BOVINE UTERINE PRESSURE AND THE RESPONSE TO OXYTOCIN AS MEASURED BY A NEW APPARATUS

A thesis presented in partial
fulfilment of the requirement for the degree of
Master of Philosophy
in Veterinary Science at
Massey University

Sai San Aik
1980

(This thesis represents 40% of the assessment of the candidate)

ACKNOWLEDGEMENTS

Many people contributed to the completion of this thesis; and to them all I owe my thanks for the assistance provided. Especially I wish to express my heart-felt thanks to my supervisor, Dr. B.S. Cooper, who provided encouragement, understanding, close supervision and valuable criticism. I am also much indebted to Professor E.D. Fielden, Dean of the Faculty of Veterinary Science, whose continued help, guidance, and interest made this study possible. Likewise I thank Dr. L.S. Forbes, Department of Physiology & Anatomy, and Professor A.N. Bruere, Head of the Department of Veterinary Clinical Sciences, for advice and encouragement.

I am grateful to Mr B.T. Pickett for helping in the development of apparatus, for technical assistance, and for his friendliness throughout. A word of thanks should also go to the clerical staff of the Department of Veterinary Clinical Sciences, and the staff of the Large Animal Hospital, for their assistance.

My thanks are due to Sharon and the Armitage family for the warmth and kindness they showed throughout my stay in New Zealand. My appreciation also goes to the library staff of Massey University, especially for the assistance provided by the interloan department and the translation work of Mr Chandler.

Finally, I would like to thank the Government of the Socialist Republic of the Union of Burma for financial support through the World Bank loan programme.

ABSTRACT

A new apparatus for measuring uterine pressure (UP) was developed from an artificial insemination tube, modified by sealing one end and creating a side opening which was covered by a rubber diaphragm. It was a wholly pneumatic system. Because every recording tube was different, each was tested *in vitro* using a syringe-barrel apparatus which could apply a range of pressures and measure the resistance factor of the diaphragm. By simple calculation, the resistance factor for each diaphragm could be used to standardise the *in vivo* recordings and so provide an estimated force or pressure of the uterine musculature. The sensor diaphragm was sensitive and responded to applied pressure on a linear scale up to 450mmHg.

The UP comprised uterine tonicity pressure (32-1010mmHg) and uterine contraction pressure (0-110mmHg). The UP therefore was much higher than the traditional intra-uterine pressure which had been interpreted by research workers previously to consist of the uterine contraction pressure alone.

The uterus of the normal cow was more active and responsive to oxytocin during estrus. In the diestral period, the uterine activity was reduced and there were times when the uterus was quiescent. In spayed cows, the uterine activity was much reduced, and in many recordings there were no active uterine contractions. Estrogen could produce estral behaviour in spayed cows and it slightly increased the uterine activity. Such activity was much less in degree than that observed during estrus in normal cows.

Urination, defecation, bellowing or arching of the back had no long-lasting effect on uterine contraction tracings, though either urination or defecation produced a transient fall in uterine tonicity. Environmental disturbances such as a tractor operating nearby did not affect the pattern of uterine activity which had a contraction frequency ranging from 0 to 22 per 10 minutes.

Some intramuscular injections of oxytocin (5 of 16) did not produce any action on a uterus which was later found to be responsive to intravenously administered oxytocin. The epidural route of oxytocin administration accounted for fewer failures (1 of 13), but the intravenous route was most consistent of all provided the uterus was undergoing regular contactions.

Five units of oxytocin given intravenously did not produce any significant increase in uterine activity whereas 10 - 20 units given 40 minutes later produced a maximum increase. Doses of 40 - 60 units given after another 40 minutes, did not produce a further increase in uterine activity.

The duration of action of oxytocin lasted for more than 9 hours when the recording was taken continuously; but when the animal was allowed rest periods interspersed with recording periods, the duration was found to be always greater than 1 hour and in 7 out of 15 recordings, more than 3½ hours.

The effect of adrenaline on uterine activity was stimulatory for a few moments only and this was followed by 2 - 4 minutes of inhibition. Xylazine in the form of "Rompun" had a stimulatory effect on uterine activity.

There are indications that the spontaneous muscular contractions of the non-pregnant uterus are governed by a complex of influences including endogenous hormones. To determine the relative importance of the different components would seem to be an interesting and important field of study that might possibly lead to the more rational use of therapeutic drugs about the time of parturition.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

APPENDICES

CHAPTER 1 INTRODUCTION

There is limited information available on the pattern of uterine activity and its modification by the action of oxytocin during various stages of the estrous cycle. Most of the studies have been concentrated around the time of parturition and more studies have been conducted in man than in animals. Various reports have presented somewhat conflicting facts about uterine contractions and the response of the organ to oxytocin, and it is clear that the significance of the normal uterine movements in cows, still needs much more attention.

The aim of this study was to develop a better understanding of activity displayed by the non-gravid uterus, in the hope that specialised equipment and preliminary findings might be of use later in investigating uterine contractions about the time of parturition. It was anticipated that the uterine movements and the responsiveness of the uterine muscle to oxytocin, might well be different at various stages of the estrous cycle, and in order to more closely determine the time of estrus, artificially stimulated spayed cows were used in addition to intact animals undergoing naturally occurring cycles.

An investigation of uterine activity *in vivo* was chosen, rather than a study on the uterus freshly removed, because any findings would not require subsequent confirmation *in vivo* and in the knowledge that all endogenous influences had been operational at the time measurements were made. Studies might have been undertaken using small laboratory animals, but again, any significant results would have needed confirmation later in cattle; and the cow's anatomy lends itself particularly well to direct observations being made on uterine activity.

In the past, research workers approaching the same problems have used a variety of pressure recording devices. These have consisted mainly of

either air-filled or water-filled balloons, connected to a pressure recording manometer. Initially, similar pieces of equipment were tried out for this study and found to be very sensitive, but they suffered from a range of deficiencies. Eventually a simple and robust piece of apparatus was constructed locally and subsequently used to provide the main results of this thesis.

By careful observation of the cow under experiment, all extraneous movements on the recording tracing were identified and it was found that characteristic uterine pressure patterns could be recognized. Details of how these differed between animals and during various stages of the estrous cycle are described in the thesis and these form the main part of the study.

Oxytocin has been widely used over many years to stimulate uterine contractions; particularly about the time of parturition. Some workers have restricted their interests to measurements of the effects of oxytocin on the non-pregnant cow. In the present study, it was possible to continue this approach and to extend the investigations to include a comparison of the oxytocin effects following different routes of administration.

CHAPTER 2 REVIEW OF THE LITERATURE

2.1 ANATOMY OF THE BOVINE UTERUS

2.1.1 Macroanatomy

In studying the movements and pressure exerted by the uterus, an understanding of its structure and position relative to other organs contained in the abdominal cavity, is important, because even slight changes in the abdominal pressure, could have some effect on uterine pressure characteristics.

The uterus of the cow is a hollow muscular organ, which is continuous with the fallopian tubes anteriorly and opens into the vagina posteriorly.

In the adult, the horns or cornua of the uterus are situated entirely in the abdomen. They appear to vary considerably in position but commonly they are pressed up against the sublumbar muscles by the intestine. They are cylindrical when moderately distended, and each is about 25 cm in length. The anterior extremity of each forms a blunt point which receives the fallopian tube. Posteriorly they increase somewhat in calibre, converge, and unite with the body. The ventral border is slightly concave and is attached to the sublumbar region by the broad ligament. The dorsal border is convex and free.

The body of the uterus is situated partly in the abdominal, and partly in the pelvic cavity. It is cylindrical, but considerably flattened dorsoventrally, so that in cross-section it is eliptical. Its average length is 3 to 4 cm and its diameter, when moderately distended, about 10 cm. Its dorsal surface is related to the rectum and other parts of the intestine. Its ventral surface is in contact with the bladder, and has inconstant relations with various parts of the intestine. The term *fundus uteri* is applied to the wide anterior part from which the

cornua diverge.

The neck or cervix of the uterus is the constricted posterior portion which joins the vagina. It is about 5 to 8 cm in length, and 3 to 5 cm in diameter. Part of it projects posteriorly into the cavity of the vagina.

The cervix is a heavy, smooth muscle sphincter which is tightly closed except during estrus or at parturition. Its inner surface is arranged in a series of circular ridges or rings, sometimes called annular folds.

The tunica muscularis of the cervix consists of inner circular and outer longitudinal smooth muscle layers. Elastic fibres are prominent in the circular layer. The muscle layers of the cervix are continuous with those of the fundus anteriorly and the vagina posteriorly. In the cow's cervix, there are four large circular folds and 15 to 25 longitudinal folds, each with many secondary and tertiary folds (31).

The cavity of the uterus is largely obliterated in the non-pregnant state by the contraction of the wall and by folds of the mucous membrane lining. At the extremity of each cornu it communicates with the fallopian tube by a minute opening on a small papilla. Posteriorly the cavity of the neck of the uterus is termed the cervical canal. It opens into the vagina by the *orificium externum uteri*, and into the body of the uterus by the *orificium internum uteri*.

The outermost layer of the uterus is a serous membrane which is part of the reflected peritoneum and known as the broad ligament. Under that, there is myometrium, the muscular portion of the wall of the uterus. It consists of a thick inner circular layer of smooth muscle and a thinner outer longitudinal layer of smooth muscle, separated from one another by a vascular connective tissue layer. The innermost layer, the mucous membrane lining the uterus, is a highly glandular structure called the

endometrium (42).

2.1.2 Innervation

Uterine function is controlled both by the autonomic nervous system and various hormones. A better understanding of uterine innervation would help to explain the pattern of uterine contraction observed in the normal cow and the effects of exogenous hormones on both intact and spayed animals. Unfortunately, definitive information on bovine uterine innervation is still quite limited. Accordingly evidence obtained from other species has to be studied in the hope of providing some basic understanding common to a range of animals including the cow.

The chief nervous ganglia which distribute nerves to the visera and vessels of the abdominal and the pelvic cavities are 2 in number, the coeliac (sympathetic) and the pelvic (parasympathetic) (88).

It is known that, the ganglion supplying the ovary and the cornu of the uterus is a different one to that supplying other parts of the uterus and vagina but both share branches from the aortic ganglia.

The nerves supplying the uterus from the pelvic ganglion leave this structure alongside other branches which supply the bladder and upper part of the vagina, although the rectum, bladder, urethra, and lower vagina receive a separate innervation by way of the pelvic nerve (80).

Stimulation of either the sympathetic or the parasympathetic nerves can elicit increased activity of the uterus of man, but denervation causes little overall change in uterine rhythmic contractility. Since the inherent activity of the uterus is largely independent of its motor innervation, autonomic nervous system blocking agents have little effect although both alpha and beta adrenergic receptors are clearly demonstrable in the myometrium of some mammalian species. A regional variation in the density of adrenergic nerves could be demonstrated in

man, the number of fibres being fewer in the fundus than in the cervix where the number was three times higher than elsewhere in the uterus (94).

Uterine contractility is inhibited by local anaesthetics, and by direct-acting smooth muscle relaxants such as papaverine, nitroglycerine, and caffeine (49).

Anatomical studies have made it fairly clear that different parts of the uterus such as the fundus, corpus, cornua and cervix; have separate nerve supplies (both afferent and efferent) (74).

2.2 UTERINE PHYSIOLOGY

Myometrial activity and ovulation.

More research has been undertaken on aspects of contraction of the human uterus than on the cow uterus, and accordingly it is important to have some understanding of human uterine physiology before any extrapolation of findings to cattle can be considered.

In studying the physiological information obtained from man and cattle with particular reference to contractions of the uterus, it was found that at estrus and menstruation there are certain important similarities.

Menstruation is a periodic discharge of a sanguineous fluid from the uterus, occurring throughout the period of a woman's sexual maturity from puberty to the menopause. Estrus is the female animal's mating period during which the male will be accepted. At the same time changes are taking place in the uterine mucosa under the influence of hormones involved with maintaining the estrous cycle.

Basic similarities in the signs and symptoms of estrus and menstruation, and in uterine activity at estrus and menstruation, are supported by the following evidence.

Uterine activity of the cow varies with the stages of the estrous cycle (52). Contractions during estrus and a few days afterwards are marked, while contractions during the diestral period are slight (33). During the diestral period, the uterus is largely at rest (99).

It is always possible to record contractions in the human non-pregnant uterus. These contractions are frequent, regular and weak in the first half of the menstrual cycle; after about the 16th day, they become stronger, but less regular, and during menstruation, the contractions reach their peak (71).

From the above points, it is noticeable that, the human uterus is most active during menstruation i.e. some 14 days before ovulation, whereas the cow's uterus is more active during estrus, and coincides with ovulation.

At times when uterine activity shows significant differences in the cow, corresponding changes can be recorded in the electrical potential of the myometrium. During diestrus, brief, weak, irregular bursts of potentials were recorded. During proestrus, increases in amplitude and frequency of contractions were accompanied by a grouping of activity into bursts of 5 to 10 seconds duration. At estrus, the "trains" of potentials, were mingled with bursts to form prolonged phases lasting 1 to 7 minutes and these occurred two to three times every hour. During metestrus, shorter and more frequent phases of activity were recorded, but their amplitude was reduced (82).

The average estrous cycle length in cattle is about 21 days. It is slightly shorter in heifers than in mature cows, and cycles within the range of 18 to 24 days are considered normal (92).

The period of estrus is arbitrarily defined as the interval during which an animal will accept mounting by another cow or bull. The average duration of estrus for both dairy and beef cattle is 18 hours, and

periods of 12 to 24 hours are considered normal. Heifers have slightly shorter periods than mature cows. The average length of estrus is reported to be as short as 12 to 13 hours in cows of European breeds in a subtropical climate.

Ovulation occurs 10 to 11 hours after the end of estrus in both beef and dairy breeds, and periods of 5 to 15 hours are considered normal. Late ovulations do not appear to occur very frequently, and attempts to improve fertility in repeat breeder cows by ovulation-hastening treatments given during estrus have met with little success (92).

Changes in the cervix: During estrus there is an increased vascularity of the cervix accompanied by edema, and relaxation in the cervical muscle tone.

The rigidity of the cervix, at estrus, is due to the edema and not due to vascular engorgement since excessive quantities of blood are not found. During metestrus and diestrus the cervix and the interior prominences are quite soft. Changes in the cow's cervix could be associated with alternate hardening and softening of the tissue, rather than with relaxation and constriction (32).

As the cervix shares some smooth muscle with the body of the uterus, the activity and reactivity to drugs are similar in the cervix and the corpus of cattle. Mostly the frequency of muscular contractions of the cervix and the corpus are similar, but sometimes the difference is as great as 6:1 (39).

2.3 HORMONES AFFECTING UTERINE ACTIVITY

Bovine uterine activity changes with the various stages of the estrous cycle, which in turn is governed by a number of factors involving the hypothalamus (producing specific hormone-releasing factor), two

gonadotropins from the anterior pituitary gland (follicle stimulating hormone and luteinizing hormone) and two hormones from the ovaries (estrogen and progesterone). Hysterectomy in cattle prolonged the life span of the corpus luteum; this fact suggests that the uterus which contains prostaglandin $F_{2\alpha}$ may also play a vital role in regulating the estrous cycle (48).

Thus there are numerous factors influencing uterine activity, but in this study only oxytocin and estrogen were used experimentally and a brief description of the activities of these two hormones is given below.

2.3.1 Oxytocin

Pharmacodynamics

The hormone oxytocin is formed in the cell bodies located principally in the paraventricular and supraoptic nuclei and it then migrates along the axons of these cells to perivascular nerve endings in the posterior lobe. The hormone accumulates in the nerve endings largely in "neurosecretory granules", within which oxytocin is bound to protein (49).

Until recently, the oxytocin available for therapeutic use and for investigation was in the form of purified posterior pituitary extracts that were contaminated to a small and variable degree by antidiuretic hormone (ADH). Pure synthetic oxytocin is now used, and the effects to be described are those of the pure hormone. However, it should be borne in mind that even oxytocin itself has slight, but not significant, antidiuretic and vascular activity that may become manifest when large doses are used (49).

Absorption, fate and excretion: If given orally, oxytocin is inactivated by chymotrypsin. However, it is effective after

administration by any parenteral route (49).

The half-life of oxytocin in plasma is less than 10 minutes (49). Its rapid removal from plasma is accomplished largely by the kidney and the liver. Mammary tissue which is highly responsive to oxytocin, also attracts oxytocin from plasma. Cell-free extracts of liver and kidney show far higher oxytocin inactivating activity than do extracts of other tissues. A very small portion of the oxytocin extracted by the kidney reaches the urine in active form (49).

During pregnancy, two additional factors take part in the pharmacokinetics of oxytocin. These are:

(1) Oxytocin inactivation occurs in plasma. The functional significance of the plasma oxytocinase of human pregnancy has not been established but it is thought that, it accounts for only a small part of the inactivation of the hormone.

(2) High oxytocinase activity is found in the tissue of the pregnant uterus and in the placenta.

Effects on uterus:

Oxytocin stimulates both electrical and contractile activity in uterine smooth muscle. With higher concentrations, decreases in the resting membrane potential occur (49). These effects are highly dependent on the presence of estrogen and when estrogen levels are low, the effects of oxytocin are much reduced (49).

Although progesterone antagonises the stimulant effect of oxytocin *in vitro*, the corresponding effect in the pregnant human uterus has been difficult to demonstrate. The sensitivity of the human uterus to oxytocin gradually increases during gestation and then increases sharply very shortly before parturition (49).

Either stilbestrol or progesterone increases the response of the uterus of the ovariectomised cow to oxytocin; stilbestrol being the more

effective (52).

Hays and Vandemark (52) claimed that, oxytocin increased the uterine activity in each of 28 observations on intact cattle. The reaction started in 10 to 12 seconds after intravenous injection and was usually characterised by a stronge tetanic contraction which increased the intra-uterine pressure 30 to 70 mm Hg from a base of approximately 20 mm Hg and lasted for 1 to 3 minutes. This was followed by a gradual reduction both in tone and contractions.

The response of the uterus to oxytocin in ovariectomised cows was slight, but after treatment with stilbestrol, the response was closer to that of the normal cow (52).

In addition to the hormones referred to above which affect the activity of the uterus, adrenaline produces a momentary stimulatory effect. On intravenous injection, a large contraction results and this preceeds a period of reduced activity. In the ovariectomised cow, the uterus reacted to epinephrine in a similar manner but the response was greatly reduced (51).

2.3.2 Estrogen

The principle female sex hormones (estrogens) in most species of animals are, estradiol - 17β, estrone and estriol. The adrenal cortex and follicle of the ovary produce some estrogens, and the placenta in late pregnancy produces large quantities.

The uterine smooth muscle is usually subject to endocrine influence especially that of the estrogens (49). The estrogens increase myometrial tone and spontaneous motility but they do not cause the forceful contractions which are partly responsible for the birth process (58).

The relaxing effect on the cervix and the sensitizing of the uterus to the ecbolic effect of oxytocin, has led to the use of estrogens as an

aid in removing the retained placenta (17).

Pinto *et al.* (76) showed that the stimulatory action of estradiol - 17β seemed to be a direct local action on the myometrium rather than acting through any other pathway. He administered estradiol - 17β by the intra-amniotic route in patients close to term, stimulated contractions of the uterus and conditioned the cervix for parturition; similar effects to those observed when the estradiol was injected intravenously in the same dose.

Jones (58) pointed out that the half-life of exogenous estrogen was very short from his experiments on dogs. When the aqueous solution of an estrogen was injected intravenously into dogs, about 90% of the dose disappeared within a few minutes. Only a small amount could be recovered from the urine.

It is known that estrogens increase the activity of the uterus whereas full enlargement after sensitization by estrogen takes some time to develop; a fact which was demonstrated by Kroc *et al.* (62) in rats.

Real growth of the uterus increased dry weight and nitrogen content in spayed immature rats treated with estrogen. This was generally preceded by a transient increase in uterine water content.

The rat uterine wet weight increased 1-2 hours after hormonal stimulation and reached a maximum at 4-5 hours. After declining slightly at 9-12 hours, a second increase occurred, which was maximum between 15-30 hours (44).

In spite of the definite response observed in the rat uterus, in none of the 33 human patients studied, was there observed any immediate change in the uterine contraction patterns, following intravenous administration of estrogen or saline (59).

