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The Effects of Relaxin on The Composition of Bovine Milk

Chris H. Nissen

THE EFFECTS OF RELAXIN ON THE COMPOSITION OF BOVINE MILK

By

Chris H. Nissen

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THE EFFECTS OF RELAXIN ON THE COMPOSITION

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

Thesis Adviser

Head of the Major Department

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INTRODUCTION

Protoplasm is a fragile substance keeping its homeostatic properties by many physiochemical phenomena. Much is known about the properties of protoplasm; but it is, perhaps, only meager when compared to what is yet to be learned. Such physical changes as electrical impulses, diffusion, and oxidation - to mention a few - are necessary for protoplasm to retain its integrity. In higher animals, particularly, for our purposes, mammals, protoplasm is sustained by different body systems working harmoniously together to supply raw materials, that remove waste, and govern all endeavors. One should not infer that single celled animals have a more simple type of protoplasm than multicellular animals, for the composition of all protoplasm is essentially the same. Single celled animals differ from multicellular animals in their methods of attaining protoplasmic precursors to the cell proper and removal of waste.

The major systems of the body that help to maintain homeostasis are: circulatory, respiratory, nervous, muscle, urinary, and endocrine. The endocrine system supplies hormones which are chemicals that function as an indispensible link in the chain of events that occur in the dynamics of protoplasm and hence, the whole organism.

Some of the hormones have been vaguely identified, their properties understood, and their functions known. Some are a complete mystery; others are just in the infancy of scientific study. The major work presented in this thesis is an investigation into the mysteries of one such hormone, relaxin.

The review of literature will be categorized into four main divisions: History, Chemistry, Extraction and Assay, and Physiology, which includes mammary development, pregnancy and parturition, pelvic relaxation, uterus function and effects on milk production and composition.

REVIEW OF LITERATURE

HISTORY

The discovery of relaxin was an outgrowth of studies concerning pelvic adaptation during parturition (59). Most work on relaxin has been done in the last decade because of its recent discovery. It was not until 1929 that Hisaw demonstrated that pelvic relaxation was under endocrine control (51,58).

Relaxin is secreted by the ovaries, the corpus luteum, the uterus and probably other extra-embryonic membranes (61). The corpus luteum is assumably the most important source for the production of relaxin,so it may be important to understand its formation from a follicle. Immediately following ovulation, the stratum granulosum cells enlarge, accompanied by an ingrowing of the theca. This structure becomes highly vascular and becomes known as the corpus hemorrhagicum or corpus luteum of ovulation. If pregnancy ensues, this organ becomes a yellowish colored structure or the true corpus luteum, remaining thus through pregnancy in all domesticated animals except the horse (21). After midpregnancy in most animals, the corpus luteum undergoes slow regressive changes. When obvious regressive changes ensue, the structure is called a corpus albicans (6).

Most of the experiments with relaxin have been conducted on small laboratory animals such as rats, mice, guinea pigs, etc. However, in recent years, more and more work is being done with relaxin on cows and sheep (19,43,74).

Relaxin displays such a profound relaxing effect on the pelvic girdle of guinea pigs that the concentration of a sample of the extract

is expressed in guinea pig units, (GPU). A GPU is the amount of the hormone which will induce unmistakable relaxation of the symphysis pubis in two-thirds of a group of 12 castrated guinea pigs weighing between 350-800 grams (59). In highly purified form, 0.035 mg. constitutes a GPU (27).

The transfusion of blood from pregnant animals into nonpregnant animals is a valuable technique for studying the effects of relaxin. When pigs were injected with blood serum from pregnant women, pelvic relaxation occurred which suggested that relaxin helped relax the symphysis pubis during pregnancy (2). Zarrow and Zarrow (91) found similar changes in the blood of rabbits following subcutaneous injections of relaxin as in normal pregnancy. In pregnant mice, relaxin has been associated with a drop in hematocrit and erythrocyte estimations with an increase in reticulocyte count (59). Relaxin has never been proven to be antigenic (59) and possesses an antiduretic action in the pregnant rabbit (88). McDonald (69) reports that relaxin in conjunction with other female sex hormones is effective in helping to alleviate urinary calculi in mice.

CHEMISTRY

Relaxin appears to be a simple protein with a comparatively low molecular weight of 9,000 as determined by ultracentrifugation and cysteine content (30). It contains approximately 12.7 percent nitrogen and 10.5 percent reducing sugar hexoseamine (33). The isoelectric point is pH 5.4-5.5 (27). Recent chemical evidence shows the presence of lycine but not arginine to be among the amino acids (84). Relaxin is susceptible

to proteolytic enzymes (31). The exposure of cysteine to relaxin abolishes any action on the symphysis pubis of guinea pigs (31,73). However, urea diminishes only the affect on the mouse (65). A 5 percent solution is negative to the Millon's, ninhydrin, and Molish tests but gives a faint violet color to the Buiret test (27).

Relaxin is soluble in aciduated ethyl alcohol, but insoluble in organic solvents such as ether, acetone, and chloroform. Thioglycolate, dithioproponal, glutathione, hydrogen sulfide, bisulfide, and tetrathionate completely inactivate relaxin (31,73). Relaxin is thermolabile and will retain potency for a week at room temperature, but if refrigerated, preparations will remain unharmed from eight to twelve months (26,27). It is not destroyed by hydrogen, carbon dioxide, or hydrogen peroxide (57). Injections of relaxin increased the labeled glycine uptake in symphysis pubis of guinea pigs from three to ten percent as compared to spayed controls (32).

EXTRACTION AND ASSAY

Different tissues vary in the concentration of relaxin. The concentration is also affected by pregnancy and more specifically by the stage of pregnancy with an increasing amount as pregnancy advances (89).

Early extractions were crude involving complex operations for recovery from the blood and urine of pregnant animals or from corpus lutea tissue (1,3,4,27).

Fresh ovaries have ten times more activity than blood or urine. Ovaries from pregnant animals have 1000-1500 times more activity than ovaries from nonpregnant animals (5,59). Fresh ovary preparations have two distinct advantages over the older types of extraction in that there was more relaxin per gram of tissue and the extraction was limited to three

steps (5).

Modern methods of extraction and purification are more effective by combining isoelectric and ethanol precipitation. The fraction recovered by this method is 500-1000 times more effective in stimulating pelvic relaxation in the guinea pig than were the former fractions (33). Relaxin has also been extracted and purified successfully by using chromatography (35).

The basis for female hormone assay is the production of definite histological changes in the female genital tract of laboratory animals similar to the changes that normally occur during pregnancy or the estrus cycle when the corpus luteum is functional. Four of these changes are the production of placentoma, the mucification of the vaginal mucosa of the rat, production of psuedo-pregnancy in rabbits, and the production of premenstrual endometrium in the uterus of monkeys (25). The application of the decidual reaction as a test response for the quanitative assay of relaxin has been found to be a useful supplement to the guinea pig assay (36).

