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Some Observations Pertaining to the Effects of Relaxin Upon the Bovine

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SOME OBSERVATIONS PERTAINING TO THE EFFECTS OF RELAXIN UPON THE BOVINE

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

The process of birth normally follows a set pattern. The main steps in chronological order are: labor, opening of the cervix, and delivering the young. The cervix serves an important function in reproduction. The cervix is the constricted portion separating the uterus and the vagina, and consists primarily of thick connective tissue especially constructed in folds forming a hollow tube. Through the cervix passes sperm, menstrual debris and in pregnancy the newborn. During pregnancy a mucous plug forms in the cervix and keeps out foreign material and bacteria. In the non-pregnant cow the opening is small. The cervix opening is constricted enough so that difficulty in inserting an artificial inseminating tube sometimes occurs.

If ova transfer can be perfected to the degree that artificial insemination is today, a revolutionary way of up-breeding a herd of cattle may result. In order for ova transfer to fulfill this role a non-surgical method of extracting and implanting must be perfected. If a non-surgical method is to be used, entrance into the uterus through the cervix is paramount. The non-surgical equipment used to collect the ova is considerably larger than an artificial inseminating tube. Passage through the cervix with this equipment is accomplished with extreme difficulty. The inaccessibility of the uterus resulting from constriction of the cervix has markedly hampered the development of ova transfer.

If the cervix can be dilated so as to allow easy entrance into the uterus, ova transfer may become of practical value in the not too distant future. A dilated cervix would allow larger and more precise collecting

instruments to be used.

Parturition is facilitated in some rodents by the action of relaxin upon the symphysis pubis and pelvic bones. Relaxin is a female hormone secreted by the placenta, uterus and ovaries and is present in the blood during gestation of many animals. Relaxin apparently has two purposes in the human; uterine quiescence during gestation and dilation of the cervix at parturition. The use of relaxin to dilate the cervix in humans suggested the possibility that dilation of the bovine cervix may be obtained by the administration of relaxin.

To further investigate the physiological properties of the cervix and the possibility of dilation in the non-pregnant cow, the following experiments were conducted.

1. The effect of relaxin upon the cow's cervix while in estrus,
2. The effect of liquid and depot relaxin upon the cow's cervix when primed with stilbestrol.
3. The effect of blood from a cow in labor, transfused into a non-pregnant, non-estrus recipient.

The milk letdown response just prior to parturition suggests that the hormones probably are influential and effective at this time. Oxytocin is known to contract the alveoli and force milk into the collecting ducts, gland cisterns, and teat sinuses. Relaxin is likewise believed to be present in quantity at parturition and might have a contracting effect on the myoepithelium around the alveoli. To determine if such action might be possible in the lactating cow, a series of experiments were conducted to determine the effect of relaxin on milk letdown.

REVIEW OF LITERATURE

The ability of the hormone relaxin to initiate the relaxation of the pubic ligaments has been known for some time. Within the last 15 years other physiological properties of the hormone have been investigated. One of these properties has brought the use of relaxin into practical importance within the last year, namely, the ability to inhibit uterine contractions.

The effects of relaxin were first observed in 1919 by Hisaw (58) in the pocket gopher. The anatomy of the female pocket gopher is such as to allow her to travel in narrow underground burrows. Because the pelvic region is disproportionally small, it is a physical impossibility for the newborn of average size to pass through the pelvic girdle. Observations on pocket gophers at parturition revealed that the pubic bone and ligaments dissolve at the end of gestation to facilitate birth. Hisaw's early observations on this phenomena stimulated many investigations pertaining to the chemistry, biochemistry, physiology, histology, pharmacology, and clinical properties of relaxin.

CHEMISTRY

Many of the chemical properties of relaxin are similar to insulin. Relaxin is amphoteric and has properties which suggest a peptide-like structure (22). An acid alcohol preparation of relaxin is both alcohol and water soluble (48). The hormone also forms water soluble salts with hydrochloric, sulphuric and acetic acids (21). Relaxin is insoluble in such non-polar solvents as ether, acetone, chloroform, petroleum ether,

cold benzol, ethyl acetate, and butyl alcohol (47,50,48). With picric acid relaxin forms a salt which is insoluble in water, alcohol and ether (21).

Relaxin is easily destroyed by both chemical and heat treatment. Relaxin is inactivated by cysteine, thioglycolate, dithiopropanol, glutathione, hydrogen sulfide, bisulfite, tertrathionate, pancreatin, trypsin, pepsin, oxidizing agents and alkalies (24,48,50). However, relaxin is stable in fairly strong acids, but concentrated acid will cause some deterioration (48). The hormone is thermolabile being destroyed by heating to 100°C for a short period of time. Exposure at room temperature will result in a gradual loss of activity. The storing of aqueous solutions for 12 months at cool temperatures resulted in no detectable decrease in activity (48,21,47).

Purified relaxin has a molecular weight of about 9000 with a structure resembling a low molecular weight protein or polypeptide. The nitrogen content of relaxin is approximately 12 percent (26). Studies of the behavior of relaxin toward precipitating agents as well as relaxin's electrophoretic behavior suggests the presence of more than one compound and more than one molecular weight (25).

BIOCHEMISTRY

The preparation of relaxin consists of extraction, purification and assay. Since relaxin is secreted by the corpus luteum, most of the early extractions were from the corpus luteum. Extraction with ether and neutral alcohol proved to be successful only part of the time. The preparations were low in relaxin activity. Saline extraction yielded consis-

tently good results, but deteriorated rapidly because of putrefaction. Acid alcohol, 95 percent ethyl alcohol acidified with hydrochloric acid proved at that time to be the best reagent to use for the extraction. After being extracted with acid alcohol, relaxin is soluble in neutral alcohol (48). When acid alcohol is used for an extraction reagent, purification consists mainly of filtration, dilution and precipitation followed by neutralization and numerous extractions with alcohol and acetone (48).

Albert (5,6) improved the method for extracting relaxin preparations. The activity of this preparation was increased 10 fold. In addition fewer steps were needed when dilute acid was used. The use of fresh whole ovaries instead of corpus lutea significantly increased the yield of relaxin. Ovaries of pregnant sows contain from 500 to 1500 times more relaxin than ovaries from non-pregnant swine (6). Relaxin exists in the ovary of the pregnant sow in a form which differs chemically from the purified hormone (27). When relaxin is extracted by the use of dilute acid the purification is as follows. After filtering the extract at pH 3.9, the relaxin is in the filtrate and may be removed by adsorption onto bentonite. The bentonite is then treated with dilute pyridinium chloride to redissolve the relaxin. Pyridine is removed by dialysis and the relaxin is further purified by repeated precipitation at pH 4.4. Bentonite will not adsorb relaxin at pH 6.5. Low temperature evaporation and isoelectric precipitation of the dialyzed filtrate after treatment with bentonite at pH 6.5 affords relaxin of comparable activity with approximately equivalent yields to that obtained by using bentonite

at pH 3.9 (27).

After observing the effect of relaxin on the pocket gopher, Hisaw directed his observations to experimental animals such as rabbits, guinea pigs, dogs and cats. The guinea pig proved to be extremely sensitive to the action of relaxin. Relaxin so effectively relaxes the pelvic ligament of the guinea pig that the potency of relaxin is measured in guinea pig units (GPU). A GPU is the miminal amount of relaxin needed to produce relaxation of the pelvic ligaments within 6 hours after a single injection into 60 percent of the animals treated (1,2,22). The pelvic ligaments in the mouse are also relaxed by the administration of relaxin. The mouse being smaller and less expensive has become increasingly more important in assay work.

