar-
ations to be used in the final stages of this study.

B. TECHNIQUES USED IN EXPERIMENTS ON RATS.

A total of 184 Sprague-Dawley female rats were used in all the
experiments carried out with this species. The majority, 162 in all,
were bilaterally ovariectomized under ether anaesthesia, using the dorsal
approach. These rats were ovariectomized in the prepubertal stage at

USED RINGER OUTFLOW TO SUCTION PUMP.

TO LEVER

OXYGEN INFLOW

BOWEL CLAMP

RINGER

PRE WARMED RINGER INFLOW
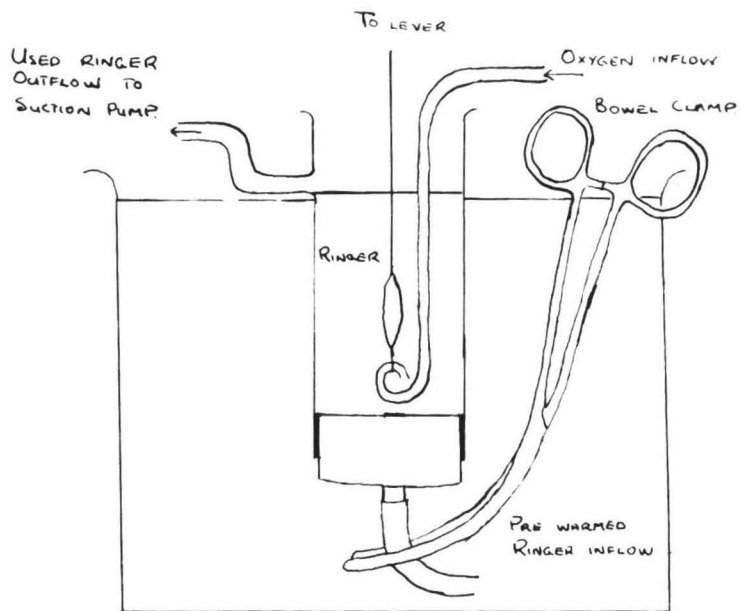
FIG. III: MODIFIED MAGNUS BATH

H   A   E   B   F

E   P

C

CM
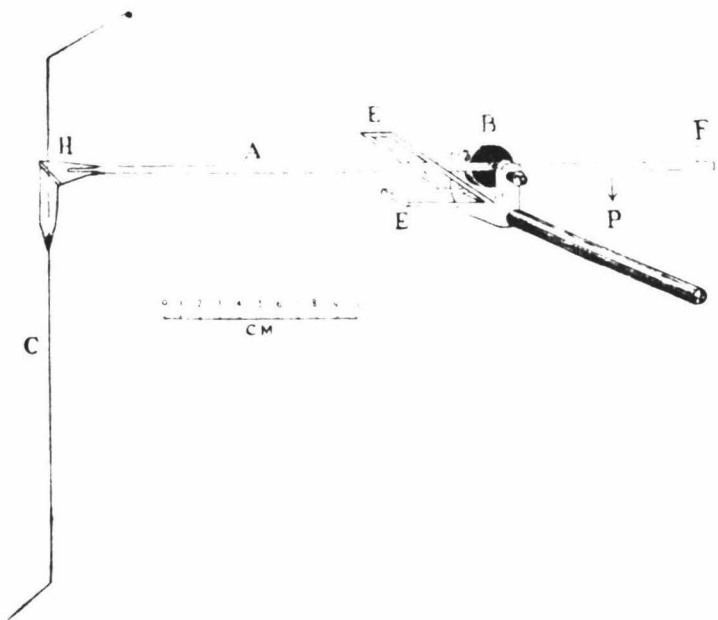
FIG. IV: SCHILD APPARATUS

approximately 21 days of age.

The remaining 22 were hemi-ovariectomized at the same stage of development, but were mated at predetermined intervals once they had reached 200 g body weight.

All rats were identified by a standard ear notching system.

1. Housing and Diet.

The rats were kept on wood shavings in metal cages, never more than six rats to a cage. They were housed in a rat colony maintained at $65^{\circ}F$, and were fed on Massey Mouse Pellets Type 2 (Lawson, 1964). Fresh drinking water was available to them at all times.

2. Treatment.

Subcutaneous injections of oestradiol monobenzoate (B.D.H.), progesterone (Organon) and arachis oil were given for assigned pretreatment periods. The hormone preparations were suspended in arachis oil at manufacture and were further diluted with arachis oil to provide hormonal concentrations suitable for experimental use.

3. Examination of Specimen.

The animals were slaughtered and the uterus excised as described for the guinea pig, but in this case, the rat uterine material was placed in de Jalons Ringer solution, once again at room temperature.

(i) Apparatus.

A 50 ml modified Magnus bath was used through which fresh prewarmed de Jalons Ringer solution could flow in at the base, when filling or changing the solutions in the bath (see Fig. III facing page 47). The Ringer solution already contained in the bath was removed through a suction tube at the top

which was connected to an aspirator pump on a water tap, and the Ringer
solution it removed passed to the sink.   During the washing process, Ringer
solution could pass from the submerged glass coils in the outer water jacket,
at a rate fast enough to displace the contents of the bath three times within
30 seconds.   These coils contained sufficient prewarmed Ringer solution for
70 seconds of continuous washing.   These modifications to the Magnus bath
ensured that the preparation was submerged in warm physiological fluid
continuously.

(ii) Ringer Solution.

The following solution at $37^{\circ}C$, as used by de Jalon et al (1945)
was adopted as the Ringer solution for work with the rat uterus.

NaCl	45 g
KCl	2.1 g
$CaCl_2$	0.3 g
Glucose	2.5 g
$NaHCO_3$	2.5 g
Distilled water	5 litres

This modified formula contains half the usual amount of glucose
and a quarter the usual amount of calcium.   This Ringer solution has been
used for all rat experiments conducted, but it was found necessary to adopt
the temperature of $32^{\circ}C$ after experiment I had been performed.   The
specially constructed 50 ml organ bath (see Fig. III) was used in place of
the Magnus type bath used in the guinea pig experiments because this had
left the preparation temporarily without Ringer solution during the washing
process.

(iii) Attachment of the Specimen.

The cervical end of the uterine strip was attached to the distal extremity of the oxygen tube by cotton thread. Initially the kymograph lever was mounted as described in the guinea pig experiments with the same attachment procedure. Later this lever was discarded in favour of the lever designed by Schild (1947).

The Schild apparatus (Fig. IV facing page 47) consisted of a frontal writing lever with a long writing point. This ensured that shortening of the uterine strip and the effect recorded on the kymograph drum bore a linear relationship to each other. Otherwise errors due to curvature would have occurred had the short writing point remained, and if the angle of excursion of the lever had become greater than 30° from the horizontal (Schild, 1947).

In the diagram of the Schilds lever (Fig. IV) E.E. represent stops which prevent this 30° angle from being exceeded. The lever itself A, was an aluminium rod fixed to a fulcrum B, which rotates around its own axis. The long writing point was of aluminium wire and was attached to a normal writing point H. This has been reproduced to scale after Burn et al (1950) who stressed the importance of distances A and C being represented by the exact dimensions depicted in the scale if faithful records of isotonic contractions were to be reproduced. The point F represents a piece of plasticine heavy enough to balance the lever when a weight of 1.3 g was applied at P, the future attachment point for the ovarian end of the preparation. A ratchet, of the type commonly used in isolated heart experiments, was added to the mounting of the fulcrum. This enabled the lever to be raised or lowered at the time of attaching or detaching the specimen. The uterine strip to be used was identified, trimmed and severed at its junction with the cervix, an entire horn thus

being selected. The cotton was threaded into the strip in Ringer solution at room temperature. It was then assembled in the organ bath, in which the Ringer solution was kept at 32°C, with the ovarian end attached to the lever.

(iv) Administration of Oxytocin.

The same proprietary preparation of this drug was used as in the guinea pig experiments: a dose range of 1 - 32 mU being employed. Washing for 30 seconds was carried out before and after administration, with a 5 minute interval allowed between doses. The period of administration varied from 30 seconds to 3 minutes between different experiments, but 45 seconds was the period selected for the majority of the work. Initially the drum was turned by hand before the administration of a fresh dose, but in the main experiment it was allowed to run continuously. All oxytocin doses were prewarmed in the outer water jacket as previously described.

4. Weight Measurements

Weights of the rats were measured on an Avery scale sensitive to 0.2 g. The slaughter weights were recorded after the rats had been killed and the uterus removed.

Uterine strip weights were measured, at the conclusion of the experiment on the particular strip in question, on a torsion balance sensitive to 0.001 g.

5. Assessment of Response.

Measurement of the highest amplitude during the period of administration was the basic method used for assessing response in all rat experiments with the exception of those conducted on material from pregnant specimens. In the latter case, measurements of the area under the trace

were made for administration and control periods.   In the main experiment, three forms of assessment were carried out:

(a) Highest amplitude during period of administration.

(b) Highest amplitude, following the administration of a dose, that occurred before the next administration.

(c) Area under trace during administration minus area under trace for a similar period prior to administration.   Areas were measured with a planimeter.

6. Statistical Analysis.

Analyses of variance (Snedecor, 1956) were used to test the significance of treatment effect.   A factorial experiment was employed to evaluate differences between the effects of nine hormonal treatments.

C. EXPERIMENT I.

"To determine the sensitivity to oxytocin of uterine strips from spayed rats pretreated with steroid hormones (treatment effect) and arachis oil (control)."

This experiment was conducted in two parts, and methods A and B represent the two dosing regimes used.

1. Method A.

(i) Method.

Eight mature female rats were ovariectomized five weeks prior to experimentation, when their weights ranged from 175 - 210 g.   They were allotted to four cages with a heavy and light rat in each cage.   Two heavy

TABLE III: BODY WEIGHT, UTERINE STRIP WEIGHT AND UTERINE STRIP RESPONSE TO OXYTOCIN (Method A).

ANIMAL PAIR	TREATMENT CONTROL	T C	BODY WT. (g)	WT. OF BOTH HORNS (mg)	*AVERAGE RESPONSE (mm) DOSE OF OXYTOCIN			TOTAL
					0.5 mU	1 mU	2 mU	
I	T		210	285	42	69	96	207
	C		225	247	8	9	14	31
II	T		210	221	23	51	64	138
	C		188	183	14	17	47	78
III	T		202	187	18	17	27	62
	C		168	197	27	35	49	111
IV	T		178	282	37	63	91	191
	C		175	192	0	0	0	0
MEAN ± S.E.	T		200±13.1	244±40.6	30	50	70	150±56.6
	C		189±22.0	205±24.9	12	15	28	55±42.6

*Average of 4 readings (2 from each horn) except for the treated rat in the
IIIrd pair where only 3 readings were taken due to a power failure.

rats and two light rats were allotted to the treatment and control groups
so there was a representative of each group in each cage.

The control rats received 0.05 ml arachis oil daily for six days;
the treatment rats received 1 mg progesterone in 0.05 ml arachis oil for
six days, with 2 µg of oestradiol on days five and six. Cages 1 and 2 were
examined on day 7. and Cages 3 and 4 on day 8. Both uterine horns were
used from each rat and doses of 0.5 mU, 1 mU, and 2 mU of oxytocin were used,
in that order, each dose being given twice and the average response recorded.

(ii) Results.

The results are shown in Table III facing page 52. For the
analyses of variance see Tables III(a) and III(b) facing page 53

The difference between contraction responses for the two groups
approached significance ($0.1 > P > 0.05$) but there was a wide variation between
preparations within these groups. There was no significant effect ($P > 0.1$)
of pretreatment on uterine strip weight.

(iii) Comment.

Following four days of pretreatment leakage from injection sites
started to occur. The oestradiol dose levels were low and as they had been
given at about the time that this leakage was noted, it seemed likely that
the pretreated rats had received ineffectual amounts of this hormone. It
was decided therefore to reduce the pretreatment period to four days in the
second part of the experiment, and to increase the amount of oestradiol
administered on days 3 and 4.

The process of assembling the strips in the organ bath proved time
consuming, but increased haste at this stage was contraindicated if all
preparations were to be examined with equal precision. It was considered

TABLE III(a):   ANALYSIS OF VARIANCE OF AVERAGE RESPONSE TO ALL DOSES OF
OXYTOCIN

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	17861	5.33	$0.1 > P > 0.05$
Error	6	3347		

TABLE III(b):   ANALYSIS OF VARIANCE OF COMBINED UTERINE HORN WEIGHTS

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	3042	1.94	$P > 0.1$
Error	6	1564		

TABLE IV(a):   ANALYSIS OF VARIANCE OF ALL RESPONSES TO 2, 4 & 8mU OXYTOCIN

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	49828	4.55	$0.1 > P > 0.05$
Error	6	10938		

TABLE IV(b):   ANALYSIS OF VARIANCE OF UTERINE HORN WEIGHTS

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	15313	5.32	$0.1 > P > 0.05$
Error	6	2873		

advisable therefore to reduce the number of preparations assembled by the random selection of one uterine horn only from each of the experimental animals.

2. Method B.

(i) Method.

Eight rats were allotted to four cages as in the previous part of the experiment. Rats under treatment were given 1 mg progesterone in 0.1 ml subcutaneously each day for four days, and on days 3 and 4 they were given 10 μg oestradiol subcutaneously in addition. The controls were given 0.1 ml arachis oil daily for four days. Alternate horns were selected from each rat on the day of the experiment and assembled as previously described. Oxytocin doses of 0.5 mU, 1 mU, and 2 mU were again employed but on preparations which gave little or no response at these levels doses of up to 16 mU were applied.