For economical reasons, mice are most frequently used for assay purposes (20).

One tenth cc. of blood serum from rabbits late in pregnancy gave response equivalent to that obtained from one gram of fresh sows' corpus luteal tissue (68). An experiment on 26 women during pregnancy showed an increase from a level of 0.2 GPU per cc. of serum at 7-10 weeks of pregnancy to a maximum concentration of 2.0 GPU per cc. at 38-42 weeks of pregnancy (89). Relaxin was found in the blood of rabbits as early as three days post copulation and in urine five days after mating (68). Similar results were obtained for guinea pigs (87).

PHYSIOLOGY

Mammary - It has been known for quite sometime that hormones are necessary for mammary gland development (24,75). Experiments conducted at the beginning of the 20th Century demonstrated that the ovaries are necessary for mammary gland development. Mammary gland development ensues when estrus producing extracts are given to rats, mice, guinea pigs, and monkeys in both castrated males and females. Estrogen induced duct growth in all species with some showing partial alveolar growth and complete development in guinea pigs and monkeys. Diethylstilbestrol initiated mammary gland growth in the goat; but histologically, it was unlike normal development. Progesterone given with estrogen stimulated alveolar growth in animals that showed only duct growth with estrogen alone (24).

When immature ovariectomized mice received daily injections of 0.83 ug. of estradiol, 1 mg. of progesterone, and 25 GPU of relaxin, considerable mammary growth and lobulation developed (52). Relaxin acts as a potentiator of estrogen working on the latter to produce a greater and more complete mammary gland (37). Trentin (81), however, in his work on mammary development, found no increase in positive mammary alveolar response in mice given relaxin in conjunction with progesterone and estrogen over a combination of the two steroids.

Smith (76) made a more complete investigation of the effects of relaxin on mammary gland development. His findings, among other things, verified that estrogen and progesterone were necessary for mammary growth. Like Garrett's (37) work on guinea pigs and rabbits, he found relaxin to be essential in combination with estrogen and progesterone for more normal mammary development in rats. In these experiments, similar results

were obtained whether pure relaxin or a crude extract was used. Histochemically, the mammary gland showed little difference whether growth was stimulated by the steroid-relaxin combination or just the steroids. A steroid-relaxin combination produced heavy concentrations of sudanophilic material in the alveolar epithelium, and the amount of lipids was related to the degree of lobular-alveolar development. Mammary developments on rats injected with only estradiol were composed mainly of ducts and buds, and these epithelial tissues were relatively devoid of sudanophilic material. Glands treated with a steroid-relaxin combination or just the steroids, effective in producing lobular-alveolar growth, were alike with respect to the sudanophilic materials and their response to prolactin. The mammary glands of mature rats were less responsive than immature rats.

The addition of relaxin to progesterone-estrogen treated animals does not display any elaborate addition in the mammary spreading factor in guinea pigs (22).

Accelerated mammary gland development has been produced in very young dairy heifers by hormone injections. In these experiments, no attempt was made to study relaxin. Pituitary extracts accelerated the effects of a combination of estrogen and progesterone, and the udder appeared more mature, structually, than in controls not receiving pituitary extracts (79).

Mention should be made that other hormones have secondary effects on the development of mammary glands. The importance of the pituitary gland has already been described. It would indeed be a naive conclusion if one would disregard hormones from glands such as

the thyroid, adrenal, parathyroid and others (24).

Pregnancy and Parturition - The duration of gestation is an hereditarily fixed characteristic terminating at parturition. The gestation period varies widely among species, but narrowly with individuals of the same species and with the same individuals during different pregnancies.

The ovaries were once believed to be indispensible for initiating parturition, but more likely the true control of parturition rests with the placenta since the injection of ovarian extracts into pregnant ovarectomized animals failed to induce parturition (55). An old concept that the growth of the fetus and consequent expansion of the uterus with pressure on sensory nerve endings in the cervix had to be abondoned when VanWogenen showed that following removal of the fetus, the placenta alone induced parturition at term. Evidently, the fetal placenta develops to an age that is characteristic for the specie and, when thus matured, initiates parturition. Probably, it then releases some substance that stimulates the endocrine as well as the nervous control system of the birth mechanism. Necrotic changes and a partial separation of the placenta from the uterine wall have been considered to expedite the process.

One may distinguish three main components of the process of parturition. First, the approach of birth is recognized by several phenomena of relaxation (6,42,85). The absence of such relaxations would cause stretching, tearing, and inflammations that might ultimately cause prolapsation of the uterus, cystoceles, and sterility (18). The cervical canal and the entire birth passage begin to widen. The

pubic symphysis of the pelvis loosens, and the ossified connective tissue transforms into an elastic ligament. Hisaw's work on the guinea pig resulted in the identification of a special relaxation hormone. The second phase of parturition involves the contraction of the uterus and abdominal muscles which cause the expulsion of the fetus. The third phase involves the removal of the fetal membranes, placenta, and decidua, which is of no concern here (85).

In mice, ovariectomized on the 14th-15th day of pregnancy, daily injection of 1.0 mg. progesterone maintained pregnancy in 90 percent of the cases; whereas, 0.5 mg. per day maintained pregnancy in 50 percent of the cases and .25 mg. per day was ineffective in maintaining pregnancy. Many fetuses were aborted and/or died in utero. Because the pelvis did not open, some pups were found dead wedged in the pelvic canal. None of the mothers reared her young. If injections were continued past the 18th day, delivery was likely to be delayed and/or difficult (47).

The addition of 1.5 ug. of estradiol per day did not alter the effects of progesterone. There was, however, a slight increase in the number of live pups born, perhaps due to a partial relaxation of the pelvis, and four mothers reared their young. The addition of relaxin to progesterone treated animals did not improve the ability of progesterone to maintain pregnancy, deliver living young, or accept the young. When estradiol and relaxin were given simultaneously with progesterone treated animals, the effect was almost the same as normal pregnancy and parturition. There was an increase in the number of fetuses per uterus maintained in a healthy condition and a great increase in the number of young reared by the mothers (47).

Strength of the fetal membranes of rats shows a rise from the fifteenth to a maximum the eighteenth day post conception. This is followed by a linear increase in collagen content. The sudden weakening is thought to be caused by a substance analogus to the substance that produces relaxation of the cervix and symphysis pubis during pregnancy. There is a parallelism between the strength of the placental membranes and the ratio of collagen to hexosamine (53).

