

South Dakota State University

## Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange

---

Electronic Theses and Dissertations

---

1962

### A Histological Study of the Effects of Relaxin on the Bovine Cervix

Carroll J. Eggee

Follow this and additional works at: <https://openprairie.sdstate.edu/etd>

---

#### Recommended Citation

Eggee, Carroll J., "A Histological Study of the Effects of Relaxin on the Bovine Cervix" (1962). *Electronic Theses and Dissertations*. 2814.

<https://openprairie.sdstate.edu/etd/2814>

This Thesis - Open Access is brought to you for free and open access by Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. For more information, please contact [michael.biondo@sdstate.edu](mailto:michael.biondo@sdstate.edu).

152  
A HISTOLOGICAL STUDY OF THE EFFECTS OF RELAXIN ON  
THE BOVINE CERVIX

BY

CARROLL J. EGGER

A thesis submitted  
in partial fulfillment of the requirements for the  
degree Master of Science, Department of  
Entomology-Zoology, South Dakota State  
College of Agriculture  
and Mechanic Arts

August, 1962

**A HISTOLOGICAL STUDY OF THE EFFECTS OF RELAXIN ON  
THE BOVINE CERVIX**

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and is 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.

~~Thesis Adviser~~

~~Head of the Major Department~~

2661m

## ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to several persons who contributed to this study in various ways:

To Dr. Arthur E. Dracy, Professor of Dairy Husbandry and the author's thesis advisor, for his suggestions and council throughout the study.

To Miss Nelle A. Hartwig, Professor of Entomology-Zoology, for her suggestions concerning staining techniques.

To Dr. Raymond J. Greb, Associate Professor of Entomology-Zoology, for his aid in mitotic interpretation.

To Gerald W. Clark, Dairy Technical Assistant, for his ideas and encouragement and for making the sample collecting possible.

To Dr. Robert M. Pengra, Associate Professor of Bacteriology, for his assistance in taking and developing pictures.

To Dr. H. E. Calkins, Associate Professor of Bacteriology, for proofreading the rough draft.

To Mrs. Duane Bullis for typing this manuscript.



## TABLE OF CONTENTS

	Page
INTRODUCTION . . . . .	1
OBJECTIVES OF THE STUDY . . . . .	3
REVIEW OF LITERATURE . . . . .	4
EXPERIMENTAL METHOD . . . . .	11
HISTOLOGICAL OBSERVATIONS . . . . .	15
Histological Picture of Untreated Cervical Biopsies . .	15
Effects of Di-ethyl Stilbesterol and Relaxin . . . . .	19
Effects of Stilbesterol, Progesterone and Relaxin . . .	21
Effects of Progesterone and Relaxin . . . . .	23
Effects of Relaxin . . . . .	25
Histological Picture Following Parturition . . . . .	26
DISCUSSION . . . . .	31
SUMMARY AND CONCLUSIONS . . . . .	33
LITERATURE CITED . . . . .	37
APPENDIX . . . . .	39

## LIST OF TABLES

Table	Page
1. Identification Numbers of Cattle and Treatments Consisting of Combinations of Injections of Di-ethyl Stilbesterol, Progesterone and Relaxin . . . . .	11
2. A Series of Biopsies of Nontreated Animals Taken to Observe the Effects of the Estrus Cycle on Cervical Tissue . . . . .	12
3. Biopsies From the Reproductive System of Cow No. 36 at 36 Hours and 38 Days Following Natural Parturition . . . . .	13
4. Mitotic Figures Present in Cervical Biopsies of Animals Primed With Combinations of Di-ethyl Stilbesterol and Progesterone and Injected With Relaxin. These Samples Were Taken Five Hours After Relaxin Injection . . . . .	40
5. Mitotic Figures Present in Cervical Biopsies of Animals Primed With Combinations of Di-ethyl Stilbesterol and Progesterone and Injected With Relaxin. These Samples Were Taken Nine Hours After Relaxin Injection . . . . .	41
6. Mitotic Figures Present in Cervical Biopsies of Untreated Animals and of One Animal Following Parturition. Whole Cross-Sectional Areas of Cervical Tissue Are Also Included . . . . .	42
7. A Comparison of the Quantity of Glycogen Present in Cervical Biopsies of Cattle Sensitized With Various Combinations of Di-ethyl Stilbesterol and Progesterone Followed by Relaxin Injection. These Samples Were Taken Five Hours After the Injection of Relaxin . . . . .	43
8. A Comparison of the Quantity of Glycogen Present in Cervical Biopsies of Cattle Sensitized With Various Combinations of Di-ethyl Stilbesterol and Progesterone Followed by Relaxin Injection. These Samples Were Taken Nine Hours After the Injection of Relaxin . . . . .	44
9. A Comparison of the Quantity of Glycogen Present in Cervical Biopsies of Untreated Animals and of One Animal Following Parturition. Whole Cross-Sectional Areas of Cervical Tissue Are Also Included . . . . .	45

## LIST OF FIGURES

Figure	Page
I. Normal tissue (100 x) . . . . .	29
II. Infiltrated tissue (100 x) . . . . .	29
III. Normal tissue (430 x) . . . . .	29
IV. Infiltrated tissue (430 x) . . . . .	29
V. Modified bone curette . . . . .	30
VI. Cervical fold (100 x) . . . . .	30
VII. Uterine tissue (100 x) . . . . .	30
VIII. Areas of mucus concentration (100 x) . . . . .	30

## INTRODUCTION

The cervix is a thick walled tube between the vagina and the uterus which serves as a passageway for sperm to enter and for the products of birth to be expelled. The tube itself is about four inches long with walls almost one inch thick and is composed of a dense stroma of connective and muscle tissue. There are three heavy collagenous rings, one near the external opening, one adjacent to the internal orifice and one midway between. The cervix, because of its muscular nature, is distinctly marked off from the uterus by the internal os and from the vagina by the external os. Sisson and Grossman (1938) describe the lumen as "spiral, tightly closed and difficult to dilate." The canal is composed of many folds of tissue which run parallel to the canal.

In cross-sectional view the cervix is lined with one layer of columnar epithelial cells, each with a nucleus at its base and mucus droplets in its apical parts. The layer appears to lie in branching folds called "arbor vitae" or "plicae palmatae," Maximow and Bloom (1952).

Beneath the columnar layer one finds the endometrium. To quote Velardo, (1958) who wrote with reference to the primate cervix:

The endometrium, a mucus membrane, is approximately 0.5 cm in thickness at the greatest period of its growth during the proliferative phase. Contained within the endometrium are the stromal cells and uterine glands. These glands lie within and are separated by the endometrial stroma. The cells of the stroma are composed of irregularly stellate processes and contain a conspicuous ovoid nucleus. These stromal cells may

anastomose one with another, but they are intermixed with macrophages, granular leukocytes, and cells of the glandular epithelia.

The innermost layers of myometrium are exclusively muscle cells while the peripheral layers of the muscular mass between the serosa and the muscularis contain numerous elastic connective tissue networks which extend inwardly between the muscular bundles. These elastic fibers plus collagenous elements give the cervix its tough, fibrous consistency.

The increase in diameter at parturition is much too great to be explained simply as stretching of the tissue due to pressure exerted by the advancing fetus. Apparently some other mechanism is involved.

Several investigators have reported that relaxin causes an observable and measurable cervical dilation. Whether this hormone, which has been assayed only in the pregnant female of both humans and animals, is the one which initiates and facilitates the birth process has yet to be determined.

### OBJECTIVES OF THE STUDY

The purpose of this study was to make histological and histochemical comparisons of the effects of relaxin alone and in combination with the sensitizing agents, di-ethyl stilbesterol and progesterone, on the cervix of the nongravid bovine. The results were then compared with normal, nonsensitized, nongravid bovine cervical tissue and with bovine cervical tissue following parturition.