(ii) Results.

The results are shown in Table IV facing page 54. For the analyses of variance see Tables IV(a) and IV(b) facing page 53. The effect of pretreatment either on response or on uterine horn weight was not clearly significant ($0.1 > P > 0.05$).

(iii) Comment.

In this part of the experiment there was a greater apparent difference between the oxytocin responses of the two groups. The high S.E. of the responses of the pretreated group however reduced the significance of this observation to a level below that obtained in method A. This was due in the main to the high reading obtained for the pretreated preparation

TABLE IV: BODY WEIGHT, UTERINE STRIP WEIGHT AND UTERINE STRIP RESPONSE TO OXYTOCIN (Method B).

ANIMAL PAIR	TREATMENT T CONTROL C	WT. OF RAT (g)	WT. OF ONE UT. HORN (mg)	RESPONSE TO ONE READING (mm) DOSE OF OXYTOCIN						TOTAL (2,4,8 mU)
				0.5 mU	1 mU	2 mU	4 mU	8 mU	16 mU	
I	T	175	235	0	0	0	25	32	–	57
	C	215	50	0	0	7	9	16	–	32
II	T	210	90	0	0	125	123	132	127	380
	C	160	25	0	0	5	2	5	10	12
III	T	175	81	–	–	59	60	66	78	185
	C	160	25	–	–	0	0	0	3	0
IV	T	176	86	–	–	4	20	57	82	81
	C	166	42	–	–	0	0	2	14	2
MEAN ± S.E.	T	184±15.0	123±64.5	–	–	47	57	72	96	176±127.3
	C	175±23.0	36±10.8	–	–	3	3	6	9	12±12.6

in group II.

The threshold for oxytocin appeared to have been raised in both pretreated and control groups in method B of this experiment. The increased oestradiol level is a possible reason for this having been observed amongst the pretreated preparations, but the elevated threshold in the controls remains unexplained.

No leakage from the injection site was noted during the four days of pretreatment, and as this time sequence had produced strip weight differences that approached significant levels, the four-day period was selected for future use.

3. Conclusions from Rat Experiment I.

Although the analyses showed no significant differences between the oxytocic responses of uteri pretreated with steroid hormones and those pre-treated with arachis oil, it seemed clear that an increased sensitivity to oxytocin had occurred in the uteri that were pretreated with the steroid hormones. Except for the horn in method A on which only one set of readings could be taken, the oxytocin response of each hormone treated uterus was greater than that of the arachis oil treated control.

Improvements in pretreatment technique yielded an effect on uterine weight that approached significance in method B; but it appeared that further refinements in technique would be necessary to reduce the S.E. of the oxytocin response. The main problem was still that of spontaneous activity in the uteri of the animals that received hormonal treatment, and it was decided to reduce the temperature of the organ bath in order to combat this effect.

A lack of response in the control rats was expected as a natural consequence of their deprivation from steroid hormones. These hormones have always been considered necessary for the maintenance of uterine

TABLE V: BODY WEIGHT, UTERINE STRIP WEIGHT AND UTERINE STRIP RESPONSE TO OXYTOCIN

ANIMAL PAIR	PRETREATMENT A PRETREATMENT B	WT. OF RAT (g)	WT. OF UT. HORN (mg)	RESPONSE TO ONE READING (mm) DOSE OF OXYTOCIN				TOTAL (2, 4, 8 mU)
				2 mU	4 mU	8 mU	16 mU	
I	A	180	66	45	45	54		164
	B	190	102	6	27	39		92
II	A	195	70	0	0	30	38	50
	B	167	60	0	34	12	38	66
III	A	190	110	0	23	54	66	97
	B	197	79	0	0	0	56	20
IV	A	154	73	2	0	5	34	27
	B	190	63	11	2	12	16	45
MEAN ± S.E.	A	180±16.1	80±17.6	12	17	36	35	85±52.4
	B	186±11.4	76±16.6	4	16	16	28	56±24.5

contractability. This presented no problem however as untreated controls would not be required in future experiments. All further comparisons would be made between the relative effects of various hormonal levels.

D. Experiment II.

"To determine the relative sensitivity to oxytocin of uterine strips from spayed rats pretreated with two different combinations of steroid hormones".

(i) Method.

Eight ovariectomized rats were allotted to four cages and randomly assigned to pretreatment A of 1 mg progesterone in 0.1 ml arachis oil subcutaneously daily for four days with additional subcutaneous injections of oestradiol 20 μg in 0.05 ml arachis oil on days 3 and 4; or to pretreatment B of 1 mg progesterone daily as before but with 5 μg oestradiol in 0.2 ml on days 3 and 4.

A temperature of 32°C was adopted in the organ bath throughout the experiment as it was considered, based on experience with the guinea pig experiments, that spontaneous activity would be reduced at this lower temperature.

(ii) Results.

These are shown in Table V facing page 55. An analysis of variance for uterine responses to oxytocin stimuli appears in Table V(a) below:

TABLE V(a): ANALYSIS OF VARIANCE OF ALL RESPONSES TO 2,4,8 mU OXYTOCIN.

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	1653	2.61	P>0.1
Error	6	631		

The differences between the sensitivities of the two pretreatments were not significant (P>0.1), but the higher level of oestrogen pretreatment appeared to produce a preparation more sensitive to oxytocin. There was no significant difference between the uterine strip weights of the two groups, probably because identical progesterone pretreatments were used.

(iii) Comment.

The S.E. of oxytocin response within pretreatments was still high, particularly in pretreatment A. It was considered that differences in tension between preparations could be responsible for some of the between preparation variation. Tension appeared to alter on occasions when the isotonic lever descended to the bottom of the kymograph drum. In these instances the uterine preparation was obviously stretched.

The Schilds lever was therefore adopted in subsequent experiments as this lever prevented over-stretching of the specimen in a manner previously described (see page 49).

E. Experiment III.

"To determine the relative sensitivity to oxytocin of uterine strips from ovariectomized rats pretreated with different combinations of steroid hormones using the Schild's apparatus."

Note. Prior to this experiment a preparatory experiment using the Schilds lever was carried out. In this preparatory study a uterine strip from a 200 g rat sensitized for 48 hours with 0.2 mg stilboestrol was used, and a wide range of oxytocin doses were employed. Each oxytocin dose was left in the $32^{\circ}C$ organ bath for 45 seconds before washing.

The following results were obtained:

<u>Response to Oxytocin (mm)</u>

Dose	0.5 mU	1 mU	2 mU	4 mU	8 mU	16 mU	32 mU
1st Series	2	2	3	5	6	11	16
2nd Series				6	8	8	14

Although the lever lost tension between the 1st and 2nd series of oxytocin doses it was not over-stretched at any stage. The 45 seconds administration period gave a record of the contraction that was more clearly defined. This technique was adopted for the next series of experiments.

(i) Method.

As progesterone was in short supply a dose sequence was adopted in which the amount of progesterone administered to each group was markedly different. Eight ovariectomized rats were allotted to four cages, one member of each cage being allotted to treatment A, and the other to treatment B. The treatments were:

DAY		1	2	3	4	5
TREATMENT A	Progesterone (mg)	2	5	5	5	DAY OF EXPERIMENT
	Oestradiol (μg)	-	-	20	20	
TREATMENT B	Progesterone (mg)	0.5	1.25	1.25	1.25	DAY OF EXPERIMENT
	Oestradiol (μg)	-	-	20	20	

Schild's apparatus with the lever adjusted to apply a constant tension of 1.3 g to the muscle preparation was used, and the period of

TABLE VI: BODY WEIGHT, UTERINE STRIP WEIGHT AND UTERINE STRIP RESPONSE TO OXYTOCIN

ANIMAL PAIR	TREATMENT A OR B	WT. OF RAT (g)	WT. OF HORN (mg)	RESPONSE TO ONE READING (mm) DOSE OF OXYTOCIN						TOTAL
				0.5 mU	1 mU	2 mU	4 mU	8 mU	16 mU	
I	A	176	57	3	10	18	24	31	48	134
	B	176	49	4	2	1	2	4	8	21
II	A	196	107	37	37	27	49	46	55	251
	B	198	55	0	7	6	12	20	32	77
III	A	227	95	77	51	29	80	83	87	407
	B	214	75	0	0	7	17	16	32	72
IV	A	164	85	37	33	45	57	53	68	293
	B	181	57	0	16	13	20	24	47	120
MEANS ± S.E.	A	191±23.8	86±9.5	39	33	30	53	53	65	271±97.7
	B	192±15.0	59±9.7	1	6	7	13	16	30	73±11.3

oxytocin administration adopted was 45 seconds timed with a stop watch.

(ii) Results.

These are shown in Table VI facing page 58. Analyses of variance are shown in Tables VI(a) and VI(b) facing page 59.

The uterine strips from rats pretreated with the higher level of progesterone were more sensitive to all administrations of oxytocin than those treated with the lower level. This difference was significant ($P < 0.05$). The strip weights also were greater in the group that received the most progesterone but this effect was significant only at the 10% level. It appeared that the technique adopted would be satisfactory for use in further experiments of this nature.

Conclusions from Preliminary Experiments.

Although many variables can affect the results obtained with this experimental procedure when applied to between animal comparisons these preliminary experiments have shown that, with sufficient refinement in technique, the effects of pretreatment changes on the uterine strip response to oxytocin can be measured.

The literature indicates, (see Csapo and Corner, 1952, 1953; Van Demark and Hays, 1952; Csapo, 1956a; Schofield, 1961), that variable oestrogen and progesterone levels are associated with the sensitivity of uterine muscle in the living animal. The evidence also suggests that an interaction of these hormones decreases the oxytocic threshold of uterine smooth muscle towards the end of the gestation period. These however are subtle changes, liable to be detected only if careful replications are made.

A factorial experiment was therefore designed in an attempt to determine how different combinations of exogenous oestrogens and progesterone

TABLE VI(a): ANALYSIS OF VARIANCE OF ALL RESPONSES 0.5-16 mU OF OXYTOCIN

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	79003	11.0	$P < 0.05$
Error	6	7180		

TABLE VI(b): ANALYSIS OF VARIANCE OF UTERINE STRIP WEIGHTS

Source of Variation	d.f.	M.S.	F.	
Pretreatment	1	1458	4.92	$0.1 > P > 0.05$
Error	6	290		

would affect the sensitivity of the rat uterus to oxytocin stimulus and also, whether the technique which had been developed would be capable of measuring these relatively subtle differences. If successful a similar approach could be considered for examining material from pregnant animals.

FACTORIAL EXPERIMENTS WITH THE RAT.

A. INTRODUCTION.

The preliminary experiments in rats indicated that pretreatment with progesterone at levels of 1 mg to 10 mg and oestradiol at levels from 2 ug to 20 ug could be expected to affect uterine response at the dose levels of oxytocin being used. The following dose levels were selected from this range for further study.

Progesterone   1 mg, 5 mg, and 10 mg

Oestradiol $5\,\mu g$, $10\,\mu g$, $20\,\mu g$

An experiment based on a factorial model, (Snedecor, 1956), was used to study the effects and possible interactions of nine different combinations of these levels of oestradiol and progesterone on the response of uterine strips from ovariectomized rats to an oxytocin stimulus.

The model selected is represented algebraically as follows:

$$X_{ijkm} = u + B_i + P_j + O_k + (PO)_{jk} + E'_{ijk} + C_m + E''_{ijkm}$$

Where B_i represents the effect of the ith block (i = 1 to 4 or 5),

P_j represents the effect of the jth level of progesterone (j = 1,2,3),

O_k represents the effect of the kth level of oestradiol (k = 1,2,3),

$(PO)_{jk}$ represents the interaction of the jth level of progesterone and the kth level of oestradiol

E'_{ijk} is the "between animal" error (Error I),

C_m represents the mth contraction in response to the mth dose of oxytocin (m = 1,2,3,4), and

E''_{ijkm} is the "within animal" error (Error II).

Nine ovariectomized rats in each block were randomly allotted to one of the nine treatment combinations.

B. GENERAL EXPERIMENTAL METHOD.

All rats receiving the same oestradiol dose were kept together in one cage. Each rat in each group was receiving a different level of progesterone. The pattern of dosing of the rats with progesterone and oestradiol is as shown in Plan I. The progesterone was suspended in arachis oil so that the required amount was contained in 1 ml. Oestradiol was suspended in arachis oil so that the required amount was contained in 0.1 ml. Both substances were suspended immediately before use and were injected subcutaneously.

Plan I.

GROUP I.

Oestradiol (μg)	Progesterone (mg)	Day I	Day II	Day III	Day IV
5	1	P	P	P + Oe	P + Oe
5	5	P	P	P + Oe	P + Oe
5	10	P	P	P + Oe	P + Oe

GROUP II.

Oestradiol (μg)	Progesterone (mg)	Day I	Day II	Day III	Day IV
10	1	P	P	P + Oe	P + Oe
10	5	P	P	P + Oe	P + Oe
10	10	P	P	P + Oe	P + Oe

GROUP III.

Oestradiol (μg)	Progesterone (mg)	Day I	Day II	Day III	Day IV
20	1	P	P	P + Oe	P + Oe
20	5	P	P	P + Oe	P + Oe
20	10	P	P	P + Oe	P + Oe

On the day of examination the preparations for the blocks under treatment were assembled as previously described. All the procedures used were as described for preliminary experiment III for the rat, (see page 56).

The doses of oxytocin used were 1, 2, 4, and 8 mU. Each dose was administered for 45 seconds and then washed out. Four minutes later the specimen was again washed and after a further minute the next dose of oxytocin administered. Doses were given in ascending order five minutes apart. The above process was repeated for each dose of oxytocin.