Pelvic Relaxation - Because of the relatively small diameter of the birth channel, in comparison to the fetus, the parturient process is effective only by the enlargement of the pelvis (80). In cows, ewes, rats, and rabbits, this enlargement is facilitated by the relaxation of the sacroiliac joints and not so much the actual spreading of the symphysis pubis. However, in man, guinea pigs, and mice, the birth channel is enlarged both by relaxation of the sacroiliac and symphysis pubis (13).

The maturation and ultimate differences in the morphology of the male and female pelvic girdle plays an important part in their respective reactions to different stimuli. The development of the male and female pocket gopher symphysis pubis are the same until puberty. The adult male has a well developed symphysis pubis; whereas, in the adult female, the pubic bones are widely separated. This sexual dimorphism seems to result from post-pubertal sex hormones since ovarian graphs in males stimulate resorption of the pubic bones while spayed females with testicular graphs prevented resorption of the pubis (55).

In a similar experiment carried out with mice, some gonadectomized at birth and some post-pubertal, there was no significant difference between male and female interpubic separation when 3 daily injections of 2 ug. of estradiol benzoate were followed by 2 daily injections of 100 GPU of relaxin. Male animals castrated post-pubertal showed a intrapubic gap of .9 mm. and females 2.9 mm. when treated similarly. When comparing age of gonadectomy to reaction of the treatment, it was shown that the males gonadectomized at birth showed a 50 percent greater gap than the ones gonadectomized at 4 months. On the other hand, the females gonadectomized at 4 months showed a 240 percent increase over those spayed at birth (14).

Both pregnant and nonpregnant mice receiving 1-2 mg. of progestrone per day with 100 GPU of relaxin injected on the 13th, 16th and 19th day post-coitum produced an intrapubic gap of 3-4 mm. in pregnant animals; whereas, in nonpregnant animals, the gap was only 1 mm. wide. Parturition always followed this administration of relaxin (77).

Electrical polarity of surface membranes is important in the dynamics of pelvic relaxation. In unrelaxed symphysis pubis cartilage, there is a high density of immovable negative charges in the ground substance, which changes to lower densities during relaxation. Then hormones, by their action on connective tissue, may effect the distribution of sodium and potassium ions through selective interaction of cations with ground substances and water (11).

During normal parturition, the relaxation of the pelvic region is at its ultimate; but there are also symptoms of relaxation during the estrus cycle in mice. Experiments conducted by Crelin and Honeyman (16)

showed that during estrus there is an increase in the interpubic separation, increased flexability of the symphysis, and a swelled fibrocartilage matrix of the symphysis pubis.

The uterus and progesterone are indispensable for the formation of relaxin. Castrated female guinea pigs given 250 mgs. of estradiol on four consecutive days will show pelvic relaxation in 42-67 percent of the cases between 72-96 hours following a single injection of 5-10 mgs. of progesterone on the 5th day. In hysterectomized castrated females, relaxation did not ensue. In neither case did the estradiol alone cause relaxation. In both cases, relaxation occurred within 6 hours after relaxin was injected. Normal female rabbits in estrus, when given 5 mg. of progesterone for 9 days, have one unit of relaxin per 5 cc. of blood serum. During the last week of normal pregnancy, .1 cc. of serum contained one unit of relaxin. Relaxin appeared in the blood of the estrogen-conditioned castrated females between 48-96 hours following a single injection of 10-20 mg. of progesterone (60,61).

Until the 13th day of the first pregnancy, the symphysis pubis of mice is inflexible. After which, the symphysis becomes completely flexible with the appearance of an intrapubic gap which gradually increases until parturition. The flexibility of the sacroiliac joints increased at the 14th day and remained status quo until parturition. In gonadectomized mice receiving 3 daily injections of 2 ug. of estradiol benzoate followed by 300 GPU of relaxin, it was demonstrated that the female symphysis became flexible 8 hours after treatment and increased in flexibility up to 120 hours. Whereas, in male mice, the

initial flexibility of the symphysis pubis was 18 hours (17).

One GPU of relaxin will cause pelvic relaxation in guinea pigs within two hours with a maximum in six hours followed by a disappearance of any response in nine hours. An increase in dosage does not shorten the initial period, but shortens the other two (38,59).

During the last week of normal pregnancy in mice, the pelvis undergoes lateral displacement at the symphysis pubis and the narrow, ridged, intensely metachromic cartilaginous symphysis pubis is replaced by a pliable band of connective tissue. In pregnant spayed mice and guinea pigs that were injected with estrogen, progesterone or relaxin or a combination of the three, it showed that progesterone alone showed no lateral displacement of the pelvis (9,29,48). Estrogen alone, although failing to completely relax the pelvis, produced more relaxation than progesterone. Relaxin given with progesterone caused progressive relaxation, but the bridge was not cytologically normal. When all three hormones were given simultaneously, a somewhat normal relaxation occurred; but again, the bridge was abnormal (48). Prolongation of administration and increased dosage increased the size of the gap between the two halves of the pelvis (49). In estrone treated animals, the relaxation of the pelvis is increased 20 fold by daily injecting .2 cc. of an extract from pregnant rabbit serum. From this, it seems that one hormone is necessary for the fundamental action and one is a potentiator (50). It is believed that estrone produces the fundamental action (45). The optimum duration of estrone priming when given in 1.5 ug. daily injections for maximum response to 0.2 cc. of relaxin was 8 days. Once the pelvis has reacted to relaxin, even though

it has since closed, it becomes subsequently more sensitive to the action of estrone (44).

When one mg. of progesterone is injected into estrogenized mice, the effects of subsequent or concurrent injections of relaxin are greatly reduced. This assumably is a result of an antagonism between progesterone and estrogen. The relaxation of the pelvis in normal pregnancy apparently depends on a narrow progesterone/estrogen ratio (45).

Ligament growth in the pelvis may not be a result of pure relaxin but of a more insoluble protein-like substance which is easily separated from relaxin. The effects of injecting relaxin show a higher potency when absorption from the site of injection into the blood stream is delayed (64).

When using a delicate staining technique, Hall (46) revealed some important concepts concerning the physiology of pelvic relaxation. In uninjected ovariectomized mice, the matrix of the symphysial cartilage, like outside the symphysis, is intensely metachromic and basophilic. Following nine daily injections of 1.5 ug. of estradiol, metachromasia disappeared from the caudal part and decreased in the cranial part of the symphysis, accompanied by swelling of the matrix. Cartilage outside the symphysis was unaffected (46). As the matrix swelled, the mature chondrocytes of the symphysial hyaline and fibracartilage underwent mitotic division. During normal pregnancy, the first mitotic figures appear at the 13th and 14th day of pregnancy; this time is greatly reduced when relaxin is applied (12). Hyaluronidase banished metachromasia from the symphysial and extra-symphysial cartilage, but the matrix did not swell. Disappearance of metachromiasia

is attributed to depolymerization or breakdown of the molecules of chondroitin sulfate resulting in a more plyable ground substance. When progesterone was given simultaneously, estradiol was less effective in abolishing metachromasia. Swelling is attributed to the uptake of water. An increase from 75-85 percent water was observed by Heringa (82). One injection of relaxin at the end of such pretreatment produced a gap in the middle of the symphysial cartilage by lateral displacement of the two halves of the innominates. Subsequent injections increased the displacement. Estrogen, perhaps by a local liberation of an enzyme, breaks down the polysaccharides of the symphysial cartilage and makes the matrix sufficiently pliable to respond to the tensile forces set up by relaxin during the last part of pregnancy (15,34,46).