## REVIEW OF LITERATURE

In 1926 F. L. Hisaw, for whom the story of relaxin represents a splendid personal achievement, discovered a substance in the blood of pregnant rabbits which, when injected into virgin guinea pigs during oestrus, caused relaxation of the symphysis pubis to create a condition similar to that of pregnancy (Martin and Schoenbach, 1959). It was reported by Fevold, Hisaw and Meyer in 1930 that the substance appeared in the blood of pregnant rabbits at approximately the seventh day of gestation in sufficient quantity to give a positive test when 2.0 cc of serum were injected into virgin guinea pigs; however activity of the substance disappeared within twelve hours after birth of the young. This substance was found so constantly present and so uniformly detectable that it could be used for pregnancy tests for rabbits. This group of workers also discovered that the animal must first be sensitized by follicular hormones to put the animal in the proper physiological condition to exhibit ligamentous relaxation. The name "relaxin" was proposed because the term "designates its physiological activity and also adheres to the generally accepted nomenclature for hormones."

Fevold, Hisaw and Meyer (1930) extracted the hormone from sow corpora lutea with acid alcohol and purified it by fractional elimination of proteins, phospholipins and other fatty substances to yield a water soluble extract, containing the active principle in concentrated form. The hormone was then obtained by crystallization of the evaporated aqueous extract from glacial acetic acid or by precipitation from solution by means of picric acid.

Albert, Money and Zarrow (1946) eliminated the estrogens and progesterone by extracting them from fresh frozen sow corpora lutea with methanol. They then pressed the residue into semi-dry pellets and extracted the relaxin with water, ammonium sulfate and cold, dilute acid. Albert, Money and Zarrow (1947) reported an improved method of extracting with HCl, precipitating with NaCl and NaOH, adsorbing the substance to bentonite, then dissolving it in pyridine.

Fresh, whole ovaries were found to yield an extract ten times more potent than had previously been obtained, either from fresh corpora lutea or from defatted luteal tissue residue. This finding led Albert and associates to hypothesize that the estrus state of the sow might influence the relaxin content of the ovary.<sup>1</sup> The results verified this hypothesis since ovaries from pregnant animals yielded several hundred times more relaxin activity than those from nonpregnant animals.

Frieden and Hisaw (1950) partially nullified the value of the bentonite method of purification by reporting that the bentonite-purified relaxin did not represent the true relaxative substance of the sow ovary.

Fevold, Hisaw and Meyer (1930) described the hormone as a pseudo-globulin containing about 11 per cent  $N_2$ , soluble in glacial acetic acid, slightly soluble in distilled water and in 95 per cent alcohol, insoluble in 99 per cent alcohol and in other organic solvents. It was soluble in aqueous alkaline or acid solutions. Its activity was impaired in

---

<sup>1</sup> Hitherto only unselected ovaries had been used.



alkaline solutions and was destroyed by trypsin, pepsin, potassium permanganate, heating and drying.

In 1961, Hall defined relaxin as a "water soluble polypeptide of rather low molecular weight, about 13 amino acids having so far been identified."

Fevold, Hisaw and Meyer (1930) wrote as follows:

It is a matter of common knowledge that the pelvis of several species of mammals is modified during pregnancy to facilitate the birth of young. These pelvic modifications commonly involve the ligaments of the symphysis pubis and its ilio-sacral unions in a fashion which enables these bones to move apart and thus increase the diameter of the cervical canal. In some animals only the ilio-sacral unions are changed while in others both these and the symphysis pubis are affected. One of the most striking examples is found in pregnant guinea pigs. Both joints begin to loosen about the middle of pregnancy and become more pronounced with the approach of parturition. At termination of pregnancy the ilia can be freely moved, and a finger can be placed between the pubic bones at the symphysis. The term, for want of a better definition, is called ligamentous relaxation.

In 1930 Fevold and associates proceeded to establish a unit of measurement and designated as a guinea pig unit "the minimum amount of hormone which causes a definite loosening of the ligaments within 10-12 hours after a single injection," using virgin female guinea pigs and subcutaneous route of injection.<sup>2</sup> In 1944, Abramowitz and associates redefined the guinea pig unit as follows: Twelve ovariectomized guinea pigs weighing from 350 to 800 g received estradiol for four days and an injection of relaxin on the fifth. A guinea pig unit was specified as the amount of hormone that produced relaxation as determined by manual

---

<sup>2</sup>This information was reported by Martin and Schoenbach in 1959.

palpation in two thirds of the animals at six hours.

In 1950 Frieden and Hisaw recorded the following:

The relaxin content of pregnant sow ovaries has been examined by Hisaw and Zarrow (1948) who reported values of 10,000 units/g for individual glands collected during the latter part of gestation. They also found that the ovary of the nonpregnant sow contained a small amount (2.5-5.0 units/g) during the luteal stage of the cycle.

Hall (1947) described the changes that took place in the pubic symphysis of the mouse during pregnancy and achieved the same phenomena artificially by the injection of pregnant rabbit serum. She reported that during the last six days of pregnancy, the pelvic bones separated and a ligament 4-6 mm long occupied the interpubic gap. This gap began to close immediately after parturition, and four days later the ligament had shrunk to half its length. The ligament was formed by proliferation of articular hyaline cartilage, readsorption of the bony medial ends of the pubis, lengthening of the ligament by formation of new cartilage and reversion of cartilage to collagenous connective tissue.

In 1960 Hall described the histological changes in the cervix of mice following estrogen sensitization and relaxin injection as a heavy deposition of glycogen in the myometrium (particularly the circular layer), edematous transformation of the endometrium, wide separation of muscle fibers, and thinning of the more loosely woven collagen fibers of the lamina propria. She observed synergism between estradiol and relaxin on the hypertrophy of epithelial cells in the outer cervix but apparently not on muscle enlargement. Heavy infiltration of polymorphonucleocytes was recorded. Augmentation of cell hypertrophy was observed when progesterone was added to the estrogen and relaxin injections.

Frieden and Hisaw (1950), concerned with the possibility of a change in sensitivity following very pronounced relaxation, segregated animals which had recovered from 1-2 units of relaxin into two groups according to their degrees of response. Two weeks later each group received an injection of two units per animal. The total response of the two groups differed only slightly, the variation being no more than that usually encountered between two groups of randomly selected animals.

Frieden (1958) continued the investigation by comparing the responses of guinea pigs to relaxin from pregnant rabbit serum and to relaxin from sows' ovaries before and after repeated injections of the latter. The guinea pigs were injected every other week for four weeks. After  $3\frac{1}{2}$  months a decreased response was noted. Greatest sensitivity to the hormone occurred 2-4 weeks after the primary injection. The sensitivity remained high for two months. Then it began to decline. The refractoriness did not occur unless the dosage was high. It was also noted that the marked (fifteenfold to twentyfold) diminution at three months to sow ovary relaxin was accompanied by (twofold to fourfold) diminution in response to pregnant rabbit serum. This finding plus the observation that the active agent was not agglutinated led the investigators to postulate that the development of the refractory state was due to the formation of an antihormone of limited species specificity.

Graham and Dracy in 1952 sensitized cattle with stilbesterol and injected relaxin at three dosages; 250 G.P.U., 1,500 G.P.U., and 8,500 G.P.U., and obtained cervical dilation of 0.93 inches, 1.27 inches and 1.31 inches respectively. The cervixes of nonestrus control cattle and of those receiving only di-ethyl stilbesterol could not be dilated.

Zarrow, Sikes and Nehen (1954) reported results similar to those obtained by Graham and Dracy in a study using young, castrated sows and heifers. Histological examination revealed depolymerization and increased permeability of the ground substance and increased water content in the cervix of relaxin-treated animals.

