THE EFFECT OF THE HORMONE RELAXIN ON THE PERIODONTAL TISSUES

by

Bruce Horace Rice

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I hereby recommend that this dissertation prepared under my direction by BRUCE H. RICE entitled THE EFFECT OF THE HORMONE RELAXIN ON THE PERIODONTAL TISSUES be accepted as fulfilling the dissertation requirement of the degree of DOCTOR OF PHILOSOPHY.

After inspection of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

- Wm. J. McCaully 4/26/60
- Raymond E. Reed 4/26/60
- Mitchell B. Davis 4/26/60
- James W. Berry 4/26/60
- John V. Slater 4/26/60

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SIGNED: Bruce W. Rice
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INTRODUCTION

Gingival disease is one of the most vexing and prevalent problems that the dental profession and the population as a whole have to face. During the early years of life dental caries causes the greatest proportion of loss of teeth with all the consequent sequelae. By middle age periodontal disease takes on a greater significance and in later life causes the loss of teeth in a much greater proportion than any other oral disease. This poses one of the greatest public health problems with which our nation has to contend. It has been stated that if every practicing dentist did nothing but fill the existing cavities in the teeth of every individual in the United States it would take at least four years to remove the backlog, to say nothing of the new cavities that had developed in the meantime. This leaves out entirely many of the other fields that are concerned with the oral health of the populace, i.e., oral surgery, periodontology, prosthodontia and others.

All periodontal diseases except certain degenerative types follow a similar pattern of development and all start with gingivitis. Many factors influence the resistance or susceptibility of the gingivae to disease processes among which are the nutritional status of the
individual, various systemic diseases, local oral hygiene procedures, and nonreplacement or nontreatment of previously existing lesions in the teeth themselves.

Some of the more important systemic factors are general debilitating diseases, disturbances in metabolism, hormonal imbalances, etc. Pregnancy, while it is not a disease process, causes a transient change in the hormone balance of the female organism. Consequently, during the course of the pregnancy a condition develops among a considerable number of women which has been termed "pregnancy gingivitis". Most investigations agree with the general figure that this occurs in approximately 25%-35% of pregnant females.

Two tissues are primarily affected in pregnancy gingivitis, the gingival epithelium and the underlying connective tissues. Most of the studies in the past have been concerned primarily with the response of the epithelium to varying conditions of hormone balance. A few recent investigations have mentioned the possible role of connective tissue. Also, more or less recently much work has been done on the hormone, Relaxin, that is produced during pregnancy and which causes changes in the connective tissues which allow for extreme degrees of relaxation of the public ligaments in some species of animals.

It was because this hormone is produced during pregnancy and has been well-demonstrated to have an effect on the connective tissue
of the uterine cervix and the pubic ligament that this investigation was undertaken.

The research reported in this dissertation was carried out to determine the effect of relaxin on the connective tissues of the periodontal ligament because:

(1) Relaxin is produced during pregnancy.

(2) It has been well-demonstrated to have effects on various tissues in experimental animals including the public ligament, uterus, uterine cervix, and mammary gland development.

(3) The gingival lesion produced during pregnancy is concerned with the state of the connective tissue.

A. Pregnancy Gingivitis

In its limited sense, the term "gingivitis" refers to inflammation of the gingiva. It is common practice to use the term gingivitis when referring to all forms of gingival disease and suitably qualifying it according to either its clinical features or etiology. For example, such terms as "acute necrotizing ulcerative gingivitis" or "chronic desquamative gingivitis" are indicative of clinical features, whereas "pubertal gingivitis" or "Pregnancy gingivitis" ostensibly refer to etiology.
There is some justification for the common usage of the term gingivitis to connote all forms of gingival disease because inflammation is almost a universal clinical feature. It is important, however, to recognize that inflammation is not necessarily always the primary change, but that in some cases it may be secondarily superimposed upon an underlying noninflammatory form of gingival disease.

With the exception of acute inflammatory involvement, generalized diffuse gingival disease is generally systemically initiated. Marginal changes are provoked by superimposed local factors. The severity and extent of the marginal change are subject to modification by systemic influences (32).

In reviewing their own and other previous contributions on the various aspects of the gingivae in pregnancy, Ziskin and Nesse (65) suggested a classification for pregnancy gingivitis based on the clinical appearance.

From their examinations of animals they also believed that epithelial hyperplasia was the primary change in pregnancy.