Estrogen alone can produce a small degree of pelvic relaxation, but is physiologically different from the way relaxin functions. Separation by prolong treatment of estrogen is caused by the resorption of bone on either side of the symphysis and does not involve the lateral displacement of the two bones (46). In estrogen treated animals, there is an increase in mucoid and alkaline phosphate but whether these play a part in pelvic relaxation or not is problematical (59).

Blood serum from pregnant rabbits or guinea pigs injected into virgin guinea pigs during early estrum produces a noticeable relaxation within 6-8 hours. The blood of parturient females looses this effectiveness rapidly during the first 8 hours post-partum (56,57). Males fail to respond to relaxin unless first feminized by ovarian graphs or

prolong estrogen treatments (57).

Extracts from the corpus luteum stimulate pelvic relaxation in female guinea pigs whether they be spayed, virgin, or immature (10).

Anesthetized guinea pigs showed a smaller gap in the symphysis pubis than unanesthetized animals. Similar results are obtained when the spinal cord is sectioned at the twelfth thoracic vertebra. Isolated symphysis pubis incubated with relaxin cannot be extended by stretching. All these results suggest some kind of a nervous correlation associated with pelvic relaxation (82).

Spayed ewes estronized with diethylstilbestrol and then treated with relaxin exhibit no pelvic relaxation (7).

Experimental results suggest that other compounds can produce pelvic relaxin. Guinea pigs primed with estrone showed pelvic relaxation when 1.5 mg. of desoxycorticosterone was injected (23). This being a steroid perhaps displays some properties of the steroid estrogen and consequently causes a small degree of pelvic relaxation.

Acetylcholine produces or in some way catylyzes the process of pelvic relaxation. In 97 tests on oophorectomized guinea pigs primed with theelin and injected with acetylcholine approximately two-thirds of the animals showed pelvic relaxation (86).

Uterus - Dogs receiving ovarian extracts containing relaxin developed hypotension, bradycardia and inhibited uterine motility. A tachyphylaxis soon developed to these reactions. Hypotension was attributed to the release of histamine or histamine-like substances liberated by the extraction (70).

The decidual reaction which normally follows traumatization of

the uterus was inhibited by injecting 60-160 GPU of relaxin per day to castrated and pseudopregnant rats that were primed with progesterone (36).

Animals receiving serum from pregnant animals developed a hypertrophy of the endometrium. This has been used as a specific test for pregnancy (83). The effects here are thought of as being caused by relaxin.

Young virgin guinea pigs receiving 0.1 mg. of diovacylin per animal for 7-12 days primed with estrone had a hypertrophied uterus comparable to 20 days of pregnancy (67). It is highly possible that relaxin helps keep the uterus quiescent during pregnancy since as little as .001 GPU per cc. of a reasonably pure preparation of relaxin from sow ovaries caused a perceptible decrease in the amplitude of uterine contraction in estrus rats. When the dosage was increased, all contraction stopped and recovery was slow (73).

Relaxin does not inhibit the response of the uterus to oxytocin or acetylcholine, but does increase the electrical threshold (73).

Subcutaneous injections of 1 mg. of relaxin per day for a week cause no increase in the weight of the male adrenal gland, seminal vesicles, ventral prostates or testicles of the rat, nor of the female adrenals or ovaries. The uterus, however, showed a marked enlargement over nontreated animals. The greatest enlargement is noted in immature animals (62).

Cervical dilation in the bovine has been observed by Graham and Dracy following relaxin treatment (40). This was confirmed by Zarrow, Sikes, and Nehen, who also found vulvular edema when castrated

heifers were treated with stilbestrol and with relaxin. Histochemically, there is an increase in premeability and depolymerization of the ground substance of the cervix (90). A very interesting experiment was conducted by Harkness and Harkness which very plainly demonstrates the changes occurring in the cervix during pregnancy. Parallel rods were placed into the cervix of a recently conceived rat. A force of 50 grams was applied in keeping the rods in parallel lines. The behavior of the cervix was unchanged until the 13th-14th day of pregnancy at which time thereafter the cervix became progressively more elastic until parturition. There was a slight increase in total collagen of the cervix over the period of increased distensibility, but no change in the percentage of hexosamine (54).

Milk Production - The heretofore study of relaxin has been confined to the physiology of the body with no mention of its effects on milk production, the primary concern of all dairymen. The amount of literature available concerning the effects of relaxin on milk production is limited and to account for this deficiency, it is necessary to review the effects of other compounds on milk secretion and postulate unverified probabilities. The effects of feeds, hormones, and therapeutics on the composition of milk have long been a concern of dairymen. Particularly important are the effects of mastitis therapeutics on the composition of milk because of the great possibilities of contamination for human consumption (24).

Many types of drugs have in the past and perhaps will in the future be used in an attempt to increase total milk production, fat yield, or both. Under certain conditions, strychnine (nux vomica),

arsenic (sodium cacodylate and Flower's solution), nicotine, and camphor stimulate the central nervous system. Muscarine, pilocarpine, physostigmine and atropine act upon the terminal nerve junctions through which nerve impulses enter the gland. Pituitrin (oxytocin and vasopressin), yohimbine, and barium chloride act on smooth muscles (24). Insulin, by producing serum hypoglycemia, caused a reduction in fat and lactose percentage which leads one to assume a common synthesis pathway for the two. However, this drop could be partially due to the reduced energy of fat and lactose producing cells resulting from the hypoglycemia (39). Phloridzin displays similar but additional effects. It lowers the renal threshold for glucose retention causing glucosuria and hypoglycemia which, like insulin, reduces the percentage of lactose. Phloridzin stimulates the body to "burn" protein rather than carbohydrate, thus causing a hypernitrogenuric condition. It tends to depress blood potassium, but increases the phosphorous level (24,39).

The glycogen content of excised mammary was increased from 25.11 mg. percent to 231.7 mg. percent when perfused with a high concentration of glucose solution. Incubation of tissue slices at 37.5°C. for six hours stimulated lactose formation. Lactose was also formed by incubating mammary tissue slices with glucose, glucose and lactic acid, and maltose (66).