Zarrow and Yochim (1961) reported, in rats, a gradual increase of dilatibility, water content, and cervical weight during pregnancy, with a sudden and extreme rise in these factors at parturition. A part of the distensibility at parturition was due to stretching induced by the fetus as it traversed the birth canal. Tensile strength and collagen content of the cervix decreased throughout pregnancy. Within 48 hours after birth distensibility and tensile strength returned to figures comparable to those obtained for nonpregnant rats.

Kroc, Steinetz and Beach (1959) reported parallel increases in cervical dilatibility and water content in response to multiple injections of relaxin in estrogen-primed mice. Mice which had been primed with estradiol followed by a single injection of relaxin showed a rapid transient rise in glycogen which paralleled but was a little longer in duration than the increase in water concentration. Only slight effects were observed in unprimed, relaxin-treated mice. Progesterone appeared to decrease water uptake but to increase glycogen uptake. The addition of progesterone to the primed, relaxin-injected mouse increased cervical dilatibility.

In 1954 Frieden and Martin reported that symphyseal connective tissue of castrated guinea pigs was relatively inert with respect to

both glycine incorporation and oxygen consumption, that estradiol caused a pronounced increase in both, and that relaxin stimulated glycogen incorporation equivalent in amount to that of estrogen. The results of oxygen uptake were inconclusive. Interestingly enough, it was observed that relaxin seemed to stimulate both growth and glycine uptake without estrogen sensitization, even though relaxation did not occur under these conditions.

Frieden (1956) continued the study by injecting 150,000-400,000 CPM/100 gm body weight of glycine- $l$ -C<sup>14</sup> into normal rats and into rats previously treated with estrogen and with relaxin. After 24 hours negligible radioactivity was noted in the symphyseal collagen of the control animals. In estrogen-treated animals glycine uptake was increased threefold to twentyfold, and the administration of relaxin for only two days had an effect approximately the same as that of estrogen, while longer treatment with relaxin brought about an even greater increase. Estrogen and relaxin administered together showed slight augmentation of glycine uptake. Other organs examined (liver, xiphoid process and skeletal muscle) appeared to be unaffected.

## EXPERIMENTAL METHOD

The experimental animals used were all nonpregnant, lactating cattle on a normal ration of alfalfa hay and grain. All were acclimated to and subsequently exposed to the rigors of midwestern weather. The age of the animals and their previous number of calves was unknown. The majority of the animals were Holstein.

The replications were set up as shown in Table 1. Sensitization consisted of a series of three subcutaneous injections of the sensitizing agents, di-ethyl stilbesterol or progesterone or both, given on each of three days immediately preceding the date of the biopsies. Relaxin was given in the morning of the day of biopsy, and the tissue samples were obtained five hours after the administration of the relaxin and again nine hours after the relaxin.

Table 1. Identification Numbers of Cattle and Treatments Consisting of Combinations of Injections of Di-ethyl Stilbesterol, Progesterone and Relaxin

Date <sup>1</sup>	Control Cow No.	Stilb. <sup>2</sup> Relaxin Cow No.	Stilb., Prog. Relaxin Cow No.	Prog. <sup>3</sup> Relaxin Cow No.	Relaxin Cow No.
July 12, 1961	220	2248	986	198	
Dec. 15, 1961	2169	2232	2111	218	
April 6, 1962	986	2169	218	2111	2232
May 7, 1962	2232	36	2169	986	2111
June 8, 1962	222	169	36	2169	2232

<sup>1</sup>Dates are those on which cervical biopsies were taken.

<sup>2</sup>Di-ethyl stilbesterol

<sup>3</sup>Progesterone

Estrogenic effects were achieved by the administration of 20 mg of di-ethyl stilbesterol suspended in cottonseed oil according to the method of Graham (1952) and Graham and Dracy (1952). The stilbesterol was first dissolved in ethyl alcohol. This was then added to cottonseed oil and the mixture heated to 68 degrees centigrade until the alcohol had evaporated.

Fifty mg doses of progesterone were administered in an aqueous vehicle. Progesterone was obtained from The Upjohn Company as a solution containing 25 mg/cc.

The relaxin was administered as a suspension in beeswax containing 1,500 guinea pig units per cc. The material was melted under hot, running water and taken up into a heated hypodermic syringe, which was kept hot by being wrapped in a hot, wet towel to keep the wax from solidifying. The injections were given as quickly as possible after the syringes were filled.

Table 2 shows the identifying numbers of the cattle from which biopsies were taken to determine the histological changes which take place during the estrus cycle.

Table 2. A Series of Biopsies of Nontreated Animals Taken to Observe the Effects of the Estrus Cycle on Cervical Tissue

March 1, 1962	2232	2111	169	986	218
	cow no.	cow no.	cow no.	cow no.	cow no.

Samples were taken from the middle cervix, deep cervix and uterus of an animal 36 hours after parturition. Another sample was taken 38 days following parturition. These samples were taken to compare the changes brought about by parturition with artificially induced cervical dilation.

Table 3. Biopsies From the Reproductive System of Cow No. 36 at 36 Hours and 38 Days Following Natural Parturition

---



---

March 1, 1962 -- 36 hours	middle cervix	deep cervix	uterus
April 6, 1962 -- 38 days	middle cervix		

---



---

Whole cervixes were obtained from four slaughtered animals. A wedge was cut from each in order to study the complete cross-sectional area of the cervix.

A bone curette (Figure V) was modified and extended to an overall length of 18 inches. The instrument was used to obtain samples in the following manner:

1. The cervix was grasped via the rectal wall.
2. The curette was inserted into the cervix via the vagina.
3. The instrument was manipulated until the cutting edge was against the desired portion of the wall of the cervical canal.
4. The handle of the curette was depressed, forcing the cutting edge to move forward.

The samples of approximately 2 x 1 x 1 mm were immediately placed in a mixture of formalin, alcohol and acetic acid. The tissue was



allowed to remain in this for 24 hours, dehydrated, cleared, imbedded, sectioned and affixed to glass slides according to the method of Brauer (1952).

Slides were stained for gross histological study by means of the Harris hematoxylin stain for nuclei, followed by the acidophilic counterstains, eosin, erythrosin, or orange G for the cytoplasmic components.

Mitotic activity was determined after simple staining with iron hematoxylin (Guyer, 1917) which exhibits selectivity for chromosomal components by precipitation of the metal oxide. Mitotic activity was also studied by Foulgen's reagent, the chemical basis of which is described by Davenport (1960).

DNA occurs chiefly in the nuclei where it is conjugated with protein to form a nucleoprotein. This protein is polymerized to form macromolecules which, when subjected to controlled hydrolysis, exposes and renders active aldehyde radicals which can be identified when subjected to Basic Fuschin Stain.

Glycoprotein comparisons were made by employing the Periodic Acid-Schiff's reaction, the chemistry which Davenport also elucidated as follows:

Periodic acid oxidizes the substrate and attacks carbon chains at sites of adjacent hydroxy, hydroxy amino and hydroxy imino groups. The adjacent groups are oxidized to aldehydes, with concomitant breaking of the carbon chains. These free aldehyde groups then react with Schiff's reagent.

The polysaccharide concentration in the tissues was also measured by careful visual colorimetry of slides stained with carmine.

## HISTOLOGICAL OBSERVATIONS

## Histological Picture of Untreated Cervical Biopsies

A columnar epithelial layer was first to be observed in viewing the cross-sectional area of the whole cervix. Beneath it lay a mucosal area of connective, secretory and reticular tissue with cells which displayed large ovoid nuclei. The endothelial layer appeared to be of uniform density with the reticular fibers composed of cellular projections. These fibers were oriented so that they radiated out from the cervical lumen and blended into the muscular cells which were oriented in the same manner. Blood vessels were prominently displayed both in cross-section and in lateral view. They were no larger than capillary size in the endometrium. Deeper areas contained vessels of venule and arteriole size as well as the smaller ones.