The influence of estrogens over uterine activity, albeit a delayed effect, is further born out by blood levels found during different phases

of the bovine estrous cycle. Estradiol 17β levels increased during the 3 days preceding estrus in cattle and attained a peak value (17 ± 1.9 [S.E.M] ng/100 ml), 4 hours before estrous behaviour could be detected. The level declined steeply during the day of estrus to reach a nadir (0.8 ± 0.11) before the time of ovulation. A minor rise was observed on day 4 and a more sustained increase on days 10-13, with a peak on day 11 (8.1 ± 3.6) (86).

2.4 TECHNIQUES USED FOR RECORDING UTERINE ACTIVITY

Over the past 100 years, man has made attempts to measure uterine activity, using a large number of different techniques with variable results. The various techniques applied have been based on the same concept and have employed only minor modifications in apparatus.

(1) Intra-uterine water bag

As long ago as 1872, an elaborate paper was published by Schatz, who reported on his use of an intra-uterine water bag (80 ml), connected to a Mercury manometer and simple kymograph, to obtain tracings of uterine action (71).

In 1938, an intra-uterine Voorhees bag was used by Salerno, for measuring uterine pressure at term. About (800 ml) bichloride solution was injected into the bag, which was connected to a Mercury manometer by a water-filled tube (83).

(2) Intra-uterine balloon

Instead of a bag, a small rubber balloon (2 ml) was used by Westermark in 1893. The concept was the same as that of the water bag, but a balloon of smaller size was used to avoid disturbing the uterine contraction pattern.

Both the intra-uterine bag and manometer had been improved in some

details by Moir (71). He described it as a bag, though the size ranged from 1.5 to 3 ml capacity. A 15 ml balloon filled with air was used by Wilson and Kurzrok (22) and a 5 ml balloon filled with liquid was used by Bickers (27).

(3) Intra-uterine granular carbon sensor

Karlson (26), in an attempt to overcome some of the shortcomings of previous tocometers, used a sensor, consisting of a granular carbon microphone, which was introduced into the uterine cavity by means of a probe. Through the probe, the wires from the sensor were connected to the current, the load resister and the recording system. He used several of these devices to make a closer study of movements within the various parts of the uterus.

(4) Open-end catheter

In 1964, Hendricks introduced the intra-uterine open-end catheter technique. It was a thin fluid-filled polyethylene catheter which was inserted into the cavity of the non-pregnant uterus, and its outer end was connected to a recording system. Similarly Alveraz (20) used a water filled, 15 gauge needle for recording intra-amniotic pressure.

(5) Strain gauge sensor

Internal. The strain gauge wire tocometer is a slender cylinder, 3 cm in length, 0.3 cm in diameter. The strain gauge wire on its lucite core was enclosed inside a thin silico-rubber sheath. The tocometric cylinder was inserted so that its tip rested against the fundus of the uterus (60).

External. In 1950, Caldeyro *et al.* used a seven channel tocometer, in which, three were strain gauges, placed along the midline of the uterus, but externally on the abdominal wall. For measurement, the soft steel probe and conducting wires were connected to a Wheatstone Bridge 6-volt battery, amplifier osillograph (19).

(6) Rubber membrane pick-up

The remaining four channels of Caldeyro's apparatus were rubber membrane pick-ups. They too were placed on the abdominal wall, and connected, by rubber tubes, to a sensitive chart-recording system.

(7) The guard-ring tocodynamometer

Smith (89) claimed that the balloons and catheters used at the time of parturition, caused some degree of inconvenience to the patient and demanded special attendance of the obstetri tian. The abdominal tocometers had weakness too in that they did not record true intra-amniotic pressure and they were influenced by skin tension.

He made a new abdominal tocometer intending to overcome the defects of the earlier instruments. It consisted of a pressure plate in which a pressure sensor strain gauge was situated. The pressure plate was surrounded by a guard plate which flattened the abdominal wall so that uterine pressure on the recording plate was more uniform.

(8) Intra-uterine sensor and transmitter (radiotelemetry)

The wireless pill was a small radio-frequency (0.4 Mc/s) transistorised transmitter with self-contained battery and switch. It was hermetically sealed in "Perspex". It was cylindrical, 9 mm in diameter, and 19.3 mm long. The pill was switched on by shaking prior to insertion, and after recovery could be switched off and reclaimed for further use. A thread was attached to the pill for ease of removal. Changes in frequency of the signal emitted by the pill were monitored by a nearby aerial and the radio-receiver was connected to a continuous recording system (90).

(9) Intra-myometrial microballoons

A plastic tube of 18 gauge and 2½ ft long, was heat sealed at one end and an 1/8 ml balloon was blown near the end. A hole was made in the tab at the sealed end. A silk thread of 0 gauge, was inserted into

this hole and tied. The plastic tube was filled with water. The micro-balloon was placed surgically in the uterine muscle.

The tube assemblies fed into a Statham strain gauge which was held in place on the side of the cow by a metal bracket. The strain gauge in turn fed into a Brush BL-300 amplifier and recorder (46).

(10) The sponge-tipped catheter

Bengtsson (10) was not satisfied with the open-end catheter. He reported that, it was difficult to maintain the column of water, and endometrial fragments might occasionally obstruct the inner-tip. To overcome these defects, a sponge-tipped catheter was made, by capping the catheter with a 5 mm cylinder piece of sponge bored from a synthetic wash-sponge.

(11) Mikro-tip catheter pressure transducer

The mikro-tip catheter pressure probe is a flexible and accurate miniature transducer. The gold plated high frequency sensor surface (1.67 mm x 4 mm), at the tip of the catheter, provides a carrier to which the semiconductor strain gauge is bonded. The body consists of machined pure titanium, which is known for its compatibility with body tissue and fluids.

It is a direct measuring system with a capacity in the range of 0 - 300 mm Hg and a limit of 750 mm Hg. The transducer can be directly connected to an amplifier and recorder.

CHAPTER 3 MATERIALS AND METHODS

3.1 DEVELOPMENT OF A RECORDING DEVICE

3.1.1 Preliminary trial

Air system. The FOLEY CATHETER (Bard International Ltd., England) which consists of a double tube, one of which passes to a cuffing balloon and the other to an open-end, was tried initially. It was introduced as far as the bifurcation of the cow's uterus, using an internal stilette to maintain rigidity. The balloon at the tip of the catheter was then inflated from the exterior, and the tracings of uterine contractions were obtained. But the baseline kept on falling, indicating an air leakage somewhere in the system. It was calculated that, the internal pressure of a 3.5 ml balloon was 300 mm Hg.

There appeared to be a series of disadvantages inherent in the system. Because of high pressure it was difficult to prevent leakage of air at tube connections, the system was relatively insensitive, the internal pressure was far too high for the transducer and it was feared that the pressure exerted by the balloon might cause changes in the uterine muscular movements.

Water system. The same FOLEY catheter was used with both the tubes involved. After insertion of the catheter, the cuffing balloon was inflated, the internal open-end tube of the catheter filled with water and then the external end of the inner tube connected to the water filled transducer.

Tracings of uterine contractions were obtained, but whenever the water column was disturbed by movements of the animal, recordings of the uterine patterns were obscured. It was more difficult to use the

water system than the air system. Since it was an open-end catheter, uterine mucus was liable to block the opening, and change the sensitivity of the fluid column.

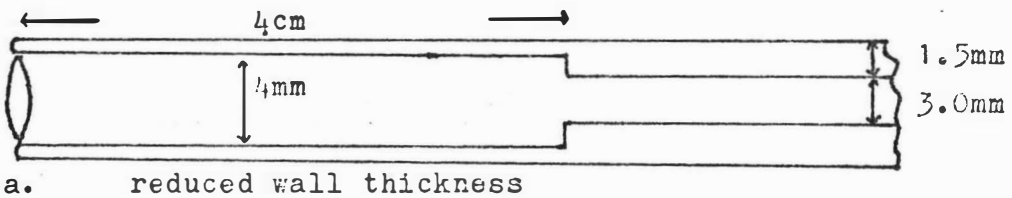
3.1.2 Closed catheter with rubber sensor diaphragm.

Many techniques are available for the measurement of uterine contractions (Chap. 2.4). The technique to be discussed below, utilised a modification of the balloon catheter and eliminated many of the problems associated with the balloon system. It had the advantages of, a rugged construction and of being reuseable a number of times.

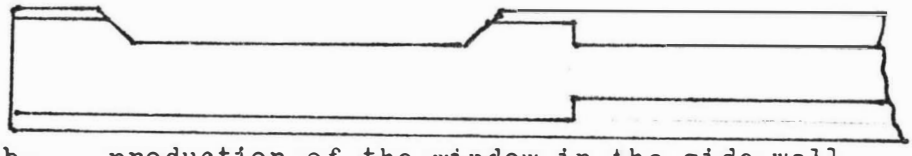
Construction. The catheter was based on the standard artificial insemination tube (National Dairy Association, New Zealand). These are semiflexible plastic tubes, 40 cm long, with an outer diameter of 6 mm (inner 3 mm) and a wall thickness of 1.5 mm. At one end the inner diameter was drilled out to 4 mm for a length of 40mm (Fig.1a). After it was drilled, a window was filed out of the reduced wall thickness as shown in Fig. 1b.

A piece of rolled cotton wool was pushed into the lumen of the drilled end of the tube, and aryldite adhesive was applied to seal it off. As it set, the material was shaped to produce a smooth cone (Fig. 1c). A diaphragm was made from a rubber finger stall which had been slit along its length, laid flat and cleaned with 70% alcohol. A cyanoacrylate adhesive (Loctite Limited, England) was applied to the filed edges of the cutaway portion of the tube as shown in Fig. 1c. The rubber sheet diaphragm could then be gently stretched over the glued area and held for a few seconds to adhere. Excess rubber sheet was carefully trimmed off afterwards with a scapel in order to produce a smooth surface.

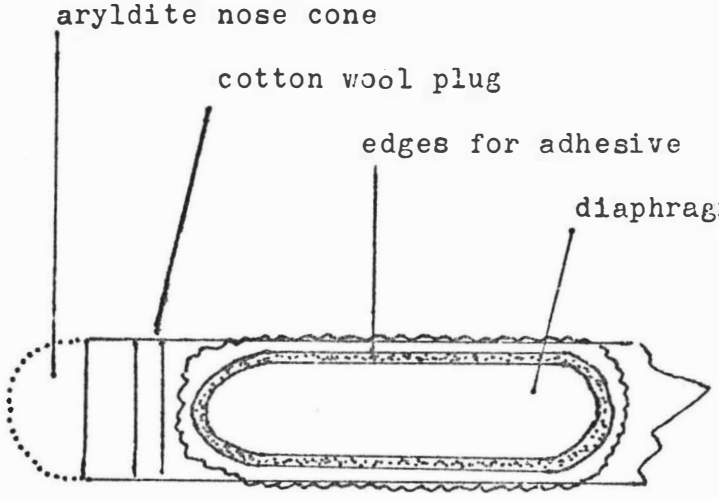
The other end of the tube was fitted to a two-way adaptor by a tight-fit plastic tube (Fig. 1d). On to the exposed part, a 21-G needle



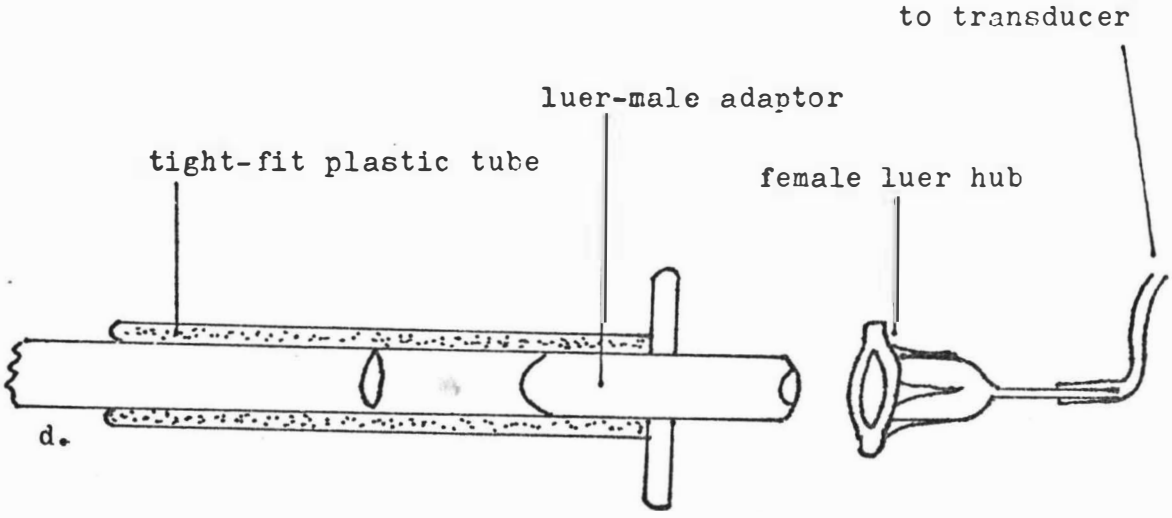
a. reduced wall thickness



b. production of the window in the side wall



c.



d.

Fig.1.CONSTRUCTION OF THE CLOSED CATHETER WITH RUBBER SENSOR DIAPHRAGM

hub was fitted after the needle had been cut and ground smooth. The needle in turn fitted into a polyvinyl tube connecting at the other end with the transducer (Fig. 1d). The electrical signal generated at the transducer was continuously plotted on a chart recorder (Devices, England).

Testing. The end of the tube bearing the diaphragm was immersed under water and 0.5 ml of air was injected into the tube using a tuberculin syringe. The diaphragm was expanded and any leakages could be detected by the escape of air bubbles. The air-tight equipment was later subjected to testing for the "resistance factor" of the diaphragm (Chap. 3.2).

The equipment described, overcame many of the disadvantages of the balloon catheter and proved rugged enough to be used on 11 occasions. Calibration drift was minimal once the catheter had reached the body temperature of the experimental animal. The small diameter of the fine tubing minimised external temperature effects. Vigorous movements of the animal could be tolerated without alteration of the base line which was the most serious defect in the fluid-filled system. Construction was simple and required no special skills.

3.2 TECHNIQUE FOR MEASURING THE RESISTANCE OF THE DIAPHRAGM

Assuming that internal air temperatures remain constant, the pressure changes in the recording tube (internal pressure) depend on the force exerted (external pressure) on the sensor diaphragm, the area of the diaphragm and the tension of the surface. It was shown that the external pressure applied had a positive association with the internal pressure as shown by the height of the contraction wave (Fig.3B-f). Tests for a relationship between external and internal pressure were carried out at applied pressures up to 1200 mm Hg but at the highest pressures, difficulties arose with air leaks past the seal. However this

relationship was linear up to at least 450 mm Hg.

Several tubes were constructed and used at different times. The aim of the study was to compare the resistance of different diaphragms and of individual diaphragms over a period of time in use. When a constant pressure was applied to different diaphragms of unequal area and of differing tensions, different amplitudes were observed on the recording tracing. On dividing the applied constant pressure by the height of the contraction wave (mm), a standard factor or criterion of resistance for each tube (the external pressure required to produce 1 mm amplitude) was obtained.

Thus when a recording tube of known resistance was used in the cow, the amplitude of the contraction wave could be used to calculate the supposed pressure of the cow's uterus.

Charles' Law states that, the pressure, the volume and the temperature of air in a limited space are all interdependent, or:

$$\frac{\text{Pressure}_1 \times \text{Volume}_1}{\text{Temperature}_1} = \frac{P_0 V_0}{T_0} = \text{Constant}$$

or $PV = \text{Constant} \times T$

During each experiment, the temperature of air trapped within the system inside the cow, could be assumed to remain constant, once equilibrium was reached. Due to possible changes in atmospheric temperature, which could affect the air volume in the exposed polyvinyl tube; the pressure of the uterus that was calculated, could not be claimed to be an absolute pressure, but rather a relative pressure.

When the wall of the uterus pressed on the diaphragm, the air in the sytem was compressed, the pressure in the system was raised and working through a transducer the amplitude of the contraction was heightened. As the relationship between height and pressure of each

uterine contraction in the lower range wave was a constant for any given recording tube, the "resistance factor" for each diaphragm was determined using the apparatus shown in Fig. 2 and Plate 1.

The apparatus consisted of four parts:

(1) The plunger of a 20 ml plastic syringe was removed, the rubber cap was taken off, a small hole made in its centre and the recording tube carefully pushed through (Fig. 2a-1). A much enlarged drawing of the construction is shown in Fig. 2b. The cap was fixed just inside the barrel of the syringe, and the diaphragm of the recording tube came to lie about halfway down the syringe barrel. A hard plastic support was screwed to the wings of the syringe, to make a robust and air tight seal (Fig 2a-2).

(2) A similar but unmodified syringe (Fig. 2a-3) had a T-piece of pressure tubing mounted on the nozzle and this connected the two syringe barrels (Fig. 2a-4).

(3) A pressure recording gauge (Fig. 2a-5) was affixed to the end of the third arm of the T-piece.

(4) A polyvinyl tube, about one-half meter long, with a diameter of 1 mm, was attached to the free end of the recording tube (Fig. 2a-6) and connected further forward to a transducer (Fig. 2a-7). The transducer (Statum P23 BB, Devices) in turn, transposed the pressure variation into an electrical signal received on a single channel recorder (Devices, England) (Fig. 2a-8).

In vitro Measurement. The recorder was set up using the equipment shown in Plate 1, and allowed to warm to operating temperature over 15 minutes. As soon as the recording was started, before connecting the recording tube to the transducer, a straight line (*in vitro* baseline) was obtained (Fig. 3A-a). Immediately after the tube was connected to the transducer, due to slight pressure applied to the column of air in

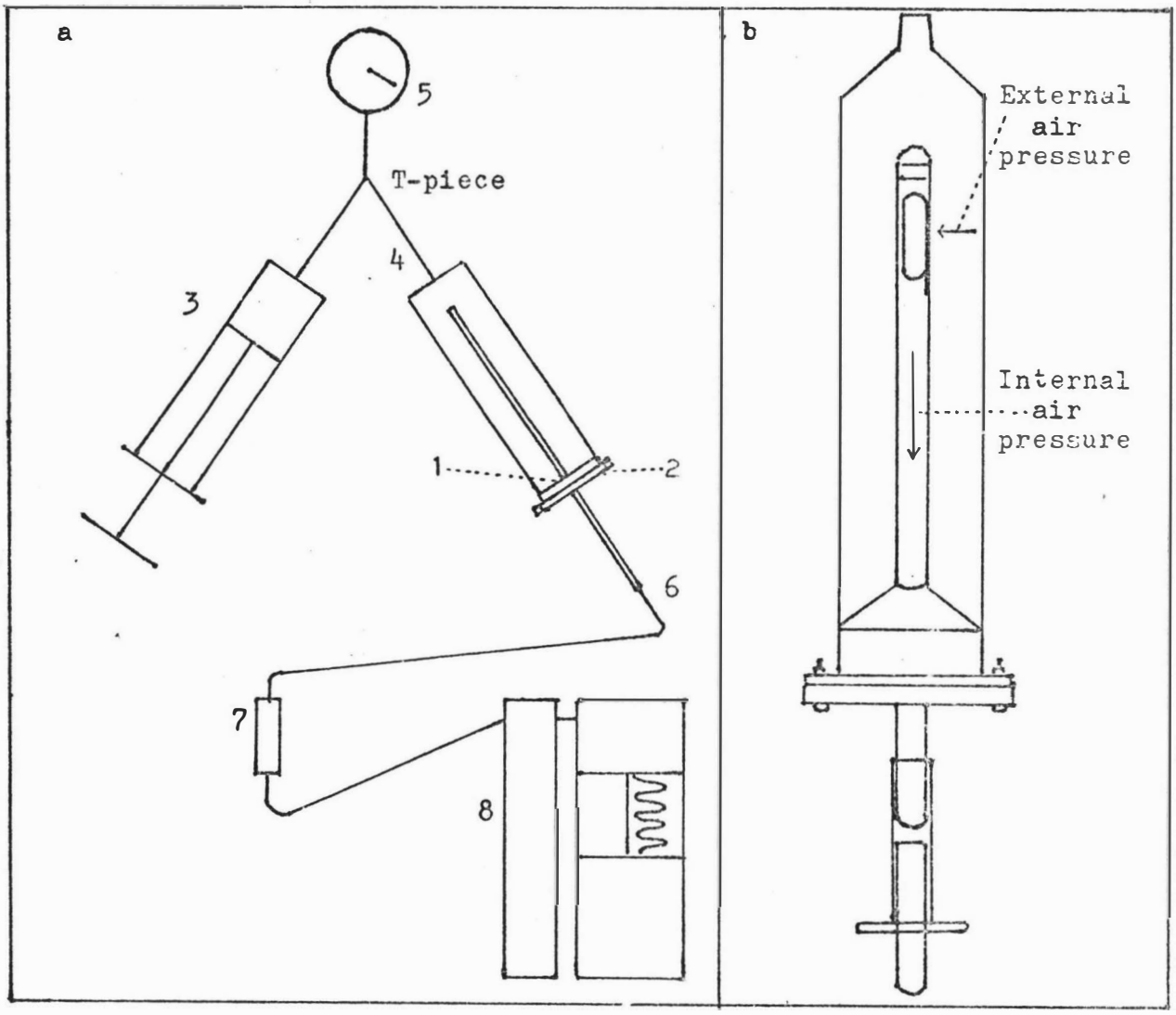


Fig.2. APPARATUS USED TO DETERMINE THE RESISTANCE FACTOR OF THE SENSOR DIAPHRAGM.

