

## THE EFFECT OF RELAXIN AND MECHANICAL DILATION ON THE BOVINE CERVIX<sup>1</sup>

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If the transfer of ova is to become practical, simple non-surgical techniques must be employed. One of the greatest obstacles encountered in nonsurgical techniques for obtaining ova is the difficulty of dilating the cervix at the appropriate time to allow the use of instruments in the uterus. To facilitate entrance into the uterus, some mechanical or hormonal means of dilating the cervix is desirable. Experiments on small laboratory animals suggest the possibility of using relaxin, which has the profound effect of relaxing the symphysis pubis (1, 2, 4, 5, 7) and may relax the cervical muscle. However, according to Hisaw (6) the animal must be under the influence of the follicular hormone before relaxin can produce symphyseal relaxation. At that time, Hisaw did not know that corpus luteum produced both progesterone and relaxin. Later work by Albert, Money, and Zarrow (3) showed that other parts of the ovary also contain relaxin. Also, Frieden and Hisaw (5) demonstrated some very important physiological actions of relaxin upon connective tissue.

In order to obtain information as to the possibility of dilating the cervix, a series of experiments was devised to answer the following questions:

1. Does the administration of relaxin in conjunction with the estrogen, diethylstilbesterol, serve to relax the cervical muscle?
2. What is the minimum dosage of relaxin which may be effective? the optimum? Is estrogen alone sufficient to relax the cervix?
3. Can a mechanical dilator be made to dilate the cervix?
4. Is the process of mechanical or hormonal administration accompanied by harmful effects to the tissue of the cervix or uterus?

### EXPERIMENTAL AND RESULTS

Eight cows were used in this investigation. Three cows were assigned to an experimental group, three to a control group, and two were used for a pilot investigation.

To determine the minimum amount of relaxin to use on the experimental cows, a series of injections was given to a pilot cow. She was given injections of relaxin with an increasing quantity at each successive treatment. She was sensitized with an estrogen 3 days in succession prior to each of the relaxin treatments.

Received for publication February 17, 1953.

<sup>1</sup> Approved for publication by the Director of the South Dakota Agricultural Experiment Station as Journal Series No. 282.

<sup>2</sup> These data are taken from a thesis presented to the graduate school of South Dakota State College by the senior author in partial fulfillment of the requirement for the Master of Science degree.

This was done by giving diethylstilbesterol at the rate of 20 mg. per day. Cervical dilation was estimated by manual palpation of the cervix as constricted, intermediate, or relaxed. This was determined by placing the hand in the vagina and estimating the cervical relaxation by entering the cervix with the finger. If the finger could not be forced into the external os, the cervix was considered constricted. If the finger could go only past the first cervical ring, the cervix was considered intermediate. If the finger could go past the second ring, the cervix was considered relaxed. There was no response to the injection of 25 or 50 G.P.U.<sup>3</sup> of relaxin; the cervix was considered constricted on examination following each dosage. A small response was noted following the injection of 100 G.P.U. of relaxin, and the cervical tonus was considered as intermediate. An injection of 250 G.P.U. of relaxin appeared to produce a definite response, and the cervical tonus was considered as relaxed. These injections were given at a time when the cow was not in estrus. The cervix was palpated before the beginning of each series of injections and was in all cases found to be constricted. The cervix was palpated also just prior to the injection of relaxin and was found to be constricted. From these data a dosage of 250 G.P.U. of relaxin was chosen as a minimum for the experiment.

A second pilot cow was used to determine the effect upon the cervix of injecting 20 mg. of diethylstilbesterol 3 days in succession with no relaxin. No relaxation was palpable following this treatment.

As relaxin relaxes the cervical muscles, some means of actually dilating the cervix in conjunction with the relaxed muscles is desirable. Therefore a mechanical instrument was made to open the cervix so that some type of ova collector could be inserted into the cervix.

Five different instruments were designed for this purpose. Each of these instruments was tested on animals not assigned to this experiment so that the cows on the experiment could be treated in the same manner with the instrument best suited.

The instrument found to be best suited was made of carefully machined bronze. The body of the instrument consisted of 1¼-in. tubing, 6 in. long. Four tool steel prongs were inserted into the head of the tubing. The prongs, which were ¼-in. wide and 8 in. long, were bent from the head to meet 5 in. from the end. The ends of the rods were joined to make one smooth rod 5/16 in. in diameter. A plunger then was placed inside the bronze tubing with a wheel and screw on the posterior end to force the plunger against the inside of the four prongs, forcing them to open. The prongs were held tightly together by a rubber band. This precision-made instrument (Figure 1) was satisfactory.