The myometrium consisted of compact bundles of smooth muscle fibers surrounding the blood vessels. Between these bundles, fibers passed in the general direction of the cervical canal. Cylindrical bundles of muscle fibers were oriented at right angles to the radial fibers. These cylindrical muscle cells constituted the longitudinal smooth muscle layers. There was a subtle blending of the layers because fibers often passed from one layer into another. The blood vessels were laterally flattened along the axis of the plicae palmitae. This was most apparent in the larger blood vessels.

The deeper reaches of the myometrium indicated a sharper deliniation in muscle pattern. The bundles were markedly separated one from

another and from the long fibrous bundles. Cylindrical packets existed which did not contain a blood vessel at the apex. The muscle layers surrounding the blood vessels were extremely thick and dense. The vessels were no longer laterally flattened. The muscle cell nuclei in this area were of a peculiar nature. They were no longer spindle shaped as was observed in the cells of the more superficial areas but appeared longer and thinner, almost needle-like in appearance. This pattern is similar to the muscle orientation of the uterus. The uterus, however, has no intermuscular connective tissue. There was a gradual transition in muscle arrangement toward the peripheral areas of the myometrium. The longitudinal muscle bundles became smaller in size and fewer in number. The radial fibers became more prominent until they were the sole fibers terminating in the serosa.

The external surface of the cervical tube was moderately folded and contained many tiny irregularities produced by the endings of muscle fibers. In places the squamosal cells of the peritoneum were faintly discerned.

The biopsy was a small piece of tissue adjacent to the cervical lumen. It usually encompassed the end of one plicae palmatae. In some samples a portion of one of the cartilaginous rings was included.

The typical biopsy of the control animals showed a deeply ridged surface lined with columnar epithelial cells. At intervals, "rosettes," of columnar cells possibly of glandular nature could be observed. These were more numerous in deep cervical biopsies than in those from the middle and outer cervix.

The cells of the epithelial layer were columnar with spindle shaped nuclei lying deep in the basal area. The cells occasionally became taller, swollen with mucus which visibly issued from the apical surface of the cell, and the nucleus became ovoid and migrated to a more medial position. Two to three layers of cuboidal epithelial cells could occasionally be observed directly below the columnar tissue. The epithelial layer varied between the secretory and nonsecretory state, depending upon the stage of the estrus cycle.

The endometrial layer ranged from 0.05 to 0.1 cm in depth. The stroma was made up of large irregularly stellate cells with a conspicuous ovoid nucleus. The processes of these cells made up the reticular fibers which connected and supported the blood vessels. The cells toward the lumen of the cervix were large and filled with globules approximately the size of an erythrocyte and to all appearances identical with mucoid globules found in the mucus of the cervical canal. At intervals, in the endometrium, rounded areas of mucoidal activity were observable. The perimeters of these areas were ill-defined with apparent increase in glycoprotein concentration as one moved toward the center of the area. In these islands of increased glycoprotein there was noted the presence of mucoid globules, apparent separation of stromal cells due to a decreased number of reticular nuclei, and an increased affinity of the substance for the counterstain. In these areas the reticular fibers seemed to be squeezed together and elongated, indicating that the mucus was intercellular rather than intracellularly located. The nuclei were not distorted. The deeper endometrial cells

did not give the appearance of globules nor did they appear to be quite as distended as in the more superficial areas. Rather than appearing swollen, they looked flaccid and irregular.

The endometrial blood supply was copious with all of the blood vessels of capillary size with walls one cell layer thick. The vessels were branched to form a thick capillary bed but there appeared to be no coiling as is found in primate endometria.

The transitional area between the endometrium and myometrium showed occasional spindle shaped smooth muscle cells extending into the area of stellate cells. Gradually the ratio of smooth muscle cells to endometrial cells became greater until the area was almost exclusively smooth muscle. The over-all picture appeared to be that of finger-like projections of smooth muscle reaching into the endometrium to form the interface.

The myometrium consisted predominantly of smooth muscle fibers which contained long, spindle shaped nuclei. These fibers were oriented along the axis of the fold terminating in the endometrium at the innermost ridge of the plicae palmatae. The muscle fibers departed from their general pattern to surround the blood vessels. The vessels had a tunica intima one layer thick and were supported by the compact bundles of muscle fibers which surrounded them. Although the blood vessels were quite large, their muscular construction allowed them to be collapsed. Consequently their lateral margins lay in close approximation. In general the vessels were oriented along the same plane as the fold.

One of the control samples showed a section of the cartilaginous ring which was composed of globular noncellular material with areas of nucleate connective tissue running through it. From the thinner, more delicate tissue adjacent to the connective tissue, it may be assumed that collagen was laid down by the reticular cells. A network of collagen appeared to be laid down followed by deposition of a substance with a great affinity for the counterstain. This appeared to be a progressive phenomenon, older tissue containing a greater deposition of the hardening substance. The cartilaginous ring was separated from the cervical lumen by a thin layer of endometrial tissue and the lining layer of columnar epithelium.

#### Effects of Di-ethyl Stilbesterol and Relaxin

Biopsies of cervixes from animals which had been sensitized for three days with di-ethyl stilbesterol and injected with relaxin, at five hours following relaxin, showed a columnar epithelial layer displaying moderate to light secretory activity with the nuclei remaining deep in the base of the cells.

The endometrial layer of this tissue approached 0.10 cm in thickness and indicated the presence of a great deal of fluid in the intercellular spaces. The cells maintained their irregular perimeters and gave no indication of swelling; however the nuclei were greatly enlarged. The projections of the stromal cells were forced into long thin strands as a result of stretching or of extracellular fluid pressure. The endometrial capillaries were enlarged and, in some cases.

were filled with blood.

A wide area existed in which there was a mixture of reticular tissue and smooth muscle fibers. The cells of both types were in a tangled, disrupted state. The muscle cells of this area had changed from a spindle shape to a fibrous pattern with much elongation. The nuclei of these cells were ovoid in shape.

The myometrium showed bundles of cells as well as interstitial cells which were fibrous, long, wavy strands with a slight amount of separation between the individual cells. The nuclei of the muscle cells were ovoid. A preponderance of blood vessels was apparent. The vessels did not, however, appear to be excessively dilated. As one moved deeper into the cervical wall, the muscle stroma gradually became more dense, in some cases approaching the appearance of the nontreated cervix.

At nine hours a picture of secretory epithelial activity was presented. Mucus, occasionally containing large numbers of leucocytes, was in the cervical lumen.

The spaces between the cells of the endometrium were small but the cells appeared to be greatly swollen indicating that the fluid which was extracellular at five hours had moved into the cells at nine hours. The nuclei were large and ovoid. The blood vessels were distended and numerous.

The myometrial cells were elongate, fibrous strands with ovoid nuclei. The blood vessels were greatly distended with blood. The vasodilation may have come from relaxation of the cells which comprised

the walls of the blood vessels or it may have been due to the pressure of an increased volume of blood shunted into the area. The picture of infiltration and cell elongation had extended throughout the tissue.

#### Effects of Stilbesterol. Progesterone and Relaxin

Animals which had been primed for three days with di-ethyl stilbesterol and progesterone, then injected with relaxin, most nearly simulated the natural hormone level of parturition. The epithelial layer appeared to be in a variable condition. In some samples it showed no activity. In others it displayed extreme secretory activity. The endometrial layer showed moderate fluid infiltration both intracellularly and extracellularly. The nuclei of the reticular cells were very large. The rich capillary bed showed extreme vasodilation.

The myometrial muscles seemed to have separated from one another and lengthened from a spindle shape to an elongated fiber. The fibers had become quite disorganized. The size of the nucleus had increased tremendously, indicating that the absorbed fluid had crossed the nuclear membrane. The lateral diameter of the fiber did not appear to have increased; in fact, it may have decreased. The nuclei had enlarged so that they occupied almost the complete width of the fiber. The longitudinal measure of the individual fiber had increased tremendously. The observation of fewer nuclei present per unit area would augment the hypothesis of cell expansion though not necessarily of cell elongation and slenderization.