1 = RUBBER CAP HOLDING THE RECORDING TUBE

2 = PLASTIC SUPPORT SCREWED TO THE WINGS OF THE SYRINGE

3 = SECOND SYRINGE TO PRODUCE EXTERNAL PRESSURE

4 = T-PIECE MOUNTED ON THE NOZZLE OF THE SYRINGE

5 = PRESSURE GAUGE

6 = POLYVINYL TUBE CONNECTING THE RECORDING TUBE AND THE TRANSDUCER

7 = TRANSDUCER

8 = CHART RECORDER

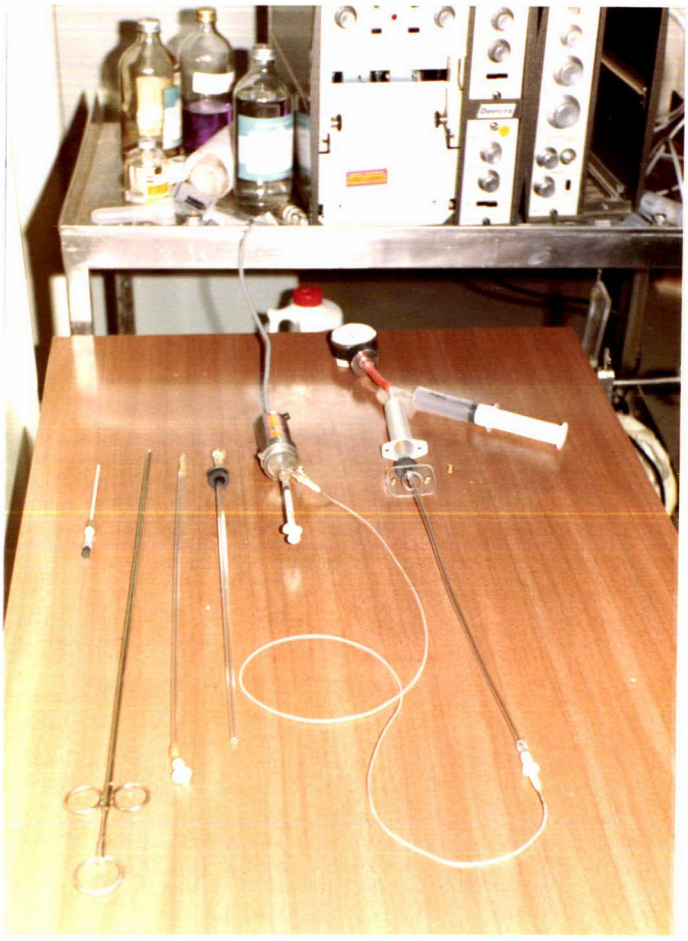


PLATE 1. *IN VITRO* TESTING OF THE RESISTANCE FACTOR OF THE RECORDING TUBE, USING THE EQUIPMENT DESCRIBED IN Fig. 2.

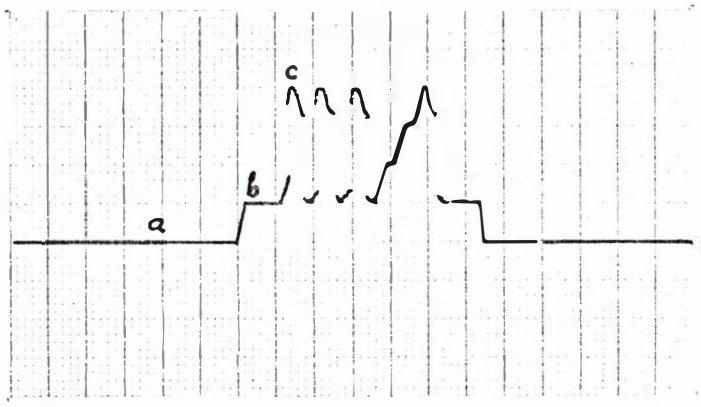


Fig.3A. IN VITRO TESTING OF THE RESISTANCE FACTOR OF THE RECORDING TUBE

a =IN VITRO BASE LINE

b =IN VITRO EXPERIMENTAL STARTING LINE

c =IN VITRO PEAK OF THE CONTRACTION

a-b =PRESSURE DUE TO AIR COMPRESSION IN THE RECORDING TUBE

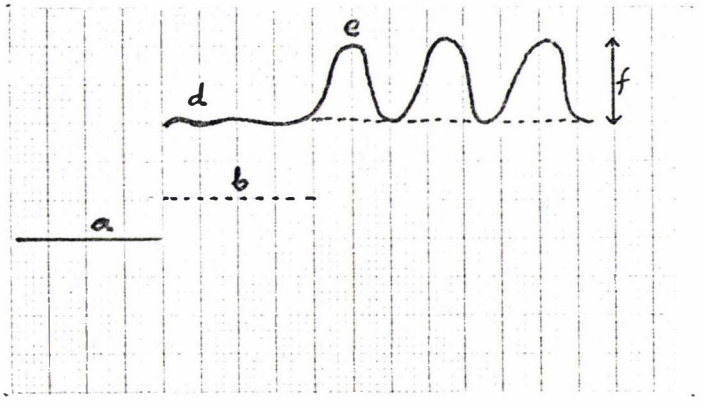


Fig.3B. IN VIVO MEASURING OF THE UTERINE PRESSURE OF THE COW

a =IN VITRO BASE LINE

b =IN VITRO EXPERIMENTAL STARTING LINE

d)=IN VIVO TONICITY LINE OR LOWEST POINT OF THE UTERINE CONTRACTION

e =IN VIVO PEAK OF THE CONTRACTION

UTERINE PRESSURE(b-e)=TONICITY PRESSURE(b-d)+ CONTRACTION PRESSURE(d-e)

f =AMPLITUDE OF THE CONTRACTION

the recording tube and its connecting tubing, the line was raised (Fig. 3A-b). The latter level or line was taken as the *in vitro* experimental starting line.

Air was injected into the T-piece by the second syringe (Fig. 2a-3), held constant at 300 mm Hg, and the recorder needle rose to a corresponding peak (Fig. 3A-c). In this manner the resistance factor of the sensor diaphragm, in terms of the height of the recorded wave, was obtained before each *in vivo* experiment.

Any recorded changes in the amplitude (Fig. 3B-f) could be described either in mm height from experimental starting line (Fig. 3B-b) or in mm Hg pressure because each increased relative to the other. But the wave amplitude produced was due to the pressure of air *within* the tube, which was in turn exerted through the sensor diaphragm by the pressure applied *outside* the tube (Fig. 2b).

No reference in the literature could be found to similar experiments having been carried out on uterine pressure recording apparati, although Braaksma *et al.* tested different catheters for degree of accuracy, in a closed fluid system (16). No mention was made of internal or external pressures.

All the uterine pressures quoted in this thesis were calculated from *in vitro* experiments as described, carried out immediately before the recording tube was used on any cow. The uterine pressures referred to in this work may be different to those quoted by previous authors, because instead of estimating the internal pressure changes in the tube, the external pressure exerted on the sensor diaphragm was used as the base reference.

3.3 RECORDING PROCEDURE

3.3.1 Introducing the recording tube into the uterus.

Following preliminary tests on the sensor diaphragm (Chap.3,2), the chosen recording tube was half immersed in methylated spirit for twenty minutes. Two ties of silk, about one half meter in length, were attached to the other end and Spencer Wells forceps tied by the finger grips. The tube was tested for air leaks or damage and then well lubricated with gynaecological lubricating jelly (Johnson & Johnson) before use.

The rectum of the cow was emptied and the hind parts thoroughly cleansed. The left hand of the operator, in a shoulder length lubricated glove, was introduced into the rectum and the recording tube guided into the vagina and through the cervix into the uterus. Occasionally, difficulty was experienced in introducing the recording tube through the cervix - this was particularly so in cows that had been spayed for some time, and in certain other intact cows during the luteal phase of the estrous cycle.

The sensor diaphragm was always placed at the bifurcation of the body of the uterus and it was shown that the direction faced by the diaphragm did not make any difference to the patterns of uterine contraction recorded. The tube was finally held in the intra-uterine position by clamping the forceps, referred topreviously, to the skin hairs over the sacral region (Plate 2).

Polyvinyl tubing (1mm external diameter) connected the free end of the recording tube (Fig. 2a-6) to a transducer which had a direct electrical connection with a single channel chart recorder.

The hind parts of the cow, the connecting tube and the rest of the recording apparatus were all kept under shelter, to avoid marked changes in tube air temperature from sun light, or the effects of inclement

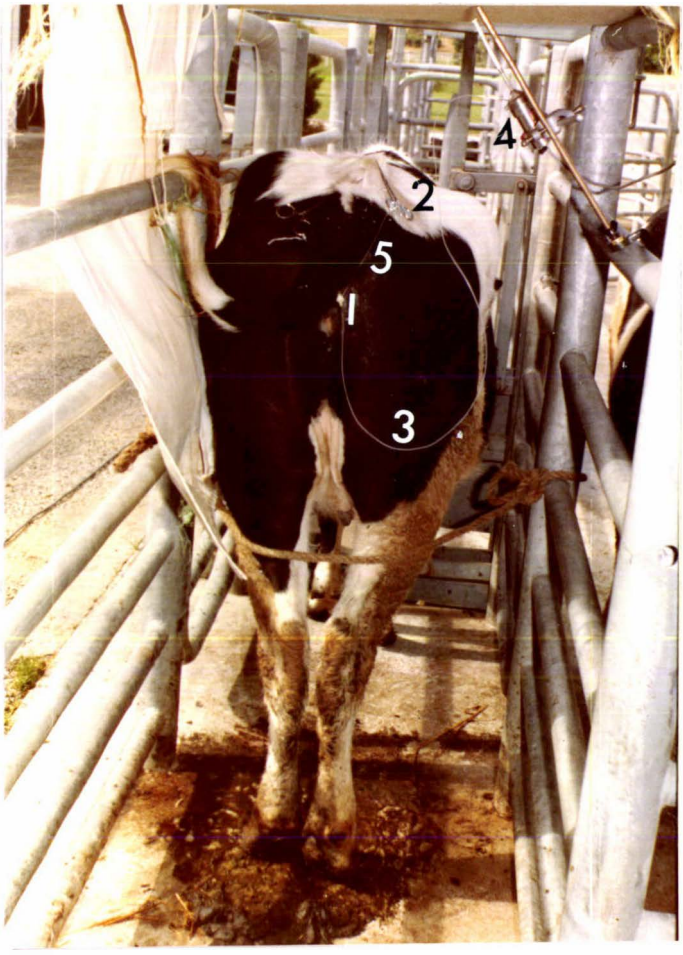


PLATE 2. *IN VIVO* MEASUREMENT OF THE UTERINE PRESSURE

Key:

1 = Recording tube in position

2 = Forceps with thread holding the tube

3 = Polyvinyl tube connecting the recording tube and the transducer

4 = Transducer

5 = Thread

weather (Plate 2).

3.3.2 Procedures used in obtaining recordings of uterine muscle activity.

Introduction. The main objective of this study was to compare the speed of onset, duration of activity and the threshold dose of oxytocin on the bovine uterine musculature, after administration by different routes. Before studying the effects of oxytocin, it was important to consider the normal variations in uterine contractions occurring at different stages of the estrous cycle.

In spayed cows, artificial estrus was produced by estradiol benzoate. The cows on experiment were kept in pairs for company, and to avoid any unnecessary fright and excitement.

Most of the experiments were conducted in the Large Animal Hospital of the Faculty of Veterinary Science. Two cattle were brought in on each occasion and kept together in one box. They were allowed 5-7 days to become accustomed to the new environment before any experimental procedures were started. They were kept on straw bedding and hay fed, one bundle (approximately 25 kg) for two per day. Water was not restricted.

Patterns of uterine contraction

After the recording tube was correctly placed in the uterus (Chap. 3.3.1), a period of 5 minutes was allowed for the cow's body temperature to have raised the tube temperature and to have stabilized the air pressure within the tube. The *in vivo* recording was started by connecting the tube to the transducer.

The level of the starting point (Fig. 3B-d) was much higher than the *in vitro* experimental starting line (Fig. 3A-b). The line at the lowest point of the contractions was interpreted as the uterine muscular tone and termed the tonicity line (Fig. 3B-d). Therefore, the distance

from the *in vitro* base line to the peak of the contraction (Fig 3B-e), consisted of the pressure due to the compression of air in the recording tube when the apparatus was connected (between *in vitro* base line and *in vitro* experimental starting line), the pressure of the tonicity of the uterus (between *in vitro* experimental starting line and the lowest point of the contraction) and the pressure of the uterine contraction (between the lowest point of the contraction and the peak of the contraction) (Fig 3).

In this work, the pattern of uterine contractions studied, included the rhythmic uterine contractions in addition to the uterine tonicity, and therefore the term *uterine pressure* has been used to describe the sum of the pressure of tonicity and the contraction pressure of the uterus.

Depending on the uterine pressure, sensitivity ranges of 250 and 100 on the chart recorder were mostly used. Sometimes, the peak of the contraction rose so high that the marking pen had to be brought down by off-setting the machine. The off-setting adjustment was calibrated as a percentage and in making calculations of pressures, allowance could be made for such changes afterwards.

Factors affecting the patterns of the uterine contraction

(a) The presence of the tube in the uterus. It was felt that the insertion or the presence of the recording tube in the uterus might cause local irritation sufficient to influence the contractions. This possibility was tested for by removing the tube after a period of recording, and later reinserting it. Different periods of recording and different periods of rest i.e. time when the recording tube was not in the uterus, were used in a series of studies but on no occasion was any local irritation or influence on uterine activity demonstrable on the chart recorder.

(b) Major body movements. Rumination, coughing, eating (chewing), arching of the back, urination and defecation, could each produce some changes in abdominal pressure which in turn might have influenced the recorded tracings of uterine muscular activity. Therefore such major body movements were carefully watched for and the event recorded on the appropriate tracings.

(c) Rectal manipulation. The contraction of one part of the uterus is different from another (1, 19) and to avoid differences arising from movements of the recording tube, checks had to be made periodically by rectal palpation to confirm that the tube was still correctly positioned. In case such interferences had any influence on the tracing, marks were made when the hand was just in the rectum without touching the uterus as well as when the uterus was actually palpated.

(d) Vaginal distension. Normally it was not difficult to guide the recording tube into the external opening of the cervix, but if there was any abnormality in the cervical position, it was sometimes necessary to put a hand into the vagina and locate the cervix manually. During the operation, the changes on the tracing before and after the cervix had been touched, were carefully noted.

(e) Milking. Unexpectedly, two spayed heifers, which had received hormone treatment, began to lactate: there was development of both udder and teats. As milk was seen to be dripping from the teats, the animals were hand-milked and the opportunity was taken to make observations on the uterine contractions while this was in progress.

(f) Rompun: brand of Xylazine (Bayer, New Zealand). In fixing the intravenous Longdwel Catheter (Page 32), a sedative dose of 0.75 ml (0.05 mg/kg body weight) of "Rompun" was used to enable the operation to be completed under local anaesthesia but with the cow in the standing position. The opportunity was taken to record uterine contractions while

the animal was still under the sedative influence.

(g) Noise effect. The environment where the experiments were carried out was not often quiet; for example, a tractor frequently passed by the bail, and such distractions were thought to possibly have some effect on uterine contractions through stimulation of the sympathetic nerv ous system. Recordings were carried out with, and without the tractor noises in close proximity.

(h) Adrenaline. Various authors refer to the effect of adrenaline on uterine motility. As a corollary to the foregoing effects of noise, adrenaline was administered intravenously as a 3 ml dose of 1:1000 dilution.

Oxytocin administration

(a) Intravenous route. To obtain a smooth injection of the drug, without disturbing the animal, a "Longdwel I.V. Catheter Needle" (14G, 10cm) (Becton, Dickinson and Company, N.J.) was introduced under sedation. Four small holes had previously been drilled in the wings of the needle hub and a nylon thread was passed through all the holes and anchored to the underlying skin (Plate 3a). A drip-set tube filled with heparinized saline (125,000 U in 100 ml) was attached to the needle hub and led dorsally to a three-way adaptor. The tubing was anchored by enclosing it within a fold of the neck skin (Plate 3b).

After withdrawing heparinized saline present in the drip-set, the tubing was filled with blood, and then oxytocin (Butocin, Burns-Biotec) was introduced and flushed into the vein with heparinized saline. The saline in the drip-set tube was replaced once daily and patency of the needle assured.

(b) Epidural route. Local anaesthesia for epidural injection was obtained using Xylocaine: brand of lignocaine (ASTRA, Australia) 2% solution, 2 ml. After 5 minutes, an epidural needle (18G, 9 cm) was

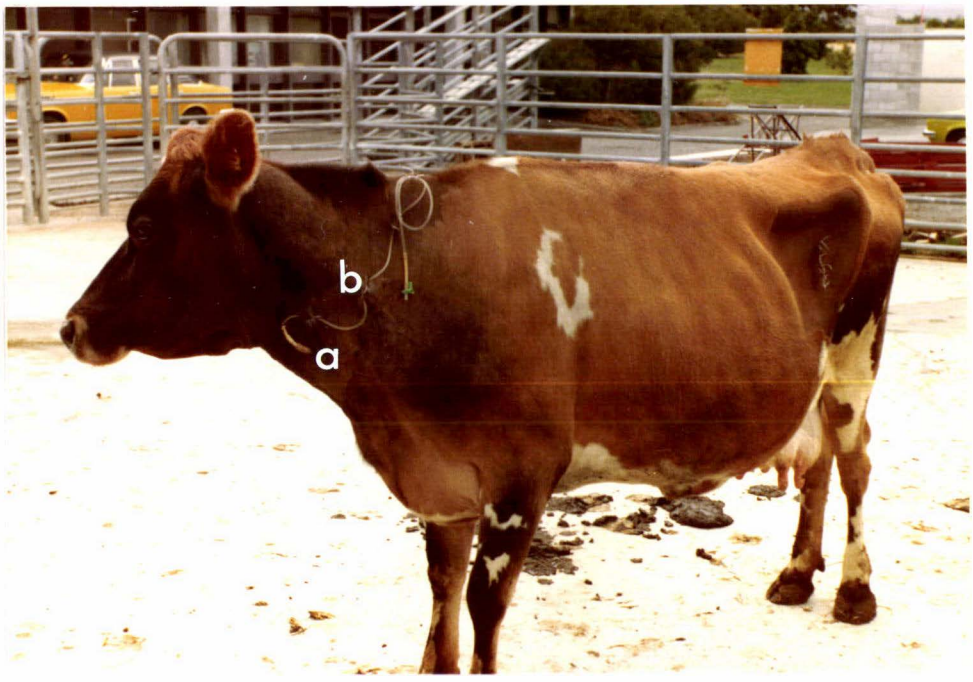
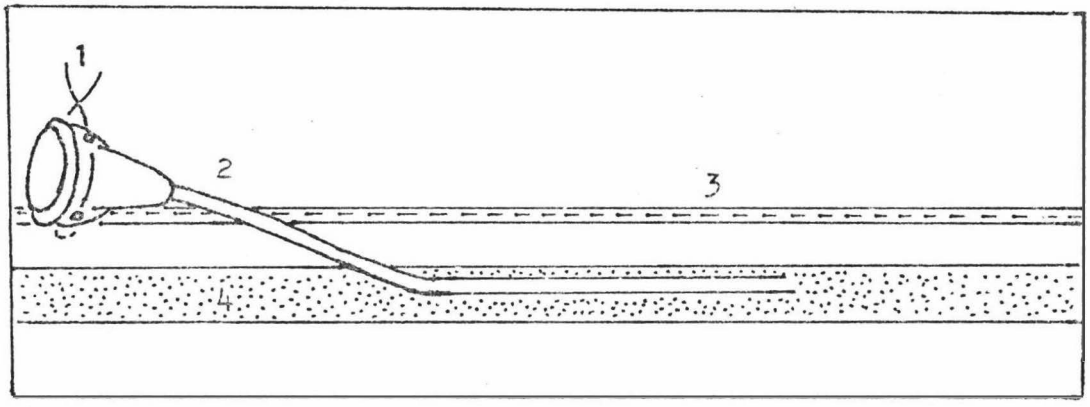


PLATE 3. THE LONGDWEL INTRAVENOUS NEEDLE AND DRIPSET TUBE WITH ADAPTOR IN POSITION



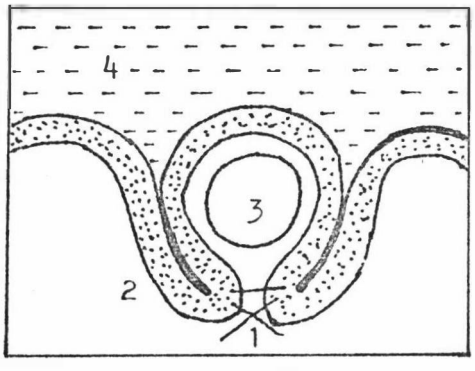
ENLARGED DIAGRAM OF PLATE 3(a) SHOWING THE LONGDWEL INTRAVENOUS NEEDLE IN POSITION

1 = Nylon suture

2 = Longdwel needle

3 = Skin

4 = Vein



ENLARGED DIAGRAM OF PLATE 3(b) SHOWING ANCHORAGE OF THE DRIPSET TUBE WITHIN A FOLD OF THE NECK SKIN

1 = Nylon suture

2 = Skin fold

3 = Dripset tube

4 = Subcutis

inserted into the first intercoccygeal space and the oxytocin solution was injected after removal of the stilette. During the operation, the animal's kicking, arching of the back, urination, defecation, tail flicking and bellowings caused slight disturbances in the pattern of uterine contractions, but these returned to normal as the animal settled.