C. FACTORIAL EXPERIMENT I.

"To compare the relative sensitivity to oxytocin of uterine strips from four blocks of nine ovariectomized rats, each block member having been pretreated with one of nine combinations of newly prepared steroid components".

(i) Method.

Each of the four blocks were examined as described in the general experimental method.

(ii) Results.

These are listed in tabular form in Table VII facing page 62. The analysis of variance is shown below in Table VII(a). A preliminary analysis indicated that subclass means and standard deviations were related and the data were therefore transformed by expressing the results in logarithms (Kempthorne, 1952). (see over page)

TABLE VII: RAT UTERINE STRIP RESPONSES (mm)[+] TO OXYTOCIN FOLLOWING VARIOUS STEROID HORMONE PRETREATMENTS

	1 mU dose response				2 mU dose response				4 mU dose response				8 mU dose response			
	*B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
**P_1O_1	50	6	26	4	57	12	20	5	62	15	27	7	81	21	42	9
P_1O_2	25	19	7	6	22	27	15	7	29	30	23	5	35	32	31	10
P_1O_3	0	30	56	0	13	30	64	5	19	37	70	8	31	47	71	10
P_2O_1	19	15	13	0	9	13	13	11	14	17	13	20	24	26	17	30
P_2O_2	4	16	20	0	6	15	14	18	6	18	23	22	6	24	30	26
P_2O_3	16	10	33	12	16	11	31	13	27	12	34	11	27	17	43	17
P_3O_1	44	4	24	17	42	8	14	18	42	23	26	22	46	38	32	23
P_3O_2	22	26	16	22	26	38	26	25	29	40	22	29	32	60	37	33
P_3O_3	10	12	2	18	11	14	5	16	15	16	9	22	16	22	14	24

* B1-B4 = Blocks.

**P_1O_1-P_3O_3 = Treatment combinations of oestrogen and progesterone.

[+] Response is the highest point of the contraction above the base line following each oxytocin administration (mm).

TABLE VII(a):   *ANALYSIS OF VARIANCE OF UTERINE STRIP RESPONSES

Source of Variation	d.f.	M.S.	F	
Blocks	3	0.4788	2.01	P>0.05
Pretreatments	8	0.1879	0.79	P>0.05
Error I:				
Blocks x Pretreatments	24	0.2584		
Contractions	3	0.6681	23.77	P<0.001
Contractions x Pretreatment	24	0.0162	0.58	P>0.05
Error II:				
Blocks x Contractions +				
Blocks x Pretreatment x				
Contractions	81	0.0281		

*Data coded and transformed according to the expression $Y = 1 + \log(x+0.2)$ where Y was analysed and x was the response in cm^2.

The analysis of variance indicated a highly significant difference between the responses produced by each administration level of oxytocin and in most preparations there was a clear increase in response when the oxytocin dose was increased. The analysis did not, however, indicate a true pretreatment effect on oxytocin response owing to the considerable interaction between blocks and pretreatments.

(iii) Comment.

The insignificant effect of the different pretreatments may have been due to too great a similarity between the different hormone levels used, but the variation in response between animals on the same

treatment was larger than anticipated from earlier experiments. It was considered that this might in part, be due to variations in mechanical stimulation of the preparation during assembly. Modifications of the assembly process were considered for future investigations.

The large variation due to blocks was also difficult to explain as there were no apparent differences in the technique. It was possible that insufficient mixing of the pretreatment drugs with the diluting arachis oil may have led to variations in pretreatment in individual blocks.

In order to examine the possibility that inadequate mixing of the hormones had been of significance, five more experimental blocks were examined without any major changes in technique, except that all the oestradiol and progesterone dilutions were made up prior to the commencement of pretreatment on the first block in the new series.

It was also felt that the simple measurement of amplitude of contraction could obscure subtle differences and other measurements were considered for subsequent blocks.

D. FACTORIAL EXPERIMENT II.

"To compare the relative sensitivity to oxytocin of uterine strips from a series of five blocks of nine ovariectomized rats per block, each block member to be pretreated with one of nine steroid combinations, the components of which had been made up prior to the beginning of the experiment".

(i) Method.

Each of the five blocks were pretreated as described in the general experimental method except that sufficient quantities of the steroid dilutions for all five blocks were made up prior to the pretreatment

TABLE VIII: RAT UTERINE STRIP RESPONSES (mm)[+] TO OXYTOCIN FOLLOWING VARIOUS STEROID HORMONE PRETREATMENTS.

	1 mU dose response					2 mU dose response					4 mU dose response					8 mU dose response				
	*B_{1A}	B_{2A}	B_{3A}	B_{4A}	B_{5A}	B_{1A}	B_{2A}	B_{3A}	B_{4A}	B_{5A}	B_{1A}	B_{2A}	B_{3A}	B_{4A}	B_{5A}	B_{1A}	B_{2A}	B_{3A}	B_{4A}	B_{5A}
**P_1O_1	36	50	0	7	17	40	92	11	9	22	62	94	19	22	40	72	100	30	32	53
P_1O_2	14	37	6	17	7	46	69	20	20	10	55	92	40	35	27	77	100	64	75	32
P_1O_3	10	2	2	2	3	17	8	4	7	25	25	20	8	12	40	32	40	14	18	35
P_2O_1	25	8	13	1	50	36	20	15	2	52	32	35	18	3	60	46	56	32	40	55
P_2O_2	55	41	2	7	7	57	71	17	22	38	62	107	40	20	57	77	111	52	20	46
P_2O_3	75	6	4	2	15	55	16	23	8	60	43	37	36	20	60	29	57	39	70	55
P_3O_1	30	7	15	3	20	45	10	27	13	42	37	15	40	22	44	29	26	60	40	90
P_3O_2	65	10	15	7	7	84	15	27	10	0	46	20	56	13	77	60	30	65	12	97
P_3O_3	58	4	0	3	10	82	5	11	8	35	104	7	16	32	57	109	10	24	32	57

* $B_{1A}-B_{5A}$ = Blocks.

**$P_1O_1-P_3O_3$ = Treatment combinations of oestrogen and progesterone.

[+] Response is the highest point of the contraction above the base line following each oxytocin administration (mm).

of the first block.   The progesterone and oestradiol dose levels were
unaltered, but the arachis oil dilution of the progesterone was reduced
so that each dose of this hormone was suspended in 0.5 ml instead of 1 ml,
to avoid the distress of bulky injections.   Also after the first block
in this experiment had been examined the kymograph drum was allowed to
run continuously in order to make a more detailed record of oxytocin response.

       During assembly the cotton connection from the specimen was allowed
to drape over the point of attachment on the lever under the weight of 1 g
of plasticine to which it had been attached.   This avoided uneven tension
being applied to the preparation during the assembly process.   The 1 g
weight was cut off prior to the lever being raised to the horizontal position.

(ii) Results.

       These are listed in tabular form in Table VIII facing page 65.
The analysis of variance is shown below in Table VIII(a).   The data was again
transformed by taking logarithms of the responses as in Factorial Experiment I.
Tests of significance were made on oestradiol and progesterone effects and
their interaction, in addition to testing for overall treatment effect.

TABLE VIII(a):   * ANALYSIS OF VARIANCE OF UTERINE STRIP RESPONSES

Source of Variation	d.f.	M.S.	F	
Blocks	4	1.2921		
Pretreatments	8	0.3042		
Progesterone	2	0.1348		
Oestradiol	2	0.5157	2.95	$P < 0.08$
Progesterone x Oestradiol	4	0.2832		
Error I:				
Blocks x Pretreatments	32	0.1754		
Contractions	3	3.1222	60.39	$P < 0.001$
Contractions x Pretreatments	24	0.0285		
Error II:				
Blocks x Contractions + Blocks x Pretreatment x Contractions	108	0.0517		

* Data transformed - see footnote Table VII(a).

Progesterone did not produce a significant effect, neither did its interaction with oestradiol.   The oestradiol effect was significant at the 8% level of probability.   The oestradiol pretreatment means ($\pm$ S.E.) in transformed units were:

Low Oestradiol	1.448	$\pm$ 0.108
Medium Oestradiol	1.500	$\pm$ 0.108
High Oestradiol	1.320	$\pm$ 0.108

An analysis of covariance with uterine strip weight was carried out on the 4th contraction using both transformed and untransformed data. Both methods improved the F ratio and reduced the S.E. but the changes were not sufficiently great to justify the covariance analysis being carried out on all contractions.

(iii) Joint Analysis of Factorial Experiments I and II.

The data from the nine blocks (81 rats) which had been used in Experiments I and II were combined and subjected to an analysis of variance. The combined analysis did not improve the oestrogen effect.   This effect of oestradiol dose, significant at the 8% level in Table VIII(a), was clearly not significant ($P > 0.1$) in the combined analysis.

An analysis of covariance with uterine strip weight was carried out on all contractions for all nine blocks.   This reduced the error term by 17.6%, but did not bring the treatment effect within the limits of significance.

E. ALTERNATIVE METHODS OF MEASURING RESPONSE.

I. Highest amplitude following Administration.

During the recording of the last four blocks the kymograph drum was allowed to run continuously.   A clearly defined contraction peak was
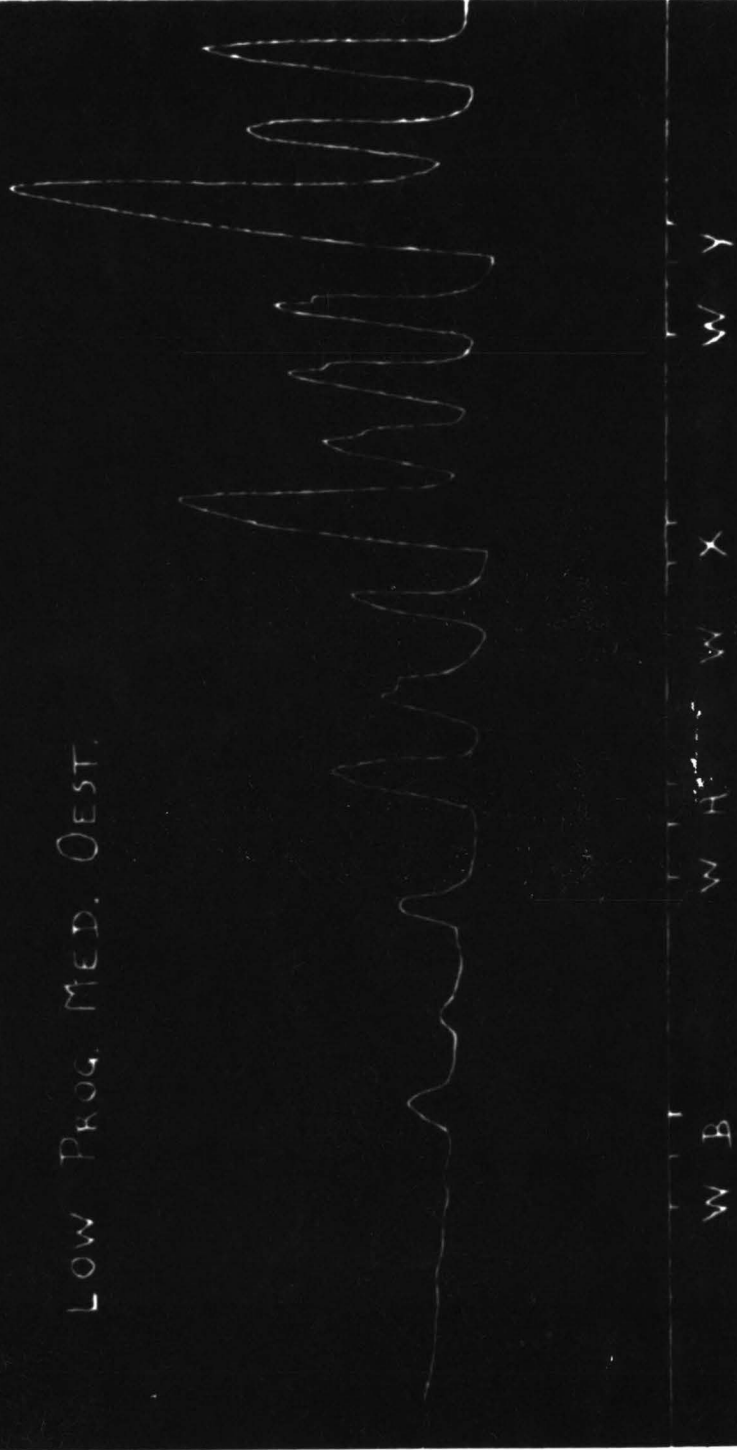
FIG. V: TRACING FROM UTERINE STRIP OF RAT (retouched) SUBJECTED TO LOW PROGESTERONE MEDIUM OESTROGEN PRETREATMENT (FACTORIAL EXPERIMENT II).

W = wash with Ringer Solution

B, A, X, Y = 1,2,4 and 8 mU Oxytocin respectively

recorded after each oxytocin administration (see Fig. V facing page 67). The peaks of these responses occurred after the oxytocin had been washed from the organ bath, and their height bore a relationship on visual examination, to the magnitude of the oxytocin dose that had been administered.

The possible significance of these responses in relation to pretreatment was evaluated by measuring the highest amplitude that occurred following the administration of each dose of oxytocin and before the administration of the next for each preparation. Consequently this system of evaluation did not entail a set time interval during which measurements were made as had formerly been the case where measurements had been taken during the 45 second administration period.