Before relaxation of the pelvic ligaments can be obtained in the guinea pig or mouse by the administration of relaxin, the animal must be primed with estrogen. This estrogen may come from endogenous or exogenous sources. The endogenous source being from the ovary when the animal is in estrum. The animal may be pre-treated with injections of estrogen in some form so as to bring the animal into an artificial estrum (13,44).

The ovary plays an important role in the hormonal balance in the pregnant and non-pregnant animal. While the uterus is active during pregnancy it is relatively inactive when the animal is not pregnant. To avoid the interference of the ovarian secretion in most assay work the animals are ovariectomized.

While the corpus luteum contains 1 GPU per gram of tissue (1), the whole ovary of a pregnant sow may range up to 10,000 GPU per gram of

ovarian tissue (49). The ovaries range from 2.5 to 5 GPU per gram of dry tissue during estrus. Soon after pregnancy the relaxin content in ovarian tissue increases rapidly and reaches a maximum concentration when the fetuses are 5 to 6 inches long. At this time the concentration is about 10,000 GPU per gram of tissue. The placenta contained 0.5 to 2.5 GPU per gram of tissue with no correlation with length of gestation. Assays show the uterus does not contain relaxin. The concentration of relaxin in the blood of pregnant animals is 2 GPU per ml. of serum (49). While the main source of relaxin is the ovary of the pregnant sow, work on the rabbit suggests that the uterus and placenta are the principal sources for that animal.

Assay on the uterus, placenta and ovaries of the pregnant rabbit revealed a relaxin content per gram of fresh tissue of 10 GPU for the uterus, 50 GPU for placenta and 25 GPU for the ovaries (95). Zarrow in later work obtained a relaxin content of 25 GPU per gram for the fetal placenta and 300 GPU for the maternal placenta per gram of fresh tissue (97).

Ovariectomized pregnant rabbits at midterm and supplemental maintenance of progesterone levels resulted in normal blood levels of relaxin which are approximately 12 GPU per ml. of serum. This suggests that the ovary is not the major source of relaxin in the pregnant rabbit (1,95,97).

Since relaxin produces distinct pelvic relaxation in the guinea pig, it is interesting to note the relaxin concentration of the various tissues during different stages of pregnancy. The uterus contains approximately 10 GPU per gram of tissue on the 56 and 63rd day of pregnancy where-

as the placenta shows 5 GPU per gram of tissue on the 56th day and 25 GPU on the 63rd day. Relaxin appears in the blood around the 21st day of pregnancy at which time relaxation of the pubic symphysis may first be detected by palpation. The concentration of relaxin reaches a peak of 0.5 GPU per ml. of serum on the 28th day and is maintained until the 63rd day after which it declines to 0.33 GPU per ml. of serum. Immediately after parturition a sharper decline takes place. A correlation exists between the concentration of relaxin in the blood and in the urine. The concentration in urine at 42 and 56 days is 0.5 GPU but drops to 0.25 GPU by the 63rd day. The drop in blood serum relaxin in the latter part of pregnancy results from a decreased output from the placenta (93).

Many of the physiological investigations on relaxin were carried out before methods for obtaining highly purified extractions of the hormone were known possible. This is fortunate because in 1951 Kliman (55) discovered there are two types of relaxin. One type will produce relaxation in the guinea pig but has little effect on the mouse, while the other type has the opposite effect. Assays using crude extracts of relaxin on guinea pigs and mice will give the same results when 10 GPU are equal to 1 mouse unit. One mouse unit is that amount of relaxin required to produce a 1.0 mm. increase in the mean separation of the pubic symphysis of eight mice. By fractional procedures substantially all of the mouse activity precipitates with an equal volume of ethanol while almost all of the guinea pig activity remains in the filtrate. The two types of relaxin are similar in heat resistance and inactivation from cysteine. A six molar urea solution will inactivate the mouse active portion but not

guinea pig active substance (55,57).

PHYSIOLOGY

In 1930 the only physiological property of relaxin known was the ability to cause relaxation of the pelvic ligaments. Because of this physiological phenomena Hisaw et al (22) named the hormone relaxin. They proposed that a heretofore unknown but specific hormone was the cause of pubic relaxation at parturition. Other workers denied the existence of a new hormone, reasoning that estrogen alone or in combination with progesterone could produce relaxation of the pelvic ligament. Confident that an unknown hormone was responsible for this relaxation, Hisaw conducted many experiments to prove relaxin existed. Until procedures were devised to remove all of the estrogen and progesterone from relaxin preparations, he was hampered by the presence of small quantities of these hormones. With pure relaxin preparations Hisaw was able to offer conclusive evidence that relaxin was a separate hormone.

An aid to the exact determination of the effects of relaxin was the use of X-rays. Not only could more accurate measurements of pubic relaxation be acquired but also other effects such as bone density could be studied. Since 1930 much information concerning the physiological properties of relaxin has been obtained. Investigations have shown a relationship of relaxin to the mammary gland, relaxation of the pelvic ligaments and the effects on the uterus.

Mammary gland. In the later stages of pregnancy the mammary gland grows rapidly and becomes active. The over-all picture as to why the mammary

gland begins growth and starts to secrete milk shortly before giving birth is not clear. The possibility of an association between the development of the mammary gland and relaxin being a hormone of pregnancy exists. Investigations with this in mind have been done on the rat, mouse, guinea pig and rabbit.

The interaction of estradiol, progesterone, and relaxin when given together to the ovariectomized rat will stimulate growth of the mammary gland. By the 13th day the mammary gland of the mouse has the appearance of those in late pregnancy. This growth was not obtained when only two of the hormones were administered. In hypophysectomized rats there was no mammary development when treated with the three hormones (41).

In the mouse there is no increase in alveolar development by the addition of relaxin to estrogen and progesterone (87). Work by Elliott et al has shown that the addition of relaxin to estrogen and/or progesterone failed to produce a significant elaboration of the spreading factor of the mammary gland (19). Likewise extracts of mammary glands from rats pregnant 8 to 14 days when injected into primed guinea pigs produced no pelvic relaxation (18). Estrogen alone or in combination with progesterone will not produce lobulation of the mammary glands of ovariectomized rats. A combination of relaxin, estrogen and progesterone will produce lobulo-alveolar growth which is indistinguishable histologically and histochemically to that seen between the tenth and twentieth day of pregnancy. Mammary glands of hypophysectomized rats showed negative ductual and lobular responses to relaxin-steroid combinations (77).

In the guinea pig, an increase in the degree of development of the

mammary gland was achieved by the addition of relaxin to estrogen, while in the rabbit the addition of relaxin transferred the emphasis of growth from the primary ducts to the entire ramifying duct system without inducing significant alveolar growth. This possibly acts as a potentiator for estrogen and stimulates growth (31).

Relaxation of Pelvic Ligaments. The process of birth is generally considered to be approximately the same in all animals. There are some specific characteristics for each species. One part of the reproductive system may play an extremely important function in one species while in another it may function very little. The pelvis of the pocket gopher is almost completely dissolved at parturition. But the pelvic girdle of many domesticated animals undergoes relatively little change.

The symphysis pubis is the junction between the pubic bones. In the male and immature virgin girl, guinea pig, mouse, sheep, bovine and swine, little if any movement of the pubic bones is possible. In non-pregnant multiparous animals there is some movement possible, but it is relatively small to the movement possible at parturition (62,61,35,45, 11,10,30).

Cantin (14) in 1899 reported that pregnancy normally produces a relaxation of the symphysis pubis during pregnancy of the human female. Within a few months after delivery, the pubic symphysis retrogresses to approximately the same width as in the non-pregnant state (65,43,4,11,88). Relaxation occurs early in pregnancy and increases rapidly until about the 7th month after which time little relaxation takes place (4,43). Some relaxation occurs during labor and delivery (88).