(c) Intramuscular route. The gluteal muscles were chosen as an injection site because the cow was restrained in the crush and after several attempts it was realised that access to the neck site was too difficult. Since the oxytocin used was a solution, only a narrow bore needle (20G, 4cm) was necessary.

The response dose of oxytocin

Oxytocin in doses between 5 to 60 U were administered intravenously followed by flushing the indwelling catheter with up to 20ml of heparinized saline. Saline was also given independently on several occasions in a dose of 20 ml.

Duration of action of oxytocin

After each method of oxytocin administration, observations of recordings were continued up to nine hours to determine whether the uterine contractions returned to the pretreatment pattern.

To test the possibility that the duration of action of oxytocin might be short and that the contraction pattern would return to pretreatment levels during a rest period (with the recording tube removed from the uterus), a programme of experiments was arranged using different periods of recording interrupted by different periods of rest according to the following schedule:

(1) ½ hour recording, ½ hour rest then recontinued;

(2) ½ hour recording, 1 hour rest then recontinued;

(3) 1 hour recording, 1 hour rest then recontinued;

(4) 1½ hours recording, 1 hour rest then recontinued;

(5) 1½ hours recording, 1½ hours rest then recontinued;

(6) 2 hours recording, 1½ hours rest then recontinued, and

(7) 2 hours recording, 2 hours rest then recontinued.

CHAPTER 4 RESULTS AND DISCUSSIONS

4.1 PATTERNS OF BOVINE UTERINE ACTIVITY

4.1.1 Differences between cows and between days

It was found that the bovine uterine contraction pattern was different from day to day (Fig. 4a, b), from animal to animal (Fig. 4a, c) and sometimes even within the same hour in the same animal. The contractions differed in amplitude, frequency, duration and pattern, and the uterus itself in its tonicity. Sometimes the duration of contractions was prolonged, as was the period of relaxation (Fig. 4d), sometimes the contractions were of short duration (Fig. 4b) and sometimes there was no distinct contraction at all.

During overt estrus in normal cows, the uterine contractions were usually slow and forceful (Fig. 5b; 6b, c). Other animals demonstrated high amplitude and increased frequency in contractions (Fig. 5a). Estrus was recognised by the swelling of the vulva, vaginal discharge, mounting behaviour and bellowing. After estrus, the uterine activity moderated (Fig. 6d). During diestrus, there were times when the uterus was at rest. The uterus of the cow D41 did not produce any contractions on day 8 after estrus; cow 350, day 10; and cow D542, day 13 after estrus. Generally as the time of estrus approached, the uterine activity increased again (Fig. 6a).

In spayed cows, at least 7 days after ovariectomy, the degree of uterine activity was much diminished in comparison to intact cows (Compare figure 4 and 7). Out of a total of 54 recordings carried out on spayed cows, only minimal uterine activity was found, even after estrogen treatment (Table 1).

a.Cow D41 18.12.78

b.Cow D41 10.1.79

c.Cow D38 23.4.79

d.Cow D38 24.4.79

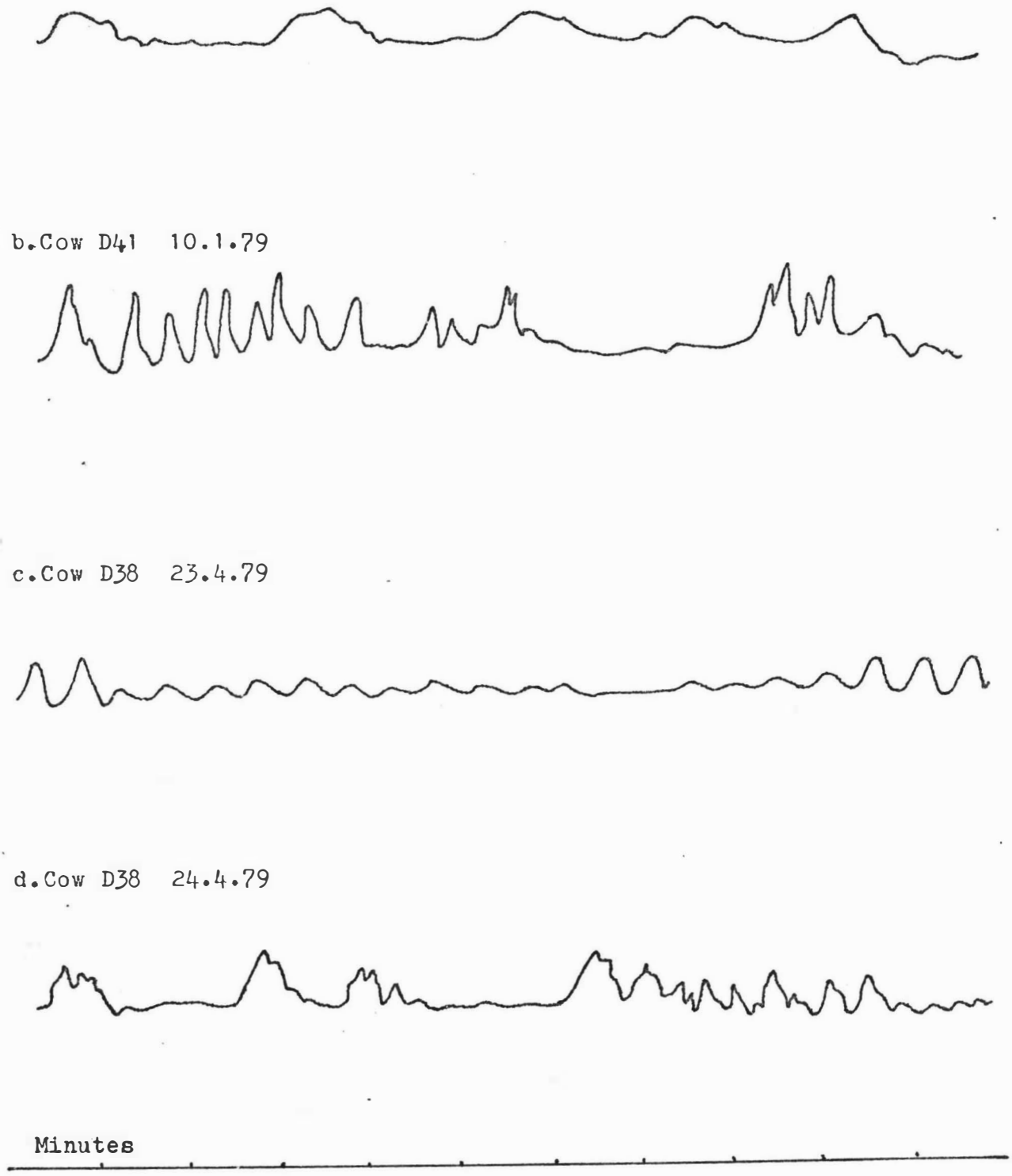
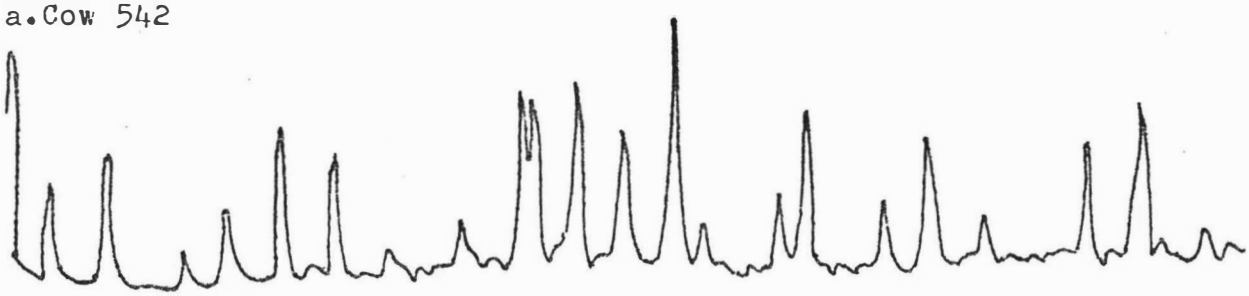


Fig.4.PATTERNS OF UTERINE ACTIVITY OF NORMAL COWS SHOWING DIFFERENCES BETWEEN COWS AND BETWEEN DAYS

a. Cow 542



b. Cow D318

Fig. 5. PATTERNS OF UTERINE ACTIVITY OF NORMAL COWS AT ESTRUS SHOWING DIFFERENCES BETWEEN COWS
(Note variation in amplitude and difference in frequency)

a.Cow D41 (approximately 2 days before estrus)

b.Cow D41 (at estrus) 20.12.78

c.Cow D41 (at estrus) 21.12.78

d.Cow D41 (approximately 1 day after estrus)

Minutes

Fig.6.PATTERNS OF UTERINE CONTRACTION IN NORMAL COWS SHOWING DIFFERENCES BETWEEN DAYS ACCORDING TO THE STAGE OF THE ESTROUS CYCLE

a.Cow D41 6.3.79(36)days after spaying.

b.Cow D41 7.3.79

c.Cow D41 9.3.79

Minutes

Fig.7.PATTERNS OF UTERINE ACTIVITY OF SPAYED COWS
SHOWING DIFFERENCES BETWEEN DAYS
(Note-no exogenous estrogen treatment)

Number of recordings	No estrogen treatment			After estrogen treatment		
	Uterine contractions			Uterine contractions		
	moderate	weak	nil	moderate	weak	nil
54	2	14	18	0	16	4

Table I UTERINE RECORDINGS OF SPAYED COWS BEFORE AND AFTER ESTROGEN TREATMENT.

4.1.2 The lack of effect of local irritation or body movements

Repeatedly removing and replacing the recording tube (Page 30) in the uterus, did not alter the contraction pattern over a series of four recordings. Sixteen rectal manipulations and seven vaginal distensions by hand during recordings, did not cause any changes in uterine contraction unless the cervix or the uterus itself was touched or handled (Fig. 8a, b).

Coughing (Fig. 8c) and bellowing (Fig. 8c, 8d) produced an abrupt and marked response in uterine pressure lasting less than one second. Arching of the back (Fig. 9a), urination (Fig. 9b) and defecation (Fig. 9c), all caused a transient rise in uterine pressure which persisted for 2-5 minutes. After urination or defecation (Fig. 9 b,c) there was generally a transient fall in tonicity of the uterus. Other factors such as flicking of the tail (Fig. 10a), chewing (Fig. 10b) or disturbance from noise (Fig. 10c), were found not to have any lasting effect on the general uterine contraction patterns.

4.1.3 Uterine pressure

The amplitude of the contraction produced by the uterus was compared with the amplitude of contraction produced in an *in vitro* testing apparatus (Page 26) using the same sensor tube. By the procedure referred to above, the physiological tonicity pressure ranged from 32 mm

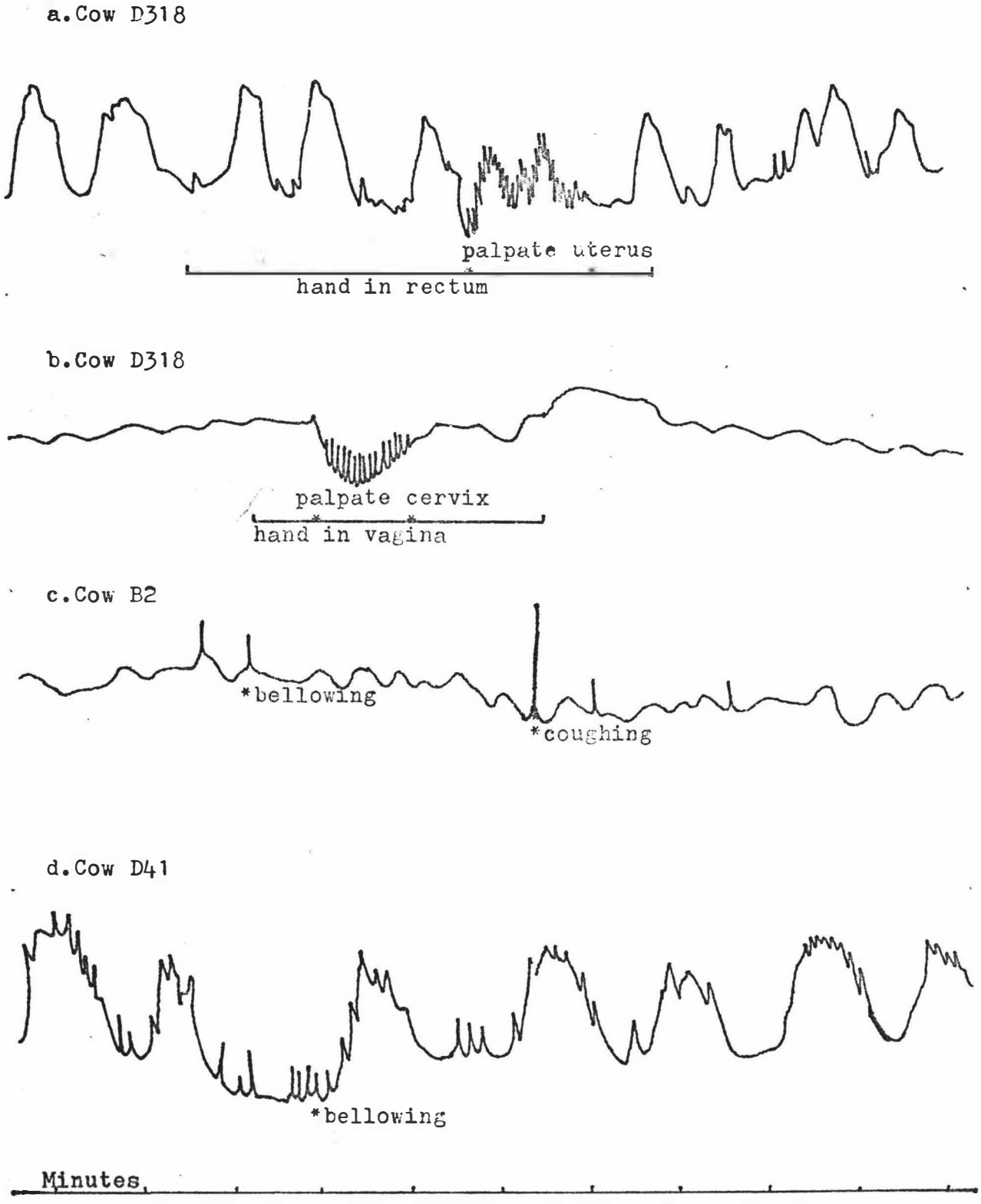


Fig.8.PATTERNS OF UTERINE ACTIVITY OF NORMAL COWS SHOWING MOMENTARY EFFECT OF UTERINE PALPATION(a), CERVICAL PALPATION(b), COUGHING(c) AND BELLOWING(c and d).

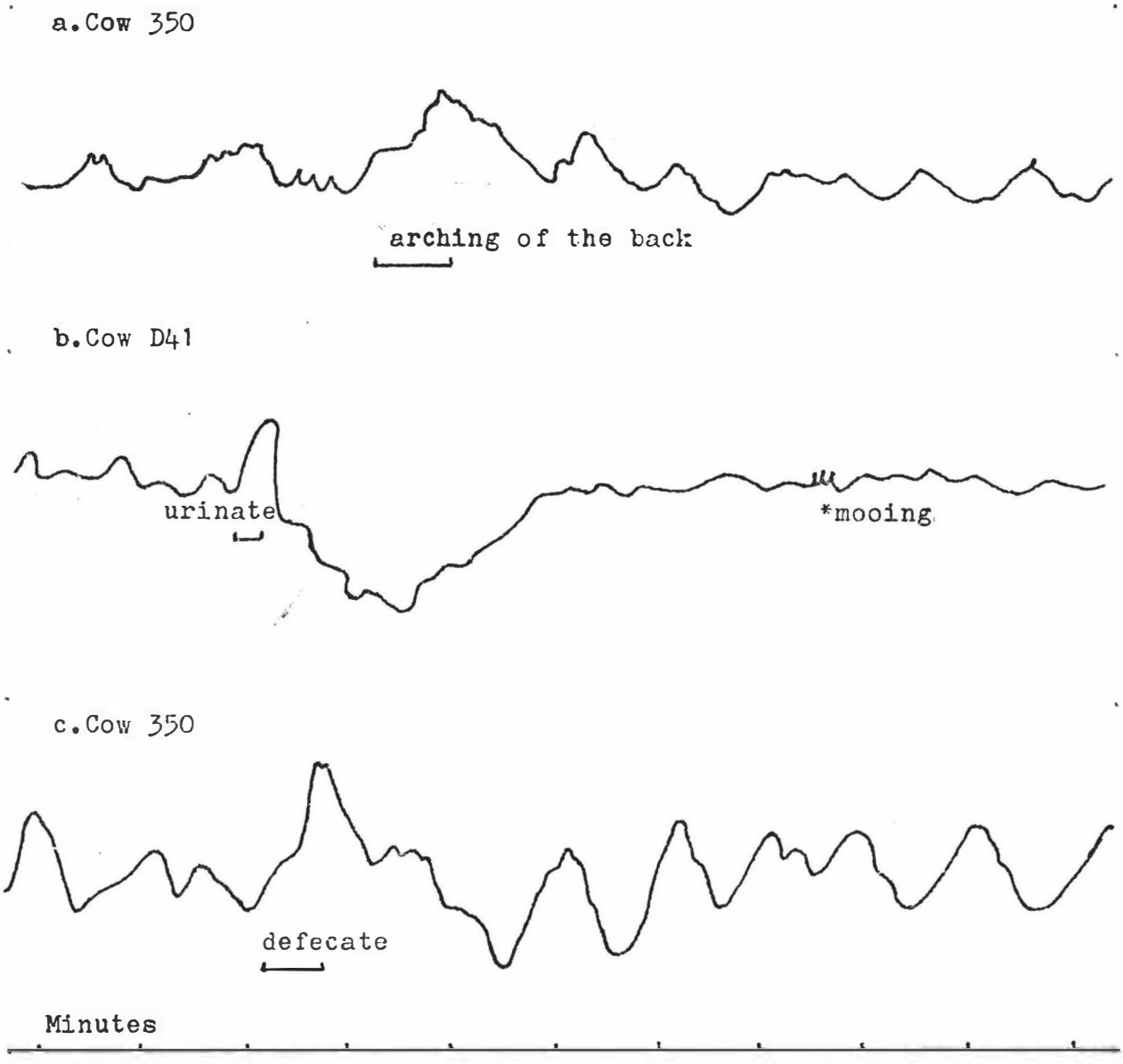
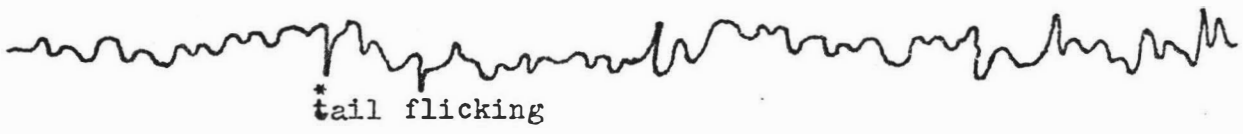
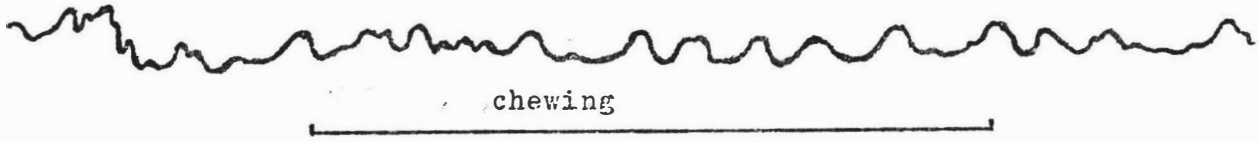


Fig.9.PATTERNS OF UTERINE ACTIVITY INTERRUPTED BY ARCHING OF THE BACK(a),URINATION(b) AND DEFECATION(c).

a.Cow X88167



b.Cow D41



c.Cow 350

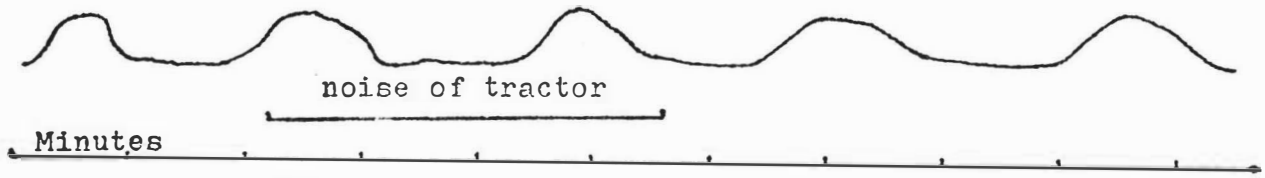


Fig.10.PATTERNS OF UTERINE ACTIVITY SHOWING LACK OF EFFECT OF BODY MOVEMENTS AND ENVIRONMENTAL NOISE:TAIL FLICKING(a), CHEWING(b) AND DISTURBANCE BY A TRACTOR(c).