It is the opinion of some dairymen that estrus decreases milk flow. Experimental evidence fails to confirm this. Gestation in itself does not influence the composition of milk, but it may indirectly cause the cow to go dry. The reason for this is not endocrinologically

important, but a matter of energy distribution, e.g. when a cow is pregnant some of the energy that is normally used in milk secretion is needed for growth and maintainance of the fetus which is accomplished at the expense of total milk production. This decrease in milk production, obviously, shows an increase in fat percentage (24).

Folley reported a temporary inhibition of lactation in lessening milk production by administering estrogenic hormones to lactating cows. There was an increase in fat and solids not fat, perhaps from diminished production. The protein content was unaffected which was not true in experiments carried out by DeFremery (1936) on goats where true milk was converted to colostrum by treating with estradiol benzoate (28).

A sure way to demonstrate the effects of a hormone on milk secretion is the removal of an endocrine gland. Thyroidectomized cows went dry in 180 days after the thyroid gland was removed whether the thyroidectomy preceded pregnancy, during gestation or post-parturient. Total milk production decreased 75 percent. Partial removal of the thyroid temporarily depressed milk secretion with a gradual return to normal. Fat, lactose, nitrogen, and specific gravity were unaffected (78).

In 1930, Becker and McGilliard demonstrated that the internal stimulus produced by pregnancy and parturition of a non-fecunal lactating cow was the largest factor controlling maximum daily milk yield. The more rapid decline of milk flow in normal lactation than in the non-fecunal lactation is attributed to a gradually diminishing internal or hormonal influence arising from reproduction. The internal stimulus is necessarily a factor in determining persistency of milk flow (8).

An average increase of 8.59 percent total production was noted by Reineke and Turner in an experiment where, in fourteen feeding trials, cows were fed 50-100 grams of thyrolactin for a period of three days, in declining stages of lactation. In six trials, a fat analysis was run which showed an increase in two but no appreciable changes in the other four. In four trials, the heart rate of the cows were observed. Interestingly enough, there is a parallel increase with the increase in production. After the effects of thyrolactin wore off, the production level gradually declined and leveled off at a level that was lower then the pre-experimental average (72).

In a similar experiment conducted by Hauffman and Turner, an attempt was made to integrate synthetic estrogenic activity with thyroid activity. Feeding thyroprotein to dairy cows and goats during declining stages of lactation produced an increase in total milk production. After a time, however, milk secretion began to decline again at the rate of persistency inherent in the individual animal (63,72). 0.25 mg. of diethylstilbestrol daily injected into a goat initiated lactation, but if this dosage was increased to 1.0-4.0 mg., a definite depression in milk secretion was noted. This depression could be counteracted by 10 mg. of thyroxine. A combination of thyroxine and diethylstilbestrol increased milk yield 145 percent; and after six months, the production level was still 100 percent above the original level. It is believed that the estrogenic hormone was responsible for an increase secretion of the lactogenic hormone which is important in maintainance and persistency of lactation. Dairy goats receiving, in their feed, 60 mg. of dimethyl

ether of diethylstilbestrol and 1.5 grams of thyroprotein per day three weeks post-parturient showed an average increase of 55 pounds per lactation per animal over normal animals. Cows receiving 200 mg. of stilbestrol and/or 15 mg. of thyroprotein, in their feed, produced an average increase of 204 pounds per lactation if only thyroprotein was fed. If stilbestrol and thyroprotein were fed concurrently, the milk production decreased on an average of 150 pounds per cow per lactation. Note should be made of the fact that cows receiving only the thyroprotein lost on the average 153 pounds body weight during the lactation period. The ones receiving both hormones lost on the average of 138 pounds; whereas, the control group lost 38 pounds per cow.

Mention should be made of the effects of relaxin on milk ejection. In an experiment with sheep, 39.1 percent of their total milk was obtained with no injections. An additional 43.5 percent of the total milk was obtained after 500 GPU of relaxin was injected; whereas, 17.4 percent of the total milk was recovered when 10 I.U. of oxytocin, the normal "let down" hormone, was injected (74). Donker (19) and Hall (43), failed to confirm these findings in cattle. When 750-1800 GPU of relaxin was injected intravenously into three cows on two occasions, there was no noticeable increase in milk ejection over normal let down (19).

Anaesthetized lactating rats that were injected with .02, .05, or .1 U.S.P. of synthcinon (synthetic oxytocin) per kilogram body weight resulted in milk let down after a latent period of 5-8 seconds and persists 4-5 minutes. This was approximately the same result as

normal oxytocin. When some of the rats in this experiment were treated with 600-1200 GPU of relaxin, there was no indication of milk let down even with a latent period of 30 minutes (41).

EXPERIMENTAL PROCEDURE

The animals used in this experiment were normal, healthy, lactating cows, three of which were Holsteins and one a Guernsey. They were all managed similarly with no pretreatments of any kind. Their ration consisted of one pound of grain mixture for every three pounds of milk, alfalfa hay (ad lib.), and thirty pounds of corn silage per animal per day.

Relaxin was furnished by the Warner-Chilcott Laboratories. The relaxin solutions were made up by two methods. One was made with a beeswax base where one ml. constituted 1200 GPU. Animals E-123, E-125 and E-167 received injections of this preparation. The other used saline as a base. This was compounded so that one ml. contained 2000 GPU of relaxin. E-158 received relaxin of this kind.

Control milk samples were collected and analyzed every other day from each cow until four analyses were run. This was used as a standard. Then the animal was injected, intramuscularly, with the desired quantity of relaxin every day with concurrent sampling and analyses until four analysis were made on milk from cows receiving relaxin.

The following analyses were determined in accordance with accepted procedures:

1. pH - Beckman pH meter
2. Specific gravity - Quevenne lactometer
3. Fat - Babcock

4. Total solids - $\frac{\text{C.L.R.}}{4}$ [(1)] + 1.2 x B.F.

5. Solids not fat = total solids - fat

6. Ash - sample heated in muffle furnace for 5 hours at 450-500°C.

7. Protein - modified Kjeldahl procedure, using a factor of 6.38 times nitrogen.

8. Lactose - picric acid method as described by Perry and Doan (71).

(1) Corrected lactometer reading which is attained by adding .1 to the reading for every degree the milk is over 60°F. or subtracting .1 below 60°F.

RESULTS

For the purpose of clarity, the results have been summarized and expressed as average composition for four pre-injection sample periods and four post-injection samples. An average was also established for all the cows for the pre-injection and post-injection periods.