The myometrium had become a web of gigantic blood vessels filled to capacity. The muscle fibers which made up the vessel walls were elongate. The nuclei were ovoid in shape. The intermuscular connective tissue showed large intercellular spaces. The reticular cell processes of these cells were long and fibrous.

At nine hours following relaxin injections the epithelial layer was nonsecretory. The endometrium was wider than it had appeared at five hours with cavernous extracellular spaces. The cells appeared to have been either stretched excessively or hydrolized. Fragments of membranes remained clumped around the nuclei. The enucleated areas invited speculation that there may have been some lysis of nuclear membranes. In some superficial areas the endometrial reticular rays appeared to be elongated parallel to the irregular cervical folds. The stress of this orientation apparently caused the reticular cell nuclei to elongate. The blood vessels were not dilated.

The cells of the transitional area showed fibrous muscle cells containing ovoid nuclei. The fibers of both reticular and muscle type were thin, stringy, separated from one another, and in a tangled state instead of in regular patterns. The blood vessels were not large. The vessels were filled with blood which showed a high proportion of leucocytes.

The fibers of the myometrium were elongate and separated from one another. No disorganization of muscle pattern or disruption of cells was apparent. The nuclei were ovoid. The blood vessels of the

area were not distended. The deep myometrium showed areas in which the smooth muscle fibers appeared to be shortened and spindle shaped, interspersed with areas of elongate fibrous cells. The nuclei of the deep myometrium were long and spindle shaped.

#### Effects of Progesterone and Relaxin

The biopsies from cattle primed for three days with injections of progesterone followed on the fourth day with an injection of relaxin produced cervical tissue in which the columnar epithelium showed light secretory activity with the nuclei in a basilar position. The columnar cells were compact and showed no distension; however a small amount of mucus issued from the apical surface of the layer.

The endometrium, especially right under the epithelial layer, showed a tremendous increase in fluid. The spaces between the cells were extremely large. The processes of the cells were long and fibrous giving the reticular cells a spider-like appearance. The nuclei were not particularly enlarged, leading one to conclude that the imbibed fluid was extracellular. The relatively few nuclei and fibers per unit area augmented the idea of distortion and fluid infiltration. Dispersed among the endometrial stroma were groups of elongate smooth muscle cells. A rich vascular capillary bed was evident. The vessels contained many leucocytes but were not distended.

The interface between endometrium and myometrium was vague as the cells involved were disrupted.

The myometrial cells had separated so that each one was isolated from all others. The cells were elongate, fibrous, wavy strands of uniform diameter. They were three to four times the length of the control cells and the ends were not tapered as they were in the controls. The nuclei were elongate ovoid. The separation of cells caused them to appear somewhat entangled and disrupted, rather than oriented in the precise patterns of the control samples. In some areas fluid increase was so great as to give the appearance of connective tissue. The infiltration had extended to the bundles adjacent to the blood vessels. In some cases these vessels were distended and engorged with blood which was low in leucocytes.

The infiltration phenomenon was most apparent directly beneath the columnar epithelial layer and became progressively less as one moved away from the superficial area.

At nine hours moderate to heavy secretory activity was displayed by the epithelial layer. The nuclei either elongated or moved into a medial position in the cell.

The endometrial layer was broad with large amounts of fluid in the intercellular spaces. The fibers were stretched parallel to the cervical folds. The capillaries, although large, did not appear to be filled. In some of the samples an invasion of granular leucocytes appeared to have taken place.

The transitional cells were separated from one another, elongated in a fibrous form. The reticular processes were long and fibrous.

The myometrial tissue showed separation of cells and elongation into a fibrous form. The blood vessels were enlarged and full of blood.

In one case the sample showed a lesser degree of infiltration at nine hours than at five hours. The rest of the samples showed activity similar to or greater than at five hours.

#### Effects of Relaxin

A single injection of relaxin produced biopsies at five hours with epithelial layers which were variable in secretory activity.

The endometrial layers were consistently infiltrated. The nuclei of the endometrial cells were extremely thin and appeared to make up most of the cell body. A thin endometrial layer directly below the columnar layer showed cavernous intercellular spaces, but abruptly therein the density increased tremendously. The transition appeared to be due not so much to a change in the consistency of the cells as in the size of the spaces between the reticular rays of the cells.

The deep endometrial tissue gradually blended with the dense muscle fibers. No clearly defined interface between endometrium and myometrium was seen.

The fibers of the myometrium showed a great deal of elongation and a moderate amount of separation. The blood vessels were large but not distended.

At nine hours following the injection of relaxin the epithelial layer presented a picture of light to moderate secretory activity.

The endometrium, although it varied in thickness, was made up of a loose stroma with gigantic intracellular spaces. The reticular fibers were long and numerous and stretched around the areas of intercellular fluid. Cell bodies were infrequent. Isolated areas showed concentrations of mucus.

The transitional area indicated recovery stages. It was composed of tangled, twisted muscle strands and reticular fibers.

The myometrium showed elongated, separated, wavy muscle fibers which retained the pattern characteristic of the control. The connective tissue in vascular areas and the muscle tissue which made up the tunica media of the vessels did not appear to be distended with extracellular or intracellular fluid.

One of the samples at nine hours included a section of the middle cartilagenous ring. It showed an area of dense collagenous material with pockets which indicated an invasion of leucocytes. Others were filled with an extensively vasculated connective tissue. Occasionally a honeycomb of tough collagen could be observed. The collagenous tissue was imbedded in endometrial tissue directly beneath the epithelial layer.

#### Histological Picture Following Parturition

A cervical biopsy was taken from an animal 36 hours after parturition. (See Table 3). No epithelial cells were present except in one

small area where a few rugged, but frayed, individuals remained.

The endometrial layer was very thin, in some areas no deeper than one cell thick. The nuclei of these cells were large and had a great affinity for stain. The cell bodies were thick, and the cellular projections were very short.

The myometrial layer extended almost to the cervical lumen. The cells were thick, short and quite separated from one another. The nuclei were ovoid. The muscle cells were oriented in concentric layers surrounding the cervical canal.

The plicae palmatae were flattened indicating that the cervical folds had temporarily been stretched into nonexistence.

Deeper myometrial areas showed muscle fibers which were extremely elongated.

The fibers differed from those in the myometrium of the treated cow in that the fibers were thicker, the strands did not show the wavy condition resulting from extreme elongation, and the nuclei were long and thin rather than ovoid. At no place in the myometrium did fibers appear to be in bundles. All had elongated and straightened in response to the stretching process. The blood vessels were small and elongated in a circular manner in compliance with the muscular orientation.

A biopsy of the deep cervix at 36 hours after parturition showed an intact, secretory, columnar epithelium, a large amount of cervical mucus and a few leucocytes.

The plicae palmatae were shallow but evident. The exposed surfaces of the folds were extremely irregular indicating normal folding.

The endometrial layer was broad and showed an excess of both intracellular and extracellular fluid.

The myometrial blood vessels, although not unduly distended, were filled with blood. The muscle cells had a long, stringy, wavy, fibrous appearance characteristic of the treated animal. A certain amount of fiber disorganization was also apparent.

The uterine biopsy showed an intact, nonsecretory layer. The endometrium averaged 0.39 cm in thickness. A layer one cell thick beneath the epithelial layer contained gigantic intercellular spaces. Beneath this a denser stroma continued on to terminate abruptly in the myometrium.

The cells of the myometrium were extremely elongate fibers which had separated from one another. The fibers were wavy, but thicker than those in the treated animal.

At 38 days following parturition the cervical columnar epithelium was complete and nonsecretory.

The endometrial area, although unusually wide and infiltrated in one small area, showed complete normality in all other areas.

The myometrial area displayed complete recovery in all respects.