Several studies have been made as to the incidence of pregnancy gingivitis. Looby (43), in a group of 475 young women in their first pregnancy, reported slight gingivitis in 40%; hypertrophic gingivitis in 10%; and pregnancy tumors in 2%. In a series of 416 women Ziskin
and Nesse (65) reported the following gingival changes: pregnancy gingivitis - 37.9%; hypertrophic gingivitis - 7.0%; raspberry-red gums - 40%; combination - 1.8%.

In a series of 530 pregnant women Maier and Orban (44) reported the following gingival findings: no pathologic change - 44.6%; mild inflammation - 35.9%; moderate inflammation - 17.5%; severe inflammation - 1.5%; tumor formation - 0.5%.

In pregnancy there is a tendency for the response of the gingiva to local irritation to be exaggerated. Differences in the appearance of the gingiva in pregnant and nonpregnant individuals in the presence of local irritation results from the modifying effect of pregnancy upon the response to local irritation. In the absence of local irritation, the gingivae in pregnancy present no notable clinical changes. However, in an electrometric study, Gans et al. (31) found that almost all patients studied showed evidence of a disaggregation of the gingival connective tissues even if clinical changes could not be observed. Their conclusion was that the gingival tissues participate in the generalized somatic reaction during pregnancy.

Aside from the discrete, tumor-like masses, gingival changes in pregnancy may be marginal or diffuse, localized or generalized. The marginal changes appear as an accentuated inflammation. The gingival
margin is notably edematous, shiny, somewhat friable and tends to be
discolored with a bluish tinge. Marginal ulceration and pseudomem-
brane formation are sometimes seen. The gingival margin or inter-
dental papillae, or both, may be involved. There is also an increased
tendency toward gingival bleeding. In some cases the gingivae are
extremely red in color and present a marginal, raspberry-like
appearance. This extreme redness represents a markedly vascular
response which occurs with superficial materia alba or superficial
bacterial involvement. Another gingival change frequently observed
is a diffuse surface shininess and an anoxemic, bluish appearance.
The gingivae lose their normal resilience and feel soft to digital pres-
sure.

The above changes in varying combinations and degree comprise
the clinical features generally referred to by the term "pregnancy
gingivitis" (Figs. 1 and 2).

For the purposes of this investigation the term pregnancy
gingivitis will be used and will be understood to include any or all of
the described changes.

Most of the studies of the past have been concerned with either
the clinical appearance, course, and treatment of this condition, or
with the inflammatory aspects of it. Glickman (32) pointed out that,
Figure 1. A clinical photograph showing the characteristic features of pregnancy gingivitis. The interdental papillae are edematous and enlarged with marked hyperemia. Patient; age 20 years, primipara in third trimester of pregnancy.

Figure 2. A clinical photograph showing the extreme gingival hyperplasia encountered in the so-called "pregnancy tumor". Patient; age 32 years, gravida III. This tumor was evident to some extent during the first pregnancy after which it did not completely regress. It became larger with each succeeding pregnancy until the time of presentation when it interfered with function. The whitish appearance on the surface is due to ulceration caused by contact with the upper anterior teeth.
based on existing evidence, neither the individual microscopic features nor the composite picture are specific enough to be pathognomonic of pregnancy. He further pointed out, however, that this opinion is subject to possible modification by further developments in histochemical methods whereby subtle tissue alterations, which are not demonstrable by existing histologic staining techniques, may be elucidated.

Further studies by other than microscopic means (31) have shown that there actually are adaptive responses in the periodontal tissues during pregnancy that are not encountered in the normal non-pregnant state.

Based on biochemical evidence some facts may be examined with the viewpoint in mind as to how they may affect these adaptive responses.

Connective tissues are labile in response to the influence of hormones and changes in the internal environment. This is determined by changes in the state of aggregation of the negatively charged colloids including the mucoproteins of the ground substance. The oral tissues, especially the gingivae and periodontal ligament, participate in these responses of connective tissue.

The first mention of the possibility that the hormone relaxin might be implicated by its modification of the internal environment
came in 1952 (22). Since that time it has been mentioned, but so far no studies have been reported which were designed to analyze specifically the modifying role it may play. Practically all studies with relaxin have been made in respect to the response of the pubic ligament (13, 14, 15, 16, 17, 18, 35, 37, 38, 42), uterine cervix (4, 8, 58), mammary gland (39, 59), and some of the blood elements (64, 52). As yet no studies have been done on other connective tissues and more specifically of the connective tissues of the periodontal ligament.