The young guinea pigs are born in a relatively mature condition with a very large head. It is a physical impossibility for the head to pass through the pelvis of a non-pregnant animal (78). Relaxation begins about the middle of gestation and increases rapidly before parturition (85). The symphysis pubis relaxes to such an extent that locomotion is difficult during the last two weeks of pregnancy (89). During parturition the distance between the pubic bones may be nearly one and one-half inches but within 72 hours after parturition there remains only slight separation (15). This separation remains and never forms a true symphysis (85).

In the mouse, separation of the symphysis pubis occurs chiefly during the last week of pregnancy. The gap closes rapidly after parturition but a small permanent separation remains (39). No separation of the pubic bones occurs during pseudopregnancy (56). To determine whether separating the pubic symphysis was necessary for the birth of the young, Crelin (17) sutured the innominate bones of virgin mice. Although the symphysis pubis remained closed there was some movement possible and the sacroiliac joints had gained the normal increased amount of flexibility and normal parturition occurred. Normally the sacroiliac joints become flexible during pregnancy much as the pubic symphysis opens for animals such as the human, cow, sheep and mouse (8,61,11,17,9).

Pubic relaxation was first produced in estrus virgin guinea pigs by the injection of serum from pregnant rabbit or guinea pig blood. After continuing injections of the follicular hormone long enough to produce an estrus, the injection of pregnant rabbit serum initiated a relaxation of the pubic symphysis (46). Hisaw (47) in 1927 isolated the substance pres-

ent in the blood of pregnant guinea pig, rabbits, cats, dog, mare, rabbit placenta and corpora lutea of the sow that would cause relaxation in the guinea pig during estrus. Ovariectomized guinea pigs brought into artificial estrum would also show relaxation of the pubic symphysis when injected with these preparations.

The existence of a separate hormone causing pubic relaxation was denied in 1932 because relaxation could be produced by treatment with estrogen (23). Brouha (12) demonstrated that pubic relaxation could be induced by estrogen when given in sufficient amounts and for long duration, but 1 mg. of corpus luteum extract containing no estrogen would produce relaxation within 24 hours in animals pretreated with estrogen. The injection of 1.5 micrograms of estrogen daily for eight days is the optimum duration for estrogen priming but treatment for 5 days is sufficient for assay purposes (35).

Trials on ovariectomized mice demonstrated that a small quantity of symphysis pubis relaxation could be obtained by estrogen but a highly significant increase was obtained by 0.1 ml. of pregnant rabbit serum in estrogen primed mice, thus indicating the presence of a specific hormone (38). This has also been observed by other workers (40,84,7).

Another hormone of the corpus luteum which plays a role in pregnancy is progesterone. The injection of progesterone into estrogen primed ovariectomized guinea pigs will produce relaxation of the symphysis pubis within three days (42). The maximum spread is obtained after treatment for 10 to 15 days. Progesterone stimulates the uterus to produce relaxin. Hysterectomy nullifies all effects of progesterone (84). In the

primed ovariectomized mouse progesterone will not cause relaxation. Relaxation produced by relaxin or continued estrogen treatment is inhibited by the presence of progesterone (36).

Reproductive Organs. The production of relaxin during pregnancy must be related to the reproductive organs. The reproductive organs must secrete relaxin during pregnancy or act upon other organs of the body so as to initiate the secretion of relaxin.

In the guinea pig the uterus plays the most important role in connection with relaxin secretion while in the mouse the ovaries are also important. In several experiments, investigators (16,52,51,92,94) have discovered that relaxation of the pelvic ligaments in the ovariectomized guinea pig may be produced by estrogen, estrogen and progesterone in presence of the uterus or estrogen and relaxin. Relaxation will take place by injecting estrogen into the ovariectomized and ovariectomized-hysterectomized animal but requires a considerable length of time. The administration of the hormone relaxin to the ovariectomized and ovariectomized-hysterectomized estrogen primed guinea pig will produce relaxation by the 6th hour after treatment approaching that at parturition.

Progesterone injected into ovariectomized virgin guinea pigs will produce relaxation within 12 days but will not cause relaxation to occur in the ovariectomized-hysterectomized animals. These experiments (94,52, 51,92,16) revealed that the ovaries are not necessary for relaxation of the pelvic ligament. Nor is the uterus necessary for treatments of estrogen or relaxin. Relaxation will only occur following treatment with progesterone if part of the uterus is present (16). Progesterone initiates

the uterus to produce relaxin thereby causing relaxation of the pelvic ligaments (52,94,84,80). Further indication concerning the importance of the uterus in progesterone treatments is the observation that progesterone in small doses which are ineffective when placed elsewhere will cause relaxation if applied locally to the uterus (63).

The administration of relaxin into estrogen primed or prolonged estrogen treatment will cause the pubic ligament to relax in ovariectomized mice (40,50). Progesterone given concurrently with either of these hormones inhibits their ability to produce relaxation of the symphysis pubis (36,50). If pregnant mice are ovariectomized after a pubic gap has occurred the opening will close even if estrogen and progesterone are injected daily (40) thus suggesting the necessity of additional secretions from the ovaries for relaxation of the symphysis pubis to occur.

Effect on the Uterus. Parturition is facilitated by strong uterine contractions. Unduly strong uterine contractions during pregnancy may initiate premature birth or abortion. The factor or factors responsible for uterine quiescence during pregnancy until parturition are extremely important. Investigations on uterine contractions *in vivo* and *in vitro* using relaxin suggest that this hormone may be responsible for the quiescence of the uterus until parturition.

Riley (70) tested commercial aqueous corpus luteum solutions containing 0.25 to 0.5 percent chlorobutanol and an aqueous corpus luteum extract without chlorobutanol. The commercial aqueous corpus luteum containing chlorobutanol reduced uterine activity while no quiescence effect

was produced by the aqueous corpus luteum extract without chlorobutanol. From these findings he concluded that the chlorobutanol inhibited uterine motility. However, this conclusion is not substantiated because later work established that the quiescent effect was produced by relaxin (60,74, 75).

In tests on the rat uterus *in vitro* relaxin has the ability to inhibit uterine contractions. The rat uterus *in vitro* normally shows strong contractions while in estrus or pregnant. When the uterus is suspended in Locke's solution to which relaxin is added; as little as 0.001 CPU per ml. of total solution will cause a perceptible decrease in uterine motility. As the relaxin content of the solution is increased the strength of the contractions is proportionally decreased to the point of complete uterine quiescence. Recovery from complete quiescence is slow. This inhibition is not a result of such impurities as histamine or epinephrine nor does relaxin inhibit the responses from pitocin or acetylcholine. The ability of relaxin to inhibit uterine contractions has proven beneficial for preventing abnormally strong contractions initiating premature births in humans between the 29th and 31st week of pregnancy (3).

Frieden *et al* (28,29) have conducted experiments to determine the effect of relaxin upon decidual reactions after traumatization of the uterus in ovariectomized and psuedopregnant rats pretreated with progesterone. Traumatization of the uterus followed by treatment with progesterone resulted in the formation of a decidua. Low potency relaxin preparation will enhance this effect but larger amounts inhibit the formation of a decidua. The inhibitory effects are proportional to the relaxin

content of the preparation over a 100 fold range. The potentiating effect of low relaxin content serums can be destroyed by heat while the inhibitory effects are increased. Deamination will also decrease the potentiating effect. Since the potentiating effect is over-ridden by larger quantities of relaxin and can be destroyed by deamination and heat without affecting the inhibiting portion, it appears that the poteniating effect is caused by an impurity. It appears that relaxin has the ability of inhibiting the decidual reaction which normally follows traumatization of the uterus in ovariectomized pseudopregnant rats.