Test for pH, specific gravity, fat, total solids, solids not fat, ash, lactose, and protein during the pre-injection period are summarized in table 1. Table 2 is a summary of the composition during the post-injection period. An average of all cows was made for pre-injection and post-injection periods (table 3) which demonstrates no appreciable change in milk composition. The pH for the pre-injection period was 6.6, post-injection 6.6, specific gravity pre-injection 1.032, post-injection 1.032, fat pre-injection 3.9, post-injection 3.9, total solids pre-injection 12.61, post-injection 12.47, solids not fat pre-injection 8.61, post-injection 8.63, ash pre-injection .674, post-injection .683, lactose pre-injection 5.34, post-injection 5.32, and protein pre-injection 3.17, and post-injection 3.20.

Table 1

Average composition of milk before relaxin treatment

Cow No.	GPU Relaxin	pH	Specific Gravity	Fat	Total Solids	Solids not fat	Ash	Lactose	Protein
E-123	None	6.5	1.032	3.5	12.10	8.57	.729	5.36	2.87
E-158	None	6.6	1.032	3.8	12.80	8.66	.683	5.34	3.40
E-125	None	6.7	1.031	3.7	12.42	8.38	.667	5.41	3.18
E-167	None	6.5	1.033	4.4	13.13	8.83	.616	5.28	3.21

Table 2

Average composition of milk after relaxin treatment

Cow No.	GPU Relaxin	pH	Specific Gravity	Fat	Total Solids	Solids not fat	Ash	Lactose	Protein
E-123	1800	6.5	1.032	3.4	11.95	8.60	.723	5.32	2.87
E-158	2000	6.3	1.031	3.8	12.36	8.56	.686	5.33	3.30
E-125	2000	6.8	1.031	3.8	12.32	8.49	.670	5.35	3.25
E-167	2000	6.6	1.033	4.4	13.26	8.86	.653	5.27	3.37

Table 3

Average composition of milk from all cows pre-injected and post-injected

Time of injection	pH	Specific Gravity	Fat	Total Solids	Solids not fat	Ash	Lactose	Protein
Pre-injection	6.6	1.032	3.9	12.61	8.61	.674	5.34	3.17
Post-injection	6.6	1.032	3.9	12.47	8.63	.683	5.32	3.20

DISCUSSION OF RESULTS

The results of this study suggest that relaxin, in the amounts administered, has little or no effect on the composition of bovine milk during normal lactation. The slight decrease in total solids and lactose (table 3) and an increase in solids not fat, ash, and protein during the post-injection period rather than being a consequence of the hormone is probably a normal fluctuation. Although the comparison of different levels of relaxin was limited, it appears that if the hormone does affect milk composition, in intact animals, it is either secreted in larger quantities or with other intrinsic factors of the organism that were over-looked during this experiment.

Since relaxin is secreted in largest quantities with increasing concentration as pregnancy advances, it seems logical to assume a correlation between relaxin and colostrum secretion. DeFremery (28) found a change from normal milk to colostrum following administration of estradiol benzoate in normal lactating goats.

Whether or not these so-called "pregnancy hormones" induce colostrum formation or not is problemathical. The results of this study fail to reveal, even to the slightest degree, any change from normal milk to colostrum when relaxin was used alone. If, therefore, relaxin plays a part in colostrum formation, unknown factors are necessary.

SUMMARY AND CONCLUSIONS

The results of these experiments are not definite nor conclusive, but suggests that relaxin has no effect upon the composition of cows' milk.

1. Control milk from four lactating cows was collected every other day and analyzed for pH, specific gravity, fat, total solids, solids not fat, ash, lactose, and protein.

2. From the ninth day following the beginning of the control period, each cow was injected with relaxin and the same milk analyses run as on the controls.

3. The results from these experiments did not reveal any differences between the control and experimental periods.

LITERATURE CITED

(1) Abramowitz, A. A., Hisaw, F. L., Kleinholz, L. H., Money, W. L., Talmage, R. V. N., and Zarrow, M. X. The preparation and biological assay of relaxin. Anat. Rec.(Absts.) 84:456-457. 1942.

(2) Abramson, D., Hurwitt, E., and Lesnick, G. Relaxin in human serum as a test for pregnancy. Surgery, Gynec. and Obstet. 65:335-339. 1937. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(3) Albert, A., and Money, W. L. Methods for concentraction of relaxin from blood serum and urine. Endocrinology. 38:56-57. 1946.

(4) Albert, A., Money, W. L., and Zarrow, M. X. Preparation of relaxin from sow corpora lutea residue. Endocrinology. 39:270-272. 1946.

(5) Albert, A., Money, W. L., and Zarrow, M. X. An improved method of extraction and purification of relaxin from whole ovaries of the sow. Endocrinology. 40:370-374. 1947.

(6) Arey, L. B. Developmental anatomy. W. B. Saunders Company. 1954.

(7) Bassett, E. G., and Phillips, D. S. M. Pelvic relaxation in sheep. Nature. 174:1020-1021. 1954.

(8) Becker, R. B., and McGilliard, P. C. Measuring the influence of hormone and mechanical stimulation by means of a non-fecunal lactation. J. Dairy Sci. 13:372-379. 1930.

(9) Brouha, L. Studies on the relaxation of the pubic symphysis of immature guinea pig. Compt. rend. Soc. belge biol. 113:406-408. 1933. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(10) Brouha, L., and Simonnet, H. Experimental production of mobility in the pubic symphysis of the guinea pig. Compt. rend. Soc. biol. 99:1769-1771. 1928. (Biol. Absts. 4:17924, 1930. Summary from Warner-Chilcott Laboratory. Original not seen.)

(11) Catchpole, H. R., Joseph, N. R., and Engel, M. B. Electo-chemical state of symphysial conn. tissue under hormone stimulation. Amer. J. Physiol. 167:774. 1952.

(12) Crelin, E. S. Mitosis of mature chondrocytes. Anat. Rec. (Absts.) 127:282-283. 1957.

(13) Crelin, E. S., and Brightman, M. W. The pelvis of the rat, its response to estrogen and relaxin. Anat. Rec. Absts. 128:467. 1957.

(14) Crelin, E. S. and Grillo, M. A. The conditioning effect of puberty on the response of the pubic symphysis in mice to estrogen and relaxin. Anat. Rec. Absts. 127:447. 1957.

(15) Crelin, E. S., and Honeyman, M. S. The inhibition of interpubic ligaments formation by progesterone in pregnant mice. Anat. Rec. Absts. 127:407. 1957.

(16) Crelin, E. S., and Honeyman, M. S. Pelvic joint changes during estrous cycle in mice. Anat. Rec. Absts. 127:408. 1957.

(17) Crelin, E. S., Wood, P. B., and Honeyman, M. S. Flexibility changes of pelvic joints in pregnant and hormonally-injected mice. Anat. Rec. Absts. 127:408. 1957.