B. Description of Teeth and Periodontal Ligament

All the teeth are held in their position in the jaws by two factors, i.e., the physical shape of the roots of the teeth themselves and the periodontal ligament which has its fibers buried in the cementum of the tooth and also in the bone of the alveolar process of the jaw.

The fibers can be roughly grouped into several groups depending on their position on the tooth and the structures to which they are attached at the end opposite the tooth. These fibers have been classified as:

(a) Free gingival group - the fibers running coronally from the tooth and ending in the free gingiva.

(b) Transseptal group - these fibers extend interproximally over the crest of the alveolar bone and are attached to the adjacent tooth. These are only evident in the molar region.
(c) Alveolar crest group - these fibers extend horizontally from the tooth and insert into the bone at the crest of the alveolar process.

(d) Horizontal group - extend horizontally from the tooth to the alveolar bone in the region just apical to the alveolar crest group.

(e) Oblique group - extend from the tooth in a coronal direction to the alveolar bone. These fibers compose the bulk of the periodontal ligament and are arranged to act as a sort of "sling" to support the tooth against the masticating forces which are applied in an apical direction.

(f) Apical group - these fibers radiate out from the end of the root of the tooth in a fanwise arrangement and are attached to the bone in the fundus of the socket.

Within the confines of the periodontal ligament space and near the bony margin of the tooth socket is a rich network of capillaries which becomes more abundant and contains larger vessels in the apical region and also in the gingival region of the ligament.

The fibers of the ligament are attached to the tooth and the bone by the extension of the substance of the fiber itself into the hard tissue. When these fibers become embedded in the cementum or bone they become known as Sharpey's fibers.
In addition, the periodontal ligament space also contains less regularly arranged connective tissue fibers which are found between the principal fibers and are termed the interstitial connective tissue. The blood vessels, lymphatics, and nerves are enclosed within this tissue.

C. Relaxin

The discovery of relaxin was the outcome of studies concerning pelvic adaptations associated with parturition. The phenomenon of "pubic relaxation" or separation of the symphysis pubis in different mammals during pregnancy and parturition has been an established fact for many years. Reference to the separation of the "ossa pubis" during labor in women may be found in the writings of Hippocrates. In the eighteenth century two schools of thought arose with reference to symphyseal separation. Mauriceau (1727) denied the concept that pubic bones separated but stated . . . "others are of the opinion that these bones thus separate at the time of labor, are thereto by degrees a little before disposed by the slimy humours which flow forth from the womb and these mollify the cartilage which at other times joins them firmly." (34)

During the next century a number of anatomical studies were carried out on the pubic symphyses of women, which definitely proved
that relaxation occurred. In 1905 Goldthwait and Osgood (33) reported
for the first time that not only did pelvic relaxation occur in women
during pregnancy but also to a slight degree during menstruation.
Chamberlain (12) measured the degree of pubic separation in pregnant
and nonpregnant women by roentgen examination. He found that definite
relaxation occurred by the fourth week of pregnancy. Other investiga-
tors, using the same types of techniques, showed the separation of the
pubic bones in pregnant women and closure of the symphysis by six
weeks postpartum. The maximum separation occurred prior to the
last two months of pregnancy. Thoms (57) concluded that relaxation
of the pubic symphysis of women occurs in normal pregnancy and is a
progressive phenomenon.

In addition to the human, pelvic changes have been reported by
many investigators in other species such as the guinea pig, mouse,
dog, mole, cow, ewe, seal, and pocket gopher (34). The most exten-
sively studied have been the guinea pig and the mouse.

For the purpose of this study the mouse was selected as one of
the experimental animals because of the availability of a purely objec-
tive method for measuring or evaluating the effect of the hormone
preparations on the organism, i.e., the increase in the length of the
pubic ligament which is easily measured by gross dissection.
1. Chemistry of Relaxin

Relaxin has been extracted from the corpora lutea of sow's ovaries by several different methods. In the initial experiments of Hisaw et al. (35, 36) and Fevold et al. (29), luteal tissue was ground and then extracted with 2% HCl in 5% ethanol. On neutralization and evaporation of the filtrate to semidryness a starting material was obtained from which the hormone was further concentrated by a procedure that involved primarily repeated fractionations with ethanol and ether. Abramowitz et al. (2) and Albert, Money, and Zarrow (7) extracted dried, defatted luteal tissue with aqueous 2% HCl and concentrated the hormone by fractional precipitation with (NH₄)₂SO₄ and ethanol. These procedures yielded preparations that contained 20 to 30 guinea pig units per milligram of dry weight.