The analysis of variance for the sum of four contractions is summarised below in Table IX.

TABLE IX: *ANALYSIS OF VARIANCE OF UTERINE STRIP RESPONSES

Source of Variation	d.f.	M.S.	F	
Blocks	3	0.1992		
Pretreatment	8	0.1719	1.74	
Progesterone	2	0.0167	0.17	
Oestrogen	2	0.1827	1.85	
Progesterone x Oestrogen	4	0.2442	2.48	$0.1 > P > 0.05$
Error				
Block x Pretreatment	24	0.0986		

* The highest amplitude of the contraction following each oxytocin stimulus was measured (mm). The results were analysed after summing contractions for each pretreatment and transforming the totals to logs.

The levels of significance attained using this method of measuring

response were little if any better than the previous method used and the technique was not investigated further.

II. Area Measurement for Period of Administration Minus Area Measurement for a Similar Period of Control.

All kymograph tracings recorded when the drum was allowed to turn continuously indicated peak contractions for each oxytocin administration. These peak contractions appeared to vary in size depending on the pretreatment that the particular uterine strip had received but these observations had not been substantiated by the analyses so far conducted. Duration of contraction, however, had not been taken into consideration to this point and as duration as well as amplitude might very well be interpreted as a component of the response to a stimulus, an effort to make an analysis taking this into consideration was made.

Planimeter measurements of the area beneath the trace that coincided with the period of each oxytocin administration were considered as a basis for this measurement. It seemed that the adoption of this method would allow both the extent and duration of each contraction to be proportionately represented.

(i) Method.

Areas on the kymograph recordings from the last four blocks where tracings were continuous were outlined as follows. The kymograph paper was placed on a drawing board, and perpendicular lines were drawn through the points at which oxytocin was administered. Two other perpendicular lines were drawn at a distance equivalent to 90 seconds time on either side of the administration line. The two areas enclosed by these lines represented intervals during which, in one case, oxytocin was exerting its influence (it had been in contact with the strip for the first 45 of the 90
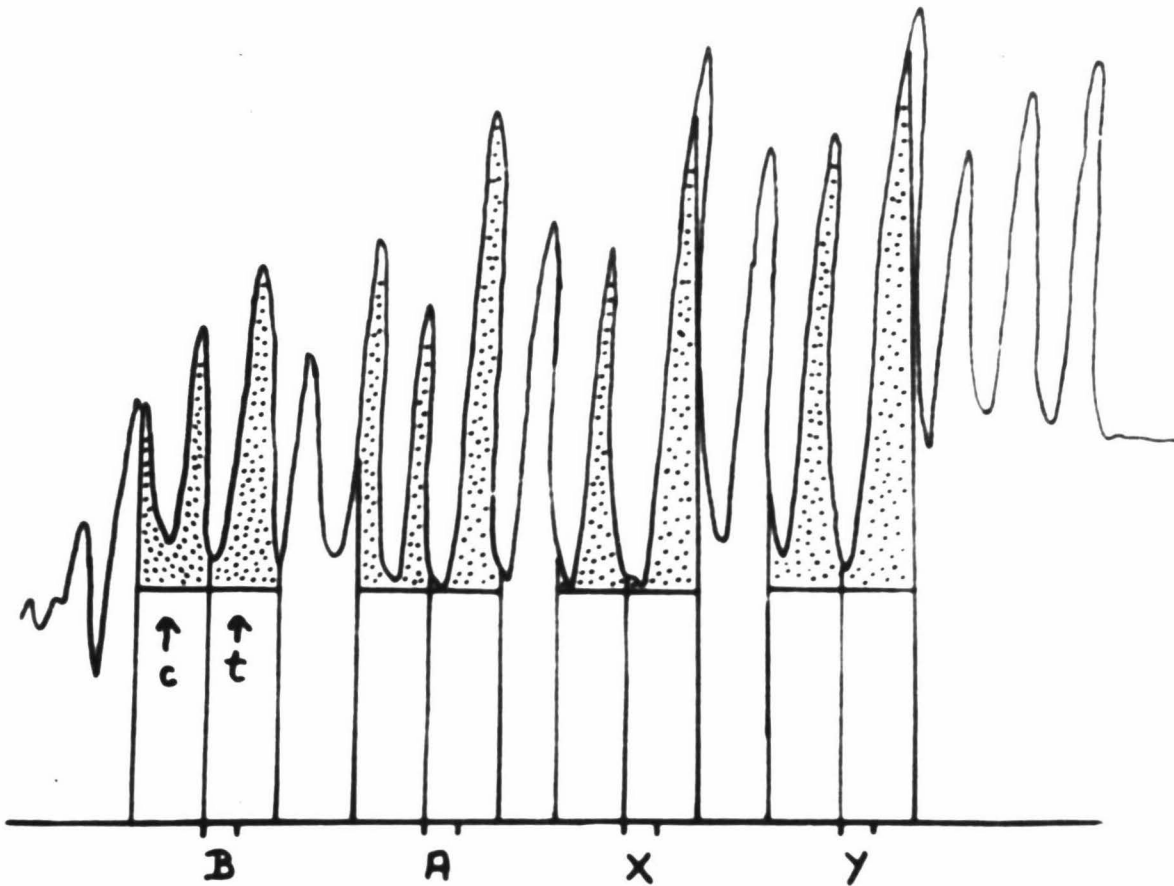
FIG. VI: DIAGRAM OF TRACING FROM RAT UTERINE STRIP (FACTORIAL
EXPERIMENT II).

This illustrates the method used in taking the area
measurements with a planimeter.

c - control area before oxytocin stimulus.

t - treatment area after oxytocin stimulus.

B,A,X,Y = 1,2,4 and 8 mU oxytocin respectively

second period), and in the second oxytocin had been washed from the preparation 3 minutes 30 seconds before the measurement was made i.e. only "carry over" effects from the previous stimulus, if any at all, were being recorded.

The base line for these two areas was constructed by drawing a line across ~~them that ran~~ through the two lowest points of the kymograph record (Fig. VI facing page 69). Both areas were measured in square centimeters for each oxytocin administration. Each individual area was measured twice with the planimeter, and if the measurements differed by less than 0.004 cm^2 a mean of the two readings was accepted as a record of the area concerned. If the first two readings differed by more than this accepted error, they were measured until two readings were made that coincided with this ruling.

The control readings, (prior to oxytocin administration for the particular dose in question), were subtracted from the contraction readings. (during and after a particular dose of oxytocin had been given), and the differences gave a measurement of oxytocin response. These measurements were transformed by taking logarithms, after coding to remove negative readings.

TABLE X: ANALYSIS OF VARIANCE OF UTERINE STRIP RESPONSE*

Source of variation	d.f.		M.S.	F	
Blocks	3		0.0724		
Pretreatments	8		0.1486	2.78	(P<0.05)
Progesterone		2	0.1911	3.57	(P<0.05)
Linear response **		1	0.2227	4.17	(P<0.10)
Non-linear response		1	0.1594	2.98	(P<0.10)
Oestradiol		2	0.1175	2.20	(P>0.10)
Linear response		1	0.0407	0.76	
Non-linear response		1	0.1943	3.63	(P<0.10)
Progesterone X Oestradiol		4	0.1429	2.67	(P<0.10)
Linear responses		1	0.2144	4.01	(P<0.10)
Non-linear responses		1	0.2375	4.44	(P<0.05)
Linear and Non-linear responses		2	0.0598	1.12	(P>0.10)
Blocks X Pretreatments (Error I)	24		0.0535		
Contractions	3		0.4563	14.30	(P<0.001)
Contractions x Pretreatments	24		0.0523	1.64	(P≑0.05)
Contractions x Progesterone		6	0.0412	1.29	(P>0.1)
Contractions x Oestradiol		6	0.0823	2.57	(P<0.05)
Contractions x Prog. x Oest.		12	0.0429	1.34	(P>0.1)
Blocks x Contractions) Blocks x Pretreatments x) Contractions) (Error II)	81		0.0319		

* Response measured as the difference between two areas (see text): Xcm^2; analysis of the data for Blocks B_{2a} - B_{5a} in the transformed and coded form: $Y = 1 + \log (X + 1)$.

** Response to log-dose of steroid.

(ii) Results.

The effects of steroid pretreatment on the response to oxytocin, measured as the difference of two areas, are given in detail in Appendix V. The analysis of variance of these data, after transformation by taking logarithms, is presented in Table X facing page 70.

(a) Differences between contractions.

As in previous analyses of variance (see Tables VIIa and VIIIa) the mean square due to differences between contractions was highly significant ($P < 0.001$). In the present analysis this effect was examined further by a t-test of differences between the four means (see Table X(b) facing page 71). The mean response to 8 mU oxytocin was greater than that to 4 mU ($P = 0.05$) and this response in turn was greater than that to 2 mU and 1 mU ($P < 0.001$). The mean responses to the two lowest doses did not differ significantly ($P > 0.1$). The administration of oxytocin, as a series of ascending doses with each uterine preparation, achieved its intended purpose; a series of increasing contractions which would allow the detection of different responses to pretreatment with oestradiol and progesterone (see also subsection (c)).

(b) Effects of pretreatment with oestradiol and progesterone.

The mean responses for individual treatment combinations and for each level of oestradiol and progesterone are shown in Table X(a) facing page 71.

The results of the analysis of variance (Table X(a)) clearly indicated a significant effect of level of progesterone treatment ($P < 0.05$) which was not a simple linear response to log. dose of progesterone; both higher doses of progesterone depressed the uterine strip response. The total mean square for the effect of oestradiol was not significant, but there was evidence ($P =$ approximately 0.08) of a non-linear response, with

TABLE X(a):   *MEAN RESPONSE ACCORDING TO PROGESTERONE AND OESTRADIOL

Oestradiol level (μg)	Progesterone level (mg)			
	1	5	10	All levels
5	1.303	1.086	1.141	1.177
10	1.356	1.310	1.160	1.275
20	1.226	1.133	1.295	1.218
All levels	1.295	1.176	1.199	

*Mean response for 4 contractions for each of 4 animals or 12 animals (Blocks B_{2a} - B_{5a} Factorial Experiment II). The means relate to data coded and transformed: $Y = 1 + \log (X + 1)$ where X was the difference between two areas in cm^2 (see text).

TABLE X(b):   MEAN RESPONSE *ACCORDING TO LEVEL OF OXYTOCIN AND OESTRADIOL
PRETREATMENT

Oestradiol level (μg)	Oxytocin dose level (mU)			
	1	2	4	8
5	1.075	1.181	1.137	1.314
10	1.131	1.257	1.339	1.374
20	1.119	1.025	1.350	1.378
All levels	1.108	1.115	1.275	1.355

*See footnote to Table X(a). In this table the significance of differences between the means for various levels of oxytocin is indicated by underlining the means which do not differ significantly.

the intermediate level of this hormone causing a greater response than either the low or high dose.

Apart from these "main effects" there was also evidence of interaction between the two steroids ($P < 0.10$). The analysis of variance indicated that the overall interaction was largely a matter of interaction between the linear responses to the two hormones ($P < 0.10$), on the one hand, and interaction between the non-linear responses ($P < 0.05$) on the other hand. The former effect can be seen in the differences in response to the two extreme doses of oestradiol in combination with low progesterone as against high progesterone. In combination with the low dose of progesterone the responses to high and low doses of oestradiol were similar, but in combination with the high dose of progesterone the high level of oestradiol gave a greater response than the low level (see Table X(a)) The latter effect indicated that the response to the intermediate dose of oestradiol, relative to the extreme doses, differed when given with the intermediate dose of progesterone from that when given with either of the other doses of progesterone. In combination with the intermediate level of progesterone, but not with other levels of progesterone, the intermediate dose of oestradiol gave a greater uterine response than either the low or high dose (see Table X(b)). This latter difference was large and resulted in the non-linear response to oestradiol over all levels of progesterone noted above.

The modifying effect of oestradiol on the response to progesterone can be examined in another way; by using a t-test to detect significant differences between the means for oestradiol levels within each progesterone level in Table X(a). The results of this examination are summarized below:

$$
\begin{vmatrix} P_1 O_3 \\ P_1 O_1 \\ P_1 O_2 \end{vmatrix} \quad \begin{vmatrix} P_2 O_1 \\ P_2 O_3 \\ P_2 O_2 \end{vmatrix} \quad \begin{vmatrix} P_3 O_1 \\ P_3 O_2 \\ P_3 O_3 \end{vmatrix}
$$

The symbols P and O designate progesterone and oestradiol and the subscripts 1, 2 and 3 the low, intermediate and high dose respectively. Within each column the mean responses are in increasing order from top to bottom. Means not joined by the vertical line differ significantly ($P < 0.05$). At the lowest dose of progesterone, which gave greater responses than the other two levels of this hormone, the level of oestradiol did not have a significant effect. As the level of progesterone increased, a significant opposing effect of increased level of oestradiol appeared. At the intermediate level of progesterone, the intermediate level of oestradiol offset the depressing effect of increased progesterone whereas at the high level of progesterone only the high level of oestradiol offset the depressing effect of the other steroid.

(c) Interaction between steroids and oxytocin levels.

There was a significant interaction between contractions and pretreatments (Table X). When this interaction was partitioned only the interaction between oestradiol level of pretreatment and oxytocin dose ("contractions") was significant ($P < 0.05$). The relevant means for this interaction are presented in Table X(b) and these have been further examined by a t-test. The overall means for each dose of oxytocin are also included in this table.