Mitotic activity (See Tables 4, 5 and 6) was indicated by absence of the nuclear membrane, presence of long chromosomal fragments and apparent chromosomes moving toward centrioles at opposite ends of the cell.

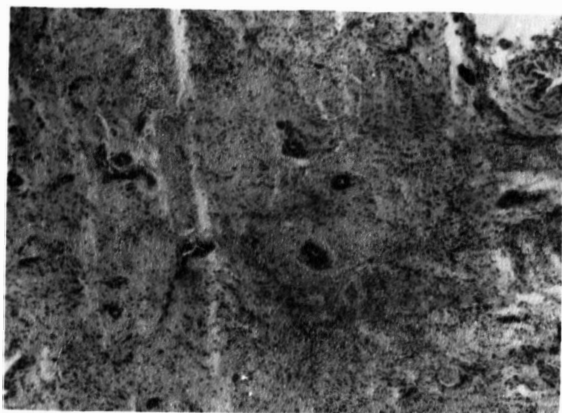


Figure I. Normal tissue  
(100 x)

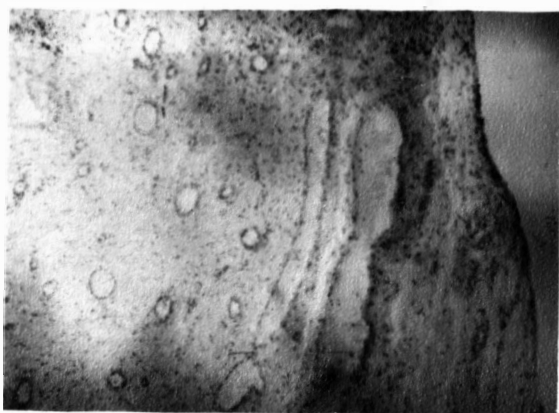


Figure II. Infiltrated tissue  
(100 x)

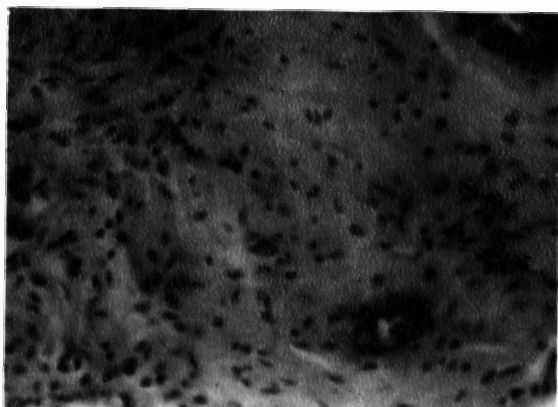


Figure III. Normal tissue  
(430 x)

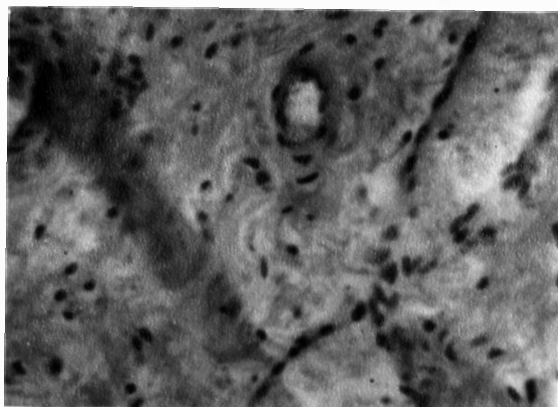


Figure IV. Infiltrated tissue  
(430 x)



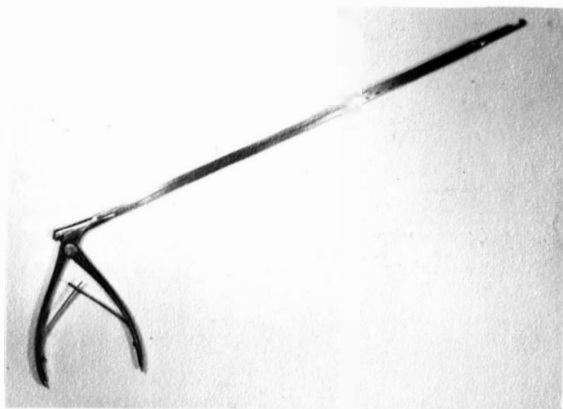


Figure V. Modified bone  
curette

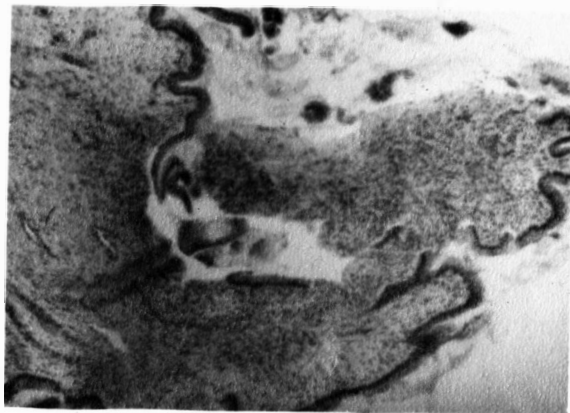


Figure VI. Cervical fold  
(100 x)

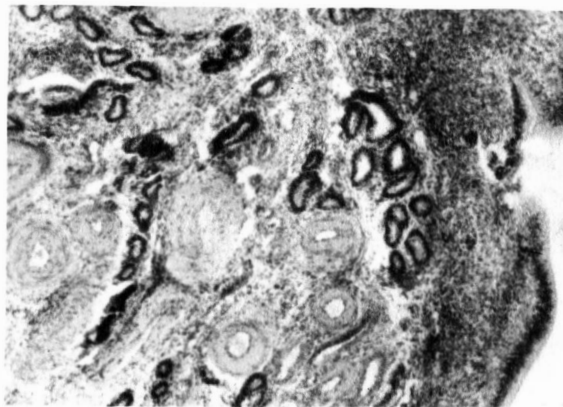


Figure VII. Uterine tissue  
(100 x)

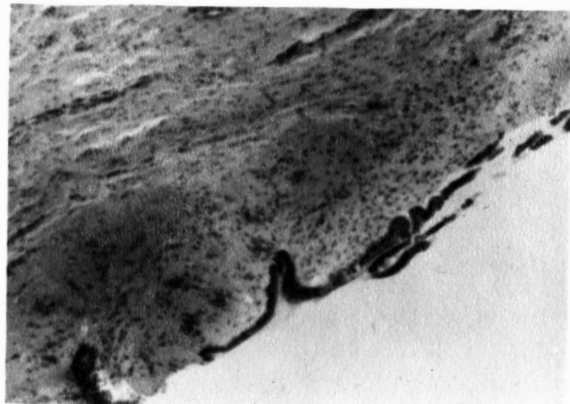


Figure VIII. Areas of mucus  
concentration (100 x)

## DISCUSSION

The effects of trauma due to biopsy were considered and an attempt was made to minimize these by the use of a sharp cutting blade in the hands of a skilled operator. The problem of infection was minimized by cleaning the curette with hot, soapy water and alcohol between samples. In two cases the presence of much mucus and thousands of leucocytes, predominantly neutrophils, in the nine hour sample, indicated either infection or the remote possibility of an extremely augmented estrus period.

According to Cole and Cupps (1959) the uterine columnar epithelial cells are tall and secretory with basal nuclei during proestrus and estrus, and again during an interval between the ninth and twelfth day of the cycle. Between these periods the cells are low with ovoid nuclei. These authors also report uterine edema and cellular elongation during estrus. Results of this study disagree with Cole and Cupps in that cervical columnar epithelial secretory activity is observed to be accompanied by a medial translocation of the nuclei. Also in cervical control samples there is very little fluctuation in anything but the epithelial layer to indicate the stage of the estrus cycle.