The chief chemical characteristics of relaxin, as judged by these earlier extracts were its isoelectric point of 5.4 to 5.5, stability in acid solutions, and its insolubility in the common nonpolar solvents. Alkalis, oxidizing agents and proteolytic enzymes such as trypsin and pepsin destroyed the activity of the hormone. The preparations were amphoteric in nature and had a nitrogen content of 11%. Doczi (20) has since purified the extract by ion exchange chromatography and found that the isoelectric point is around pH of 8. He used the ion-exchange resin IRC-50 because it could be buffered in this pH range.
Experimental evidence obtained by dialysis (26) and ultracentrifugation (24) indicated the molecular weight of relaxin to be in the neighborhood of 9,000. This figure is also indicated by the cystine content which is 2.7%. It shows positive reactions to the common protein reagents.

Relaxin contains either 12.7% nitrogen as pointed out by Frieden and Hisaw (26) or as stated previously about 11% (2, 7). Upon hydrolysis it has also been shown to contain about 10.5% reducing sugar about half of which is hexosamine.

It is rapidly and completely inactivated by cysteine, thioglycolate, dithiopropanol, glutathione, hydrogen sulfide, bisulfite, and tetrathionate. It is sensitive to reducing agents, acetylation, methylation, the action of iodine and digestion by proteolytic enzymes (23, 24). An appreciable proportion of the total nitrogen is free amino nitrogen and nitrous acid does not significantly reduce its activity.

It also appears that the relaxin molecule contains a single essential disulfide group (24).

Albert, Money, and Zarrow (6) reported a simplified procedure for the extraction of relaxin from fresh, whole ovaries of the sow. They discovered that the ovaries are a better source than dried corpora lutea which makes it less difficult to prepare in highly potent preparations.
It was originally shown that relaxin was present in the blood of various animals during pregnancy. In 1930 Fevold et al. (30) indicated that the hormone could be obtained from the serum by evaporating to semidryness and extraction with a solution of acidified 95% ethanol. Further work with sodium sulfate as a precipitating agent showed the relaxing activity to be present in the globulin fraction and specifically in the pseudoglobulins.

Abramson et al. (3) fractionated serum with ammonium sulfate and found relaxin present in all three fractions, i.e., globulins, pseudoglobulins, and albumin. They concluded that an acid alcohol extract gave the most potent preparation.

Albert and Money (5) devised a method for extracting relaxin from urine. The urine was dialyzed against running tap water and distilled water, then reduced to one half its original volume by evaporation in a current of warm air, and poured into four volumes of cold acetone. Precipitation was facilitated by adding a few drops of saturated sodium chloride, the precipitate collected by centrifugation and suspended in saline so that 1 ml. was equivalent to 100 ml. of urine. This procedure gave a 10% loss in relaxin activity, which probably occurred during dialysis.

Frieden, Layman, and Stone (27) have isolated three active components from a sample that was analyzed by countercurrent distribution.
The amino acid content of each was determined by paper chromatography and the following amino acids were found: cystine, aspartic acid, glutamic acid, glycine, serine, histidine, lysine, arginine, alanine, valine, threonine, and leucine. These were quantitatively identical from all three samples, but the $R_f$ values for the samples were 0.53, 0.63 and 0.87, respectively. When dinitrophenyl derivatives of intact peptides were hydrolyzed and chromatographed only the epsilon-dinitrophenyl lysine could be identified. They concluded that there were several substances, closely related chemically, which were responsible for the relaxin activity of ovarian extracts. This conclusion was also supported by the results of experiments with a third solvent system.

2. Physiology of Relaxin

   a. Action on the mammary gland

   Hamolsky and Sparrow (39) reported a synergistic action of relaxin on the effects of estrogen and progesterone on the development of the mammary glands in rats. Treatment with estradiol, progesterone, and relaxin causes growth and lobulation of the mammary glands in immature castrated rats comparable to that seen in pregnancy whereas estradiol and progesterone alone gave only slight growth.
b. Action of the vaginal mucosa

Dewar et al. (19) reported a potentiation of the vaginal response to estrone by relaxin. Using the "cross over" test in castrated mice and vaginal cornification as an index of activity, these investigators found that the effect of estrone was increased from 7.5 to 50 fold by the addition of 2.5 guinea pig units of relaxin that had been extracted from pregnant rabbit serum.