Although, as indicated above, the overall means show a stepwise increase with oxytocin dose, increases which were significant apart from that between the two lowest doses, this effect was not uniform between levels of oestrogen pretreatment. This interaction was, however, confined to the relative position of the two intermediate levels of oxytocin. At all levels of oestradiol the highest level of oxytocin gave a mean response which was significantly greater than that of the lowest level.

<u>Summary of Area Measurement Findings.</u>

The technique of measuring the response by determining areas under the response curve (for description see text page 68) resulted in a number of significant findings in this experiment.

Pretreatment with steroid hormone combinations had a significant effect upon oxytocin response. This overall effect of pretreatment on all contractions was due largely to the progesterone component. Significantly greater responses occurred at low progesterone doses (Table X(a)), than occurred at medium and high levels of this hormone. Oestradiol, at appropriate dosage, tended to overcome the depressing effect of the higher doses of progesterone and although the effect of the two higher doses of progesterone was similar, a higher level of oestradiol was required to counteract the depressing effect of progesterone at the higher level.

The area measurement method though extremely time consuming, gave results upon analysis that were of greater significance than the results obtained from the amplitude methods. As this technique was an estimate, at least to some degree, of duration as well as amplitude, (both components of a response to stimulus in this type of preparation), it was considered the best approach for measuring and analysing the response in future work of this type.

F. <u>VARIANT OF FACTORIAL EXPERIMENT II.</u>

This additional experiment was run in conjunction with each of the last four blocks by examining the opposite horn to the one used in the Factorial Experiment II. A second observer participated in this experiment using a modified method of stimulation of the uterine strip.

(i) Method.

Six of the pretreatment levels of the factorial were selected,

$P_1O_1; \quad P_1O_3; \quad P_2O_2; \quad P_2O_3; \quad P_3O_2; \quad P_3O_3.$

The alternate horns were assembled immediately their counterpart, selected at random for the main experiment, had been removed from the organ bath. A similar method of assembly of the horn was used except that it was done by a separate observer, and, a 5 g as opposed to a 1 g weight was used when the free end of the cotton was draped over the writing point lever.

Only two doses of oxytocin, 1 mU and 4 mU, were administered, but for a 3 minute not a 45 second period and with 10 minutes between each dose. The preparation was washed twice before the second oxytocin administration, once at the removal of the 1 mU dose and again within five minutes, allowing a further five minutes to elapse before the 4 mU dose was given. This washing routine was also followed before administration of the 1 mU dose.

The response was measured by determing the area under the kymograph tracing during the administration period, plus the area under the tracing for the 3 minute period immediately following this administration (cm^2).

The area for the six minutes immediately prior to the administration of oxytocin was deducted from the sum of areas for the two three minute periods described. The difference represented the response for the level of oxytocin administered and was compared with the responses to these levels for the appropriate pretreatments in the main experiment.

(ii) Analysis of results.

An analysis of variance was carried out for each of the doses of oxytocin used and these are shown on the following page (75):

TABLE XI: ANALYSIS OF VARIANCE OF RESPONSES TO 1 mU DOSE OF OXYTOCIN

Source of Variation	d.f.	M.S.	F
Pretreatment	5	0.0553	
Block	3	0.0605	
Observer	1	0.0547	
Pretreatment x Block	15	0.0232	$\doteqdot 1$ N.S.
Pretreatment x Observer	5	0.0261	$\doteqdot 1$ N.S.
Block x Observer	3	0.0179	<1 N.S.
Residual	15	0.0253	

TABLE XI (a): ANALYSIS OF VARIANCE OF RESPONSES TO 4 mU DOSE OF OXYTOCIN

Source of Variation	d.f.	M.S.	F
Pretreatment	5	0.0780	
Block	3	0.0561	
Observer	1	0.8034	
Pretreatment x Block	15	0.0377	<1 N.S.
Pretreatment x Observer	5	0.0524	1.21 N.S.
Block x Observer	3	0.0333	<1 N.S.
Residual	15	0.0431	

There was no significant interaction between pretreatment and block, nor between pretreatment and observer, nor between block and observer for response to oxytocin at either dose level.

(iii) Comment.

These analyses showed that there was no significant difference between the results obtained by two different observers using slightly different methods. When the responses to pretreatment were ranked for each observer these did not differ.

Conclusions from Factorial Experiments.

The variation in oxytocin response between rat uteri attached to an isotonic system in vitro is high, but this response was altered to a detectable degree in preliminary experiments by pretreatment with steroid hormones. The differences in levels of steroid hormones were relatively great and their effect on oxytocin response could be detected by simple amplitude measurements. On the other hand when a more limited range of dose levels were used simple measurements of amplitude of contraction did not enable detection of significant differences even with 9 replications.

The method of measuring the area of a contraction and subtracting from it the corresponding control area for each oxytocin dose gave significant results (with only 4 animals per treatment) with nine steroid pretreatments used in a factorial experiment. These results did not differ from those of another observer who employed a similar method on each occasion, using the opposite horn of each preparation from six of these pretreatments.

FURTHER COMPARISONS OF THE EFFECTS OF STEROID HORMONES ON THE RESPONSES
OF THE UTERINE MUSCLE OF THE RAT

A. INTRODUCTION.

Evidence from the factorial experiment showed that greater uterine
response to oxytocin occurred when progesterone levels were low. The degree
of this activity was influenced to some extent by the amount of oestradiol
that was present. However, the oestradiol had always been injected after
the rats had received progesterone for two days. Consideration was therefore
given first to the possible effects of reversing the order of oestradiol
administration, and secondly to the influence that the arachis oil diluent
may have had on progesterone absorption when the concentrations of this
hormone were lowered.

B. EXPERIMENT I

"To compare the effects on oxytocin sensitivity of rat uteri
pretreated with either progesterone or oestradiol alone, and to compare the
effects of these hormones as a combined pretreatment given in both the
standard and reverse order."

(i) Method.

A total of 24 rats were used in this experiment. Twelve of these
rats represented the first block, in which 3 rats were randomly allotted to
each of four groups. This block was examined on experiment day 1, and five
weeks later another twelve rats were allotted to a second block in a similar
manner for examination on experiment day 2. One dose level of each steroid
was selected namely 5 mg progesterone and 10 μg oestradiol, and the pattern

of their administration is shown in Plan II.

PLAN II.

	DAY 1	DAY 2	DAY 3	DAY 4
Group I				
Oestradiol (μg)	-	-	10	10
Group II				
Progesterone (mg)	5	5	5	5
Group III				
Progesterone (mg)	5	5	5	5
Oestradiol (μg)	-	-	10	10
Group IV.				
Oestradiol (μg)	10	10	-	-
Progesterone (mg)	5	5	5	5

The procedure for examining the uterine preparations was the same as for factorial experiment II except that the 8 mU dose of oxytocin was omitted. Responses were assessed by measuring the area of contraction as outlined previously. Each uterine strip was weighed on a torsion balance sensitive to 0.001 g immediately it was removed from the organ bath.

(ii) Results.

The results are summarised in Table XII facing page 79. Because of the marked differences between groups in the variances of both strip response and strip weight, differences which could not be removed by simple transformation (see Kempthorne, 1952), analysis of variance was not attempted.

TABLE XII: UTERINE STRIP WEIGHT AND RESPONSE TO STIMULUS WITH OXYTOCIN

Pretreatment	I		II		III		IV	
	Response	Strip wt.	Response	Strip wt.	Response	Strip wt.	Response	Strip wt.
	*Y	(mg)	Y	(mg)	Y	(mg)	Y	(mg)
	40	54	29	46	50	76	45	37
	29	44	58	53	30	44	33	49
	39	50	31	51	60	100	54	55
	27	46	29	47	36	48	37	39
	30	51	41	63	26	83	56	71
	33	42	24	46	84	64	41	66
MEAN	33.0	47.8	35.3	51.0	47.7	69.1	44.3	52.8
± S.E.	±2.2	±1.9	±5.1	±2.7	±8.9	±8.4	±3.8	±5.7

* Y = total response to oxytocin stimulus transformed according to the expression

Y = 10 (x + 10) where x = difference (cm^2) between the oxytocin and control area

for each dose of oxytocin used (1, 2 and 4 mU). For details of this measuring

technique see text.

Approximate "t" tests (Cochran and Cox, 1957) were therefore used to examine the differences that would have been orthogonal in an analysis of variance, i.e. :

$$
\begin{array}{ll}
\text{Groups} & \quad \text{I } \underline{v} \text{ II)} \\
& \quad \text{III } \underline{v} \text{ IV)} \quad \text{N.S.} \\
& \text{I + II } \underline{v} \text{ III + IV) P 0.05}
\end{array}
$$

For both responses the groups (I and II) treated with only oestradiol or progesterone did not differ significantly nor did groups (III and IV) receiving both steroids but in different order. However, the groups receiving both steroids (irrespective of order of administration) had greater strip responses and strip weights than the groups receiving the single hormone only.

(iii) Comment.

There appeared to be no advantage in reversing the order of administration of oestradiol and progesterone from that used in the earlier experiments.

C. EXPERIMENT II.

"To compare the effects of two progesterone dilutions used during the pretreatment period on the response of ovariectomised rat uterine strips to a stimulus with 3 mU of oxytocin".

(i) Method.

A total of 24 rats were used in this experiment and they were randomly allotted to four pretreatment groups. The oestrogen component of each pretreatment was kept constant with 10 µg oestradiol being used on

each group.

The progesterone component was varied, 1 mg and 5 mg of this hormone were the doses selected; and each was given to one of the groups in concentrated form (0.1 ml and 0.5 ml respectively). Each of the two remaining groups received one of these dose levels diluted with 0.5 ml arachis oil. The pattern of pretreatment administration is shown in Plan III. Experimental examination of all preparations was carried out on Day 5.

PLAN III.

	DAY 1	DAY 2	DAY 3	DAY 4
Group I				
Oestradiol (μg)	-	-	10	10
Progesterone (mg) DILUTED	1	1	1	1
Group II				
Oestradiol (μg)	-	-	10	10
Progesterone (mg) CONCENTRATED	1	1	1	1
Group III				
Oestradiol (μg)	-	-	10	10
Progesterone (mg) DILUTED	5	5	5	5
Group IV				
Oestradiol (μg)	-	-	10	10
Progesterone (mg) CONCENTRATED	5	5	5	5

The same procedure was used to measure uterine contractions, except that only one dose of oxytocin, namely 3 mU, was given to each preparation. This dose was selected because small areas had been difficult to measure, while larger areas with many indentations in the contraction

TABLE XIII: UTERINE STRIP WEIGHT AND *RESPONSE TO 3 mU OXYTOCIN

| Pretreatment | I | | II | | III | | IV | |
	Response $(cm^2 \times 100)$	Strip wt. (mg)	Response $(cm^2 \times 100)$	Strip wt. (mg)	Response $(cm^2 \times 100)$	Strip wt. (mg)	Response $(cm^2 \times 100)$	Strip wt. (mg)
	3	66	21	46	19	75	55	106
	8	80	15	54	42	91	25	92
	4	48	25	55	14	85	18	96
	2	68	7	55	41	76	11	90
	1	52	19	82	37	64	47	81
	6	97	6	56	22	85	19	71
MEAN	4.0	68.5	15.5	58.0	29.2	79.3	29.2	89.3
$\pm$ S.E.	± 1.1	± 7.4	± 3.1	± 5.0	± 5.0	± 3.9	± 4.3	± 5.0

* Response as the difference between oxytocin and control area

(cm^2) coded by x100 to remove decimal figures.

line had proved laborious in terms of time. Once again uterine weights were recorded as previously described.

(ii) Results.

The results are summarised in Table XIII facing page 81. Because of marked differences in the variance of strip response to oxytocin, differences which were not likely to be removed by transforming to logs, (Kempthorne, 1952), analysis of variance was restricted to the uterine strip weights and an approximate "t" test (Cochran and Cox, 1957) was used to examine the differences in uterine strip response to oxytocin.

Using this test the comparison Groups I v II was significant ($P < 0.001$) whereas the comparison Groups III v IV was not significant ($P > 0.05$). Thus there was a marked effect of dilution at the lower dose of progesterone but no effect (the mean responses were identical) at the higher dose.

The analysis of variance of uterine strip weights is shown below (Table XIII(a)).

TABLE XIII(a):   ANALYSIS OF VARIANCE OF STRIP WEIGHTS

Source of Variation	d.f.	M.S.	F	
Pretreatment	3	1099.3	6.1	$P < 0.01$
Dilution	1	0.4		
Dosage	1	2667.0	14.8	$P < 0.01$
Dilution x Dosage	1	630.4	3.5	$0.10 > P > 0.05$
Error	20	180.1		

The effect of dose of progesterone was clearly significant ($P < 0.01$) whereas the effect of dilution of the hormone on uterine strip

weight was not significant. Nor in this case was there any clear cut interaction between dilution and dosage (0.10>P>0.05) although it was noted that the progesterone, when used in the concentrated form, produced relatively greater dose response differences (mean strip weights of 58.0 ± 5.0 mg and 89.3 ± 5.0 mg for the 1 mg and 5 mg doses of progesterone respectively) than when used in the diluted form (68.5 ± 7.4 mg and 79.3 ± 3.9 mg respectively).

Conclusions from Comparative Experiments.

Uterine strip weights were a more sensitive indication of pretreatment effect than was uterine oxytocic response. This was not unexpected in view of the opportunities for error when applying a mechanical technique on a between animal basis.

Oestradiol alone produced the lightest uteri and these appeared to be the least sensitive to oxytocin. Combined progesterone oestradiol pretreatment given in standard order produced the heaviest uteri and these appeared to be the most sensitive.