The effects of an injection of relaxin in the unprimed cow would not necessarily yield a true picture of relaxin activity for a certain amount of estrogen is always present in the normal animal. Likewise in the stilbesterol or progesterone primed animal, one could not be sure that estrogen or progesterone was not naturally present in sufficient quantity to produce at least a minimal priming effect.

Mitotic activity could only be determined as present or absent because the nuclei were extremely small. It is postulated that many mitotic figures were missed because only those could be detected in which the two future cells were aligned in the same plane. In samples from control and treated cattle at nine hours there was a definite clumping of chromatin granules. Infiltration of fluid, it seems, should have caused a decrease of chromatin granule concentration. If this effect were not normal mitotic activity, possibly the granules were artifacts, products of abnormal conditions.

The estimates of the relative amount of glycoprotein in the sections was not very accurate, as the sole criterion for these estimates was direct visual observation of the slides. See Tables 7, 8 and 9.

In drawing the conclusion that during parturition a change in muscle configuration takes place, it is assumed that relaxin is the substance which prepares the cervix for dilation and that the changes caused by artificial dilation were the same as those caused by natural parturition. This assumption has not yet been proven.

## SUMMARY AND CONCLUSIONS

The mechanism for cervical dilatability is the elongation of the muscle cells and a change in form from a spindle shape with tapered ends to an elongate fibrous strand of uniform diameter. This change is accompanied by separating the cells as a result of imbibition of great amounts of fluid.

The endometrial stroma responds to dilation-producing substances by a vast increase in fluid content which appears to be extracellular in the earlier stages, then gradually moves into the cell. The reticular cell processes become elongate and fibrous in response to the increase of fluid.

The columnar epithelial layer lining the cervical lumen shows variable activity ranging from a nonsecretory state with spindle shaped cells in a basilar position to an extremely active, swollen condition with ovoid cells in a medial position and mucus visibly extruding from the apical surface.

The effects of priming the experimental animal for three days with a combination of di-ethyl stilbesterol and progesterone prior to the relaxin injection, produced an effect greater than when the sensitizing substance had been di-ethyl stilbesterol alone. It might be that the priming effect is a synergy between di-ethyl stilbesterol and progesterone.

The endometrium at five hours was thickened than it had been in the stilbesterol primed animal. The myometrial cells were extensively elongate and separated. Extreme vasodilation was obvious. The

effects at nine hours were still more pronounced.

Injections of progesterone for three days, followed by administration of relaxin, caused effects comparable to those found in the stilbesterol-relaxin injected animal. There was evidence, indicated by one of the nine hour samples, to indicate that the effects of progesterone priming were not as long lasting as when di-ethyl stilbesterol was included.

The injection of relaxin alone, although it produced no obvious cervical dilatibility, did produce histological changes similar to those produced by either the stilbesterol or progesterone primed animal. The effects, however, were more transitory with recovery stages obvious nine hours after the injection.

In each case fluid infiltration was greatest immediately beneath the epithelial layer and gradually became less obvious as the distance from the cervical lumen increased. The samples at nine hours showed the fluid infiltration to be much more extensive. The muscle bundles adjacent to the tunica intima of the blood vessels appear last and least affected.

The birth process caused changes which were somewhat more organized and purposeful than those which occurred in the artificially dilated animal. This was possibly due to the intense stretching of the cervix caused by pressure of the advancing fetus. During the birth process the cervical folds had responded by flattening; thus stretching the endometrial layer almost into nonexistence. The epithelial layer had been scraped off with exception of a few small areas. The efficient

arrangement of muscle cells had caused all of them, including those surrounding the blood vessels, to elongate and orient themselves in a circular layer. The blood vessels were small and elongate in compliance with the muscular orientation. The lower cervical myometrial cells fit nicely into place with no disorganization or tangling of cells.

A deep cervical sample showed fibrous disorganization common to the myometrial-endometrial interface of all injected animals.

Twenty-eight days after parturition the cervix had returned to its normal histological appearance.

The observed effects of parturition were somewhat different in that there was fibrous disorganization only in the transition area between the endometrium and myometrium. The cells of the myometrium were noticeably shorter and thicker, and each cell fit into its proper pattern. There was no disorganization of cells due to excess length of the fibers.

The study of mitotic figures as indicated by data from Tables 4, 5 and 6 showed that little mitotic activity was present in the control cervix but that a greater amount was present in nine hour controls and in injected animals. Whether the effect was due to trauma or to hormones was inconclusive. Much more study needs be done.

Histologically, the injection of relaxin caused major changes which were the same whether given alone or preceded by either progesterone or stilbesterol or both. The most obvious effects were fluid infiltration and muscle cell conversion from a spindle to a fibrous condition. This effect extended also to some of the reticular fibers

of the endometrium.

Maximow and Bloom reported in 1952 that parturition caused cells to elongate by water absorption. This is partially but not totally true. In addition to separation of cells caused by increased fluid content there is an obvious change in form of the muscle cells. They change from a short, spindle shaped fiber, broad in the center and tapered at the ends, to a long fiber of uniform diameter throughout. This happens even when the fibers are not stretched as is proven by the wavy, tangled, disorganized appearance of the fibers. This disarrangement of fibers is not so obvious following true parturition because the pressure of the fetus distends the tube and keeps the muscle fibers taut.

Since there is obvious change in cell form which is not caused by fluid pressure at the actual stress of dilation, it may be daringly postulated that a change in the electrolyte balance of the absorbed fluid causes a chemical reaction which changes the configuration of the muscle myosin.

Mechanical, cervical dilatability and histological changes are independent of one another. Muscle elongation takes place with a single injection of relaxin, or with a single relaxin injection preceded by three progesterone injections, even though the cervix cannot be measurably dilated under these circumstances.

## LITERATURE CITED

- Abramowitz, A. A., W. L. Money, R. V. N. Talmage, L. H. Kleinholtz, and F. L. Hisaw. 1944. Preparation, biological assay and properties of relaxin. *Endocrinology* 34:103-114.
- Albert, A., W. L. Money, and M. X. Zarrow. 1946. Preparation of relaxin from sow corpora lutea residue. *Endocrinology* 39:270-271.
- \_\_\_\_\_. 1947. An improved method of extraction and purification of relaxin from fresh whole ovaries of the sow. *Endocrinology* 40:370-374.
- Brauer, A. 1952. *Histological techniques*. Burgess Publishing Co., Minneapolis. 47 p.
- Cole, H. H., and P. T. Cupps. 1959. *Reproduction in domestic animals*. Academic Press, New York. 651 p.
- Davenport, H. A. 1960. *Histological and histochemical techniques*. W. B. Saunders Co., Philadelphia. 401 p.
- Fevold, H. L., F. L. Hisaw, and R. K. Meyer. 1930. The relaxative hormone of the corpus luteum. Its purification and concentration. *J. Am. Chem. Soc.* 52:3340-3348.
- Frieden, E. H. 1956. The effects of estrogen and relaxin upon the uptake of glycine-1-C<sup>14</sup> by connective tissue in vivo. *Endocrinology* 59:69-73.
- \_\_\_\_\_. 1958. Non-steroid ovarian hormones: observations on the mechanism of the acquisition of relaxin resistance in guinea pigs. *Endocrinology* 62:41-46.
- Frieden, E. H., and F. L. Hisaw. 1950. The purification of relaxin. *Arch. Biochem.* 29:166-178.
- Frieden, E. H., and A. S. Martin. 1954. Uptake of glycine-1-C<sup>14</sup> by connective tissue. Effects of estrogen and relaxin. *J. Biol. Chem.* 207:133-142.
- Graham, E. F. 1952. Some mechanical and hormonal methods of dilating the bovine cervix. M. S. Thesis, S. Dak. State College.
- Graham, E. F., and A. E. Dracy. 1952. The effects of relaxin on the cow's cervix. *J. Dairy Sci.* 35:499.