c. Antidiuretic action

Preparations containing relaxin were shown by Zarrow (61) to have an antidiuretic action in rabbits. Female rabbits injected with 250 to 1,000 guinea pig units of relaxin three times daily for three days showed a 60% to 80% drop in urine output. It was found that the minimum effective dose in terms of relaxin for an antidiuretic action was about 250 guinea pig units and that the antidiuretic activity of a preparation paralleled its relaxin content. In addition it should be mentioned that dialysis studies indicated that the antidiuretic factor is not identical with the posterior pituitary hormone.

d. Action on the cellular components of blood

In conjunction with the investigation on the antidiuretic action of relaxin, hematocrit studies indicated that one of the accompanying effects was a definite shift in the water balance. This led to a study of the
changes that occur in the cellular components of the blood during pregnancy and after the injection of relaxin. Preliminary results indicated that the rabbit develops a pregnancy anemia similar to that described by Newcomber (49) for the rat. These changes include a drop in hematocrit and erythrocytes and an increase in reticulocytes. These same conditions have been observed in normal and castrated female rabbits receiving relaxin (64).

**e. Capillary permeability**

After the administration of estrogen and relaxin the uterus has been shown to undergo changes resembling acute inflammation (52). These changes include vascular dilatation and engorgement, leucocyte emigration and increased capillary permeability.

Talmage (56) showed that one of the immediate effects in the administration of estrogen and relaxin was the increase in water content of the tissues of the pubic ligament area. This was attributed to the increase in permeability of the capillaries to the fluid portion of the blood.

Storey (54) suggested that in mice the relaxation effect of relaxin is due in part to increased capillary permeability in the pubic ligament with resultant edema and unwinding of the collagen spirals.
3. Pharmacology of Relaxin

a. Time required for the onset of pubic relaxation

Abramowitz et al. (2) found that a maximum relaxation of the symphysis pubis in a group of guinea pigs produced by a subcutaneous injection of 1 guinea pig unit was obtained in 6 hours. Zarrow and Money (63) further observed that a maximum response was obtained in 6 hours after the injection of 1 guinea pig unit of relaxin by either the subcutaneous, intraperitoneal or intracardiac route. A small percentage of animals showed relaxation as early as 2 hours. Also, the time response curve was the same for the various routes of injection. By 9 hours after treatment the response began to disappear and by 24 hours the symphyses were no longer relaxed.

b. Rate of disappearance of relaxin from the blood stream

Marder and Money (46) showed that the relaxin content of the blood of pregnant rabbits rapidly decreased following parturition. In 72 hours the blood level fell from 10 guinea pig units per milliliter of serum to less than 1 unit. After a single intravenous injection of relaxin, approximately 50% was lost from the blood in 1 hour and by 24 hours the hormone was no longer detectable. It appeared that the material was very rapidly broken down in the body or for the most part excreted as a degraded and inactive substance.
c. Toxicity

Relaxin has not been obtained in a chemically pure state and until that is accomplished its toxicity cannot be determined with a high degree of validity.

d. Antibody formation

Several attempts have been made to establish whether or not relaxin had antigenic properties and so far all have given negative results. Zarrow and Money (63) failed to develop antibodies to relaxin in the rabbit after a series of treatments. This was in agreement with the fact that castrated guinea pigs could be used repeatedly for more than a year in the assay of relaxin without loss of sensitivity to the hormone or the occurrence of anaphylactic reactions. These observations indicated that relaxin was either weak or lacking entirely in antigenic potentialities.

4. Assay Procedures

a. Manual palpation

The assay procedure most commonly used for relaxin was based on the relaxation of the pubic symphysis of the guinea pig which could be determined by manual palpation.

In an attempt to quantitate the assay, Abramowitz et al. (1, 2) established a standard dose response curve which had a typical sigmoid
shape. On the basis of this curve one guinea pig unit (G. P. U.) was defined as that amount of hormone which induced an unmistakable relaxation of the symphysis pubis in two thirds of a group of 12 castrated guinea pigs weighing between 350 and 800 grams. All animals were pretreated with 0.83 grams of estradiol daily for 4 days and then the pubic symphysis was palpated 6 hours following the injection of relaxin. The authors indicated that by this procedure doses differing by 50% but not less could be distinguished.

b. X-ray

Hall (38) described an assay for relaxin using spayed mice and measuring the interpubic separation by X-ray photographs. In this study he also found no seasonal variation when guinea pigs were used.

c. Dissection technique

Most of the studies reported resorted to some means of measuring the increase in length of the pubic ligament of mice after the animals were sacrificed and the area of the ligament dissected clear and the ligament measured directly. This precludes the further use of the animal and might not be suitable to all experimental problems.