HISTOLOGY

The gross anatomy of the widening symphysis pubis resulting from long estrogen treatment or administration of relaxin to estrogen primed mice appears similar. Histological studies have shown there is a difference in the mode of action between the two hormones.

The female guinea pig's symphysis pubis is the two pubic bones which are held together by a narrow ligament, whereas in the male the pubic bones form a partial or complete union (72). During pregnancy connective tissue cells multiply and allow the symphysis ligament to lengthen. Resorption of the pubic bones progresses until extensive areas of the pubis and ischium are resorbed (73). There is a proliferation of the osteoclasts along the symphysial surface of the pubis and ischium (53).

Administration of estrogen to ovariectomized or ovariectomized-hysterectomized guinea pigs results in the absorption of the pubic bone and growth of loose fibrous heavily nucleated connective tissue. Relaxin acts

upon the symphysis ligament producing a loosening of intercellular matrix and disappearance of the collagenous fibers. Progesterone injected into estrogen primed ovariectomized guinea pigs produces relaxation similar to that of relaxin (80,81). Perl et al (67,68) suggests the mode of action for relaxin is the depolymerization of the glycoproteins of the symphysial region.

Hall (34) observed the following changes take place in a normal pregnant mouse. Growth of hyaline cartilage, resorption of the medial ends of the pubis, lengthening of the symphysial ligament by formation of new cartilage and reversion of the cartilage into collagenous connective tissue. Relaxin initiates a relaxation which is histologically similar to that observed in normal pregnancy. The administration of estrogen to the mouse simulates relaxation primarily by resorption of the pubic bones. The amount of resorption depends upon the level and length of treatment (79). A vascular, heavily nucleated tissue replaces the resorpted portion of the bone and is thought to arise from the marrow of the resorpted bone. Progesterone alone does not alter the pubic symphysis. When progesterone and estrogen are given simultaneously the effect of estrogen is inhibited (37).

Estrogen Pretreatment. There has been no explanation given for the necessity of the estrogen pretreatment if the administration of relaxin is to produce relaxation of the symphysis pubis. There have been observations made on the changes which occur in the symphysis pubis of the guinea pig during this pretreatment. In order for the symphysis to fully respond to relaxin, the water content must be approximately 74 percent by weight. The

labelling the relaxin molecule with I^{131}, Kraintz et al (59) have shown that the adrenals contain 10 times more relaxin than an equal mass of muscle, 30 minutes after an injection of relaxin. The greatest amount of concentration was found in the liver and kidneys.

Toxicity from relaxin seems to be very low. Injections of 80,000 GPU of a highly purified preparation per kg. of body weight into mice showed no harmful effects (96). Zarrow et al (96) were unable to produce antibodies to relaxin in the rabbit. Guinea pigs have been given bi-weekly injections of relaxin for over a year without developing refractivness to relaxin or showing symtoms of anaphylaxis (96). In humans up to 40,000 GPU per day for 9 days have been administered with no toxic effects except for local pain and inflammation. Also, trials of 24 hours duration showed relaxin has no effect on blood pressure, pulse, temperature, blood count or blood level of 17-hydroxycorticosteroids. In 1956 the Food and Drug Administration approved the sale of Releasin (relaxin).

CLINICAL

Many of the investigators studying the properties and effects of relaxin have done so just for the satisfaction of knowing more about the hormone. Many were not interested in the practical applications of these experiments but were interested in the academic aspects of relaxin. With an attitude such as this, investigators have been able to formulate ideas which seemed relatively unimportant but later suggested useful properties of the product. This has happened in the case of relaxin which has the

properties of exhibiting two opposite reactions at different times of pregnancy. The first property of relaxin noted was the facilitating of birth at parturition. Later, the ability to inhibit uterine contractions was discovered. Workers with the thought of making these properties of practical importance have found relaxin beneficial in treatment of dysmenorrhea and premature birth.

Dysmenorrhea (painful menstruation) may be caused by several factors. Whatever the cause, the pain associated with dysmenorrhea generally results from disorganized uterine contractions or excessive pressures developed by organized contractions. These abnormal contractions are a result of a factor which is the cause of dysmenorrhea. Some of these factors are juvenile type of uterus, scar tissue, cyclic edema, very low threshold for sensory impulses, excessive tissue fragility, emotional stress, menstrual toxins, vascular changes in the ovaries, allergy and malposition of the uterus. Many of these factors are of such a nature that surgery would be necessary to correct them. Others are only temporary conditions (86). Organized uterine contractions bring discomfort to patients with a strength ranging from 70 to 100 mm. of mercury, discomfort increases in severity with distress appearing between 140-180 mm. of mercury. Disorganized contractions will result in severe pain with pressures as low as 40 mm. of mercury. Besides the pain, disorganized contractions do not propel the flow of menstrual debris and in some cases may obstruct the normal flow (91). Resek (69) prepared an extract from the corpus luteum and administered it to patients with dysmenorrhea. Within 15 minutes 70 percent of the patients with normal endometrium had

complete relief. Although relaxin is inactivated by digestive enzymes, Dr. Abramson (58) is presently administering relaxin in tablet form. This experiment is not completed at present but preliminary results suggest that the hormone is of value in the treatment of dysmenorrhea.

The field in which the relaxin has been most successful is the prevention of premature births. In the United States approximately 200,000 babies die each year before or at birth. Premature babies born after 28 weeks of pregnancy have a very high death rate. Often in premature births late labor is reached before the cervix opens enough to allow the passage of the baby. These uterine contractions force the delicate tissues of the baby's brain against the closed cervix. The contractions frequently are strong enough to damage the brain and cause death to the baby. Dr. Leonard A. Scheele, president of Warner-Chilcott, makers of Releasin (relaxin), estimates relaxin could save 140,000 of these 200,000 babies.

Falls et al (20) successfully used corpus luteum extracts to prevent premature births. Abramson (3) administered 2000-5000 GPU of relaxin intramuscularly and repeated the treatment when necessary to five women in premature labor between the 29th and 31st weeks of pregnancy. Labor ceased and pregnancy continued in all the cases and terminated with normal delivery.

Approximately 600 women with various labor problems have been treated with relaxin. Relaxin is most effective for treating patients between 28 and 34 weeks of pregnancy. The administration of relaxin before the 28th week or after the 34th week of pregnancy has proved benefic-

ial but not to the same extent (58). In the early stages of labor, relaxin is inhibitory. However, if labor has advanced to the stage where it can not be stopped, relaxin will facilitate birth. In treatment where labor can not be stopped, relaxin apparently has saved between 50 and 95 percent of the premature babies. If pregnancy has progressed to normal term, but the baby has not become fully developed, parturition may be delayed until the baby is fully developed (90). Relaxin has proven beneficial for use in pregnancy. The usefulness of the hormone relaxin does not appear to end here. Investigations for other uses in unrelated fields are being explored.

EXPERIMENTAL PROCEDURE

The animals used for this experiment were normal, healthy cows not assigned to any other research during this period. Some of these animals were used on a stilbestrol feeding trial prior to these investigations, thus some were maintained on a high prolonged stilbestrol level. If any of the animals became infected 300,000 units of penicillin G were injected and the animals were removed from the experiment until the pathological condition was corrected. All of the cattle were maintained on a general management and feeding program.

