Histological Study of Effects of Relaxin on the Bovine Cervix

CARROLL J. EGGE and ARTHUR E. DRACY
Department of Zoology and Department of Dairy Science,
South Dakota State University, Brookings

Abstract
Cattle were treated with relaxin following sensitization with progesterone, diethylstilbestrol, or both, and a histological study of cervical biopsies undertaken to determine the cellular changes produced by relaxin. Results were then compared with cervical changes which occur during natural parturition.

Cervical tissue from cattle injected with relaxin following sensitization with progesterone showed muscle fiber elongation and a small amount of fluid infiltration; those with relaxin following injections of diethylstilbestrol showed more pronounced infiltration; and cattle which received relaxin after being primed with both progesterone and diethylstilbestrol showed muscle fiber elongation and a large amount of fluid uptake, with consequent relaxation and dilatation of the cervical passage. Cattle which received only relaxin showed muscle fiber elongation but no fluid increase. Cervical tissue from an animal following normal parturition yielded results comparable to cervical tissues treated with relaxin following sensitization with progesterone and diethylstilbestrol.

In 1926, F. L. Hisaw (9) discovered a substance which, when injected into virgin guinea pigs during estrus, caused relaxation of the symphysis pubis, to create a condition similar to that of pregnancy. It was reported by Fevold, Hisaw, and Meyer (3) in 1930 that assayable quantities of relaxin appeared in the blood of pregnant rabbits on approximately the seventh day of gestation. Its activity could be detected until 12 hr after birth of the young. These workers also discovered that the animal must first be sensitized by follicular hormones, to put it in the proper physiological condition to exhibit ligamentous relaxation. Albert, Money, and Zarrow (1) observed that ovaries from pregnant sows yielded several hundred times more relaxin activity than those from non-pregnant animals. In 1947, Hall (7) reported that in the mouse the pelvic bones separated during the last six days of pregnancy, and that a ligament 4-6 mm long occupied the interpubic gap. Cervical changes in mice following estrogen sensitization and relaxin injection were described by Hall (8) as a heavy desposition of glycogen in the myometrium (particularly in the circular layer), edematous transformation of the endometrium, wide separation of muscle fiber, and thinning of the more loosely woven collagen fibers of the lamina propria. Synergism was observed between estradiol and relaxin on the hypertrophy of epithelial cells in the outer cervix, but apparently not on muscle enlargement. Augmentation of cell hypertrophy was observed when progesterone was added to the estrogen and relaxin injections. Graham and Draey (5) sensitized cattle with stilbestrol and injected relaxin at three dosages: 250, 1,500, and 8,500 G.P.U., and obtained cervical dilatation of 0.93, 1.27, and 1.31 in., respectively. The cervices of nonestrous control cattle and of those receiving only diethylstilbestrol could not be dilated. Zarrow, Sikes, and Nehen (10) reported results similar to those obtained by Graham and Dracy, in a study using young castrated cows and heifers. Zarrow and Yochim (11) reported a gradual increase in relaxin effects in rats, with a sudden and extreme rise in these factors at parturition. The effects were no longer evident 48 hr after parturition.

Experimental Procedure
The experimental animals used were all non-pregnant, lactating cattle on a normal ration of alfalfa and grain. The majority of the animals were Holstein. None of these had calved for at least a year.

Cervical biopsies were first taken from five cattle, to determine the endometrial and myometrial changes which occur during the normal estrus cycle. These workers also discovered that the animal must first be sensitized by follicular hormones, to put it in the proper physiological condition to exhibit ligamentous relaxation. Albert, Money, and Zarrow (1) observed that ovaries from pregnant sows yielded several hundred times more relaxin activity than those from non-pregnant animals. In 1947, Hall (7) reported that in the mouse the pelvic bones separated during the last six days of pregnancy, and that a ligament 4-6 mm long occupied the interpubic gap. Cervical changes in mice following estrogen sensitization and relaxin injection were described by Hall (8) as a heavy desposition of glycogen in the myometrium (particularly in the circular layer), edematous transformation of the endometrium, wide separation of muscle fiber, and thinning of the more loosely woven collagen fibers of the lamina propria. Synergism was observed between estradiol and relaxin on the hypertrophy of epithelial cells in the outer cervix, but apparently not on muscle enlargement. Augmentation of cell hypertrophy was observed when progesterone was added to the estrogen and relaxin injections. Graham and Dracy (5) sensitized cattle with stilbestrol and injected relaxin at three dosages: 250, 1,500, and 8,500 G.P.U., and obtained cervical dilatation of 0.93, 1.27, and 1.31 in., respectively. The cervices of nonestrous control cattle and of those receiving only diethylstilbestrol could not be dilated. Zarrow, Sikes, and Nehen (10) reported results similar to those obtained by Graham and Dracy, in a study using young castrated cows and heifers. Zarrow and Yochim (11) reported a gradual increase in relaxin effects in rats, with a sudden and extreme rise in these factors at parturition. The effects were no longer evident 48 hr after parturition.