Arachis oil diluent however, interfered markedly with oxytocic response when progesterone levels were low, but it had less effect when these levels were raised. Conversely there were greater differences between the uterine weights for the two progesterone doses when the arachis oil was omitted.

It seems reasonable to conclude therefore that combinations of progesterone and oestradiol can influence uterine oxytocic response to a degree that depends upon the dose administered and the concentration in which it is contained.

CHAPTER 6.   SUMMARY AND CONCLUSIONS.

Although critical time sequence studies of the birth process have been carried out in a number of species such as man (Caldeyro Barcia and Poseiro, 1959; Jeffcoate, 1965), rabbit (Cross, 1958b; Bengtsson, 1957; Csapo, Takeda and Wood, 1963), cattle (Gillette and Holm, 1963), sheep (Hindson et al, 1965) and pigs (Jones, 1966), the events which precipitate and control parturition are as yet not completely understood.   After consideration of the various techniques which had been utilised in these and similar investigations a basic in vitro study of some of the events associated with changing activity of the myometrium was planned.

The experiments reported in this thesis were undertaken, therefore, with the objective of defining the effects of two groups of steroid hormones (oestrogens and progestogens) on the response of the myometrium to stimulus with synthetic oxytocin.

In the intact animal both nervous and endogenous endocrine factors, particularly those of the ovary and posterior pituitary gland, have been shown to exert considerable influence over the activity of the myometrium (see literature review) and as long ago as 1904 it had been shown that the excised uterus would contract when freed from its nervous connections (Kurdinowski, 1904).   Oxytocin in suitable dosage had been shown to override these nervous effects at least in the rabbit (Cross, 1958b).   Moreover, with both in vitro and in vivo preparations pre-treatment of the experimental subject with either progestogens or oestrogens has been shown to markedly modify the response to this drug (Burnstock et al, 1963; Reynolds, 1965).

With these points in mind the techniques employed in this

study involved selection of uterine strips from previously ovariecto-
mized subjects which had, subsequent to this operation, been treated
with a specified combination of the steroid hormones under test.
The uterine strips (in most cases one complete uterine horn) were
assembled in an organ bath and connected to a lever which would record
contractions on a kymograph in a conventional isotonic system.   The
responsesof the strips to graded doses of oxytocin were measured either
by determining the amplitude of the resulting contraction from a given
base line or in the later experiments by measuring the area under the
dose response trace following such a stimulus, and relating it to the
activity which had been occurring (if any) immediately prior to the
stimulus.   As numerous other factors such as temperature, tension,
ionic status of the solutions used and assembly technique were known
to affect such uterine preparations considerable care was taken in an
effort to standardise these conditions for each experiment.   Minor
modifications in method, however, were often found necessary to improve
technique from one experiment to the next - these changes are documented
and discussed in the main text of the thesis.   Initially ovariecto-
mized guinea pigs were used as experimental subjects but these were
abandoned later for ovariectomized albino rats.   Finally preliminary
experiments were undertaken with rats pregnant in only one uterine horn,
a situation that was achieved by mating after unilateral ovariectomy
(see Appendix VI).

Experiments with the guinea pig demonstrated that pretreatment
with oestradiol and progesterone did increase the sensitivity of the
uterus to oxytocin when the responses obtained were compared with those
from control animals which had not been pretreated with these hormones.
Variability of response between animals within each experimental group

was high and spontaneous activity in some preparations, particularly from control groups was a major problem. It was clear that large numbers of animals would be necessary in further studies of this type if satisfactory precision was to be obtained. The guinea pig also presented certain anatomical and physiological features that were undesirable for future work with pregnant animals and these factors, coupled with the findings of other authors that the uterus of this species was unsuitable for oxytocin assay (Holton, 1948; Stewart, 1949; Thorp, 1950; Lewis, 1964) led to a decision to continue the investigation with material from a strain of Sprague Dawley rats.

The interesting problem which remains unsolved, however, is the reason for continuing spontaneous activity in preparations from some guinea pigs which had been ovariectomized several weeks prior to the preparation being examined. Residual effects of steroid hormones known to affect uterine activity seem unlikely after this length of time; nor was there any evidence from the experiments to indicate this. The possibility of an alternative endogenous source of hormone developing after ovariectomy from tissue such as that present in the adrenal gland cannot be dismissed although no relationship between uterine response and adrenal weight was found. The influence of exogenous hormone in the diet was unlikely to account for large differences between animals. No explanation for the phenomenon apart from inherent animal variation can be given.

Preliminary experiments with the rat demonstrated that differences between pretreatments with widely divergent combinations of oestradiol and progesterone could be measured and a factorial experiment was designed to determine the relative effects of different combinations of these two hormones. Although variability of response between animals within a

given treatment group again proved to be high, adequate precision was
obtained to detect differences between the various pretreatment combina-
tions when the area technique for estimating the degree of response to
a given oxytocin stimulus was used. Where amplitude was taken as the
index of response, however, inconclusive results were obtained.

As had been anticipated the magnitude of the response obtained
to each oxytocin stimulus was clearly dependant on the dose of oxytocin
irrespective of the pretreatment combination that had been used. Even
though an interaction between oxytocin dose and oestradiol level of pre-
treatment was observed in this experiment the overall responses showed a
stepwise increase with increasing oxytocin dose, and at all levels of
oestradiol pretreatment, the highest level of oxytocin gave responses
which were significantly greater than the responses observed at the
lowest dose level of this drug.

Differences related to the particular pretreatment combination
that had been used were observed and these were largely due to the
progesterone component, greater responses being obtained at the low pre-
treatment level with this hormone than at either of the two higher levels.
Oestradiol, at appropriate dosage, tended to overcome the depressing
effect of increasing doses of progesterone although this did not, in this
experiment, appear to be a simple response. At the low level of progest-
erone for example increasing doses of oestrogen had no significant effect
on response whereas at the middle progesterone level the middle dose of
oestrogen was most effective and at the high progesterone level the highest
dose of oestrogen was necessary to produce the greatest response. Whether
other factors were operating to produce this apparent complexity of effect,
(see comments on the effect of the vehicle in which the drugs were admini-
stered in the final section of this summary), or whether the effect of

oestrogen on the myometrium is complex in this species as Gennel (1940) has suggested, could not be determined in this study. The generally held view that oestradiol activates and progesterone inhibits activity of the myometrium was therefore upheld, at least when one steroid hormone was acting in the presence of the other, and the ultimate effect obtained appears to depend on the particular ratio that is used. These results were confirmed by a second observer who used the opposite uterine horn from rats in six of the nine pretreatment combinations in the last four blocks of this experiment.

The final section of this thesis describes two experiments that were carried out with a view to determining whether the order of administration of oestradiol and progesterone was of any significance and whether concentration of the drug behind used, i.e. the presence of greater or lesser amounts of diluting vehicle, would have any effect on the responses obtained. In the first experiment no differences between oestradiol or progesterone pretreatments when given alone were observed, a rather surprising finding as Frank et al (1925) described rythmic activity of the uterus in ovariectomized rats 24 hours after administration of oestrogen, and this response has been confirmed according to Reynolds (1965) in several other species. It is possible that the vehicle used for injection of the oestradiol, arachis oil, may have been responsible for this apparent lack of response as in the rabbit the latent period is lengthened to many days before maximum motility is reached when oil is used as the suspending vehicle (Reynolds, 1965). Progesterone on the other hand cannot inhibit the uterus unless it has been treated or subjected to the influence of oestrogen according to the same author. If the threshhold to the oxytocic stimulus had been altered by either form of pretreatment differences in response were to be anticipated.

When, however, responses to treatment with either oestradiol or progesterone alone were compared to responses of strips from animals which had received both steroids, irrespective of the order of administration of these hormones, significant differences were observed and greater responses occurred with the latter form of pretreatment. The order of administration of the steroids did not appear to matter, a point which partially nullifies the argument above in regard to the possible effects of a prolonged latent period when oestradiol is given in an oily vehicle.

In the second experiment the arachis oil diluent interfered markedly with response to oxytocin when the progesterone dose levels were low but had no effect when progesterone dose levels were high. At the low dose level of progesterone responses of strips from animals which had received pretreatment with undiluted progesterone were significantly greater than responses from those where pretreatment had taken place with the hormone in the diluted form. This dilution effect was not observed when uterine strip weight was taken as the index of response although progesterone used at two different dose levels in a concentrated form resulted in relatively greater differences between uterine strip weights for each group than when it was used at the same dose rates in a diluted form.

The following conclusions were drawn following these investigations:

1. The use of an isotonic system involving excised uterine strips subjected to stimuli with graded doses of oxytocin in an organ bath, the strip being connected to a lever recording on a kymograph, appears to have severe limitations as a technique for making comparisons between

groups of animals subjected to different experimental treatments.
Isolated uterine muscle, when stretched. liberates a substance that
slowly increases uterine tone to the point of contracture according to
Jung (1966), and hence an isometric rather than an isotonic system may
provide a better measurement of the effects taking place. With an
isometric system the problems of unrecorded stretch do not arise
(Schofield, 1954; Csapo, 1954).

2. Uterine strips from ovariectomized guinea pigs are unsuitable for
experiments involving responses to oxytocin because of the wide varia-
bility between animals. The reasons for this variation are unknown
and would appear to be worthy of investigation. Pretreatment of guinea
pigs which had been ovariectomized with a combination of oestradiol and
progesterone did however increase the response obtained with a given
stimulus by oxytocin.

3. When uterine strips from ovariectomized rats pretreated with combin-
ations of these two steroid hormones are subjected to stimuli with
oxytocin responses are obtained which are greater than those resulting
from pretreatment with either hormone alone. The degree of response
obtained is related to the magnitude of the stimulus applied and depends
also on the particular combination of the steroid hormones used. In
general increasing doses of progesterone tend to decrease the response
of the strip to a given dose of oxytocin and with increasing doses of
progesterone greater doses of oestrogen are necessary to override this
effect.

4. Where relatively low doses of progesterone are used for pretreatment
of animals in this type of experimental preparation dilution with arachis
oil should be avoided as it appears to modify the response obtained

depending on the dilution used.

5.   In these preparations the most effective method of measuring the
response to oxytocin was measurement of the area under the dose response
tracing for a fixed period of time following its administration.   This
measurement was then related to the activity which had been taking place
over a similar time interval immediately prior to administration of the
stimulus and the difference between the two areas was used as a measure
of the response.   The technique approximates to a measure of the
additional work performed by the uterine strip as a result of an oxytocic
stimulus.