Four different experiments were conducted. Three of these dealt with cervical relaxation and one with the milk letdown response. The four groups are as follows:

1. The effect of relaxin upon the cow's cervix while in estrus.
2. The effect of liquid and depot relaxin upon the cow's cervix after being primed with stilbestrol.
3. The effect of blood from a cow in labor, transfused into a non-pregnant, non-estrus recipient.
4. The effect of relaxin on the milk letdown response upon lactating cows.

The relaxin administered to the cows was given in two forms. The material used in the first experiments was a preparation from pregnant sow's ovaries redissolved in saline. These saline solutions were readily absorbed and quickly lost from the blood following an intramuscular injection. In order to compound a mixture that would act as a depot reserve and remain in the animal for a prolonged time the same preparation was used but suspended in bee's wax to slow the absorption. Since depot relaxin is

a solid at room temperature, the syringe, needle, and relaxin preparation were heated to facilitate the subcutaneous injections.

For estrogen priming, diethylstilbestrol was given in two forms; implants of pellets subcutaneously and liquid suspensions of diethylstilbestrol intramuscularly. The diethylstilbestrol powder was suspended in 80 percent cottonseed oil, 20 percent ethyl alcohol solution; 12 to 15 mg. being suspended per ml. of solution. McCarthy (64) reported that diethylstilbestrol in liquid form dissapears rapidly from the blood stream. This was suggested because women report much more even response from treatment with diethylstilbestrol in oral form than daily injections. For this reason the procedure was changed from injecting the full dose in liquid form to injecting one half the dose in implants and one half in liquid form.

Cervical measurements and observations were accomplished by inserting a finger or fingers through the vagina and as far as possible into the cervical canal. The inside circumference of the cervical folds was estimated in inches by measuring the finger or fingers. Periodically a speculum was used to determine the condition of the cervix and to see if any infection was present.

To determine the effect of relaxin upon the cervix of a cow while in estrus (group 1) the animals' cervix were measured before the injection to establish the cervical dilation resulting from estrus and 8 hours later to determine the amount of dilation resulting from relaxin. Generally 1500 GPU of relaxin were injected intramuscularly immediately after palpation. A total of 18 trials were performed on 9 cows. Two animals had

been pretreated with diethylstilbestrol. Cow No. 2550 received diethylstilbestrol implants of 375 mg. per week for 9 weeks with the last implant being given 16 weeks and 6 days before the injection of 1500 GPU of relaxin. Cow No. E88 received 60 mg. per week of diethylstilbestrol implants for 9 weeks with the last implant being given 18 weeks and 6 days before her first injection on this experiment.

To investigate the action of liquid and depot relaxin upon the cervix (group 2) a considerably longer period of time was used. Three animals were assigned to this experiment. One animal, E88 had been pretreated with estrogen (see above paragraph). She had received her last implant 24 weeks prior to the time she was placed on this experiment. This estrogenic treatment left her with a high tailhead which was drawn to one side and her hip caps had dropped approximately 4 inches. During this experiment she was given depot relaxin for 6 weeks. After a two week period she was then given 1200 GPU liquid relaxin every hour for 24 hours.

Another animal (E154) received a total of 7750 mg. of diethylstilbestrol in pellet and liquid form over a 9 week 5 day period. This treatment left her a condition similar to E88 but less severe. During the last 6 weeks she received a total of 105,000 GPU of depot relaxin. On the last day she was given 1200 GPU of liquid relaxin every hour for 24 hours. The last cow (E106) was primed with 4560 mg. of diethylstilbestrol in liquid and pellet form over a period of 7 weeks 4 days and then given 1200 GPU of liquid relaxin every hour for 24 hours. For a detailed day by day account of this group see Appendix, part 2.

In order to determine the possibility of the blood containing a cervical relaxing factor, the blood from three parturient cows was transfused into three recipients. Approximately 8 liters of blood was drawn from each of three cows, (E125, E157, E158). None of these animals had been used on any previous experiment. The blood was withdrawn from the jugular or the milk vein as soon after parturition as possible from two of the animals. On the last animal (E158) blood withdrawal was started 10 minutes before the calf was born and continued after delivery. The blood was drawn into 2000 ml. flasks containing 4 gm. of sodium citrate to prevent coagulation. The blood was transfused into the jugular vein of three non-pregnant cows (E152, E106, E107). None of the recipients were in heat at the time of transfusion. The cow (E152) used for the first transfusion had been on a diethylstilbestrol experiment before being used for this transfusion. She had received a 15 mg. implant per week of diethylstilbestrol for 9 weeks with the last implants being given 19 weeks before transfusion. After transfusing 2000 ml. of blood from the freshening cow (E125) into E152, 3400 ml. of blood was drawn from E152 and later injected into E104, also in the diestrum period. After removing 3400 ml. from E152 another 6000 ml. of blood from the freshening cow (E125) was transfused into E152. Three thousand GPU of relaxin was added to the 3400 ml. of blood taken from E152 and then transfused into E104. Six hours after the transfusion E104 received an injection of 1500 GPU of relaxin.

Cow No. E106 used on the 2nd trial had been treated with a total of 360 mg. of liquid diethylstilbestrol in the 5 days preceeding the transfusion. The last animal E107 in this experiment was pretreated with a

total of 1440 mg. of liquid and pellet diethylstilbestrol during the previous 4 weeks. Cervix dilation was measured before the transfusion and at different periods afterwards. For a more detailed description of each transfusion, refer to Appendix, part 3.

An experiment (group 4) was designed to determine if relaxin produced milk letdown in lactating cows. Ten cows were milked a total of 100 times using 1500 GPU of relaxin on 50 and one half ml. of saline solution for a control on the other 50. The animals were milked prior to any injections to obtain the milk normally let down. An injection of relaxin or saline was then made and the animal was again milked to obtain any additional milk which might result from the injection. After the milk flow had again ceased, an injection of 10 units of oxytocin was given to more nearly evacuate the gland. All injections were made intrajugularly. A bucket milker was suspended on a scale in such a manner that continuous observations were observed. One experimental milking was made per day and the milkings were so arranged that the injection of either relaxin or saline was not made on consecutive days.

RESULTS

For a more clear description of the results obtained, they are presented in the four groups that were used. The data from group 1 suggested that relaxin given during estrus frequently will not produce cervical dilation more than what it normally dilates during estrus. Three trials showed some dilation. One cow's (255C) cervix dilated to such an extent that a calf could have passed through. With a speculum it was possible to look into the uterus of 255C. Her uterus was contracting and exhibited symptoms similar to those of labor. The uterus was bleeding bright red blood. Even after 4 days the cervix was dilated enough to allow the passage of a hand. At this time she was slaughtered and her reproductive tract examined. No injuries or tears were present in the cervix.

Four different levels of relaxin were tried. Since no additional relaxation was apparent from the higher levels in the cases tried, the rest of the trials were made using 1500 GPU of relaxin. For individual trial data see Appendix, part 1.

In group 2 there was no consistent pattern of results from depot relaxin. One animal (E88) showed some relaxation up to a point, after which no further relaxation was obtained while another animal's cervix (E154) closed the same amount that E88's opened. Cow No. E154 was receiving diethylstilbestrol concurrently with depot relaxin. A small amount of relaxation was obtained in two animals (E88, E154) by the administration of relaxin hourly for 24 hours. In one cow (E154) the 1st fold was ruptured during the 23rd hour of palpation. The third animal (E106) showed no relaxation from this hourly treatment. Detailed information on these

animals is given in part 2 of the appendix.

The transfusion experiments showed considerable individualism so these results are presented individually. The first transfusion was made into an animal (E152) that had been pretreated with estrogen. Her cervix dilated progressively until a fist could be passed through her cervix 15 hours after the transfusion. The cervix remained open for some time but had begun closing 12 days after the transfusion. Possibly blood vessels were broken during dilation as clotted blood was present in the reproductive tract when examinations were made. She had bleeding and straining after the transfusion similar to 255C.