Hg to 1010 mm Hg, and the contraction pressure ranged from 0 mm Hg to 110 mm Hg. Therefore the uterine pressure (UP), composed of the sum of these two, ranged from 32 mm Hg to 1120 mm Hg in non-pregnant cows (Table II). After oxytocin treatment, uterine tonicity hardly changed; uterine contraction pressure rose in all cases and contraction frequency either increased, or it decreased and in these cases contractions became of longer duration (Table II).

During any one recording session, there was invariably fluctuation in tonicity and amplitude of the contractions. To obtain an average figure, the measurement was made at the modal base line of tonicity and at the modal peak of contraction.

The uterine pressure figures obtained were approximate only because small variations in contraction amplitude were equivalent to very large changes in uterine pressure depending on the sensitivity of the sensor diaphragm.

4.1.4 Frequency of uterine contraction

The frequency of bovine uterine contractions ranged from 0 to 22 per 10 minutes. The number varied with the duration and amplitude of the contraction. Mostly the frequency was between 10-15/10 minutes. When the duration of the contraction was short, the frequency was increased; when the contraction was prolonged, the frequency was decreased. Both the duration and pattern of the contractions were inconsistent (See Table II and Figure 5).

Sometimes, the general pattern of the tracing at a contraction peak was complex and variable and caused difficulties in deciding where one contraction finished and another began.

Experiment No.	Cow Tag	Date	Uterine Tonicity (mm Hg)		Uterine Contraction (mm Hg)		Contraction Frequency (per 10 minutes)		Route of Administration
			Before Oxytocin	After Oxytocin	Before Oxytocin	After Oxytocin	Before Oxytocin	After Oxytocin	
1	350	13.12.78	38	38	0	94	0	5	IV
2	"	20. 2.79	969	969	36	118	11	9	IV
3	"	21. 2.79	72	72	72	89	22	29	IV
4	"	28. 2.79	787	787	40	79	8	20	IV
5	"	1. 3.79	395	395	27	55	11	26	IV
6	"	3. 3.79	986	986	90	150	16	19	IM
7	"	4. 3.79	1010	1010	110	157	16	13	EP
8	D41	6. 3.79	340	340	0	45	0	14	IV
9	"	15. 3.79	220	220	22	42	21	32	IM
10	"	20. 3.79	450	450	60	90	18	26	EP
11	X88	30. 1.79	99	163	16	89	14	10	IV
12	"	13. 2.79	46	46	0	26	0	21	EP
13	D38	23. 4.79	440	440	110	155	18	15	IV
14	"	26. 4.79	37	37	40	120	9	13	IV
15	"	28. 4.79	90	90	33	68	17	21	IV
16	D15	29. 4.79	180	272	60	90	9	24	IV

Table II - Pressure of uterine tonicity, pressure of uterine contraction, and frequency of uterine contractions before and after oxytocin treatment.

IM = Intramuscular injection, EP = Epidural injection, IV = Intravenous injection.

4.1.5 The intra-uterine "lumen"

Although the uterus is a hollow organ its walls are normally in contact leaving no cavity. At all stages of the cycle, it was clear from rectal palpation that the endometrium clung tightly to the recording tube even though in the cervical area the tube might feel quite loose. The pressures recorded therefore were a direct reading of uterine musculature thrust rather than the same conveyed through air pressure retained in a closed organ.

When the uterus was palpated during estrus, it was hard and enlarged whereas during other stages of the cycle, it was soft and relaxed.

4.2 DISCUSSION

The considerable variability observed in uterine activity between cows and between days, agrees with the findings of other workers (52, 54, 57, 71, 82). One explanation could be that the variation was due to fluctuations in the levels of reproductive hormones which influence uterine function. According to Caldeyro *et al.* (19), the activity of the human uterus is different from midpart to fundus, and from left to right horn. If the same is true in the bovine, day to day differences in this study, might be explained by slight variations in positioning of the sensor diaphragm. Any variation though would be slight, because care was taken to see that the diaphragm was always placed at the bifurcation of the uterine body, and there was no risk of recordings being taken from a distinctly different area of the uterus.

The transient fall in uterine tonicity, after urination or defecation, might be due to the reduction in abdominal pressure after elimination resulting from provision of more dead space.

In support of the findings reported in this study, Gillette (47) noticed that the general movements of the cow such as eating, drinking,

etc., did not interfere with recordings.

Blank *et al.* (13) claimed that vaginal distension significantly elevated the plasma concentration of oxytocin, resulting in an increase contractile activity of the uterus (29, 37, 43, 51). However during the present investigation, vaginal distension caused only momentary changes in the contraction pattern.

In ruminants, the uterine activity is largely reduced during diestrus, whereas during estrus it is marked (33, 52, 57, 63, 82, 99). The results reported here agree with those of the above workers. Other workers (71) have suggested that specific days of the cycle are closely correlated with the activity of the uterus, but for the normal cows concerned here, the days during diestrus on which the uterus was at rest tended to be variable.

In man and cattle, the uterus is more active during menstruation and estrus respectively; but Reynolds (80) has shown that the activity of the human uterus increases twice during a menstrual cycle; the other occasion being at the time of ovulation which could correspond to the bovine estrous period.

The use of the ovariectomised cows produced results that agree with those of Evans and Miller (33), i.e. that ovariectomy either abolishes motility of the uterus in the cow, or Hays and Vandemark - that activity is much diminished in both frequency and amplitude (52).

The way uterine pressure was calculated, differed from the techniques used by previous workers. The pressure changes in the measuring tube were recorded, and then interpreted in terms of the external pressure acting on the diaphragm. Other workers took the internal pressure changes in the tube, as indicative of the intrauterine pressure (IUP).

Uterine pressure has been described either in mm Hg or Montevideo

Units (MU)=Amplitude (A) x Frequency (F). Csapo's (26) measuring system of MU may be meaningful when only one of the factors (A or F) is changing, but the system becomes less useful when both factors are changing as was shown repeatedly (Fig. 5). Indeed there was so much variation that, only the pressure of a single representative contraction was measured in this work, rather than a series of uterine contractions.

Zerobin *et al.* (99) stated that, the strength of the uterine contraction of cows, although varying greatly, was normally very high; up to 60 mm Hg. In his method of calculation, he took the base line of uterine contraction as zero, so excluding any pressure arising from uterine tone (tonicity). If allowance is made for this discrepancy, Zerobin's figures are in the same range as have been found in the present study.

The frequency did not consistently increase during estrus because the force of the uterine contraction could be either strong or weak. Usually during estrus, the amplitude of the contractions was greater than at other stages. When a spayed cow was brought into estrus by estrogen treatment, the frequency of contractions increased but the amplitude of the contractions was much reduced.

The frequency of uterine contractions in the normal cow, has been studied before with conflicting results. During diestrus the contractions were less frequent. According to Gillette (47) the frequency of uterine contractions of cows ranged from 2-9 per 10 minutes. He quoted an average value but the results were so variable; it might have been better to just give the range.

Various authors in referring to intrauterine pressure (4, 26, 35, 80, 91, 99) have implied that the pressure exerted on the balloon was due to the air held inside the hollow uterus by the closed *os uteri* of the cervix. One worker even stated that the smaller the intrauterine space, the greater the intrauterine pressure (26). In the investigation

reported in this thesis, the pressure exerted on the sensor diaphragm was due to direct contraction of the uterine musculature. In many cases, the recording tube moved freely and without resistance through the *os uteri* of the cervix, which was not air tight and certainly would not have withstood pressures in the range of 32 - 1120 mm Hg.

Although the pressures recorded were the result of the direct force of uterine musculature contraction, it was not established whether this was due to the longitudinal or circular musculature of the uterus, or both.

4.3 EFFECT OF OXYTOCIN

4.3.1 Uterine response to oxytocin

Whenever the uterus of the cow showed contractions, it responded to intravenous (IV) injection of oxytocin, with an immediate sharp increase in tone followed by increased frequency of contractions (Fig. 11a). When the uterus was quiescent, there was no response to oxytocin.

The onset of action was immediate after oxytocin had been administered by the IV route. Both intramuscular (IM) and epidural (EP) injections of oxytocin also gave good responses (Fig. 11a, b), but the increased activity arose less abruptly than after IV injection. Even when the uterus was in an active stage, there were failures in response to administration by the IM and EP routes. Oxytocin given by the IM route led to more failures than following the EP route (Table III). The latent period after using the IM route ranged from 4-14 minutes and after the EP route 3-17 minutes. The different routes of administration had no effect on the duration of action.

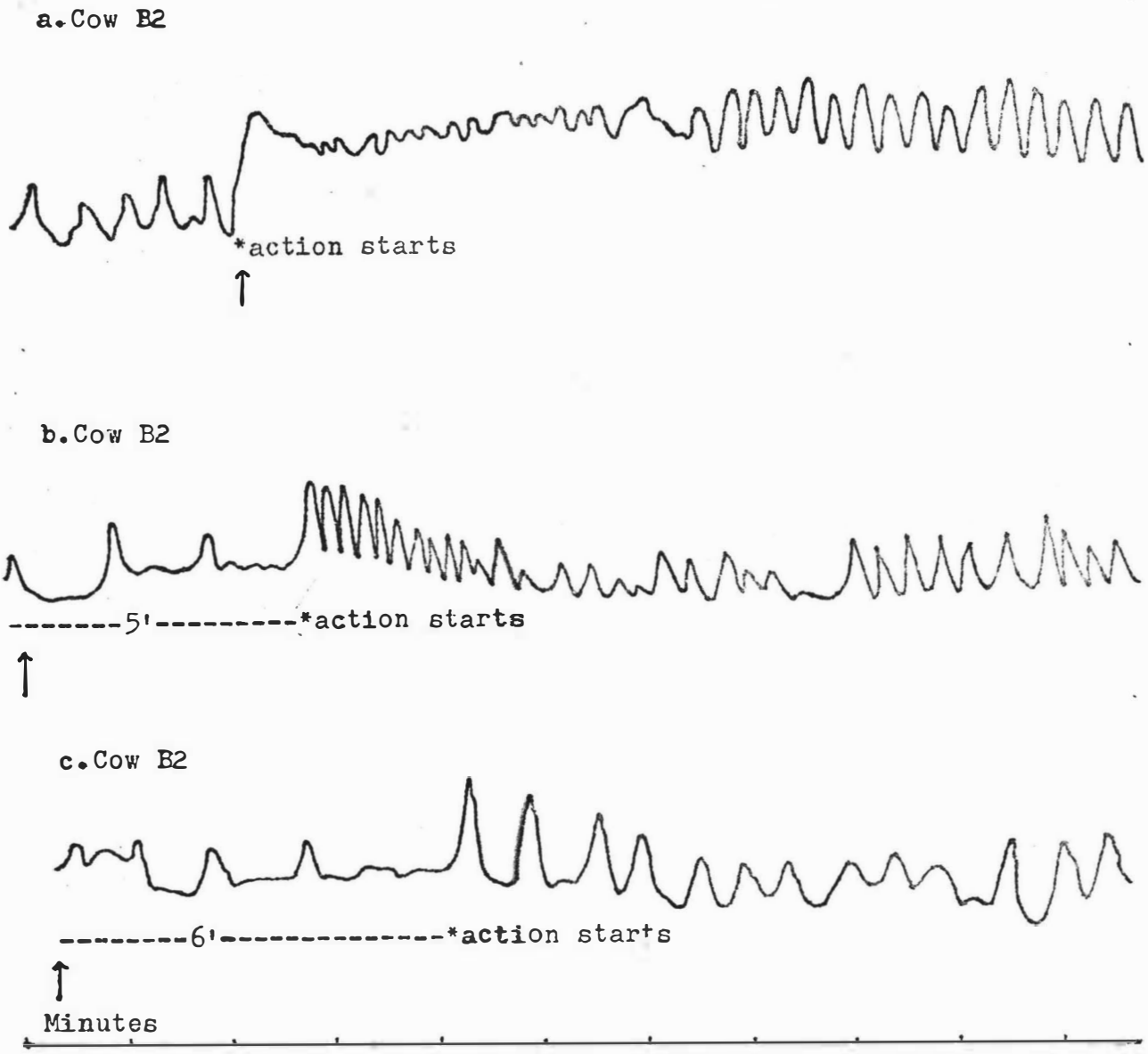


Fig.11. CHANGES IN THE PATTERN OF UTERINE CONTRACTION DUE TO OXYTOCIN ADMINISTRATION.

(a) By intravenous route

(b) By intramuscular route

(c) By epidural route.

(↑) Oxytocin administration

Route	Mean latency (range) min.	Number responded	Number failed to respond	Total experiments	Remarks
Intravenous	0 (0)	30	4	34	The 4 failures occurred in cows with initially noncontracting uteri.
Intramuscular	9 (4 - 14)	11	5	16	The 5 failures showed immediate response to oxytocin by IV route.
Epidural	10 (3 - 17)	12	1	13	The 1 failure showed immediate response to oxytocin by IV route.

TABLE III. SUMMARY OF EFFECTS OF OXYTOCIN GIVEN BY THE INTRAVENOUS, INTRAMUSCULAR OR EPIDURAL ROUTE.

4.3.2 The response dose of oxytocin

Oxytocin in a dose of 5 units (U.S.P.) given by the IV route did not produce noticeable changes in the uterine contractions. In a series of 9 experiments, IV doses of oxytocin containing 10-20 units, repeated 40 minutes after a 5-unit dose, increased the uterine activity considerably but subsequent repeated doses produced no further response (Fig. 12b, 13c).

4.3.3 Duration of action of oxytocin

After the action had started, the increased uterine activity persisted for an extended period, on one occasion for more than 9 hours during which time the recording tube remained *in utero*. From a series of 16 experiments on 3 cows, the duration of action of oxytocin (40 units) was found to be variable from at least 1 to more than 3½ hours (Fig. 14 and Table IV) provided that the cows were let free and allowed to rest in between first and second recordings.

4.4 DISCUSSION

Of the three routes of administration tried, intravenous, intramuscular and epidural, the intravenous route produced the most consistent results. The epidural route was more reliable than the intramuscular route (Table III) and should be preferred where intravenous administration proves difficult, but great care must be taken to maintain asepsis.

Spiridonov (34) claimed that epidural injection of oxytocin caused a more marked stimulation of the frequency and duration of bovine uterine contraction than subcutaneous injection. In this study, no difference in the intensity, frequency and duration of contractions could be ascribed to the route of oxytocin administration.

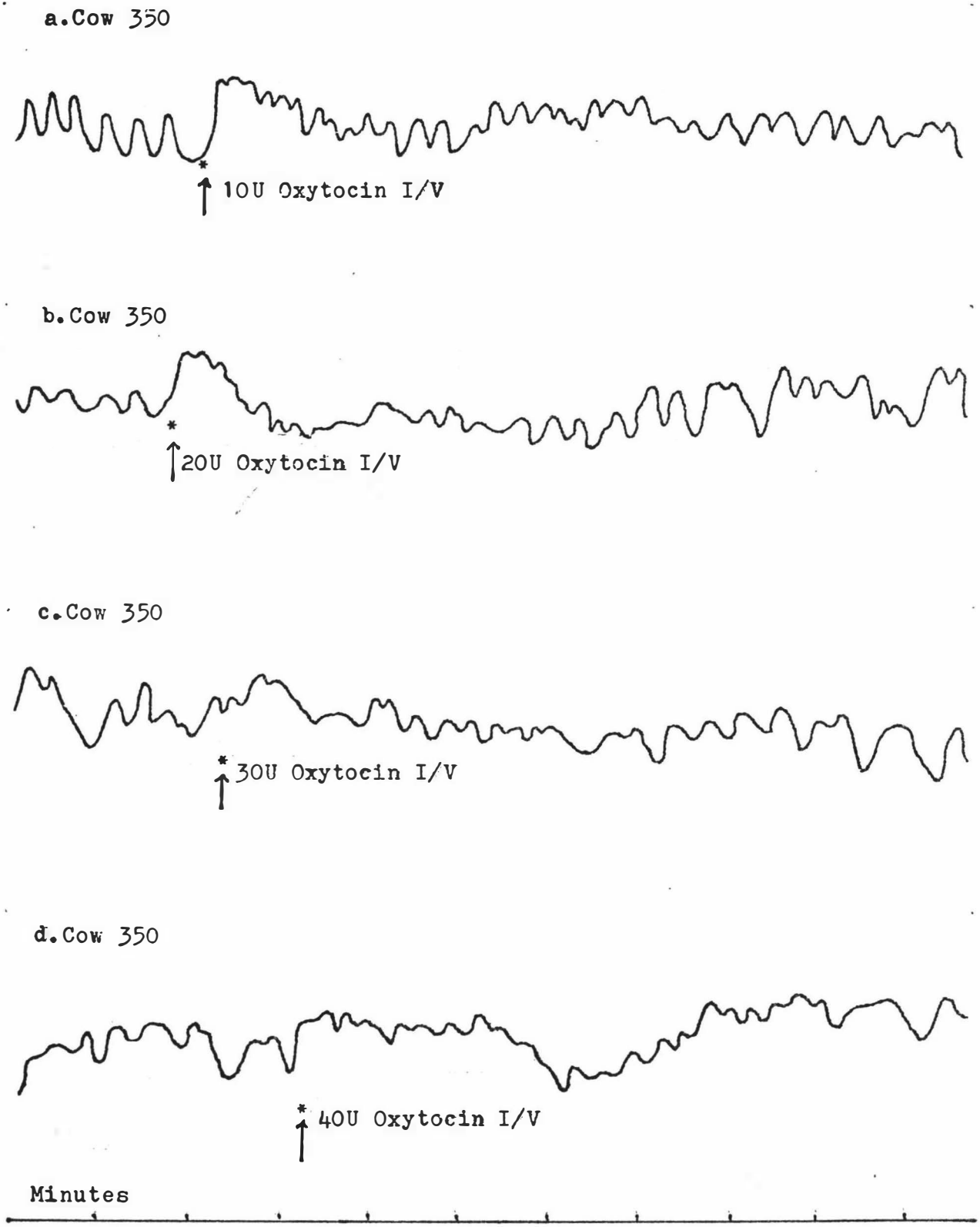


Fig.12.CHANGES IN THE PATTERNS OF UTERINE CONTRACTION AFTER DOSES OF OXYTOCIN ADMINISTERED INTRAVENOUSLY (I/V) AT INTERVALS OF 40 MINUTES.