Three cows were assigned to an experimental group to receive relaxin, and three were kept as controls. Cervical tonus was estimated by palpation during estrus, 5 days post estrus, and 10 days post estrus. The cervical dilator was used at estrus and 5 days post estrus to test the effects of relaxin injections upon the muscle of the cervix. Five days post estrus was selected as the day to give

<sup>3</sup> One Guinea Pig Unit of relaxin is the minimum quantity required to relax the pubic symphyses of approximately two-thirds of the guinea pigs in a group of twenty.

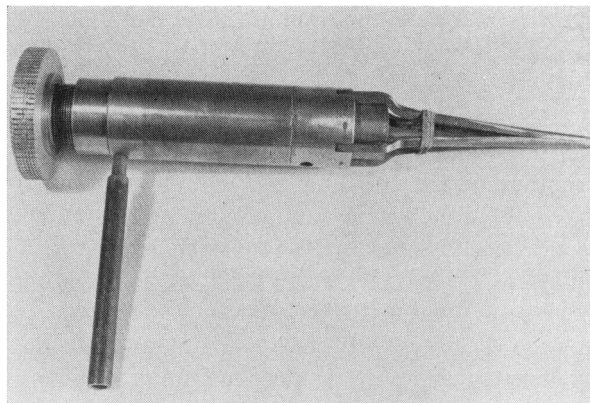


FIG. 1.

the cows the relaxin because normally the fertilized ovum enters the uterine horn about 72 hours post estrus. However, after 5 days post estrus it is necessary to open the cervix for the collection of ova.

Relaxin was given to the same three cows for three consecutive estrous cycles. The relaxin was injected subcutaneously 12 hours before the cervix was to be dilated. Three different dosages of relaxin were given. Five days post estrus of the first, second, and third estrous cycles 250, 1,500, and 8,500 G.P.U., respectively, were given.

During the three estrous cycles, a certain degree of relaxation was observable in all six animals as measured by manual palpation. The cervical relaxation was estimated as intermediate or relaxed. Five days post estrus of each cycle it was noted that the experimental cows differed markedly from the untreated control cows in the degree of palpable relaxation of the cervix. As shown by Table 1, the animals sensitized 3 days in succession with 20 mg. of diethylstilbesterol and injected with relaxin indicated more relaxation than did untreated cows. The degree of relaxation varied with the amount of relaxin given. Table 1 also indicates that 1,500 G.P.U. of relaxin gave more cervical relaxation than 250 G.P.U. but that 8,500 G.P.U. gave but little more relaxation than did 1,500 G.P.U. Palpating the cervix 10 days post estrus showed that all the control cows had a normal constricted cervix, whereas the cervixes of the cows receiving relaxin were normal or near normal during the first estrous cycle but a small general build-up of relaxation was noticed in the second and third estrous cycles.

The mechanical dilator was used to determine the actual amount the cervix had relaxed. As shown by Table 2, the control cows and the experimental cows were dilated to approximately the same extent during the first and second estrous periods. The cervixes of the experimental cows in the third estrous period were dilated an average of 1.27 inches and the controls averaged 0.85 inch. This gain probably is due to a build up of relaxin.

Five days post estrus the cervix of the control cows could not be dilated. The mechanical dilator could not be placed into the cervix. All cows receiving

TABLE 1

*The effect of varying dosages of relaxin on cervical relaxation during three estrous cycles, measured by manual palpation*

Cow No.	1st estrus 1-4-52		5 days post estrus		10 days post estrus		2nd estrus		Relaxin 1,500 G.P.U.		5 days post estrus		10 days post estrus		3rd estrus		Relaxin 8,500 G.P.U.		5 days post estrus		10 days post estrus				
	C	I	R <sup>a</sup>	C	I	R	C	I	R	C	I	R	C	I	R	C	I	R	C	I	R	C	I	R	
Experimental:																									
E 53	.	.	.	..	.	.	.	.	.	.	..	.	..	.	..	.	..	.	..	.	..	.	..	.	
E 55	.	.	.	..	.	.	.	.	.	.	..	.	..	.	..	.	..	.	..	.	..	.	..	.	
E 57	.	.	.	..	.	.	.	.	.	.	..	.	..	.	..	.	..	.	..	.	..	.	..	.	
Control:																									
E 54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E 59	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E 62	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

<sup>a</sup> C = constricted, I = intermediate, R = relaxed.  
Dots indicate degrees of relaxation.