- Guyer, M. F. 1917. *Animal micrology*. U. of Chicago Press, Chicago. 289 p.
- Hall, D. 1947. The effects of pregnancy and relaxin on the histology of the pubic symphysis in the mouse. *J. of Endocrinology* 5: 174-182.
- \_\_\_\_\_. 1960. Modification by relaxin of the response of the reproductive tract of mice to oestradiol and progesterone. *J. of Endocrinology* 20:355-364.
- \_\_\_\_\_. 1961. The present state of relaxin studies. *J. of Endocrinology* 22:22-23.
- Kroc, R. L., B. G. Steinetz, and V. L. Beach. 1959. The effects of estrogens, progestagens, and relaxin in pregnant and nonpregnant laboratory rodents. *Ann. N. Y. Acad. Sci.* 75:942-980.
- Martin, G. J., and U. Schoenbach. 1959. Historical aspects of relaxin. *Ann. N. Y. Acad. Sci.* 75:923-929.
- Maximow, A. A., and W. Bloom. 1952. *Textbook of Histology*. W. B. Saunders Co., Philadelphia. 616 p.
- Sisson, S., and J. Grossman. 1938. *Anatomy of the domestic animals*. W. B. Saunders Co., Philadelphia. 972 p.
- Velardo, J. T. 1958. *The endocrinology of reproduction*. Oxford U. Press, New York. 340 p.
- Zarrow, M. X., D. Sikes, and G. M. Nehen. 1954. Effects of relaxin on the uterine cervix and vulva of young, castrated sows and heifers. *Am. J. of Physiology* 178-9:687.
- Zarrow, M. X., and J. Yochim. 1961. Dilation of the uterine cervix of the rat and accompanying changes during the estrus cycle, pregnancy and following treatment with estradiol, progesterone and relaxin. *Endocrinology* 69:292-304.

## APPENDIX

Table 4. Mitotic Figures Present in Cervical Biopsies of Animals Primed With Combinations of Di-ethyl Stilbesterol and Progesterone and Injected With Relaxin. These Samples Were Taken Five Hours After Relaxin Injection.<sup>1</sup>

Dates	Control	S-R <sup>2</sup>	S-P-R <sup>3</sup>	P-R <sup>4</sup>	R <sup>5</sup>
Dec. 15, 1961	Absent	Possibly one	Absent	Absent	---
April 6, 1962	Possibly 2-3	Present	Present	Present	Absent
May 7, 1962	Definitely one	Present	Present	Absent	Present
June 8, 1962	Absent	Present	Present	Absent	Absent

<sup>1</sup>The stains used for these observations were Feulgen's and Iron Hematoxylin.

<sup>2</sup>Injections of 20 mg di-ethyl stilbesterol for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>3</sup>Injections of 20 mg di-ethyl stilbesterol and 50 mg progesterone for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>4</sup>Injections of 50 mg progesterone for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>5</sup>A single injection of 1,500 G.P.U. of relaxin.

Table 5. Mitotic Figures Present in Cervical Biopsies of Animals Primed With Combinations of Di-ethyl Stilbesterol and Progesterone and Injected With Relaxin. These Samples Were Taken Nine Hours After Relaxin Injection.<sup>1</sup>

Dates	Control	S-R <sup>2</sup>	S-P-R <sup>3</sup>	P-R <sup>4</sup>	R <sup>5</sup>
Dec. 15, 1961	Present	Present	Present	Present	---
April 6, 1962	Possibly 2-3	Present	Present	Present	Present
May 7, 1962	Present	Absent	Present	Present	Present
June 8, 1962	---	Present	Present	Absent	Present

<sup>1</sup>The stains used for these observations were Feulgen's and Iron Hematoxylin.

<sup>2</sup>Injections of 20 mg di-ethyl stilbesterol for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>3</sup>Injections of 20 mg di-ethyl stilbesterol and 50 mg progesterone for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>4</sup>Injections of 50 mg progesterone for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>5</sup>A single injection of 1,500 G.P.U. of relaxin.

Table 6. Mitotic Figures Present in Cervical Biopsies of Untreated Animals and of One Animal Following Parturition. Whole Cross-Sectional Areas of Cervical Tissue Are Also Included.<sup>1</sup>

Control	2232	2111	169	986	218
March 1, 1962	Absent	Absent	Absent	Absent	Absent
Parturition	Cervix	Deep cervix	Uterus		
March 1, 1962	Present	Present	Present		
Parturition	Cervix				
April 6, 1962	Present				
Whole cervix	1	2			
April 23, 1962	Possibly a few	Possibly a few			
Whole cervix	3	4	5		
June 12, 1962	Absent	Absent	Absent		

<sup>1</sup>The stains used for these observations were Feulgen's and Iron Hematoxylin.

Table 7. A Comparison of the Quantity of Glycogen Present in Cervical Biopsies of Cattle Sensitized With Various Combinations of Di-ethyl Stilbesterol and Progesterone Followed by Relaxin Injection. These Samples Were Taken Five Hours After the Injection of Relaxin.<sup>1-2</sup>

Dates	Control	S-P <sup>3</sup>	S-P-R <sup>4</sup>	P-R <sup>5</sup>	R <sup>6</sup>
Dec. 15, 1961	2	4	4	3.5	---
April 6, 1962	2.5	1	3	1	2
May 7, 1962	3.5	3	1	2.5	2.5
June 8, 1962	4	3	3	2	2

<sup>1</sup> An increasing visual scale of 1-4 (from very light to very dark) was employed.

<sup>2</sup> The stains used for these observations were carmine and Periodic Acid-Schiff's Reagent.

<sup>3</sup> Injections of 20 mg di-ethyl stilbesterol for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>4</sup> Injections of 20 mg di-ethyl stilbesterol and 50 mg progesterone for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>5</sup> Injections of 50 mg progesterone for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>6</sup> A single injection of 1,500 G.P.U. of relaxin.

Table 8. A Comparison of the Quantity of Glycogen Present in Cervical Biopsies of Cattle Sensitized With Various Combinations of Di-ethyl Stilbesterol and Progesterone Followed by Relaxin Injection. These Samples Were Taken Nine Hours After the Injection of Relaxin.<sup>1-2</sup>

Dates	Control	S-P <sup>3</sup>	S-P-R <sup>4</sup>	P-R <sup>5</sup>	R <sup>6</sup>
Dec. 15, 1961	3	2	3.5	2.5	---
April 6, 1962	2	1	1	2.5	1.5
May 7, 1962	2	1	3	1	2
June 8, 1962	---	2	3	2	2

<sup>1</sup> An increasing visual scale of 1-4 (from very light to very dark) was employed.

<sup>2</sup> The stains used for these observations were carmine and Periodic Acid-Schiff's Reagent.

<sup>3</sup> Injections of 20 mg di-ethyl stilbesterol for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>4</sup> Injections of 20 mg di-ethyl stilbesterol and 50 mg progesterone for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>5</sup> Injections of 50 mg progesterone for three consecutive days followed by 1,500 G.P.U. of relaxin on the fourth day.

<sup>6</sup> A single injection of 1,500 G.P.U. of relaxin.

Table 9. A Comparison of the Quantity of Glycogen Present in Cervical Biopsies of Untreated Animals and of One Animal Following Parturition. Whole Cross-Sectional Areas of Cervical Tissue Are Also Included.<sup>1-2</sup>

Control					
March 1, 1962	2	2	2.5	3	3.5
Parturition	Cervix	Deep cervix	Uterus		
March 1, 1962	2.5	3	1		
Parturition	Cervix				
April 6, 1962	3				
Whole Cervix	1	2			
April 23, 1962	2	2			
Whole Cervix	3	4	5		
June 12, 1962	2.5	3	3		

<sup>1</sup> An increasing visual scale of 1-4 (from very light to very dark) was employed.

<sup>2</sup> The stains used for these observations were carmine and Periodic Acid-Schiff's Reagent.