(Note: No further increase in uterine activity after 10 U)

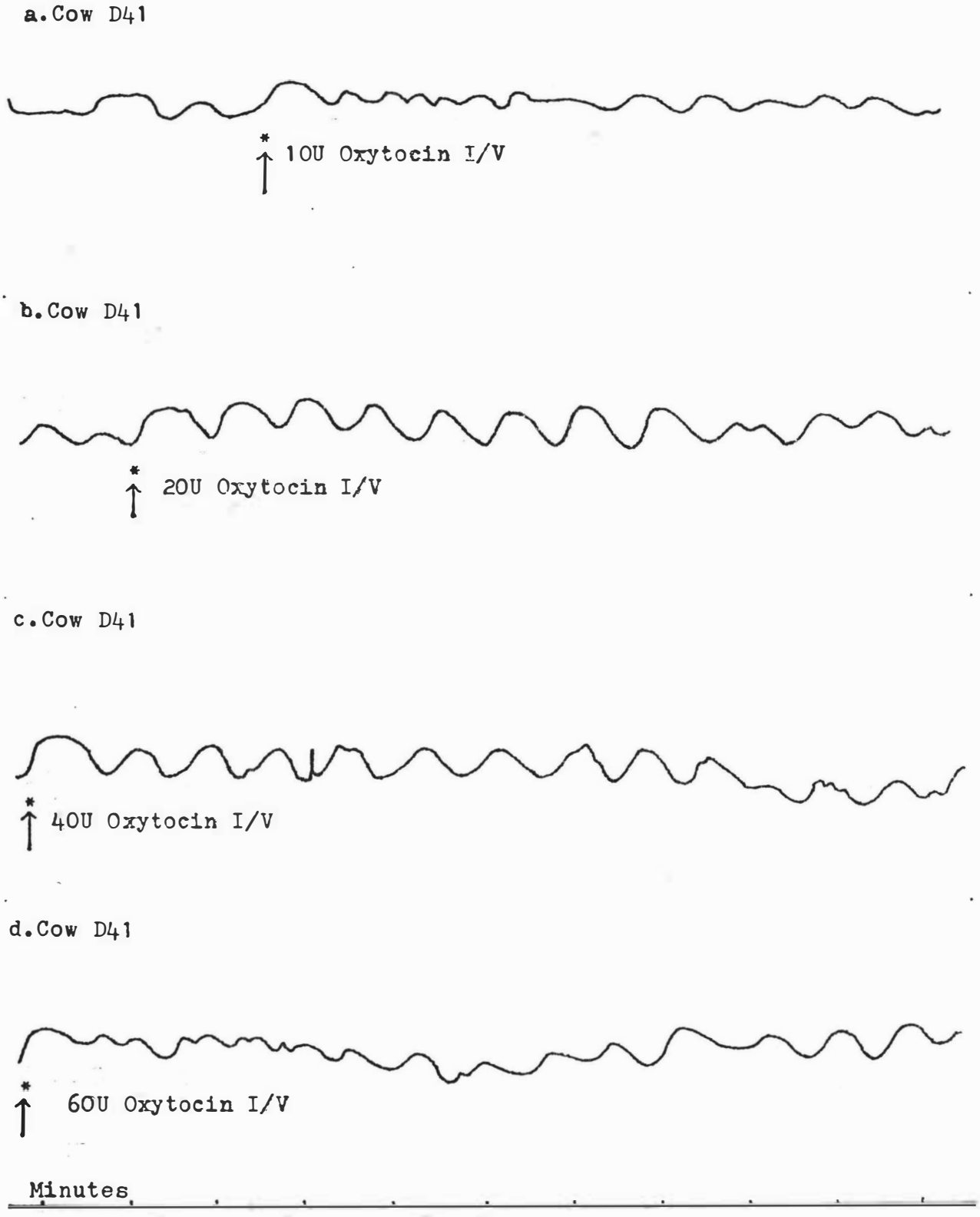


Fig.13. CHANGES IN THE PATTERNS OF UTERINE CONTRACTION AFTER DOSES OF OXYTOCIN ADMINISTERED INTRAVENOUSLY AT INTERVALS OF 40 MINUTES.
(Note: No further increase in uterine activity after 20 U)

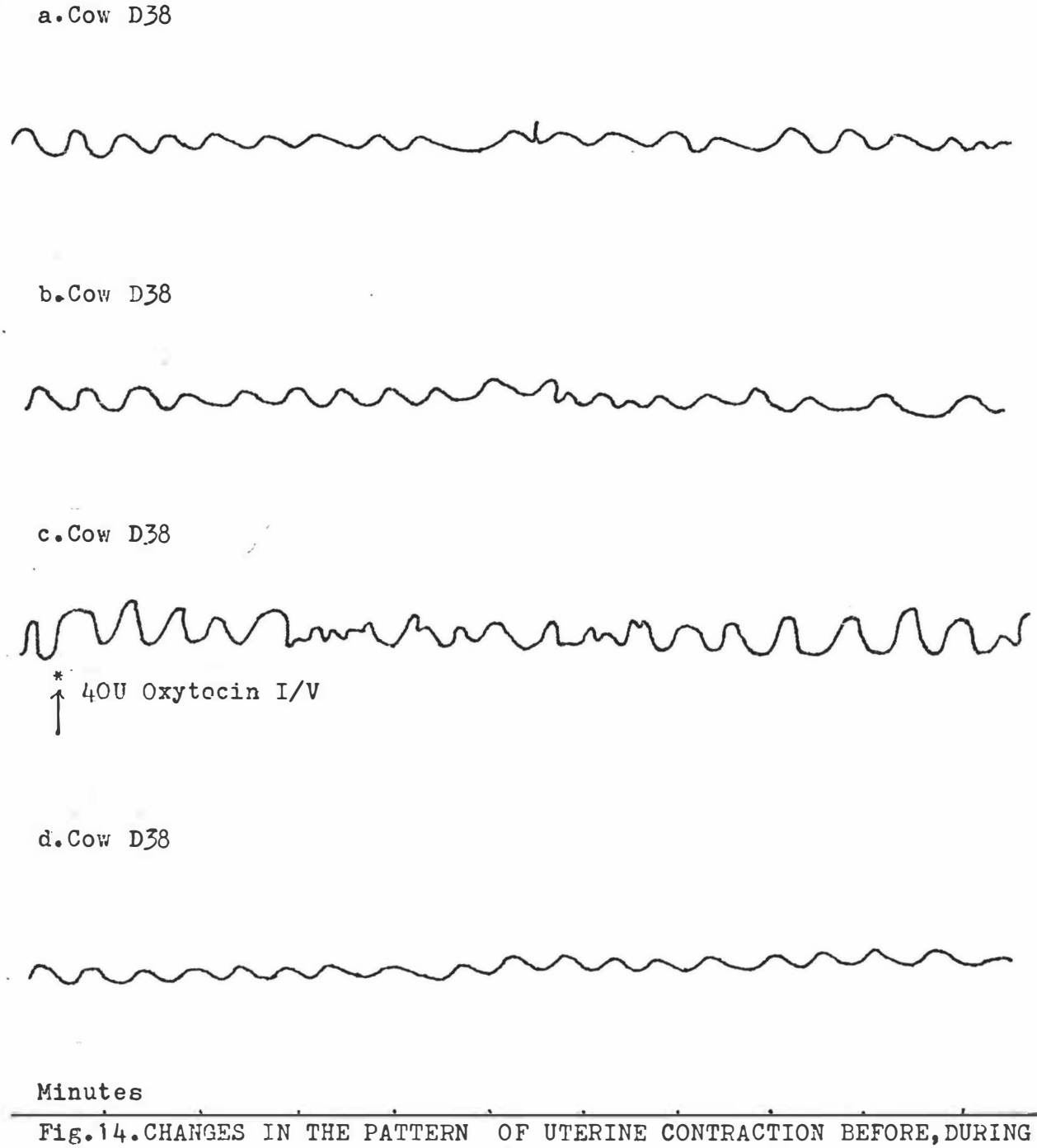


Fig.14. CHANGES IN THE PATTERN OF UTERINE CONTRACTION BEFORE, DURING AND AFTER THE ACTION OF OXYTOCIN.

(a) Uterine contractions at the begining of the experiment

(b) Uterine contractions after the recording tube was taken out and reinserted back into position

(c) The increased uterine activity due to oxytocin

(d) Uterine contractions after 1 hour ofrecording(c) and 3.5 hours of rest .

Date	Cow ear tag	Initial uterine activity	Oxytocin dose & route	Response in uterine contraction	First recording period. (min.)	Rest period after first recording. (min.)	Second recording period. (min.)	Duration of action at least	Uterine contractions at the commencement of second recording period
9. 4.79	D41	0	IV 40U Oxy.	I	-	-	-	-	-
10. 4.79	D41	+	IV 40U Oxy.	I	142	186	60	2½ hrs.	Returned to base line
11. 4.79*	D41	+	IV 40U Oxy.	I	76	127	79	1 hr.	Returned to base line
12. 4.79	D41	0	IV 40U Oxy.	I	-	-	-	-	-
20. 4.79	D38	+	IV 40U Oxy.	I	113	255	76	2 hrs.	Returned to base line
21. 4.79	D38	+	IV 40U Oxy.	I	57	63	43	1 hr .	Returned to base line
22. 4.79*	D38	+	IV 40U Oxy.	I	59	181	58	1 hr .	Returned to base line
23. 4.79	D38	+	IV 40U Oxy.	I	55	70	48	3 hrs.	Continuing
24. 4.79	D38	+	IV 40U Oxy.	I	61	121	44	3½ hrs.	Continuing
26. 4.79	D38	+	IV 40U Oxy.	I	56	90	36	3 hrs.	Continuing
28. 4.79	D38	+	IV 40U Oxy.	I	64	188	39	1 hr .	Returned to base line
1. 5.79	D38	+	EP 40U Oxy.	I	33	52	34	2 hrs.	Continuing
25. 4.79*	D15	+	IV 40U Oxy.	I	82	95	30	1½ hrs.	Returned to base line
27. 4.79	D15	+	IV 40U Oxy.	I	70	75	50	3½ hrs.	Continuing
					-	99	34	3½ hrs.	Returned to base line
29. 4.79	D15	+	IV 40U Oxy.	I	49	57	50	2½ hrs.	Continuing
30. 4.79	D15	+	EP 40U Oxy.	I	42	36	22	1½ hrs.	Continuing

TABLE IV. EFFECT OF OXYTOCIN ON UTERINE CONTRACTIONS.
(Key: 0= no activity, + = contracting, * =estrus, I = increased, IV = intravenous, EP = epidural)

Manufacturers of oxytocin have for many years recommended a dose of up to 100 units for cows and mares (41), as an aid in the management of parturition. This dose rate is supported in the 1953 British Veterinary Codex. Unfortunately there is no means of comparing the response dose at full term to that effective in the non-pregnant cow and often no source of the unit value is given. Even the British Pharmacopoeia (Veterinary) 1977 fails in this respect. Doses used by the writer, have all been reported in United States Pharmacopoeia (U.S.P.) units.

Hays and Vandemark (51) gave 5 units of oxytocin intravenously to each of 3 non-pregnant cows and these animals showed only a slight stimulation of the uterine contractions. The results of the present work agree with those of previous workers who chose 15 units of oxytocin because it gave a maximum response in all cows tried, while a dose of 10 units gave a maximum response only in some cows and a 5 unit dose always gave a submaximal response.

Significant differences in the duration of effect of oxytocin, as measured by uterine activity, were found in the literature. Most work refers to species other than cattle. In the human, the duration of effect ranged from 15 minutes to 1 hour (1,15,20,71) whereas in the pig, a dose of 0.5 unit given intravenously, produced a response from oxytocin that lasted just 15 minutes (73).

In cows, Fitzpatrick (41) recorded completion of effects of oxytocin within 30 minutes whereas Fedorov (34) using pituitary extract recorded responses up to 3 hours and using synthetic oxytocin, up to 4½ hours.

The latter study is much more in line with the results reported in Chapter 4.3. It appears that although the half-life of oxytocin in serum is as short as 10 minutes (in man) (49), the action produced is an extended one (see page 53), particularly if recording is continuous. Even when periods of recording were interrupted by periods of rest (i.e. the recording apparatus removed from the cow), the response to oxytocin continued for more than 1 hour. This suggests that oxytocin is either inactivated slowly at its site of action after being quickly distributed or it may trigger off effects by enzyme induction.

There are practical advantages to use of the intramuscular route but the associated failures experienced in this study led to the use of the intravenous and epidural routes as better alternatives. In terms of duration of effect, Fitzpatrick (41) found no difference between the use of the intramuscular or the intravenous route which agrees with results reported on page 51, while Fedorov (34) found that the epidural route produced a considerably longer duration of effect than the subcutaneous route. Depending on the exact location of a subcutaneous injection, differences in absorption from the site might be considerable.

4.5 ESTROGEN ADMINISTRATION TO SPAYED COWS

4.5.1 Palpable changes of the uterus

The uterus of normal cows in estrus is enlarged and hard, whereas in the diestral period, it becomes smaller and soft. In spayed cows, the uterus is even smaller and with a soft consistency similar to the empty small intestine.

During estrus, both the cervical canal and the *os uteri* become more relaxed than at other stages of the cycle. There were many occasions when the cervical dilator had to be used to ease the passage for the recording tube. Usually the cervical canal of heifers and spayed cows was smaller than that of normal cows. In the case of one heifer, which had been spayed six months previously, it was impossible to pass the dilator through the internal opening of the cervix.

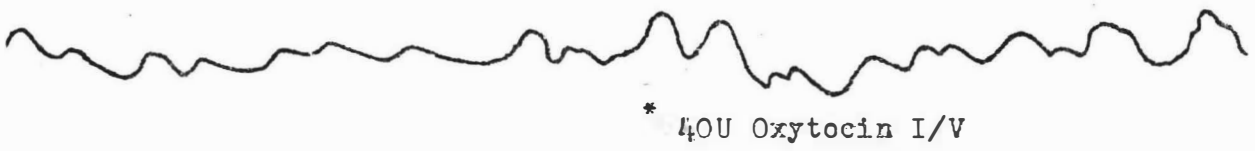
When the four spayed cattle were brought into estrus, using 15 mg estradiol benzoate, the uterus became enlarged and hard within 20 hours and overt estrus was apparent in 40 hours. Estrus lasted from 1 to 4 days.

Smaller doses of estradiol benzoate (5 mg) were sufficient to cause overt behavioural changes of estrus within 70 hours but these were accompanied by only slight uterine and cervical changes in the same spayed cows.

4.5.2 Uterine response to oxytocin

After spaying, the force of contraction of the uterus was greatly reduced; sometimes there were no contractions at all. In 3 spayed cows which initially did not show any activity of the uterus, after 15 mg of estradiol benzoate was given, and within 20 hours, the uterus began to display small contractions (Fig.15 b). Whenever the uterus

a.Cow X88167 9.2.79(11 days after spaying)



b.Cow X88167 10.2.79(20 hours after estrogen treatment)

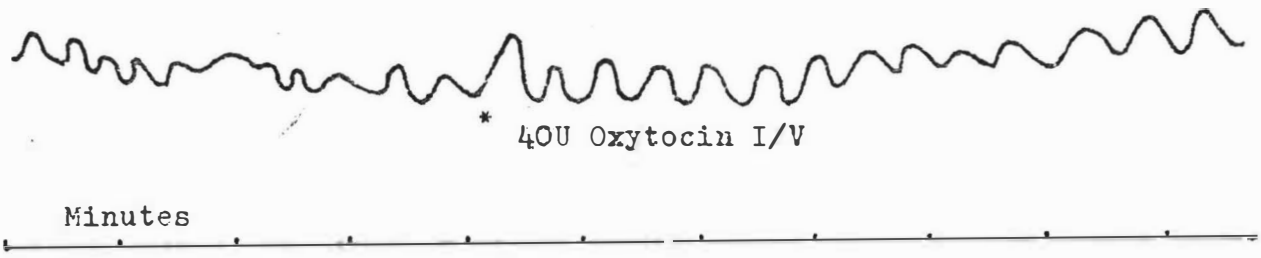
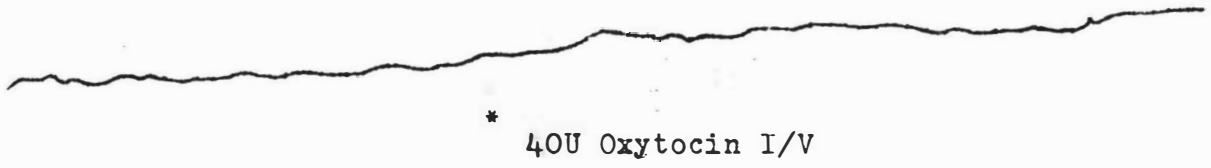
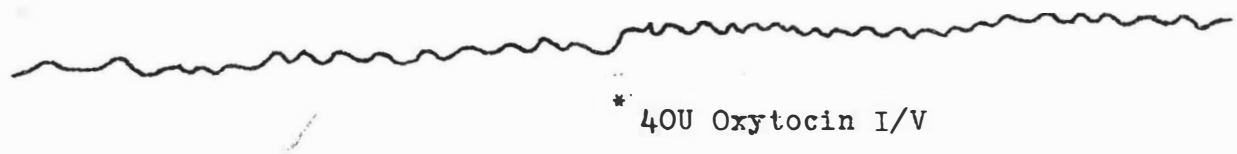


Fig.15.PATTERNS OF UTERINE CONTRACTION IN SPAYED COWS SHOWING ACTIVE CONTRACTIONS AND THE RESPONSE TO OXYTOCIN BEFORE(a) AND DURING(b) THE ACTION OF ESTROGEN.

a.Cow X88167 4.4.79(57 days after spaying)



b.Cow X88167 5.4.79(24 hours after estrogen treatment)



c.Cow X88167 9.4.79

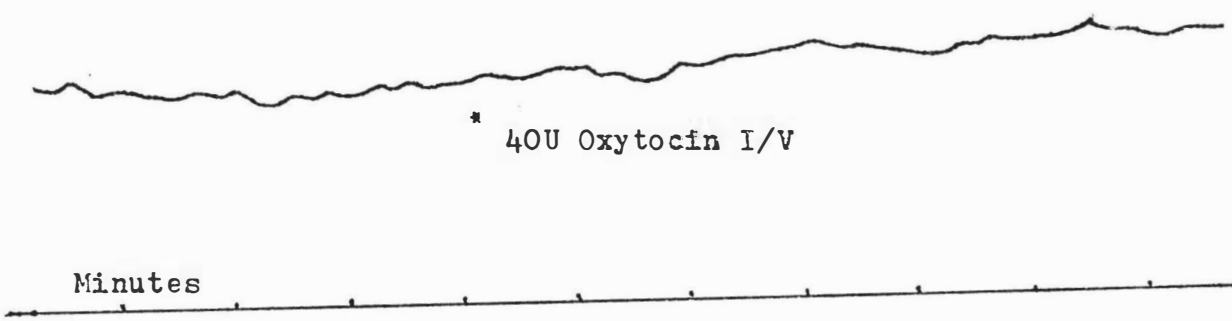


Fig.16. THE RESPONSE OF THE QUIESCENT UTERUS IN SPAYED COWS TO OXYTOCIN BEFORE, DURING AND AFTER THE ACTION OF ESTROGEN.

was active or was stimulated to activity by estrogen, it responded to oxytocin (Fig. 15 b). Sometimes the increased uterine activity subsided within 48 hours after estrogen treatment, even though at that time the animal might still be showing behavioural signs of estrus (appendix table II, IV, VII).

As the period after ovariectomy extended, so did the sensitivity of the uterus diminish to both estrogen (compare figures 15 a, and 16 a) and oxytocin (compare figures 15 b, and 16 b). In spayed cows where the uterus did show contractions, administration of both estrogen and oxytocin caused pronounced responses (Fig. 15 a & b).