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Five groups of five cattle each were then treated in the following manner: One animal received 20 mg diethylstilbestrol per day for three days and 1,500 guinea pig units of relaxin on the fourth day; one received 50 mg progesterone per day, followed by 1,500 G.P.U. of
relaxin on the fourth day; one received both diethylstilbestrol and progesterone followed by relaxin; one animal received only relaxin; and the fifth animal was a control. Several animals were used more than once; however, these animals were given several months to establish a normal pattern before reuse. Relaxin was given on the morning of the day of biopsy; tissue samples were obtained 5 hr after administration of relaxin and again 9 hr after relaxin. Estrogenic effects were obtained by administration of diethylstilbestrol suspended in cottonseed oil in a concentration of 20 mg per milliliter, according to the method of Graham (4) and Graham and Dracy (5). Progesterone in an aqueous solution of a concentration of 25 mg/ml was administered in 50-mg doses. Relaxin was administered as a suspension in beeswax containing 1,500 guinea pig units per milliliter. The suspension was melted under hot, running water, drawn into a heated hypodermic syringe, and wrapped in a hot, wet towel to prevent the wax from solidifying. Biopsies were obtained with a bone curette modified and extended to an overall length of 18 in.

Slides were fixed in F.A.A. and stained for gross histological study with Harris hematoxylin and eosin, erythrosin, and orange G counterstains. Mitotic activity was observed by staining with iron hematoxylin according to Guyer (6), and Feulgen's reagent according to Davenport (2). Glycoprotein comparisons were made by employing the periodic acid-Schiff's reaction elucidated by Davenport (2).

**Results and Discussion**

A general cross-sectional view of a nontreated cervix (Figures 1 and 3) showed a much-
folded tube lined by a columnar epithelial layer in various states of secretory activity, depending upon the stage of the estrous cycle. In places, rosettes of columnar cells could be observed. Lying adjacent to the epithelium was an endometrium of connective, secretory, and reticular tissue containing a rich capillary network (Figure 1). The myometrium consisted of compact bundles of spindle-shaped cells, often with a blood vessel in the center (Figure 3). Radial fibers separated these cylindrical bundles. The fibers passed from one bundle to another close to the cervical lumen, but became more sharply delineated and the cells appeared longer and thinner as the distance from the cervical passage increased. The external surface of the cervical tube was somewhat folded and showed many tiny irregularities produced by muscle fiber endings. One of the control samples showed a portion of the collagenous ring.

Biopsies from cattle primed for three days with injections of progesterone, followed on the fourth day with an injection of relaxin, resulted in tissue, after 5 hr, in which the columnar epithelium showed light secretory activity. A small amount of mucus was free at the apical surface. The nuclei were in a basilar position. The endometrium showed a tremendous increase in extracellular fluid. A rich capillary bed was evident. The vessels contained many leucocytes, but were not distended. Myometrial cells were separated, and had elongated to three to four times the length of control cells. They no longer appeared spindle-shaped. The cells were separated and the fibers appeared tangled and disrupted rather than oriented in the precise patterns of control samples. In some cases myometrial blood vessels were distended and engorged with blood low in leucocytes. At 9 hr, moderate to heavy secretory activity was displayed by the epithelial layer, with the nuclei moving into the cells at 9 hr. Endometrial fluid increase had resulted in the fibers stretching in a direction parallel to the cervical folds. In some samples an invasion of granular leucocytes appeared to have taken place. Myometrial tissue showed separation and elongation of cells. Some blood vessels were enlarged and full of blood. In one exceptional case the sample showed a lesser degree of infiltration at 9 hr than at 5 hr.

Biopsies of cervixes from animals sensitized for three days with diethylstilbestrol and progesterone, then injected with relaxin, at 5 hr following relaxin showed a columnar epithelial layer displaying light to moderate secretory activity. The nuclei remained deep in the base of the cells. The endometrial layer which at times was .01 mm in thickness indicated the presence of great quantities of extracellular fluid. The irregularly shaped reticular cells gave no evidence of swelling; however, the nuclei were greatly enlarged. The cellular projections were forced into long, thin strands, possibly due to extracellular fluid pressure. Endometrial capillaries were enlarged and, in some cases, filled with blood. Vasodilation may have come from relaxation of supporting cells or it may have been due to the pressure of an increased volume of blood shunted to the area. Both reticular cells and smooth muscle cells in the transition area between the endometrium and myometrium were in a tangled, disrupted state. The muscle cells of this area had changed from spindle shape to a fibrous pattern with much elongation. The nuclei had changed from round to ovoid and they were enlarged. The myometrial cells had become long, wavy strands with slight separation between individual cells. Blood vessels were numerous but not excessively dilated. Infiltration decreased as distance from the cervical passage increased. At 9 hr the columnar epithelium showed secretory activity. Mucus and leucocytes were present in the cervical lumen. The fluid which was extracellular at 5 hr had moved into the cells at 9 hr. Both cells and nuclei were enlarged. Blood vessels were numerous and distended. Myometrial cells were elongate, fibrous strands with ovoid nuclei, and blood vessels were greatly distended. The picture of infiltration and cellular elongation had extended throughout the tissue.