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TREATMENT GROUP*	GUINEA PIG	UTERINE HORN	R	R	1	2	4	8	R	1	2	4	8	1	1	2	2	4	4	8	8	1	2	4	8	TOTAL RESPONSE FOR STRIP (cm)	TOTAL RESPONSE FOR ANIMAL (cm)	WEIGHT AT OVARIECTOMY (g)	WEIGHT AT SLAUGHTER (g)	CHANGE IN WEIGHT (g)	WEIGHT OF UTERINE STRIP (g)	DAYS OVARIECTOMY TO SLAUGHTER
I	257	RIGHT	1.6	0	2.8	3.2	3.4	4.9	1.3	2.7	3.3	4.9	7.3	2.3	1.4	1.3	3.0	6.4	4.6	9.7	5.5	1.6	4.3	5.4	10.6	91.5)	192.0	460	420	− 40	RH = 0.15	28
		LEFT	0.6	0.7	2.4	3.9	5.0	12.3	0.6	2.7	2.7	4.7	7.5	2.0	1.9	2.7	2.3	4.2	5.8	13.3	6.9	1.9	3.1	4.5	8.8	100.5)					LH = 0.19	
	251	RIGHT	10.6	1.5	2.4	2.5	4.3	9.6	0.9	2.5	2.9	5.1	8.4	2.1	1.9	3.1	2.6	5.1	5.5	10.9	9.5	2.2	4.6	5.6	11.0	114.8)	149.7	465	420	− 45	RH = 0.21	29
		LEFT	0	0	1.0	1.2	1.8	3.2	0.8	1.3	1.3	1.8	2.6	1.0	1.1	0.9	1.2	1.7	2.1	2.8	2.6	1.3	1.2	1.7	2.3	34.9)					LH = 0.21	
	203	RIGHT	11.0	0.9	1.4	1.2	2.8	4.4	0.4	1.1	1.7	2.1	4.3	1.4	1.2	1.5	1.4	2.4	2.0	4.0	4.2	1.2	1.7	2.3	5.6	60.7)	75.1	530	517	− 13	RH = 0.25	40
		LEFT	0.7	0.4	0.6	0.6	0.7	1.1	0.4	0.6	0.6	0.7	1.2	0.5	0.5	0.4	0.3	0.5	0.5	0.8	0.9	0.4	0.5	0.6	1.0	14.4)					LH = 0.25	
	233	RIGHT	1.0	0.6	0.5	0.5	0.6	0.6	0.2	0.4	0.5	0.4	0.6	0.3	0.2	0.4	0.2	0.4	0.3	0.4	0.4	0	0.1	0.2	0.3	9.1)	230.6	640	706	+ 66	RH = 0.18	41
		LEFT	3.8	0.9	5.2	9.3	14.4	17.7	1.0	5.6	8.2	14.2	17.2	4.8	4.9	6.7	7.2	15.3	8.1	18.3	17.9	3.9	7.1	17.2	12.1	221.5)					LH = 0.11	
II	246	RIGHT	0	0	5.8	9.2	16.1	16.9	0	3.6	5.7	14.7	15.8	10.7	3.5	5.5	3.8	14.9	11.0	17.3	16.2	2.1	5.1	16.9	16.4	211.3)	450.9	440	470	+ 30	RH = 0.50	28
		LEFT	0	0	14.0	6.3	13.8	14.2	0	13.4	8.3	13.6	14.0	8.3	7.1	14.0	8.2	14.4	14.2	14.8	14.5	3.0	14.2	13.4	14.5	239.6)					LH = 0.44	
	250	RIGHT	0.8	0.6	1.2	1.6	2.6	4.3	0.6	1.8	2.2	4.2	6.6	2.9	2.0	2.3	2.5	3.6	3.1	7.6	3.9	2.4	1.7	4.2	11.2	73.9)	129.4	470	450	− 20	RH = 0.22	29
		LEFT	1.3	0.7	1.2	2.9	1.7	3.1	0.6	1.6	1.5	1.9	2.3	0.7	1.4	0.8	1.0	1.4	1.8	3.7	8.9	0.9	1.4	3.9	10.8	55.5)					LH = 0.26	
	247	RIGHT	4.0	4.2	2.7	2.4	3.1	3.8	1.5	1.6	2.0	3.0	4.1	1.7	1.5	2.1	2.3	3.2	3.5	7.6	5.4	2.6	3.7	6.1	7.4	79.5)	110.6	520	643	+ 123	RH = 0.21	30
		LEFT	0.7	0.7	0.9	1.1	1.2	1.5	0	0.7	1.2	1.3	2.1	1.1	1.0	1.2	1.6	1.2	1.6	2.3	2.2	1.2	1.4	2.0	2.9	31.1)					LH = 0.18	
	202	RIGHT	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	13.0	9.0	9.3	10.0	5.0	6.6	9.8	14.6	18.0	−	−	−	−	475.3)	713.6	590	729	+ 139	RH = 0.32	41
		LEFT	19.0	19.0	5.7	9.4	13.1	18.5	1.7	7.4	9.2	11.4	18.7	3.6	6.9	19.0	19.0	18.0	18.0	7.7	2.2	1.2	1.3	0	0.9	238.3)					LH = 0.32	
III	258	RIGHT	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	19.0	437.0)	676.4	670	745	+ 75	RH = 0.30	52
		LEFT	6.6	4.6	7.2	13.4	15.1	15.7	0.7	5.5	8.8	13.3	16.7	3.7	3.5	7.8	8.0	15.3	17.4	18.0	17.4	2.7	12.3	7.7	18.0	239.4)					LH = 0.24	
	256	RIGHT	1.0	0.6	0.9	0.8	1.4	1.7	0.3	0.7	0.7	1.0	1.5	0.5	0.5	1.0	0.9	1.2	2.1	2.0	1.7	0.5	0.6	1.2	1.9	33.1)	33.1	460	628	+ 168	RH = 0.17	43
		LEFT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0)					LH = 0.15	
	254	RIGHT	0.3	0.2	0.3	0.4	0.4	0.6	0	0.2	0.2	0.3	0.5	0.2	0.2	0.2	0.3	0.3	0.4	0.5	0.4	0.2	0.3	0.3	0.5	7.0)	19.1	480	625	+ 145	RH = 0.17	44
		LEFT	3.6	0	0.3	0.4	0.5	0.7	0.2	0.3	0.4	0.5	0.6	0.2	0.2	0.4	0.2	0.4	0.4	0.6	0.6	0.3	0.3	0.4	0.6	12.1)					LH = 0.20	
	255	RIGHT	1.6	0.8	3.7	3.9	15.6	15.2	0.5	1.5	2.4	15.6	6.9	1.3	1.4	3.5	2.4	16.0	3.8	14.0	6.7	1.2	2.7	4.7	16.2	141.6)	335.9	560	686	+ 126	RH = 0.49	45
		LEFT	2.1	0.3	3.1	3.1	16.7	18.3	0.7	5.8	19.8	2.2	6.1	4.7	19.7	6.2	20.0	20.4				1.3	6.5	20.4	6.7	194.3)					LH = 0.47	

*Group I − short period ovariectomy to slaughter:
 Group II − short period ovariectomy to slaughter + arachis oil:
 Group III − long period ovariectomy to slaughter.

RESPONSES TO OXYTOCIN STIMULUS (GUINEA PIG EXPT. III) APPENDIX III.

PAIR DAY	GUINEA PIG	UTERINE HORN	**RESPONSES (Amplitude, cm)						
			* R	1 mU	4 mU	R	4 mU	1 mU	R
I	270 (T)	RIGHT	2.0	6.0	8.9	1.9	10.0	9.0	1.5
		LEFT	3.7	5.6	12.7	2.1	14.8	8.6	13.0
	269 (C)	RIGHT	1.9	1.3	2.1	0.7	2.0	1.0	0.4
		LEFT	0.0	0.0	0.3	0.4	1.0	0.6	0.0
II	264 (T)	RIGHT	1.2	3.4	7.1	2.6	5.8	3.6	2.1
		LEFT	2.8	5.6	7.5	6.7	13.3	7.9	6.0
	266 (C)	RIGHT	1.5	0.8	1.3	0.3	1.2	0.5	0.3
		LEFT	0.0	0.0	0.2	0.4	0.8	0.4	0.3
III	259 (T)	RIGHT	4.3	6.5	11.5	4.3	12.3	9.5	4.3
		LEFT	7.8	2.5	10.4	8.5	2.7	6.4	7.5
	260 (C)	RIGHT	4.5	2.1	4.6	1.3	3.4	1.5	0.6
		LEFT	0.5	0.0	0.5	1.0	2.7	1.5	1.7
IV	263 (T)	RIGHT	7.5	10.0	8.3	5.8	16.3	7.3	6.3
		LEFT	11.7	5.5	19.0	5.1	10.4	7.0	5.1
	265 (C)	RIGHT	0.6	1.0	1.5	0.5	1.4	2.3	0.7
		LEFT	0.4	0.6	7.0	2.6	3.2	6.5	6.2
V	268 (T)	RIGHT	6.6	8.5	12.7	5.3	13.6	7.4	5.1
		LEFT	4.9	9.5	14.9	4.4	16.2	13.2	4.7
	261 (C)	RIGHT	14.0	14.6	15.0	15.0	15.2	15.2	3.6
		LEFT	15.6	14.9	14.6	12.1	16.6	16.8	4.7

* R = Ringer solution.   T = Treatment group.   C = Control group.
** Note the sequence of administration of the oxytocin is shown for the right horn.   The sequence was reversed for the left horn.   (See method in text).

WEIGHTS OF GUINEA PIGS, UTERINE STRIPS, ADRENAL GLANDS (GUINEA PIG EXPT. III) APPENDIX IV.

PAIR DAY	GUINEA PIG	WEIGHT AT OVARIECTOMY (g)	*WEIGHT AT SLAUGHTER (g)	CHANGE IN WEIGHT (g)	WEIGHT OF ADRENAL GLANDS (g)	*WEIGHT OF UTERINE STRIP (g)
I	270 (T)	433	440	+ 7	R 0.25) L 0.24) 0.49	R 0.38) L 0.41) 0.79
	269 (C)	515	520	+ 5	R 0.19) L 0.20) 0.39	R 0.14) L 0.12) 0.26
II	264 (T)	504	520	+16	R 0.21) L 0.21) 0.42	R 0.42) L 0.43) 0.85
	266 (C)	525	580	+55	R 0.23) L 0.24) 0.47	R 0.21) L 0.20) 0.41
III	259 (T)	539	535	- 4	R 0.22) L 0.23) 0.45	R 0.50) L 0.48) 0.98
	260 (C)	561	560	- 1	R 0.22) L 0.26) 0.48	R 0.10) L 0.12) 0.22
IV	263 (T)	533	530	- 3	R 0.21) L 0.22) 0.43	R 0.46) L 0.56) 1.02
	265 (C)	617	615	- 2	R 0.19) L 0.21) 0.40	R 0.16) L 0.16) 0.32
V	268 (T)	565	560	- 5	R 0.21) L 0.18) 0.39	R 0.37) L 0.41) 0.78
	261 (C)	667	665	- 2	R 0.19) L 0.24) 0.43	R 0.36) L 0.41) 0.77

* Weight at slaughter = live weight.   L = Left.   R = Right.   T = Treatment group.
* Weight of Uterine Strip = the uterine tissue used in the experiment.   C = Control group.

	1 mU dose response				2 mU dose response				4 mU dose response				8 mU dose response			
	B_{2A}	B_{3A}	B_{4A}	B_{5A}	B_{2A}	B_{3A}	B_{4A}	B_{5A}	B_{2A}	B_{3A}	B_{4A}	B_{5A}	B_{2A}	B_{3A}	B_{4A}	B_{5A}
P_1O_1	11	1	1	12	16	5	3	4	23	12	16	10	8	16	20	24
P_1O_2	20	4	4	5	33	3	11	1	7	24	18	11	27	28	27	15
P_1O_3	3	0	1	2	2	2	5	7	11	7	7	20	20	11	20	9
P_2O_1	-4	3	0	2	10	1	-1	6	-3	-3	1	-1	8	12	7	15
P_2O_2	11	6	1	7	23	9	3	6	37	37	1	5	26	20	3	13
P_2O_3	3	7	-1	6	8	-6	0	-10	13	26	4	8	17	17	5	16
P_3O_1	-4	4	3	4	14	1	4	8	10	15	2	-6	7	23	2	1
P_3O_2	-2	-3	1	1	1	20	3	7	5	5	4	20	3	21	-3	20
P_3O_3	1	9	-1	16	1	12	9	7	4	11	28	27	8	22	6	26

* B_{2A} - B_{5A} = Blocks.

** P_1O_1 - P_3O_3 = Treatment combinations of oestrogen and progesterone.

APPENDIX VI: PRELIMINARY EXPERIMENTS WITH PREGNANT RATS.

A. Introduction.

In the previous experiments it had been possible to detect differences in oxytocic response of *in vitro* preparations from rats that had been pretreated with steroid hormones.

These differences were not great but they were sufficient to show that the effect of pretreatment could vary with the dose of steroid that was used and with the amount of suspending vehicle in which these hormones were contained.

The next stage was to take this *in vitro* technique and use it to determine whether there were differences in oxytocin sensitivity between uteri of pregnant rats at various stages of gestation.   Minor investigations had indicated that preparations from gravid uteri would be unsuited to the present system

(a) because the size of the pregnant horn permitted only portions of it to be accommodated in the organ bath

(b) the use of a complete transverse section of the gravid horn was impractical because its bouyancy in the organ bath affected lever tension

(c) when transverse sections of a gravid horn were cut longitudinally, and thereby reduced to a manageable size, they no longer represented the composite unit of circular and longitudinal muscle, upon which all previous comparisons had been made.

It seemed desirable therefore to use rats that had become pregnant in one uterine horn only so that the non pregnant horn could be used as a suitable *in vitro* preparation.

This was accomplished by removing one ovary only from mature rats one week prior to mating.   As it was not possible to estimate the number

that would become pregnant a fixed experimental design could not be employed. Under these circumstances it was decided to consider one stage of the gestation period only, and the period 48 hours prior to parturition was selected in the first instance. Pregnancy was diagnosed by visual assessment of the rat when hanging in a head downwards position and estimates of parturition dates, although related to the mating period selected, were largely subjective.

B. Experiment 1.

"To compare the effects of approaching parturition on the oxytocic response *in vitro* of non-pregnant uterine horns obtained from pregnant rats".

(i) Method.

Six mature female rats between 130 and 160 g body weight were hemiovariectomized using the standard procedure one week prior to mating. The left ovary was removed from half the number, and the right ovary from the remainder. The hemiovariectomized rats were then randomly allotted to two cages, three rats to a cage. A male rat was introduced to each cage and allowed to remain there for nine days. Fourteen days after the removal of the males, one rat was slaughtered for photographic purposes. As the time of parturition appeared close all other rats were examined the following day; unfortunately one produced young during the night.

Preparations from the non-pregnant horn were limited to 1 cm in length, as preparatory experiments had demonstrated that these preparations stretched to a very considerable degree. Doses of 1 mU and 4 mU of oxytocin were administered, and responses were measured with a planimeter as described previously. The administration time of each oxytocin dose

was extended to 3 minutes, for it was anticipated that the frequency of lever excursions would increase with pregnant preparations. Thus planimeter measurements would be less accurate if the area response to the dose recorded on the kymograph was not increased also.

(ii) Results.

The findings from the rats which became pregnant are summarised in Table XIV below. Rat 5 failed to become pregnant and rat 6 gave birth 12 hours before the experiment began - both have been included in the Table.

TABLE XIV: WEIGHT OF RAT, NUMBER OF PUPS IN LITTER, WEIGHT AND *RESPONSE TO OXYTOCIN OF THE NON PREGNANT HORN FROM PREGNANT, NON PREGNANT AND POST PARTUM HEMIOVARIECTOMIZED RATS.

Rat	Wt. of rat (g)	Estimated hours pre-partum	No. of pups in litter	Wt. of non pregnant horn (mg)	Response to oxytocin 1 mU	4 mU
1	356	1	13	242	18	51
4	346	24	13	229	18	51
3	323	48	12	252	0	25
5	309	(not pregnant)		134	3	11
6	263	12 (post partum)	12	124	3	11

*Response = 10 x (cm^2) where x = difference between treatment and control area (for method of measurement see text page 68).

(iii) Comment.

It was interesting to note that identical oxytocin responses were shown for two of the pregnant preparations and that the two preparations that were non pregnant at the time of examination also gave identical results but at a lower level. A second trial was attempted using more

animals and employing a different mating technique to obtain a better assessment of parturition date.