4.6 DISCUSSION

The uterus and cervix of spayed cows became turgid within 20 hours after estrogen treatment, because estrogen produces vasodilation and increases the permeability of the endometrial capillaries (35). The full bulk of the uterus remained another day, and after that, the size reduced; this time-sequence of events agrees with that of Gardner *et. al.* (44), who found that the rat uterine wet weight increased 1 - 2 hours after estrogen stimulation and a second increase in wet weight occurred between 15 - 30 hours.

Hays and Vandemark (52) claimed that estrogen returned the decreased uterine activity of the spayed cow to normal. In this study, the estrogen did increase the uterine activity of the spayed cow but not to the level of that of a normal cow. The difference could be explained by the different schedule of estrogen administration; they used 5 mg stilbestrol daily for 3 days whereas in this study, estradiol benzoate 15 mg was used once only.

The fact that the quiescent uterus of spayed cows could be stimulated by estrogen, and the stimulated uterus subsequently became non-active even though the animal was still showing signs of estrus, is in conflict with some other work. In particular it does not seem to totally agree with results of Finn and Porter (35) who claimed that the myometrial activity in the rabbit is dependent upon the presence of estrogen to maintain actinomyocin concentrations in the uterine muscle cells, and to sustain the resting membrane potential and the electrical activity of the cell. A possible explanation might be that behavioural estrus is dependent upon a large number of factors in addition to estrogen and that the hormone might be only one part of a cascade reaction.

The trend showed by spayed cows towards a decrease in uterine activity with time, could be explained by a developing atrophy of the uterus. This in turn may result from the lack of exposure to regular priming doses of estrogen.

4.7 EFFECTS OF MILKING AND ADRENALINE ON UTERINE ACTIVITY

4.7.1 Milking

In 6 out of 12 experiments on 2 cows, the stimulus of milking slightly increased the frequency of uterine contraction (Fig. 17 a), but as soon as milking was stopped, the uterine activity returned to its original level.

4.7.2 Adrenaline

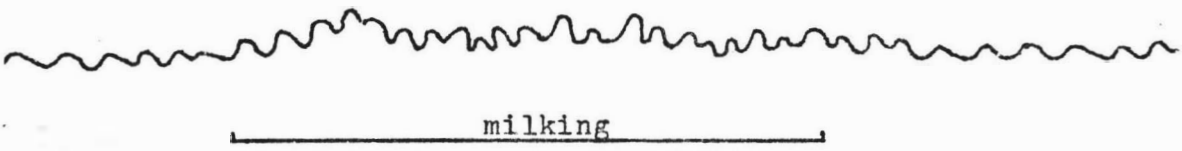
Over a series of 17 recordings, intravenously administered adrenaline increased the force or amplitude of the existing uterine contractions. The stimulus lasted for 1 - 3 contractions after which there was inhibition for 2 - 4 minutes (Fig. 17 b) before the original contraction pattern reappeared.

4.8 DISCUSSION

A number of workers (47, 51, 71, 97) have claimed that, either suckling or the milking procedure causes an increase in the production of oxytocin, which in turn stimulates uterine activity. The first point has been confirmed by Barowicz and Ewy (5) who found that the oxytocin activity in the blood plasma of 10 cows increased during machine milking. The highest concentration (236.29± 60.89) was reached one minute after application of the teat cups.

Evidence against the above view was found by Adair and Davis (1) who suggested that nursing had no effect on uterine contractions, as did half of the observations made during milking in this study. The discrepancy might be explained by either the difference in the degree of stimulus at nursing or milking, or perhaps by the stage of the estrous cycle, in particular if the time coincided with the short period of uterine quiescence.

a.Cow D41



b.Cow D41

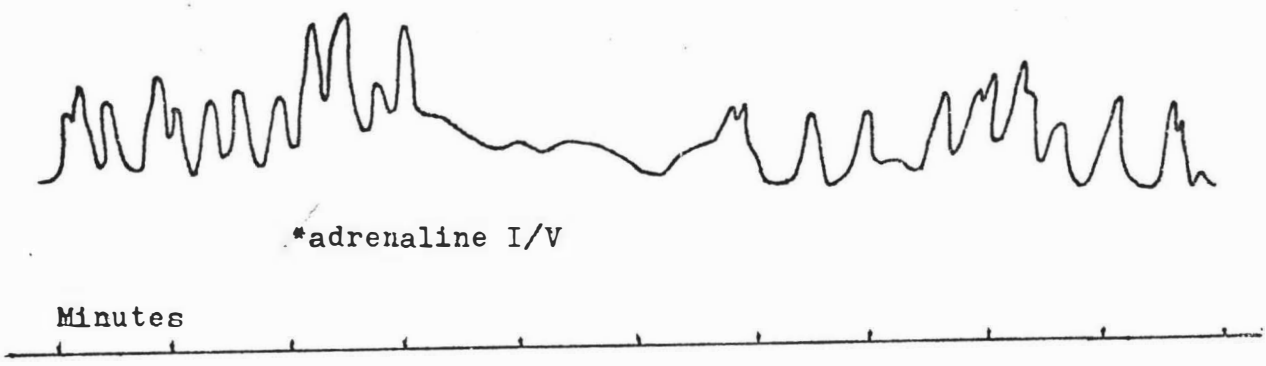


Fig.17.CHANGES IN THE PATTERN OF UTERINE CONTRACTION DUE TO THE EFFECTS OF MILKING(a) AND ADRENALINE(b).

In the present work reported in this thesis, as soon as milking was stopped, the increased uterine acitvity returned to its original level whereas had this stimulation been due to oxytocin alone, one would have expected a more extended action, because oxytocin injected into a cow exhibiting uterine contractions, causes a stimulation lasting for many hours (Chap. 4.3.3).

The possibility then must be considered that the milking operation causes a nerve-mediated stimulus of the uterine musculature, and it is this, rather that the oxytocin, which has a major effect on uterine concentrations during milking or nursing. Obviously the matter requires far more investigation.

The effect of adrenaline on uterine activity in this study was similar to the findings of Hays and Vandemark (51), Fitzpatrick (38) and Zerobin and Sporri (99), i.e. that the action of adrenaline on uterine musculature contractions, is biphasic; commencing with a brief stimulation and being followed by a slightly longer inhibition phase. Hays (35) showed that the same uterine response to adrenaline could be demonstrated using either *in vivo* or *in vitro* methods.

Cupps' (51) findings were different. From his *in vitro* experiments, he claimed that adrenaline is inhibitory to uterine motility during the estrogenic phase and stimulatory during the phase of progesterone influence. The different interpretation might be explained by the difference in technique, in that Cupps used strips of uterine muscle removed from cows at different stages of the estrous cycle. Such *in vitro* methods fail to take into account the continuing influence of endogenous hormones and therefore the *in vivo* studies reported in this thesis and by others (38, 51, 99) are more likely to be valid.

5. GENERAL DISCUSSION

A major finding arising from this investigation using the new apparatus developed, is a more relevant understanding of what contributes to uterine pressure (UP). The pressure exerted by the contraction of the uterus, has been called intra-uterine pressure (IUP) in the past (4, 26, 35, 80, 91, 99). From the finding in this study it seems more appropriate to call it UP rather than IUP, because the pressure measured was not transfered by air retained in the lumen of the uterus. Rather the pressure recorded was due to the contraction of the uterine wall directly on the sensor diaphragm of the recording tube (see 4.1.5). The uterus has potential interior space as a hollow organ but normally in the nongravid state, the uterine walls are in apposition.

The UP recorded in this study is much higher than the IUP reported by other workers because UP consists of any uterine contraction superimposed upon the pressure of uterine tonicity, whereas the latter has not been considered by other workers in their IUP. If uterine tonicity as a force is discounted, then the uterine contraction pressures recorded in this study and the IUP of others, fall in the same range. The tonicity pressure of the uterus is measurable (page 42); it should be taken into account and therefore the IUP figures put forward by others previously, may need to be reinterpreted in different terms.

The apparatus developed for this work consisted of an artificial insemination tube and a rubber sensor diaphragm. Because the same tube was used time and again the elasticity or the resistance factor of the rubber diaphragm was tested *in vitro* before each experiment, and it was found that in 14 out of 110 cases the resistance factor had changed from its previous value. These elasticity changes certainly would apply to other workers' inflated balloons but no mention was made in their publications of repeatedly checking the resistance factors. Under such conditions, though the amplitude of uterine contractions shown on the tracing paper in different experiments might be of the same height, there is no guarantee that the uterine contraction pressure to cause this, is necessarily the same.

For the purposes of recordings made in the present investigation , the recording tube described in Chapter 3.1.2, was easily managed and proved to be a sensitive yet robust instrument. The very latest equipment which consists of a microtransducer mounted on a flexible lead, may lend itself particularly well to semi-permanent implantation in the uterine wall, and recordings may be obtainable from a single cow during the non-pregnant period as well as throughout a subsequent pregnancy.

Patterns of spontaneous uterine activity have been found to vary between days and between cows, due to the changes in the reproductive cycle and possibly to the range of simple biological variation between individuals. Though the uterus of cattle produces contractions almost all the time throughout the estrous cycle, there are times which range from the 8th to the 17th day post-estrus, when the uterus is nonactive for 1 to 2 days. One possible explanation for this quiescent period could be the fluctuation of estrogen blood levels after estrus, although no measurements of plasma hormones were made during this study. Generally, the uterus contracts more forcefully during estrus, and at that time the musculature is more responsive to oxytocin.

Without the hormonal influence derived from the ovaries, the uterine activity of spayed cattle is much reduced and some exhibit no contraction at all. As the period after ovariectomy extended, the force of uterine activity became weaker and later quiescent. It seems as if the autonomic nervous system must play an important role in maintaining any spontaneous uterine activity after the ovaries are removed and the quiescent state of the uterus might be due to the development of uterine atrophy. Further physiological and morphological studies are required to establish whether such changes occur in the spayed cow.

The uterus of non-pregnant cattle is contracting at a rate ranging from 0 to 22 times per 10 minutes. These recorded contractions are not influenced by local irritation from the recording tube, sudden changes in the abdominal pressure due to coughing, bellowing or urination, nor are they modified by external disturbances such as noise of a near-by tractor. There-

fore it is understood that the body movements of the animal and external disturbances appear to have no lasting effect on the normal rhthymic contractions of the uterus.

Immediately after defecation or urination, there is a transient fall in uterine tonicity pressure, presumably due to the reduction in the abdominal pressure. During the act of elimination, the abdominal muscle contracts and later relaxes before regaining tone and reestablishing the equilibrium.

Provided the uterus was exhibiting contractions, these were always enhanced following the intravenous administration of oxytocin. Either intramuscular or epidural routes of administration were largely successful but the epidural route produced the more consistent results probably because the oxytocin was directly exposed to a better blood supply in the vertebral canal than in the muscle. The epidural route of drug administration is not without risk, but in the case of oxytocin, it is practicable as a second choice after the intravenous route.

There appeared to be little advantage in giving any more than 20U oxytocin, as larger doses repeated afterwards did not further improve the amplitude of individual contractions in normal cows. The sensitivity of the uterine musculature to oxytocin about the time of parturition may be totally different to that of the non-pregnant cow undergoing regular estrous cycles and as such it would be unwise to extrapolate findings from this study.

A surprising finding in this study was the extended duration of oxytocin effect following administration. On one occasion when recording was continuous the response to oxytocin lasted for more than 9 hours, and on numerous other occasions, when the animals were allowed a rest period between recordings, the effect was maintained for at least 1 and sometimes to 3½ hours. In view of the short plasma half-life of oxytocin which has been reported to be less than 10 minutes (49), it would seem that the hormone may act at its receptor like an off/on switch and that continuing increased activity is independent of the plasma oxytocin concentration.

In order to more closely define the timing of the estrous cycle, free from the influence of endogenous hormones on uterine contractions, spayed cattle were utilised and brought into artificial estrus by the use of estrogen. At that time, both the uterus and cervix enlarged and felt firm on palpation, and uterine contractions were apparent. Though the period of artificial estrus, in terms of behavioural changes, lasted from 1 to 4 days, on certain occasions the increased uterine activity persisted for 24 hours only. It is assumed that slightly different and additional mechanisms are responsible for each of these two phenomena of estrus.

The investigation has shown that the normal contracting uterus in the non-pregnant cow is always responsive to intravenous oxytocin. The range of the duration of this effect is surprisingly wide. Whether the variation is associated with a characteristic of the drug or is due to other endogenous influences remain to be determined.

The writer's study and findings raised a number of interesting issues which were beyond the scope of this investigation. For example, both milking in the normal cow and estrogen treatment of spayed cows, caused an initial response in uterine contractions, but activity rapidly returned to pretreatment levels. This raises the key question as to what extent uterine activity is due to hormone influences and what part is played by autonomic nervous control. The balance of these two influences in controlling of uterine activity in the non-pregnant cow; together with complementary studies undertaken about the time of parturition, are aspects of uterine physiology which must be left to other investigators.

REFERENCES

1. Adair,F.L. and Davis,M.E.(1934):Astudy of human uterine motility. Am.J.Obstet.Gynec.,27:383-394.

2. Anderson,J.N.,Peck Jr.E.J. and Clark,J.H.(1973):Nuclear receptor-estrogen complex. Endocrinology,92:1488-1495.

3. Anderson,J.N.,Peck Jr.E.J. and Clark,J.H.(1974):Estrogen-induced uterine response and growth. Edocrinology,96:160-167.

4. Bangham,D.H.,Cotes,P.M. and Parson,J.A.(1966):Measurement of intra-uterine pressure changes in the pregnant rhesus monkey by telemetric recording from pressure sensitive capsules inserted in the uterus. Mem.Soc.Endocr.,14:249-251.

5. Barowicz,T. and Ewy,Z.(1973):Oxytocic activity measurement in blood plasma with some observations on oxytocic disappearance rates in lactating cows. Bulletin de l'academic polonaise des sciences,21(9):615-618.

6. Beck,N.F.G.,Carter,M.C.,Jansen,C.A.M.,Joyce,P.L.,Krane,E.J., Nathanielsz,P.W.,Steer,P. and Thomas,A.L.(1977):A method for the quantitative assessment of uterine activity in the pregnant sheep. J.Physiol.,271:9p-10p.

7. Behrman,S.J.,Archie,J.T. and O'Brien,O.(1969):Myometrial activity and the IUCD:II propagation waves. Am.J.Obstet. Gynec.,104:123-129.

8. Bell,G.H.(1941):The movement of the unloaded uterus. J.Physiol.,99:352-363.

9. Bengtsson,L.Ph.(1957):The endocrine control of myometrial contractility in the uterus of the pregnant rabbit. Am.J.Obstet.Gynec.,74:484-493.

10. Bengtsson,L.Ph.(1968):The sponge-tipped catheter. J.Reprod.Fert.,16:115-118.

11. Berg,S.L. and Ginther,O.J.(1978):Effects of estrogens on uterine tone and life span of the corpus luteum in mares. J.Anim.Sci.,47(1):203-208.

12. Best,F.A. and Pickles,V.R.(1962):A myometrial effect of estradiol,initiated by high Mg.concentration. Proceedings of the Physiological Society,14-15 Decemb.,12p-13p.

13. Blank,M.S. and DeBias,D.A.(1977):Oxytocin release during vaginal distension in the goat.Biol.Reprod.,17:213-223.

14. Bonnycastle,D.D. and Ferguson,J.K.W.(1941):The action of pitocin and adrenaline on different segments of the rabbit uterus. J.Pharmac.Exp.Ther.,72:90-98.

15. Bourne,A. and Burn,J.H.(1927):The dosage and action of the pituitary extract and of the ergot alkaloids on the uterus in labour,with a note on the action of adrenaline. J.Obstet.Gynec.Br.Emp.,34:249-272.

16. Braaksma,J.T.,Janssens,J.,Eskes,T.,Arp,A. and Hein,P.R.(1970): Accurate pressure recording in the non-pregnant human uterus. Gynec.Invest.,1:288-302.

17. Brander,G.C. and Pugh,D.M.(1977):Veterinary applied pharmacology and therapeutics,3rd.ed.Balliere,Tindall &Cox,London.

18. Brown,W.E. and Wilder,V.M.(1943):The response of the human uterus to epinephrine. Am.J.Obstet.Gynec.,45:659-665.

19. Caldeyro,R.,Alvarez,H. and Reynolds,S.R.M.(1950):A better understanding of uterine contractility through simultaneous recording with an internal and a seven channel external method. Surg.Gynec.Obstet.,91:641-650.

20. Caldeyro,R. and Poseiro,J.J.(1957):Oxytocin and contractility of the pregnant human uterus.Ann.N.Y.Acad.Sc.,75:813-829.

21. Capraro,D.L.,Vernon,M.W.,Abrams,R.M. and Sharp,D.C.(1976): Effects of oxytocin and PGF_2 on mare uterine motility. J.Anim.Sci.,43(1):277.

22. Cibils,L.A.(1967):Contractility of the non-pregnant human uterus. Obstet.Gynec.,30:441-461.

23. Csapo,A.I. and Corner,G.W.(1952):The antagonistic effect of estrogen and progestrone on the staircase phenomenon in the uterine muscle. Endocrinology,51:378-385.

24. Csapo,A.I. and Corner,G.W.(1953):The effect of estrogen on isometric tension of rabbit uterine strips.Science,117:162-164.

25. Csapo,A.I.(1963):Model experiments and clinical trials in the controls of pregnancy and parturition. Am.J.Obstet.Gynec.,85:359-379.

26. Csapo,A.I.(1970a):The diagnostic significance of the intra-uterine pressure,part 1.Obstet.Gynec.Surv.,25:403-435.

27. Csapo,A.I.(1970b):The diagnostic significance of the intra-uterine pressure,part 2.Obstet.Gynec.Surv.,25:515-543.

28. Coutinho,E.M.(1967):Uterine activity in non-pregnant woman. Eight Int.Conf.I.P.P.F.Santiago,Chile,432-439.

29. Cross,B.A.(1957):The motility and reactivity of the estroginized rabbit uterus in vivo with comparative observations on milk ejection.J.Endocr.,16:237-260.

30. Cupps,P.T. and Asdell,S.A.(1944):Changes in the physiology of the uterine muscle of the cow in relation to estrous cycle. J.Anim.Sci.,3:351-359.

31. Dellmann,H.D. and Brown,E.M.(1976);Text book of veterinary histology,Lea & Febiger Philad.,336-345.

32. El-Banna,A.A. and Hafez,E.S.E.(1972):The uterine cervix in mammals. Am.J.Obstet.Gynec.,112:145-169.

33. Evans,E.I. and Miller,F.W(1936):Uterine motility in cow. Am.J.Physiol.,116:44-45.

34. Fedorov,A.I.(1973):Effect of subcutaneous or epidural injections of pituitrin or oxytocin on uterine contractions of cows.Leningradskii Veterinarnyi Institut,34:311-314.

35. Finn,C.A. and Porter,D.G.(1975):The uterus.Paul Elek Ltd., London.

36. Fitzpatrick,R.J.(1951):The activity and the pharmacological reactivity of the cervix and body of the uterus in ruminants. Proc.R.Soc.Med.,44:870-871.

37. Fitzpatrick,R.J.(1957):On oxytocin and uterine function. The neurohypophysis,Butterworths,London,203-220.

38. Fitzpatrick,R.J.(1957):Uterine response to adrenaline in intact cows and sheep. J.comp.Path.,67:223-238.

39. Fitzpatrick,R.J.(1957):The activity of the uterine cervix in ruminants. Vet,Rec.,69:713-717.

40. Fitzpatrick,R.J.(1958):The response of the cervix uteri of ruminants to adrenaline.J.comp.Path.,68:219-231.

41. Fitzpatrick,R.J.(1960):The reactivity of the ruminant uterus to posterior pituitary hormones.J.comp.Path.,70:36-58.

42. Frandson,R.D.(1970):Anatomy and physiology of farm animals. Lea & Febiger,Philad.,328-331.

43. Fuchs,F. and klopper,A.(1971):Endocrinology of pregnancy. Harper & Row Pub.N.Y.,247-292.

44. Gardner,R.M.,Kirkland,J.L. and Stancel,G.M.(1978):Selective blockade of estrogin-induced uterine response by the anti-estrogen nafoxidine. Endocrinology,103(5):1583-1589.

45. Gier,H.T. and Marion,G.B.(1968):Uterus of the cow after parturition:involution changes.Am.J.vet.Res.,29:83-91.

46. Gillette,D.D. and Holm,L.(1963):Prepartum to postpartum uterine and abdominal contractions in cows. Am.J.Physiol.,204:1115-1121.