The cow (E106) that received 3400 ml. of blood from the estrogen pretreated animal (E152) plus 3000 GPU of relaxin showed some relaxation 6 hours after the transfusion. The additional injection of 1500 GPU of relaxin 6 hours after the transfusion provided no additional relaxation. The 2nd trial on cow E106 produced no detectable dilation of the cervix. The 3rd trial on cow E107 entailed some complications. Examination 6 hours after the transfusion revealed some relaxation. However, the animal did not appear well as her eyes were puffed and general well being was that of a sick animal. Six hours later, (12 hours after the transfusion) the cow was dead. The extremities were still warm indicating death had occurred shortly before. The cervix was measured and no further dilation than that present at the 6 hour examination could be detected. The cause of the sickly appearance of the cow 6 hours after the transfusion and her death were not determined but differences in blood type seemed a reasonable explanation. Data on the individual trials may be found in the Appendix,

part 3.

An analysis of variance using the F table was used on the milk let-down experiment (group 4), and the T test was also used. No significant increase in the amount of milk was obtained from the injection of 1500 GPU of relaxin when compared to an injection of 0.5 ml. of saline.

DISCUSSION OF RESULTS

The results obtained from these experiments are insufficient to draw positive conclusions as to the effect of relaxin upon the cow, especially regarding cervical dilation. However, some interesting observations were made in this respect. Graham et al (32,33) produced relaxation of the cow's cervix five days post estrus with as little as 100 GPU of relaxin after priming the animals with 20 mg. per day of diethylstilbestrol for three days prior to the treatment with relaxin. A mechanical dilator was used to determine cervical relaxation. Zarrow et al (98) also produced dilation of the cervix in young castrated sows and heifers. Treatment of the heifers consisted of priming with 15 mg. of diethylstilbestrol for 7-13 days and 750-1300 GPU of relaxin three times daily for 3-5 days. Zarrow measured dilation by inserting aluminum rods of different size into the cervix. The results of this experiment when compared to previous work suggest that the estrogen level is not high enough during estrus to allow cervical dilation by the use of relaxin. However, since no great amount of relaxation was obtained by other investigators, the possibility exists that they only obtained as much relaxation as normally occurs during estrus. This in no way detracts from the value of these experiments but suggests that other factors are important in this problem if complete cervical dilation is to be produced. The extreme dilation of the cervix of 255C is unexplainable, but suggests that considerable dilation may be obtained in the non-pregnant cow if optimum conditions exist.

The use of depot relaxin seemed desirable for several reasons. In the pregnant animal, relaxin is continually present in the blood in small

quantities. This effect can best be simulated by the slowly absorbing depot relaxin. Kliman et al (54) observed the results obtained by using liquid relaxin for the proliferation of the pelvic ligament in mice. They were increased 65 fold by using depot relaxin. On the limited number of cows used for this experiment the expected results were not obtained. Additional experiments using progesterone simutaneously with relaxin may prove of value since progesterone is also present in relatively large quantities during pregnancy. The hourly injections for 24 hours possibly were not of long enough duration since a large amount of dilation was not obtained.

The large amount of cervical relaxation obtained in E152, no relaxation in E106, and only a small amount in E107 plus her death makes it impossible to draw definite conclusions with respect to the value of blood transfusions. Again it has been demonstrated that the cervix may be dilated in a non-pregnant cow. The failure of response in E106 and E107 substantiate the line of thought that a number of factors are involved and conditions must be correct before any great amount of cervical dilation may be produced.

Pretreatment with estrogen appears to be an important factor as both 255C and E152 had been pretreated although with different levels. Both animals had received diethylstilbestrol implants for the same time. These two cows suggests the need for more research work on this line.

The results of the milk letdown response experiment are controversial to those obtained by Shaffhausen et al (76) on sheep. The results obtained in this experiment suggest that 1500 GPU of relaxin will

not produce milk letdown in cattle. Further experiments using higher levels of relaxin may substantiate the work of Shaffhausen et al. The cow is conditioned to be milked and the effect if any from relaxin on the myoepithelium around the alveoli may be present but is over-ridden by the oxytocin secreted during normal milking. This would not be in disagreement with the observations of Shaffhausen et al on sheep. The sheep is not conditioned to being milked and therefore may not have a quantity of oxytocin in the blood stream during milking equal to the level in the blood stream during milking of the cow.

SUMMARY AND CONCLUSIONS

These preliminary experiments were conducted to investigate the effects of relaxin upon the female bovine, with special interest on cervical dilation and milk letdown. From the data collected the following conclusions may be drawn.

1. The cow's cervix may be greatly dilated by the injection of relaxin if the cow is in the proper state of being. However this may not normally be obtained by an injection of relaxin during estrus.
2. The use of depot relaxin over a period of a few weeks did not dilate the cervix. Coinciding treatment with diethylstilbestrol did not enhance the dilation.
3. Hourly injections of relaxin for 24 hours did not produce cervical relaxation of significance in animals which had been pretreated with estrogen for a long period of time.
4. The cow's cervix may be dilated by transfusing blood from a laboring cow into a non-pregnant cow, providing the recipient has been properly pretreated.
5. The injection of 1500 GPU of relaxin into lactating cows will not produce milk letdown.
6. This preliminary work suggests some lines of research which should receive considerable attention.

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APPENDIX

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Appendix — Part One

Appendix, Part One contains the individual trials of injecting relaxin into the cows during estrus. The quantities of relaxin administered are given. Cervical dilation was measured prior to the injection and eight hours later. The inside circumference of the cervical folds was estimated in inches. Measurements were taken of the 1st, 2nd, and 3rd folds. A total of 18 trials were conducted on 9 cows.

Table 1. The Results from Injecting Relaxin upon the Cow's Cervix During Estrus

Cow No.	Date	Injection of relaxin, GPU	Hours	Circumference in inches Dilation of cervix fold 1st	2nd	3rd
255C	1- 4-57	1500	0	2.0		
			8	20.0	20.0	20.0
E155	1- 4-57	3000	0	2.0		
			8	2.0		
E88	1-18-57	1500	0	2.5	2.0	
			8	2.5	2.0	
E155	1-20-57	9000	0	3.75	3.25	
			8	3.75	3.25	
E151	1-21-57	2000	0	5.0	4.0	
			8	5.0	4.0	
E88	1-21-57	15000	0	2.5	2.0	
			8	2.5	2.0	
E106	1-22-57	1500	0	2.0		
			8	2.0		
E86	1-22-57	1500	0	2.5	2.0	
			8	2.5	2.0	
E154	1-30-57	1500	0	2.5	2.0	
			8	2.5	2.0	
E107	1-30-57	1500	0	3.25	2.5	2.0
			8	4.00	2.5	2.0
E125	2- 4-57	1500	0	5.5	5.0	4.0
			8	6.5	5.75	4.75
E107	2-10-57	1500	0	3.75	3.25	2.0
			8	5.00	4.00	2.0
E86	2-10-57	1500	0	2.5	2.0	
			8	2.5	2.0	

Table 1. Continued

Cow No.	Date	Injection of relaxin, GPU	Hours	Circumference in inches Dilation of cervix fold 1st	2nd	3rd
E125	2-12-57	1500	0	5.0	4.0	3.25
			8	5.0	4.0	3.25
E107	2-13-57	1500	0	3.75	3.25	2.0
			8	3.75	3.25	2.0
E151	2-18-57	1500	0	4.0	3.25	2.0
			8	4.0	3.25	2.0
E125	2-25-57	1500	0	3.0	2.5	2.0
			8	3.0	2.5	2.0
E86	2-25-57	1500	0	2.5	2.0	
			8	2.5	2.0	

Appendix — Part Two

This part of the appendix is designed to give detailed data concerning the treatment and results from the injection of liquid and/or depot relaxin upon the cervix of three cows. Estrogen pretreatment of the animals is included. Six tables are used to more clearly illustrate the treatment given. Tables 2 and 6 deal with the treatment and effect of depot relaxin whereas tables 3, 5 and 7 records the observations on the 24 hourly injections of 1200 CPU of liquid relaxin.