C. Experiment II.

"A further comparison to determine the effects of approaching parturition on the oxytocic response of non pregnant uterine horns from pregnant rats".

(i) Method.

A total of 11 hemiovariectomized mature female rats were allotted, at random, two or three to each of four cages. A male rat was placed in each cage for a 3-day period. The mating period was thus six days shorter than the one adopted in the previous experiment. This enabled parturition dates to be anticipated with greater accuracy.

It was decided to record both the weight and the number of each litter, on the premise that high average foetus weight would be indicative of approaching term.

(ii) Results.

The results are summarised in Table XV on the following page (v).

TABLE XV: WEIGHT OF RAT, AVERAGE FOETUS WEIGHT, WEIGHT AND *RESPONSE TO OXYTOCIN OF THE NON PREGNANT HORN FROM PREGNANT AND NON PREGNANT HEMIOVARIECTOMIZED RATS.

| | Rat | Wt. of rat (g) | Average foetus wt. (g) | Wt. of non pregnant horn (mg) | Response to oxytocin | | |
					1 mU	4 mU	Mean
PREGNANT	2	314	3.7	275	25	60	42.0
	8	260	3.4	258	20	44	32.0
	10	203	2.3	242	27	39	33.0
	11	257	2.0	335	28	56	42.0
	3	325	1.3	299	13	30	21.5
	7	221	1.2	207	25	53	39.0
	9	255	1.1	199	26	67	46.5
MEAN		262	2.14	259	23.3	49.9	36.6
± S.E.		±16.8	±0.19	±18.4	± 1.9	±4.9	±3.1
NON PREGNANT	1	255		155	1	58	29.5
	6	236		144	28	34	31.0
	5	199		142	4	4	4.0
	4	251		139	15	36	25.5
MEAN		235		145	12.0	33.0	22.5
± S.E.		±12.8		±3.5	±6.1	±11.1	±6.3

* Response = $10 \times (cm^2) + 5$ where x = difference between treatment and control area (for method of measurement see text page 68).

(iii) Comment.

The anticipated parturition time for each hemipregnant preparation was within ± 36 hours, and it was noted that apart from rat 3 the oxytocic responses of these preparations were not widely divergent. The preparations from the pregnant rats gave greater responses to the oxytocin stimulus but this may have been a reflection of the amount of tissue used in the preparation rather than any difference in sensitivity.

No obvious relationship between average foetus weight and response to oxytocin was observed. The possibility of differentiating preparations in terms of their proximity to parturition with the present method therefore appeared to be remote. Nevertheless the next step was to compare groups at two widely separated stages of the gestation period.

Two different oxytocin doses appeared to offer little advantage in the assessment of response and it was decided to use an intermediate dose of 3 mU in the next experiment.

D. Experiment III.

"To compare the effects on the oxytocic response of non pregnant uterine horns from pregnant rats at two widely divergent stages of pregnancy."

(i) Method.

Nine hemiovariectomized rats were allotted to four different cages so that three of the cages contained 2 rats each, while 3 were contained in the fourth cage.

A male was introduced to each of the first two cages and left there for four days before removal. Four days after these males were removed, a male rat was put into each of the second two cages, and again these were allowed to remain for a four day period.

(ii) Results.

The examination of all preparations was conducted on the day after the first litter had been produced, but as another rat produced a litter early that morning and as yet another had failed to conceive, only one pregnant rat remained in the first mated group. The findings for all rats is shown on the following page (vii) in Table XVI.

TABLE XVI: WEIGHT OF RAT, AVERAGE FOETUS WEIGHT AND *RESPONSE TO
OXYTOCIN OF THE NON PREGNANT HORN FROM PREGNANT, NON
PREGNANT AND POST PARTUM HEMIOVARIECTOMIZED RATS.

Rat		Mating group	Average foetus wt. (g)	Wt. of rat (g)	Wt. of non pregnant horn (mg)	Response to 3 mU dose of oxytocin
1		I	5.1	284	94	61
4		II	4.0	227	111	1
11	PREGNANT	II	1.5	253	112	65
2		II	0.8	240	70	50
5		II	0.4	200	127	38
6	POST	I	5.0	286	92	50
01	PARTURIENT	I	7.4	267	78	90
3	NOT	II	−	200	99	96
0	PREGNANT	I	−	310	96	68

* Response = 10 x (cm²) + 42 where x = difference between treatment
and control area (for method of measurement see text page 68).

(iii) Comment.

The lack of preparturient rats in the first group to be mated due
to two rats having given birth made comparisons of oxytocin response for the
two stages of gestation impossible. Rat 1, the rat closest to parturition,
did not in fact produce the greatest oxytocic response in this case.

The tracings from all the hemi pregnant rats that were in mid-
pregnancy (group II) were similar on visual appraisal but for some reason
not understood the response to oxytocin of the strip from rat 4 appeared to
be negative i.e. less active after oxytocin stimulus than before. The
tracings of the non-pregnant preparations and that of the only preparturient
preparation were very different in terms of spontaneous activity but both
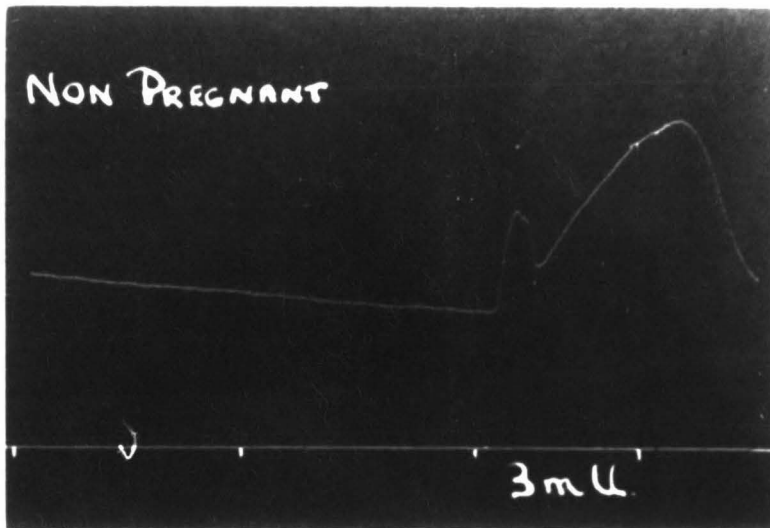responded to oxytocin stimulus and with the examination being limited to

FIG. VII: TRACING FROM NON PREGNANT RAT UTERUS
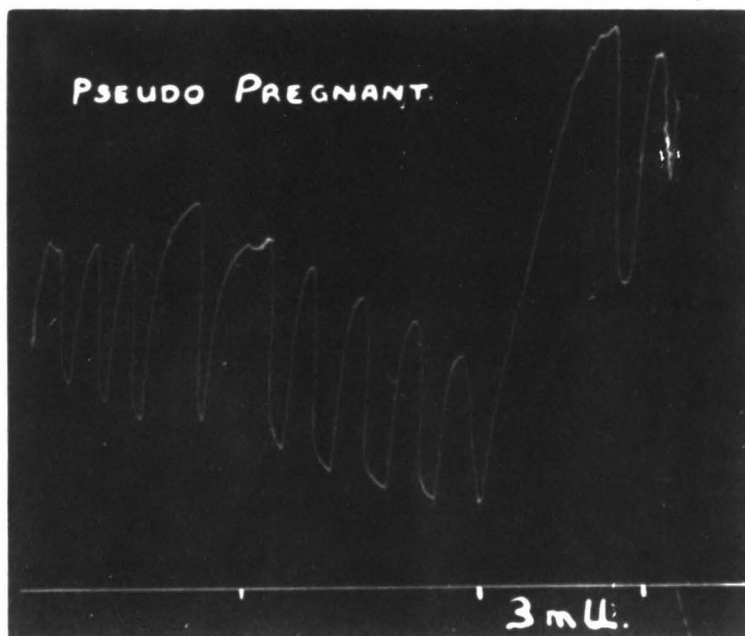(3 mU = dose of oxytocin given)

FIG. VIII: TRACING FROM PSEUDO PREGNANT RAT UTERUS
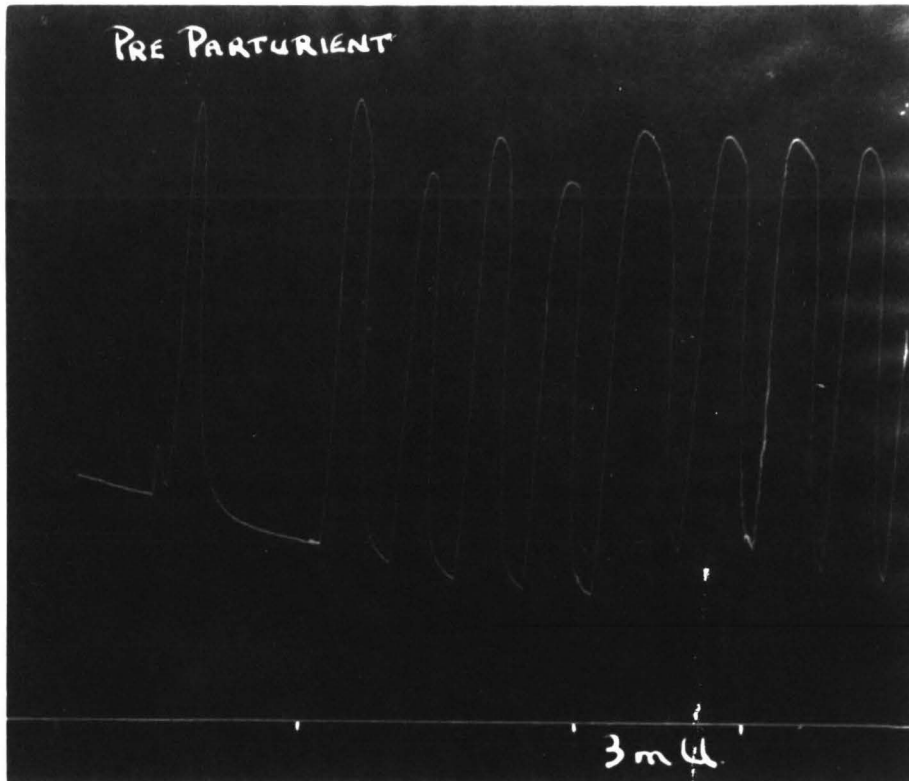(3 mU = dose of oxytocin given)

FIG. IX: TRACING FROM RAT UTERUS JUST BEFORE PARTURITION
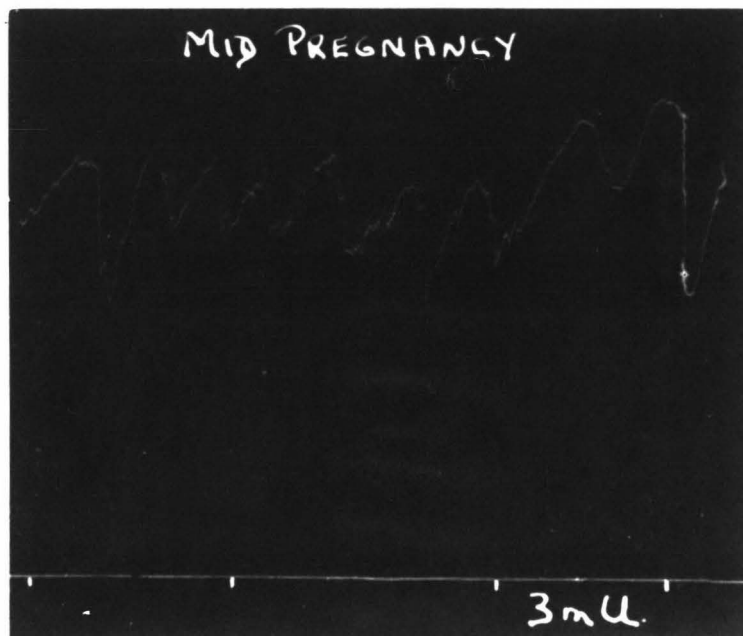(3 mU = dose of oxytocin given)

FIG. X: TRACING FROM RAT UTERUS TAKEN AT MID PREGNANCY
(3 mU = dose of oxytocin given)

so few preparations no conclusions could be drawn.

The tracing from rat 3 was noted with interest because of the probability of this animal being pseudopregnant. Less than 14 days had elapsed since mating in this instance and under these circumstances the chances of pseudopregnancy in the rat are great (Long and Evans, 1922). Examples of the tracings obtained are shown in Figs. VII-X facing page (viii).

Summary of Preliminary Experiments with Pregnant Rats.

The surgical technique of hemiovariectomy has enabled uterine preparations from pregnant rats to be used in an isotonic system for _in vitro_ recording identical to that which had been employed for non pregnant preparations. Rats subjected to this technique prior to successful mating, conceived in one horn only. The opposite and non pregnant horn from these rats provided a suitable uterine preparation for the apparatus that was used.

Non pregnant horns from these pregnant rats appeared to contract in a similar manner when taken at the same stage of pregnancy. The patterns of these contractions tended to alter between mid pregnancy and parturition, but no real changes in oxytocic sensitivity during these periods were measured probably because of the small numbers that were examined.

Thus the isotonic technique that has been used in this study appeared to be capable of detecting patterns in spontaneous uterine contractions that coincided with certain stages of gestation, but the ability of the method to detect oxytocic sensitivity changes on these occasions did not appear to be great. Further work however is necessary to prove or disprove these observations and for this many more animals would be necessary. A prerequisite to further studies of this nature should be the development of an accurate system for recording the time of mating so that parturition date estimates would become clearly defined.