Tissue from animals primed for three days with diethylstilbestrol and progesterone, then injected with relaxin, most closely resembled the natural conditions of parturition. Secretory activity in the epithelial layer was variable. The endometrium showed moderate intracellular and extracellular infiltration. The capillary bed was extremely dilated (see Figures 7 and 8). Myometrial fibers were elongated, separated, and disorganized. The nuclei of the muscle cells had increased, to occupy almost the complete width of the fiber, which had decreased in diameter as it elongated. The blood vessels were gigantic and filled to capacity. At 9 hr the epithelial layer was none secretory. The endometrium had increased in width and had cavernous extracellular spaces. The cells appeared to have been stretched excessively. Fragments of membranes remained clumped around nuclei, whereas large enucleate areas invited speculation that there may have been some lysis of nuclear membranes.

Endometrial reticular processes near the cervical lumen were elongate in a direction parallel to the cervical folds. Blood vessels were not dilated. The mixed muscle and reticular cells between the endometrium and myometrium were...
thin, stringy, separated from one another, and in a tangled state instead of a regular pattern. Blood vessels were not dilated but were filled with blood and contained a large number of leukocytes. Myometrial fibers were elongate and separated but not disorganized. No vasodilation was evident. As the distance from the cervical passage increased, infiltration became less apparent.

Five hours after a single injection of relaxin the epithelium of different samples showed such variability that no pattern could be established; in all cases, however, the thin endometrial layer directly below the columnar layer showed extremely large intercellular spaces, indicating a great deal of fluid uptake (Figure 2). On the other hand, the myometrium appeared to have a very small amount of fluid infiltration (Figure 4). Its muscle fibers had elongated tremendously, a reaction apparently independent of any stretching in response to fluid uptake. Since these fibers had no place to go, they were twisted, turned, and distorted. The small myofibrils of these muscles had lengthened and twisted, giving a total picture of disorganization. The blood vessels were large but not distended. After 9 hr the epithelium presented a picture of light to moderate secretory activity. The endometrium was made up of loose stroma with gigantic extracellular spaces (Figure 2). Isolated areas showed concentrations of mucus. The transition area and myometrium indicated recovery with
elongate, separate, wavy muscle fibers which had a pattern characteristic of the control. Blood vessels were not distended.

A cervical biopsy was taken from an animal 36 hr after parturition (Figures 5 and 6). Epithelial tissue remained in only one small area and the endometrial layer was extremely thin, possibly because the birth canal was enlarged. The cells making up the endometrial reticulum had short projections and large nuclei. Cervical folds were no longer evident and muscle cells, rather than being arranged in bundles with a blood vessel at the apex, were oriented as a circular layer around the cervical canal, with subsequent flattening of the blood vessels. The fibers differed from those in the myometrium of treated animals, in that they were thicker, the strands did not show the disorganized condition resulting from extreme elongation, and the nuclei were spindle-shaped 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. At 38 days following parturition, recovery was complete.

The effects of an injection of relaxin in the unprimed cow would not necessarily yield a true picture of relaxin activity, because a certain amount of estrogen is always present in the normal animal. This would also hold true for the stilbestrol and progesterone-primed animal. The injection of relaxin alone, although it produced no obvious cervical dilation, did produce histological changes in the muscle cells similar to those produced by the primed animals. The effects, however, were more transitory, with recovery stages obvious 9 hr after injection. Sensitizing experimental animals for three days with a combination of diethylstilbestrol and progesterone prior to the relaxin injection produced an effect greater than when the sensitizing substance had been diethylstilbestrol alone, possibly because of a synergistic reaction between the two hormones. In all cases tissue fluid infiltration was greatest immediately beneath the epithelial layer and it gradually moved out from the cervical lumen. The birth process caused changes somewhat more purposeful than those which occurred in the artificially dilated animal. During the birth process all muscles, including those surrounding the blood vessels, elongated and oriented themselves in a circular layer. Blood vessels were small, and the lower cervical myometrial cells fit nicely into place with no disorganization or tangling of fibers. The study of mitotic figures showed little activity in the control cervix, but a greater amount was present in injected animals. Whether the effect was due to trauma or to hormones was not evident.

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References