Table 2. The Effect of Depot Relaxin Upon E88's Cervix

Date	Injection of relaxin, GPU	Circumference in inches Dilation of cervix fold 1st	2nd	3rd
*	Pretreatment			
2-19-57	3000			
2-21-57	3000			
2-23-57	3000	2.00		
2-25-57	3000	2.00		
2-28-57	3000			
3- 4-57	3000			
3- 7-57	3000			
3- 9-57	3000			
3-11-57	3000			
3-13-57	3000			
3-15-57	6000			
3-18-57	6000			
3-20-57	6000			
3-22-57	6000	3.75	3.25	
3-24-57	9000			
3-26-57	9000			
3-28-57	9000			
3-29-57		3.00	2.50	2.0
3-30-57	9000			
4- 1-57**	15000	3.00	2.50	2.0
4- 4-57		3.00	2.50	2.0
4- 8-57		3.00	2.50	2.0
4-12-57		3.00	2.50	2.0
4-16-57		3.00	2.50	2.0

* 60 mg. per week implants of diethylstilbestrol pellets from 7-5-56 to 9-9-56

** 300,000 units of penicillin G for vaginal infection

Table 3. The Effect of Liquid Relaxin upon ESS's Cervix Over A 24 Hour Period

Hour	Injection of relaxin, GPU	Circumference in inches Dilation of cervix fold 1st	2nd	3rd
4:00 p.m.	1200	3.0	2.5	2.0
5:00 p.m.	1200	3.0	2.5	2.0
6:00 p.m.	1200	3.0	2.5	2.0
7:00 p.m.	1200	3.0	2.5	2.0
8:00 p.m.	1200	3.0	2.5	2.0
9:00 p.m.	1200	3.0	2.5	2.0
10:00 p.m.	1200	3.0	2.5	2.0
11:00 p.m.	1200	3.25	2.5	2.0
12:00 a.m.	1200	3.25	2.5	2.0
1:00 a.m.	1200	3.25	2.5	2.0
2:00 a.m.	1200	3.25	2.5	2.0
3:00 a.m.	1200	3.25	2.5	2.0
4:00 a.m.	1200	3.25	2.5	2.0
5:00 a.m.	1200	3.25	2.5	2.0
6:00 a.m.	1200	3.25	2.5	2.0
7:00 a.m.	1200	3.25	2.5	2.0
8:00 a.m.	1200	3.25	2.5	2.0
9:00 a.m.	1200	3.25	2.5	2.0
10:00 a.m.	1200	3.25	2.5	2.0
11:00 a.m.	1200	3.25	2.5	2.0
12:00 p.m.	1200	3.25	2.5	2.0
1:00 p.m.	1200	3.75	3.25	2.0
2:00 p.m.	1200	3.75	3.25	2.0
3:00 p.m.	1200	3.75	3.25	2.0
4:00 p.m.		3.75	3.25	2.0
5:00 p.m.		3.75	3.25	2.0
6:00 p.m.		3.75	3.25	2.0
7:00 p.m.		3.75	3.25	2.0
8:00 p.m.		3.75	3.25	2.0
9:00 p.m.		3.75	3.25	2.0

Table 4. Diethylstilbestrol Pretreatment of E106

Date	Stilbestrol Mg. Liquid	Pellet	Remarks
2-23-57	120		
2-25-57	120		
2-28-57	120		Transfusion (table 10)
3- 4-57	120		
3- 7-57	120		
3- 9-57	120		
3-11-57	120		
3-13-57	120		
3-15-57	60	60	
3-18-57	60	60	
3-19-57	60	60	
3-20-57	60	60	
3-21-57	60	60	lst fold cir. 2 inches
3-22-57	60	60	
3-23-57	60	60	
3-24-57	60	60	
3-25-57	60	60	
3-26-57	60	60	
3-27-57	60	60	
3-28-57	60	60	
3-29-57	60	60	lst fold cir. 2 inches
3-30-57	60	60	
3-31-57	60	60	
4- 1-57	60	60	300,000 U. penicillin G
4- 2-57	60	60	
4- 3-57	60	60	
4- 4-57	60	60	lst fold cir. 2 inches
4- 5-57	60	60	
4- 6-57	60	60	
4- 7-57	60	60	
4- 8-57	60	60	lst fold cir. 2 inches
4- 9-57	60	60	
4-10-57	60	60	
4-11-57	60	60	
4-12-57	60	60	lst fold cir. 2 inches
4-13-57	60	60	
4-14-57	60	60	
4-15-57	60	60	

Table 5. The Effect of Liquid Relaxin upon E106's Cervix Over A 24 Hour Period

Hour	Injection of relaxin, CPU	Circumference in inches Dilation of cervix fold 1st	2nd	3rd
4:00 p.m.	1200	2.0		
5:00 p.m.	1200	2.0		
6:00 p.m.	1200	2.0		
7:00 p.m.	1200	2.0		
8:00 p.m.	1200	2.0		
9:00 p.m.	1200	2.0		
10:00 p.m.	1200	2.0		
11:00 p.m.	1200	2.0		
12:00 a.m.	1200	2.0		
1:00 a.m.	1200	2.0		
2:00 a.m.	1200	2.0		
3:00 a.m.	1200	2.0		
4:00 a.m.	1200	2.0		
5:00 a.m.	1200	2.0		
6:00 a.m.	1200	2.0		
7:00 a.m.	1200	2.0		
8:00 a.m.	1200	2.0		
9:00 a.m.	1200	2.0		
10:00 a.m.	1200	2.0		
11:00 a.m.	1200	2.0		
12:00 p.m.	1200	2.0		
1:00 p.m.	1200	2.0		
2:00 p.m.	1200	2.0		
3:00 p.m.	1200	2.0		
4:00 p.m.		2.0		
5:00 p.m.		2.0		
6:00 p.m.		2.0		
7:00 p.m.		2.0		
8:00 p.m.		2.0		
9:00 p.m.		2.0		

Table 6. The Effect of Relaxin and Stilbestrol upon E154's Cervix

Date	Stilbestrol Mg. Liquid	Stilbestrol Mg. Pellet	Injection of depot relaxin, GPU	Circumference in inches Dilation of cervix fold 1st	2nd	3rd
2- 8-57	250					
2- 9-57	250			3.0	2.5	2.0
2-10-57	250					
2-11-57	250					
2-12-57	150					
2-13-57	150			(In constant estrus)		
2-14-57	150					
2-15-57	150					
2-17-57	150					
2-18-57*				3.0	2.5	2.0
2-18-57**				3.0	2.5	2.0
2-19-57	150		3000			
2-21-57	150		3000			
2-23-57	150		3000	2.5	2.0	
2-25-57	150		3000	2.5	2.0	
2-26-57				(300,000 units penicillin)		
2-28-57	150		3000			
3- 4-57	150		3000			
3- 7-57	150		3000			
3- 9-57	150		3000			
3-11-57	150		3000			
3-13-57	150		3000			
3-15-57	75	75	6000			
3-18-57	75	75	6000			
3-19-57	75	75				
3-20-57	75	75	6000			
3-21-57	75	75		2.5	2.0	
3-22-57	75	75	6000			
3-23-57	75	75				
3-24-57	75	75	9000			