(18) Curtis, A. H. Textbook of gynecology. W. B. Saunders Company. 1930.

(19) Donker, J. D. Lactation studies. I. Effects upon milk ejections in the bovine of various injection treatments using oxytocin and relaxin. J. Dairy Sci. 41:537. 1958.

(20) Dorfman, R. I., Marsters, R. W., and Dinerstein, J. Bioassay of relaxin. Endocrinology. 52:204. 1953.

(21) Dukes, H. H. Physiology of domestic animal. Comstock Publishing Associates. 1955.

(22) Elliott, J. R., and Turner, C. W. Relation of relaxin to steriod ovarian hormones on production of mammary spreading factor. Proc. Soc. Exp. Biol. Med. 82:234-235. 1953.

(23) Emery, F. E. Relaxation of the pubic symphysis in guinea pig following the injection of desoxycorticosterone acetate. Proc. Soc. Exp. Biol. Med. 63:100-102. 1946. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(24) Espe, D., and Smith, V. R. Secretion of milk. The Iowa State College Press 1952.

(25) Fevold, H. L., Hisaw, F. L., and Leonard, S. L. Hormones of the corpus luteum. The separation and purification of the three active substances. J. Am. Chem. Soc. 54:254. 1932.

(26) Fevold, H. L., Hisaw, F. L., and Meyer, R. H. Isolation of the relaxative hormone in the corpus luteum. Proc. Soc. Exp. Biol. Med. 27:604-606. 1930. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(27) Fevold, H. L., Hisaw, F. L., and Meyer, R. K. The relaxin hormone of the corpus luteum. Its purification and concentration. J. Am. Chem. Soc. 52:3340. 1938.

(28) Folley, J. S. The effects of oestrogenic hormones on lactation and on the phosphatase of the blood and milk of the lactating cow. The Biochemical Journal. 30:2262-2272. 1936.

(29) deFremery, P., Kober, S., and Tausk, M. Enlargement of the symphysis pubis of the female guinea pig by menformon. Acta brev. neerland. 1:146-148. 1931. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(30) Frieden, E. H. Chemical studies on relaxin. Fed. Proc. 10:184. 1951. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(31) Frieden, E. H. The effect of cysteine and other reducing agents upon relaxin. Arc. Biochem. 30:138-143. 1952. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(32) Frieden, E. H. The effect of estrogen and relaxin upon the uptake of glycine - 1-C14 by connective tissue in vivo. Endocrinology. 59:69-73. 1956.

(33) Frieden, E. H., and Hisaw, F. L. Purification and properties of relaxin. Fed. Proc. 9:44. 1950. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(34) Frieden, E. H., and Hisaw, F. L. The mechanism of symphysial relaxation. The distribution of reducing groups, hexosamine, and protein in symphysis of normal and relaxed guinea pig. Endocrinology. 48:88. 1951.

(35) Frieden, E. H., Nuall, M. W., and deFlorida, F. A. Fractionation of relaxin by chromatography and counter-current distribution. J. Clin. Investigation. 15:848. 1955. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(36) Frieden, E. H., and Velardo, J. T. Effects of relaxin upon the decidual reactions in the rat. Fed. Proc. 11:50. 1952. (Summary by Warner-Chilcott. Original not seen.)

(37) Garrett, F. A., and Talmage, R. V. The influence of relaxin on mammary gland development in guinea pigs and rabbits. J. Endocrinology. 8:336-340. 1952.

(38) Gold, J. C., and Emery, F. E. Relaxation of the symphysis pubis in monkeys. West. J. Surg. Abst. Gyn. 61:24-26. 1953. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(39) Gowen, J. W., and Tobey, E. R. The influence of insulin and phloridzin on the mechanism of milk secretion. Maine Agriculture Experiment Station, Bulletin 360. Abst. from J. of Gen. Physiology. 15:67-85. 1931.

(40) Graham, E. F., and Dracy, A. E. The effect of relaxin in the cows cervix. J. Dairy Sci. 35:499. 1952.

(41) Grosvensor, C. E., and Turner, C. W. Milk let-down activity in synthetic oxytocin (Syntocinon) and relaxin in lactating rats. Proc. Soc. Exper. Biol. and Med. 97:189. 1958.

(42) Hadely, F. B. Principals of Veterinary Science. W. B. Saunders Company. 1954.

(43) Hall, V. A. Some observation pertaining to the effects of relaxin upon the bovine. M. S. Thesis. South Dakota State College. 1957.

(44) Hall, Kathleen. Further notes on the action of estrone and relaxin on the pelvis of the spayed mouse, including a single-dose test of potency of relaxin. J. Endocrinology. 5:314-320. 1948.

(45) Hall, Kathleen. The role of progesterone in mechanism of pelvic relaxation in the mouse. Quart. J. Exper. Physiology. 35:65-76. 1950. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(46) Hall, Kathleen. An evaluation of the roles of estrogen, progesterone, and relaxin in producing relaxation of the symphysis pubis of ovariectomized mouse using the technique of metachromatic staining with toluidine blue. J. Endocrinology. 13:384-393. 1956.

(47) Hall, Kathleen. Maintenance of pregnancy, parturition, and rearing of litters in mice ovariectomized and injected with progesterone, estradiol, and relaxin. J. Physiol. 134:17P-18P. 1956.

(48) Hall, Kathleen. The symphysis pubis in mice in which pregnancy was maintained after ovariectomy by injecting progesterone alone or with estradiol and relaxin. J. Physiol. 134:3P. 1956.

(49) Hall, Kathleen, and Newton, W. H. The action of relaxin in the mouse. Lancet. 1:54-55. 1946. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(50) Hall, Kathleen, and Newton, W. H. The effect of estrone and relaxin on the x-ray appearance of the pelvis of the mouse. J. Physiol. 106:18-27. 1947. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(51) Hammond, H. Hormones of pregnancy. Progress in the physiology of farm animals. 3:878-879. 1957.

(52) Hamolsky, M., and Sparrow, R. C. Influence of relaxin on mammary development in sexually immature female rats. Proc. Soc. Exper. Biol. Med. 60:8-9. 1945.

(53) Harkness, M. L. R., and Harkness, R. D. Changes in the physical properties and in the collagen and hexosamine content of the foetal membranes during pregnancy of the rat. J. Physiol. 132:482-491. 1956.

(54) Harkness, M. L. R., and Harkness, R. D. Changes in the properties of the uterine cervix of the rat in pregnancy. J. Physiol. 131:19P. 1956.

(55) Hisaw, F. L. The influence of the ovary on the resorption of the pubic bones of the pocket gopher. Geomys Bursarius (Shaw). J. of Exp. Zool. 42:411. 1925.