47. Gillette,D.D.(1966):Placenta influence on uterine activity in the cow. Am.J.Physiol.,211:1095-1098.

48. Ginther,O.J.(1968):Utero-ovarian relationship in cattle: Physiological aspects.J.Am.vet.med.Ass.,153:1656-1664.

49. Goodman,L.S. and Gilman,A.(1965):The pharmacological basis of therapeutics,4th ed.The Macmillan Co.,London,894-897.

50. Harris,J.S. and Gillespie,E.C.(1950):Development of methods for studying human uterine contractions in pregnancy. Obstet.Gynec.Surv.,5:1-14.

51. Hays,R.L. and Vandemark,N.L.(1953):Effects of oxytocin and epinephrine on uterine motility in the bovine. Am.J.Physiol.,172:557-560.

52. Hays,R.L. and Vandemark,N.L.(1953):Spontaneous motility of bovine uterus.Am.J.Physiol.,172:553-556.

53. Hawk,H.W.,Conley,H.H. and Bolt,D.J.(1977):Comparison of *in vivo* and *in vitro* uterine contractility in the ewe. Theriogenology,7(4):215-223.

54. Henry,J.S. and Browne,J.S.L.(1943):Contractions of human uterus during menstrual cycle:effect of progestrone and posterior pituitary extract upon motility of human uterus. Am.J.Obstet.Gynec.,45:927-949.

55. Hindson,J.C.,Schofield,B.M. and Turner,C.B.(1976):Effect of a single dose of stilbestrol on cervical dilation in sheep.Res.vet.Sci.,8:353-360.

56. Johnson,W.L.,Ek,T.W. and Brewer,L.L.(1966):Motility of human uterus before and after insertion of an intra-uterine device. Obstet.Gynec.,28:526-527.

57. Jones,D.E. and Knifton,A.(1975):Spontaneous and oxytocin induced uterine motility during the estrous cycle in goats. Res.vet.Sci.,19:131-134.

58. Jones,L.M.(1968):Veterinary pharmacology and therapeutics, 3rd ed.Iowa State University Press,U.S.A.,575-766.

59. Kelly,J.V.(1961):The effect of intrvenous estrogen on uterine motility. Am.J.Obstet.Gynec.,82:1207-1210.

60. Kelly,J.V. and Schleifer,O.(1962):A new method for assessment of uterine contractions.Surg.Gynec.Obstet.,114:389-392.

61. Krantz,K.E.(1959):Innervation of the human uterus. Ann.N.Y.Acad.Sc.,75:770-780.

62. Kroc,R.L.,Steinetz,B.G. and Beach,V.L.(1959):The effects of estrogens,progestagens and relaxin in pregnant and non-pregnant laboratory rodents.Ann.N.Y.Acad.Sc.,75:942-979.

63. Lehner,A.R. and Schindler,H.(1974):Uterine motility of ewe during the estrous cycle.J.Anim.Sci.,38(1):86-88.

64. Lima,J.R. and Montenegro,C.B.A.(1972):Tocometry in obstetrics practice:the development of a pneumatic system. Am.J.Obstet.Gynec.,112:304-307.

65. Mann,L.I.(1969):Effect of IUCD on uterine motility in the ewe. Fert.Steril.,20:951-958.

66. McSporran,K.D.(1975):Some aspects of dystocia in sheep with particular reference to Romney stud ewes. Thesis,Ph.D.,Massey University,N.Z.

67. Michael,D.K. and Reinke,D.A.(1970):Rabbit uterine contractile activity in the presence of an intra-uterine device. Am.J.Obstet.Gynec.,107:188-193.

68. Milvae,R.A.,Hixon,J.E. and Hansel,W.(1976):Progestrone and LH in oxytocin treated heifers.J.Anim.Sci.,43:297.

69. Mitchell,M.D.,Flint,A.P.F. and Turnbull,A.C.(1977):Increasing uterine response to vaginal distension during late pregnancy in sheep. J.Reprod.Fert.,49:35-40.

70. Moawad,A.H. and Bengtsson,L.P.(1968):In vivo studies of the motility patterns of the non-pregnant human uterus:II the effect of estrogen on myometrial activity. Acta.Obstet.Gynec.,47:225-232.

71. Moir,C.(1933):Recording the contractions of the human pregnant and non-pregnant uterus.Trans.Edinb.Obstet.Soc.,93-120.

72. Momogan,V.G. and Schmidt,G.H.(1969):Oxytocin levels in the plasma of Holstein-Friesian cows during milking with and without a permitting stimulus.J.Dairy Sci.,53(6):747-751.

73. Ngiam,T.T.(1977):A study of the motility of the uterus of the sow during the periparturient period. Singapore vet.J.,1:13-27.

74. Nixon,W.C.W. and Smyth,C.N.(1957):Physiological and clinical aspects of uterine action.J.Obstet.Gynec.Br.Comw.,64:35-46.

75. Parkas,V. and Anderson,R.R.(1971):Pituitary and blood plasma oxytocic activity and oxytocin disappearance rates in cattle. J.Dairy Sci.,55(1):75-79.

76. Pinto,R.L.,Lerner,U.,Mazzocco,N. and Glauberman,N.(1966): The oxytocic action of intra-amniotic estradiol-17β on the pregnant human uterus.Am.J.Obstet.Gynec.,94:876-880.

77. Randy Jr.H.A. and Graf,G.C.(1972):Factors affecting endogenous plasma oxytocic activity in lactating Holstein cows. J.Dairy Sci.,56(4):446-450.

78. Rees,H.G.(1967):Some effects of oxytocin on in vitro preparations of uterine muscle.Thesis,M.V.Sc.,Massey University,N.Z.

79. Reid,C.W.S.,Melville,A.W. and Cornwall(1959):A technique for recording pressure changes in the reticulo-rumen of cattle using small electrical pressure transducers and a four channel recorder. N.Z.J.agri.Res.,3:41-62.

80. Reynolds,S.R.M.(1965):Physiology of the uterus. Hafer Pub.Co.,N.Y.,463-476.

81. Rowson,L.E.A.,McNelly,A. and O'Brien,C.A.(1972):The effect of vaginal and cervical stimulation on oxytocin release during the luteal phase of the cow's estrous cycle. J.Reprod.Fert.,30:287-288.

82. Ruckebusch,Y. and Bayard,F.(1975):Motility of the oviduct and uterus of the cow during the estrous cycle. J.Reprod.Fert.,43:23-32.

83. Salerno,J.P.(1938):Observation on intra-uterine pressure during first stage of labour.Am.J.Obstet.Gynec.,36:294-302.

84. Sharma,S.C.,Fitzpatrick,R.J. and Ward,W.R.(1972):Coital induced release of oxytocin in the ram.J.Reprod.Fert.,31(3): 488-489.

85. Sharma,O.P.(1974):Release of oxytocin elicited by suckling stimulus in mares. J.Reprod.Fert.,37:421-423.

86. Shemesh,M.,Ayalon,N. and Linder,H.R.(1972):Estradiol levels in the peripheral blood of cows during the estrous cycle. J.Endocr.,55:73-78.

87. Sibaja,R.A. and Schmidt,G.H.(1974):Epinephrine inhibition milk ejection in lactating cows.J.Dairy Sci.,58(3):344-348.

88. Sisson,S. and Grossman,J.D.(1975):The anatomy of the domestic animals,5th ed.vol.1.W.B.Saunders Co.,Philad.,946-955.

89. Smyth,C.N.(1957):The guard-ring tocodynamometer. J.Obstet.Gynec.Br.Comw.,64:59-66.

90. Smyth,C.N. and Wolff,H.S.(1960):Application of endoradiosonde or wireless pill to recording of uterus contraction and foetal heart sounds. Lancet,2:412-413.

91. Stander,R.W.(1966):An approach to quantitative analysis of intra-uterine pressure data.Obstet.Gynec.,27:110-115.

92. Swen,M.J.(1975):Dukes' physiology of domestic animals,8th ed. Cornell University Press,London,1189-1356.

93. Taketa,H.(1965):Generation and propagation of uterine activity in situ.Fert.Steril.,16:113-119.

94. Theobald,G.W.(1973):Endocrine control of uterine innervation. Butterworths & Co.,London,23-31.

95. Thind,P.S.and Gangwar,P.C.(1974):The effect of oxytocin on let down time and composition of milk and hard-to-milk buffaloes. Indian vet.J.,51(1):76.

96. Vandemark,N.L. and Hays,R.L.(1952):Uterine motility responses to mating. Am.J.Physiol.,170:518-521.

97. Venable,J.H. and McDonald,L.E.(1958):Postparturient bovine uterine motility:normal and experimentally produced retention of the foetal membrane.Am.J.vet.Res.,19:308-313.

98. Verma,O.P. and Singh,L.P.(1976):Estrogen- and progestrone-induced uterine motility in anestrous goats. Am.J.vet.Res.,37(2):233-235.

99. Zerobin,K.,and Sporri,H.(1972):Motility of bovine and porcine uterus and fallopian tube.Adv.vet.Sc.comp.Med.,16:303-332.

APPENDIX

No.	Date	Estrous cycle	Initial activity	Injections	Uterine activity after treatment: Frequency	Amplitude	Recording period Minutes	Other injection	Remark
1	6.11.78	Estrus	Uterus +	SC 40U Oxy.	I	I	-	-	Estrus on 5th.
2	9.11.78	-	Uterus +	SC 40U Oxy.	D	I	-	-	Stronger contr.
3	16.11.78	-	Uterus 0	IV 40U Oxy.	-	-	61	-	
4	21.11.78	-	Uterus +	IV 40U Oxy.	I	I	157	-	Stronger contr.
5	23.11.78	-	Uterus +	IV 40U Oxy.	I	-	80	-	Tractor noise
6	27.11.78	-	Uterus 0 Cervix +	IV 40U Oxy.	-	-	141	-	
7	13.12.78	-	Uterus +	IV 80U Oxy.	I	I	105	IM 40U Oxy.	IM no action
8	15.12.78	-	Uterus +	EP 80U Oxy.	I	I	147		
9	22.12.78	-	Uterus +	IM 80U Oxy.	-	I	93		
10	27.12.78	-	Uterus +	IM 0.75ml. Rom	I	-			Stimulate
11	31.12.78	-	Cervix +	IM 60U Oxy.	-	-			No action
12	12. 1.79	-	Uterus + Cervix +	IV 3ml. Adr. IV 20U Oxy.	- I	- I			Estrus on 11th. Both responded
13	15. 1.79	-	Uterus +	IM 40U Oxy.	-	I	124	IV 40U Oxy.	No fatigue
14	18. 1.79	-	Uterus +	EP 40U Oxy.	-	I	91		Latency 17'

APPENDIX I. EXPERIMENTS ON COW 350

(Key: 0 = no activity, + = contracting, I = increase, D = decrease, IV = intravenous, EP = epidural, Oxy. = Oxytocin, IM = intramuscular)

No.	Date	Estrous cycle	Initial activity	Injections	Uterine activity after treatment		Recording period Minutes	Other injection	Remark
					Frequency	Amplitude			
15	19. 2.79	Spayed	Uterus +	IM 0.75ml.Rom	I	I	-	IV 40U Oxy	Both responded
16	20. 2.79	-	Uterus +	IV 40U Oxy.	D	I	121	IM 5mg.Est	Cervix rigid
17	21. 2.79	-	Uterus +	IV 10,20,30, 40U Oxy.	D	I			20U optimal
18	22. 2.79	-	Uterus +	IV 3ml.Adr. IV 40U Oxy.	- D	- I	131		Responded Cervix rigid
19	27. 2.79	Estrus	Uterus + Cervix +	IM 40U Oxy.	I	-		IM 15mg.Est on 25th.	Uterus enlarged Cervix rigid
20	28. 2.79	Estrus	Uterus +	IM 40U Oxy. IV 40U Oxy.	- I	- I	215		IM no action
21	1. 3.79	Estrus	Uterus +	EP 40U Oxy.	I	I	242		Recorded 5 hrs.
22	3. 3.79	Estrus	Uterus +	IM 40U Oxy.	-	I	124		Cervix dilated
23	4. 3.79	-	Uterus +	EP 40U Oxy.	D	I	112	IV 40U Oxy.	Both responded
24	7. 3.79	-	Uterus +	IV 40U Oxy.	I	I	120		Cervix dilated
25	9. 3.79	-	Uterus +	IV 40U Oxy.	I	I	146		Cervix small
26	14. 3.79	-	Uterus +	IV 5,10,20U Oxy.	I	I			10U Optimal
27	19. 3.79	-	Uterus +	IV 40U Oxy.	-	I	50		

APPENDIX II. EXPERIMENTS ON COW 350 (SPAYED ON 29.1.79)

(Key: 0 = no activity, + = contracting, I = increase, D = decrease, IV = intravenous, EP = epidural Est. = estradiol benzoate, Oxy. = Oxytocin, IM= intramuscular)

No.	Date	Estrous cycle	Initial activity	Injections	Uterine activity after treatment		Recording period Minutes	Other injection	Remark
					Frequency	Amplitude			
1	18.12.78	-	Uterus +	EP 80U Oxy.	I	-	90	-	Prostaglandin on16
2	20.12.78	Estrus	Uterus +	EP 40U Oxy.	I	I	114		Estrus on 19 p.M.
3	21.12.78	Estrus	Uterus +	IV 40U Oxy.	I	I	180		
4	22.12.78	-	Uterus +	IV 10,20,40U Oxy.	I	I			No stimulation after 10U
5	24.12.78	-	Uterus +	IV 10,20,40, 60U Oxy.	-	I			No stimulation after 20U
6	26.12.78	-	Uterus +	IV 10,20,40U Oxy.	D	I			10U optimal
7	29.12.78	-	Uterus + Cervix +	IV 50U Oxy.					Both responded
8	4. 1.79	-	Cervix 0	IM 70U Oxy.					No response
9	8. 1.79	-	Uterus +	IM 80U Oxy.	-	I	180		Strong contract.
10	9. 1.79	Estrus	Uterus + Cervix +	IV 3ml.Adr. IV 40U Oxy.					Both responded
11	10. 1.79	-	Uterus +	IM 0.75ml.Rom	-	I			Oxytocic effect
12	17. 1.79	-	Uterus 0	EP 40U Oxy.				IM 40U Oxy.	No response

APPENDIX III. EXPERIMENTS ON COW D41

(Key: 0 = no activity, + = contracting, I = increase, D = decrease, IV = intravenous, EP = epidural, Oxy. = Oxytocin, IM = intramuscular)

No.	Date	Estrous cycle	Initial activity	Injections	Uterine activity after treatment		Recording period Minutes	Other injection	Remark
					Frequency	Amplitude			
13	5. 3.79	Spayed	Uterus 0	IM 0.75ml.Rom.	-	I			
14	6. 3.79	-	Uterus 0	IV 5,10,20, 40U Oxy.	-	I		IM 5mg.Est	
15	7. 3.79	-	Uterus +	IV 40U Oxy.	I	I	122		
16	8. 3.79	-	Uterus 0	IV 40U Oxy.					No response
17	9. 3.79	Estrus	Uterus 0	IV 3ml.Adr.				IV 40U Oxy	No response
18	11. 3.79	-	Uterus 0	IV 40U Oxy.					No response
19	12. 3.79	-	Uterus 0	IV 40U Oxy.				IM 10mg.Est.	
20	14. 3.79	-	Uterus +	IV 40U Oxy.	I	I	144		
21	15. 3.79	-	Uterus +	IM 40U Oxy.	I	I	93		
22	16. 3.79	Estrus	Uterus +	EP 40U Oxy.	I	I	125		
23	17. 3.79	-	Uterus +	IV 5,10,20, 40U Oxy.	I	I			No stimulation after 10U
24	19. 3.79	-	Uterus +	IM 40U Oxy.	I	I	114		Giving milk
25	20. 3.79	-	Uterus +	IV 40U Oxy.	I	I	137		Giving milk
26	21. 3.79	-	Uterus +	IV 40U Oxy.	I	I	120		Giving milk
27	23. 3.79	-	Uterus + Cervix 0	IV 3ml.Adr. IV 40U Oxy.					Both no response

APPENDIX IV. EXPERIMENTS ON COW D41 (SPAYED ON 29.1.79)

(Key: 0 = no activity, + = contracting, I = increase, IV = intravenous, EP = epidural, IM = intramuscular
Est. = estradiol benzoate, Oxy. = oxytocin)

No.	Date	Estrous cycle	Initial activity	Injections	Uterine activity after treatment		Recording period Minutes	Other injection	Remark
					Frequency	Amplitude			
1	30. 1.79	Spayed	Uterus +	IV 40U Oxy.	I	I	87		Spayed on 29.1.79
2	9. 2.79	-	Uterus +	IV 3ml.Adr. IV 10,20,40U Oxy.	I	I		IM 15mg Est	No stimulation after 20U
3	10. 2.79	-	Uterus +	IV 40U Oxy.	I	I	71	IV 40U Oxy.	No fatigue
4	11. 2.79	-	Uterus +	IV 40U Oxy.	I	I	61	IM 40U Oxy.	No fatigue
5	12. 2.79	Estrus	Uterus +	IM 40U Oxy.	I	I	61	IV 40U Oxy.	No fatigue
6	13. 2.79	Estrus	Uterus 0.	EP 40U Oxy.				IV 40U Oxy.	EP no action IV very slight
7	14. 2.79	Estrus	Uterus 0	IV 40U Oxy.				IV 40U Oxy.	Both very slight
8	15. 2.79	Estrus	Uterus 0	IV 10,20U Oxy.					Very slight
9	16. 2.79	-	Uterus 0	EP 40U Oxy.	-	-			Very slight
10	4. 2.79	-	Uterus 0	IV 40U Oxy.				IM 15mgEst	No response
11	5. 2.79	-	Uterus +	IV 40U Oxy.	I	I			Slight response
12	9. 2.79	Estrus	Uterus 0	IV 40U Oxy.					No response
13	10. 2.79	Estrus	Uterus 0	IV 40U Oxy.					No response
14	11. 2.79	-	Uterus 0	IV 40U Oxy.					No response

APPENDIX V. EXPERIMENTS ON COW X88167 (SPAYED ON 29.1.79)

(Key: 0 = no activity, + = contracting, I = increase, D = decrease, IV = intravenous, EP = epidural, IM = intramuscular, Est. = estradiol benzoate, Oxy. = Oxytocin)

No.	Date	Estrous cycle	Initial activity	Injections	Uterine activity after treatment		Recording period Minutes	Other injection	Remark
					Frequency	Amplitude			
1	1.2.79	Spayed	Uterus +	IV 40U Oxy.	I	I	-	IM 20mg.Est	
2	2.2.79	-	Uterus +	IV 40U Oxy.	-	I	130		Uterus enlarged Cervix dilated
3	3.2.79	Estrus	Uterus +	IV 10,20,40U Oxy.	I	I			No stimulation after 10U
4	4.2.79	Estrus	Uterus +	IM 40U Oxy.	I	I	133		Latency 4'
5	5.2.79	Estrus	Uterus +	IM 40U Oxy.	I	I	80		Latency 4'
6	6.2.79	-	Uterus +	EP 40U Oxy.	I	I	105		Latency 3'
7	8.2.79	-	Uterus +	EP 40U Oxy.	-	I	105		Latency 11'
8	12 .2.79	-	Uterus +	IM 0.85ml.Kom.	I	-		IM 40U Oxy.	IM no response
9	15 .2.79	-	Uterus 0 Cervix 0	IV 40U Oxy.					No response

APPENDIX VI. EXPERIMENTS ON COW B2 (SPAYED ON 26.11.78)

(Key: 0 = no activity, + = contracting, I = increase, IV= intravenous, EP = epidural, IM = intramuscular, Est. = estradiol benzoate, Oxy. = Oxytocin)

No.	Date	Estrous cycle	Initial activity	Injections	Uterine activity after treatment		Recording period	Other injections	Remarks
					Frequency	Amplitude	Minutes		
COW D318									
1	11.7.79	Estrus	Uterus +	IV 20U Oxy.	I	I	120		Rectal manipulation.
2	16.7.79	-	Uterus 0	IV 20U Oxy.			120		Slight response
3	24.7.79	-	Uterus 0	IV 20U Oxy.			120		Slight response
COW D542									
4	11.7.79	Estrus	Uterus +	IV 20U Oxy.	I	I	80		
5	24.7.79	-	Uterus 0	IV 20U Oxy.					Slight response

APPENDIX VII. EXPERIMENTS ON COW D318 AND COW D542

(Key: 0 = no activity, + = contracting, I = increase, IV = intravenous, Oxy. = Oxytocin)