TABLE 2  
*Amount of mechanical cervical dilation of cows treated with relaxin  
 and untreated cows during 3 estrous cycles*

Cow No.	1st estrus dilation	Relaxin treat- ment	5 days post estrus dilation	2nd estrus dilation	Relaxin treat- ment	5 days post estrus dilation	3rd estrus dilation	Relaxin treat- ment	5 days post estrus dilation
	( <i>in.</i> )	( <i>G.P.U.</i> )	( <i>in.</i> )	( <i>in.</i> )	( <i>G.P.U.</i> )	( <i>in.</i> )	( <i>in.</i> )	( <i>G.P.U.</i> )	( <i>in.</i> )
Experimental:									
E 53	0.87	250	1.06	0.62	1500	1.40	1.20	8500	1.50
E 55	0.81	250	0.87	0.75	1500	1.00	0.87	8500	0.90
E 57	0.87	250	0.87	0.90	1500	1.41	1.75	8500	1.61
Control:									
E 54	0.87	.....	none	0.62	.....	none	0.87	.....	none
E 59	0.75	.....	none	0.70	.....	none	0.87	.....	none
E 62	0.85	.....	none	0.62	.....	none	0.81	.....	none

relaxin were dilated, and with increasing dosages of relaxin an increased amount of cervical dilation was noted. In many cases by using relaxin the cervix could be dilated more than an inch, with an average of 0.90 in., with 250 G.P.U., 1.27 inches with 1,500 G.P.U., and 1.34 inches with 8,500 G.P.U.

Biopsies were taken from the cervix and uterus of each cow three times during each estrous cycle: at estrus, 5 days post estrus, and 10 days post estrus. Histological studies of the biopsies gave no indication of trauma from either relaxin or mechanical dilation.

#### DISCUSSION

The data suggest the possibility of mechanical and hormonal dilation of the cervix. The cervix was dilated with a mechanical dilator during estrus but could not be dilated post estrus unless the animal had been treated with relaxin. Relaxin definitely gave some relaxation of the muscles of the cervix; however, two cows, E 53 and E 57, responded more than did E 55.

Apparently the cervix can be dilated periodically without danger of traumatizing the epithelial wall. This is particularly important, for if the periodic collection of ova through the cervix is to become practicable, the cervix must be opened often without damaging the tissue.

#### SUMMARY

These experiments were conducted to determine the possibility of dilating the cervix to facilitate the ease of collecting ova.

The administration of relaxin in conjunction with an estrogen served to relax the cervical muscles. Estrogen alone as judged by the pilot cow gave no cervical relaxation. The minimum dosage of relaxin which gave cervical relaxation was 250 G.P.U.; the optimum, about 1,500 G.P.U. A suitable mechanical instrument was designed for dilating the cervix. However, the instrument must be used with the aid of relaxin when used 5 days post estrus. Histological studies of biopsies from the cervix and uterus indicated no signs of trauma from either the relaxin or the instrument.

## ACKNOWLEDGMENTS

The authors express their appreciation to Robert L. Kroc, Director of Biological Research, Chilcote Laboratories, Morris Plains, N.J., for supplying the relaxin-containing extract, "Releasin," prepared from sow ovaries.

## REFERENCES

- (1) ABRAMOWITZ, A. A., AND HISAW, F. L. Preparation, Biological Assays and Properties of Relaxin. *J. Endocrinol.*, **34**: 103. 1944.
- (2) ALBERT, A., MONEY, W. L., AND ZARROW, M. X. Preparation of Relaxin from Sow Corpora Lutea Residue. *J. Endocrinol.*, **39**: 270-272. 1946.
- (3) ALBERT, A., MONEY, W. L., AND ZARROW, M. X. An Improved Method of Extraction and Purification of Fresh Whole Ovaries of the Sow. *J. Endocrinol.*, **40**: 370. 1947.
- (4) FEVOLD, H. L., HISAW, F. L., AND MEYER, R. K. The Relaxitive Hormone of the Corpus Luteum, Its Purification and Concentration. *J. Am. Chem. Soc.*, **52**: 3340-3348. 1930.
- (5) FRIEDEN, E. H., AND HISAW, F. L. The Mechanism of Symphyseal Relaxation. The Distribution of Reducing Groups. Hexoseamine and Proteins in Symphysis of Normal and Relaxed Guinea Pigs. *J. Endocrinol.*, **48**: 88. 1951.
- (6) HISAW, F. L. Experimental Relaxation of the Pubic Ligament of the Guinea Pig. *Proc. Soc. Exptl. Biol. Med.*, **23**: 661. 1926.
- (7) HISAW, F. L. The Corpus Luteum Hormone. *Physiol. Zool.*, **2**: 59-79. 1929.