(56) Hisaw, F. L. Experimental relaxation of the symphysis pubis of guinea pig. Anat. Rec. Absts. 37:126. 1927.

(57) Hisaw, F. L. Experimental relaxation of the symphysis pubis of guinea pig. Anat. Rec. Absts. 37:126. 1927.

(58) Hisaw, F. L. The corpus luteum hormone. I. Experimental relaxation of the pelvic ligaments of the guinea pig. Physiol. Zool. 2:59-79. 1929. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(59) Hisaw, F. L., and Zarrow, M. X. The physiology of relaxin. Vitamins and hormones. 8:151-178. 1950.

(60) Hisaw, F. L., Zarrow, M. X., Money, W. L., Talmage, R. V. N. and Abramowitz, A. A. Importance of the female reproductive tract in the formation of relaxin. Biol. Absts. 18:10,341. 1944.

(61) Hisaw, F. L., Zarrow, M. X., Talmage, R. V. N., Money, W. L., and Abramowitz, A. A. Relation of progesterone to the formation of relaxin. Anat. Rec. 84:457. 1942.

(62) Jablonski, W. J. A., and Velardo, J. T. Effects of relaxin on uterine weight of immature rats. Endocrinology. 61:474. 1957.

(63) Kauffman, G. and Turner, C. W. The effect of combined thyroprotein and estrogen feeding upon milk production. Paper presented at 40th Annual meeting of the American Society of Animal Production, Chicago, Illinois. November 27, 1948.

(64) Kliman, B., and Creep, R. O. Effects of depot relaxin on pubic ligament growth in mice. J. Clinical Endocrinology. 15:847. 1955. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(65) Kliman, B., Salhanick, H. A., Frieden, E. W., and Hisaw, F. L. The comparison of response of the pubic symphysis of the mouse and guinea pig to extracts of pregnant sow ovaries. Reprint from Endocrinology. 53:408-409. 1953.

(66) Knodt, C. B., and Peterson, W. E. Studies of the carbohydrate metabolism of mammary gland tissue in vitro. I. Production and utilization of various carbohydrate substances. Reprint from J. Dairy Sci. 28:415-429. June 1945.

(67) Krantz, J. C., Bryant, H. H., and Carr, C. J. The action of aqueous corpus luteum extract upon uterine activity. Surg. Gynec. Abst. 90:372-375. 1950. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(68) Marder, S. N., and Money, W. L. Concentration of relaxin in the blood serum of pregnant and post-partum rabbits. Endocrinology. 34:115-121. 1944. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(69) McDonald, D. F., and Huffman, P. G. Influence of sex hormones on incidence of experimentally induced visical calculi in female rats. Biol. Absts. 31:2585. 1955.

(70) Miller, J. W., Kesley and Murray, W. J. The effects or relaxin containing ovarian extracts on various types of smooth muscle. J. Pharm. and Exp. Therapeutics 120:426. 1957.

(71) Perry, N. A., and Doan, F. J. A picric acid method for the simultaneous determination of lactose and sucrose in dairy products. J. Dairy Sci. 33:176-185. 1950.

(72) Reineke, E. P., and Turner, C. W. Formation in vitro of highly active thyroproteins, their biological assay, and practical use. University of Missouri Research Bulletin. 355:72-73. 1952.

(73) Sawyer, W. H., Frieden, E. H., and Martin, A. C. In vitro inhibition of spontaneous contraction of the uterus by relaxin containing extracts of sow ovaries. Amer. J. Physiol. 172:547-552. 1953.

(74) Shaffhausen, D. D., Jordan, R. M., and Dracy, A. E. The effect of relaxin upon milk ejection. I. The let-down effect upon sheep. J. Dairy Sci. 37:1173-1175. 1954. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(75) Smith, T. C. Influence of the adrenal on response of the rat mammary gland to ovarian steriods and relaxin. Fed. Proc. 10:129. 1951. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(76) Smith, Thomas, C. The action of relaxin on mammary gland growth in the rat. Endocrinology. 54:59-70. 1954.

(77) Smithberg, M., and Runner, M. N. The maintenance of pregnancy in prepuberal mice. J. Exp. Zool. 133:441-457. 1956.

(78) Spielman, A. A., Peterson, W. E., and Fitch, J. B. The effects of thyroidectomy on lactation in the bovine. Reprint from J. Dairy Sci. 27:441-448. 1944.

(79) Sykes, J. F., and Wrenn, T. R. Hormonal development of the mammary gland. J. Dairy Sci. 33:194-203. 1950.

(80) Tapfer, S., and Haslhofer, L. Hormonal influence on the spreading of the pelvis in animal experiments. Arch. F. Gynec. 159:313-331. 1935. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(81) Trentin, J. J. Relaxin and mammary growth in the mouse. Biol. Absts. 26:8559. 1952.

(82) VanderMeer, C. Experiments on the mechanism of action of relaxin. Acta. Endocrinology. 4:325-342. 1950. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(83) VonFels, E. Concerning the problem of the corpus luteum hormone and its specific test. Zentrable. Gyn. 55:914-920. 1931. (Summary by Warner-Chilcott Laboratories. Original not seen.)

(84) Wislocki, G. B., Weiss, L. P., Burgos, M. H. and Ellis, R. A. The cytology, histochemistry, and electron microscopy of the grandular cells of the metrail gland of the gravial rat. J. of Anatomy. 91:130. 1957.

(85) Witschi, E. Development of Vertebrates. W. B Saunders Company. 1956.

(86) Young, W. C., and Embry, F. E. Relaxation of the pelvis ligaments in guinea pigs by acetylcholine. Amer. J. Physiol. 162:606-609. 1950.

(87) Zarrow, M. X. Relaxin content of blood, urine, and other tissues of pregnancy and post-partium, guinea pig. Proc. Soc. Exp. Biol. Med. 66:488-491. 1947. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(88) Zarrow, M. X. The antiduretic action of relaxin - containing preparation. Prac. Soc. Exp. Biol. Med. 71:705-707. 1949. (Summary from Warner-Chilcott Laboratories. Original not seen.)

(89) Zarrow, M. X., Halmstrom, E. G., and Salkanick, H. A. The concentration of relaxin in the blood serum and other tissues of women during pregnancy. J. Clin. Endocrinology. 15:22-27. 1955.

(90) Zarrow, M. X., Sikes, D., and Nehen, G. M. Effects of relaxin on uterine cervix and vulva of young casterated sows and heifers. Amer. J. Physiol. 179:687. 1954.

(91) Zarrow, M. X., and Zarrow, I. G. Anemia in the rabbit following treatment with relaxin. Fed. Proc. 9:141. 1950. (Summary by Warner-Chilcott Laboratories. Original not seen.)