* 1500 GPU liquid relaxin injected
** Dilation 8 hours later

Table 6. Continued

Date	Stilbestrol Mg. Liquid	Pellet	Injection of depot relaxin, GPU	Circumference in inches Dilation of cervix fold 1st	2nd	3rd
3-25-57	75	75				
3-26-57	75	75	9000			
3-27-57	75	75				
3-28-57	75	75	9000			
3-29-57	75	75		2.0 (Tailhead drawn to one side. Cow stiff)		
3-30-57	75	75	9000			
3-31-57	75	75				
4- 1-57	75	75	15000	2.0 (300000 U. penicillin)		
4- 2-57*	75	75		2.0		
4- 2-57**				2.0		
4- 3-57	75	75				
4- 4-57*	75	75		2.0		
4- 4-57**				2.0		
4- 5-57	75	75				
4- 6-57	75	75				
4- 7-57	75	75				
4- 8-57*	75	75		2.0		
4- 8-57**				2.0		
4- 9-57	75	75				
4-10-57*	75	75		2.0		
4-10-57**				2.0		
4-11-57	75	75				
4-12-57*	75	75		2.0		
4-12-57**				2.0		
4-13-57	75	75				
4-14-57	75	75				
4-15-57	75	75				

* 1500 GPU liquid relaxin injected
** Dilation 8 hours later

Table 7. The Effect of Liquid Relaxin upon E154's Cervix Over A 24 Hour Period

Hour	Injection of relaxin, GPU	Circumference in inches Dilation of cervix fold 1st	2nd	3rd
4:00 p.m.	1200	2.0		
5:00 p.m.	1200	2.0		
6:00 p.m.	1200	2.0		
7:00 p.m.	1200	2.0		
8:00 p.m.	1200	2.0		
9:00 p.m.	1200	2.0		
10:00 p.m.	1200	2.0		
11:00 p.m.	1200	2.5	2.0	
12:00 a.m.	1200	2.5	2.0	
1:00 a.m.	1200	2.5	2.0	
2:00 a.m.	1200	2.5	2.0	
3:00 a.m.	1200	2.5	2.0	
4:00 a.m.	1200	2.5	2.0	
5:00 a.m.	1200	2.5	2.0	
6:00 a.m.	1200	2.5	2.0	
7:00 a.m.	1200	2.5	2.0	
8:00 a.m.	1200	2.5	2.0	
9:00 a.m.	1200	2.5	2.0	
10:00 a.m.	1200	2.5	2.0	
11:00 a.m.	1200	2.5	2.0	
12:00 p.m.	1200	3.25	2.0	
1:00 p.m.	1200	3.25	2.0	
2:00 p.m.	1200	3.25	2.0	
3:00 p.m.	1200	3.25*	2.0	
4:00 p.m.		3.25	2.0	
5:00 p.m.		3.25	2.0	
6:00 p.m.		3.25	2.0	
7:00 p.m.		3.25	2.0	
8:00 p.m.		3.25	2.0	
9:00 p.m.		3.25	2.0	

* 1st fold ruptured

Appendix — Part Three

To insure a more vivid picture of the transfusing of blood from a freshening cow into a recipient, individual transfusions are recorded. For each cow the estrogen pretreatment, experimental procedure and results are included. Cervical dilation is listed in the appendix as the circumference of the inside of the cervical folds in inches for the 1st, 2nd and 3rd folds, e.g. cervix dilation 3.0, 2.5, 2.0 would indicate the 1st fold of the cervix had a circumference of three inches, 2nd fold two and one half inches and the 3rd fold two inches. Other pertinent observations at the different hours are also given.

Table 8. The Transfusion of Blood from E125 into E152

Date. January 16, 1957

A. Pretreatment: 15 mg. diethylstilbestrol implant weekly from July 5, 1956 to September 9, 1956.

B. Experimental Procedure:
1. Withdrew 8000 ml. of blood from E125 starting 90 minutes after she had freshened.
2. Transfused 2000 ml. of E125's blood into E152.
3. Withdrew 3400 ml. of blood from E152 after being transfused with the 2000 ml. of blood from E125.
4. Transfused 6000 ml. of E125's blood into E152.

C. Results from transfusion into E152.

Time	Cervix dilation			Remarks
Before trans.	3.25	2.0		
2 hrs. after trans.	4.00	3.0	2.0	
6 hrs. after trans.	4.25	3.5	2.0	
15 hrs. after trans.	20.00	20.0	20.0	Cervix rather weak in texture, undetermined source of bleeding, perhaps from the uterus
36 hrs. after trans.	20.00	20.0	20.0	Large quantities of clotted blood present in the vagina and cervix
48 hrs. after trans.	20.00	20.0	20.0	Cervix becoming firmer and uterus and cervix and vagina filled with clotted blood
72 hrs. after trans.	20.00	20.0	20.0	Clotted blood present in vagina, cervix and uterus
96 hrs. after trans.	20.00	20.0	20.0	Clotted blood present. Cow off feed (ate grain but not hay or silage)
12 days after trans.	6.5	6.5	6.5	Firm cervix tone with small quantities of blood present

Table 9. The Transfusion of Blood from E152 into E104

Date. January 16, 1957

A. Pretreatment: None

B. Experimental Procedure:
1. Mixed 3000 GPU of relaxin with blood from E152.
2. Transfused 3400 ml. of blood from E152 (see table 8) into E104.
3. Injected 1500 GPU of relaxin into E104 six hours after transfusion.

C. Results from transfusion into E104.

Time	Cervix dilation	
Before transfusion	2.00	
6 hours after transfusion	3.25	2.0

D. Results from injection of 1500 GPU of relaxin.

Time	Cervix dilation	
Before injection	3.25	2.0
8 hours after injection	3.25	2.0

Table 10. The Transfusion of Blood from E157 into E106

Date. February 28, 1957

A. Pretreatment:

2-23-57	120 mg. of liquid Diethylstilbestrol
2-25-57	120 mg. of liquid Diethylstilbestrol
2-28-57	120 mg. of liquid Diethylstilbestrol

B. Experimental Procedure:

1. Withdrew 7230 ml. of blood from E157 starting 25 minutes after she had freshened.
2. Transfused 7000 ml. of E157's blood into E106.

C. Results from transfusion into E106.

Time	Cervix dilation	
Before transfusion	2.0	
8 hours after transfusion	2.5	2.0

Table 11. The Transfusion of Blood from E158 into E107

Date: March 21, 1957

A. Pretreatment:

Date	Stilbestrol Mg. Liquid	Pellet	Remarks
2-23-57	120		
2-25-57*	120		2.5 2.0
2-25-57**			2.5 2.0
2-26-57			300,000 units of Penicillin G
2-28-57	120		
3- 4-57	120		
3- 7-57	120		
3- 9-57	120		
3-11-57	120		
3-13-57	120		
3-15-57	60	60	
3-18-57	60	60	
3-19-57	60	60	
3-20-57	60	60	

* 1500 GPU of relaxin given
** Dilation 8 hours later

B. Experimental Procedure:
1. Withdrew 8000 ml. of blood from E158 starting 10 minutes before she freshened.
2. Transfused 7700 ml. of E158's blood into E107.

C. Results from transfusion into E107.

Transfusion	Cervix dilation		Remarks
Before	2.50	2.00	
6 hours after	3.75	3.25	Sickly
12 hours after	3.75	3.25	Dead. Extremities still warm