5. Therapeutic uses of Relaxin

During the past few years relaxin has been used in the treatment of several conditions and diseases. Because of its softening effect on
the uterine cervix, Stone, Sedlis, and Zuckerman (53) felt that it was a worthwhile adjunct in the medical induction of labor. On the other hand, it has been used to prevent premature labor or at least to allow the mother to carry the fetus for a longer period of time because of its property of inhibiting uterine contractility. This makes possible a better chance of delivering a viable fetus in cases of premature labor (4, 8, 45, 55, 58, 62).

Rezek (51) and Jones and Smith (40) reported that relaxin has been useful in the treatment of the dysmenorrhea symptom complex.

Boucek (9) noted that while preparing a sponge biopsy for the study of in vitro cultured fibroblasts that the skin of the experimental animals seemed to be loosened. This led Casten and Boucek (10) to use relaxin in the treatment of scleroderma with considerable success, and in Raynaud's phenomenon where they obtained healing of the ulcerations on the fingers which were due to the lack of circulation (11).

Birnberg and Abitbol (8) found that the use of relaxin combined with oxytocin would shorten the length of labor by about one half to two thirds of that obtained with oxytocin alone.

6. Mechanism of Action of Relaxin

Several suggestions have been made as to what actually happens during the process of relaxation of connective tissue under the influence of relaxin. They are briefly:
(1) Depolymerization of ground substance.

(2) Splitting and dissolution of connective tissue fibrils.

(3) Hyperplasia of the connective tissue elements.

(4) Accumulation of water.

Perl and Catchpole (50) felt that the initial change was in the ground substance. Ground substance is defined as the intercellular material surrounding the cellular and fibrillar elements and is composed of a homogenous glycoprotein of a nonfibrillar nature. It is assumed to be structurally organized at a submicroscopic level and polymerized to a degree that may vary with the physiologic state.

Stated in physical terms the depolymerization of ground substance involves the breaking down of complexly organized glycoprotein molecules into a greater number of highly reactive subgroups. In the presence of periodic acid-leucofucsin dye these subgroups react more completely and hence appear to be more deeply colored than do the compact elements of a more polymerized tissue. After having been stimulated with the hormone relaxin subsequent to a conditioning with estrogen the symphyseal ground substance of the guinea pig undergoes changes which are believed to be depolymerizing in nature. The ground substance becomes more loosely arranged, the tissue assumes a more fluid consistency and a degree of mobility is attained.
Storey (54) proposed that at an early stage of the process the fibroblasts accumulated a periodic acid schiff-staining material which is probably mucopolysaccharide in nature. The secretion of this material, which is known to be a polyelectrolyte increased the osmotic pressure and resulted in the retention of water in the connective tissue spaces. There was also an increase in capillary permeability, for Evans blue dye injected intravenously leaked out into the surrounding tissues. The area surrounding the symphysis exhibited many of the signs of inflammation; not only were the vessels dilated but there was leucocyte infiltration and accumulation of round cells. Thus, two mechanisms existed for the accumulation of fluid in the relaxing ligament.

Associated with these changes were alterations in the form of the connective tissue fibers. Although there was no evidence of dissolution, the collagen swelled so that the fibers apparently split into fibrils which were continuous and arranged in the form of loose spirals.

Hyaluronidase has not been found in the target tissues and is not capable of producing the same effects in vitro. Assays for phosphatase and glucuronidase (25) revealed no differences significant enough to implicate these enzymes. The most significant chemical finding has been that the ratio of collagen nitrogen to total insoluble nitrogen
decreased during relaxation. Collagen, as determined by alkali extraction or tryptic digestion, constituted 60-80% of the total residue protein in unrelaxed ligaments, while in the relaxed ligaments the range was 40-70% of the total residue protein.

Engel (22) stated that connective tissue under the influence of relaxin behaved in such a manner as to indicate presence of negatively charged immobile macromolecules. As relaxation proceeds, the density of these aggregates was reduced.

Crelin (17) concluded that the direct application of the hormone to target tissues had little or no enhancing effect on its physiologic action.
EXPERIMENTAL PROCEDURE

In selecting experimental animals for this study the mouse was chosen because it has been shown by many investigators (2, 37, 38, 42) that this animal is very sensitive to the action of relaxin and the effect can be easily measured grossly by the increase in length of the pubic ligament. It has also been shown that intact animals respond almost, if not equally, as well as castrated animals (21, 22).

The animals used in this experiment were Swiss albino mice (CF No. 1 strain). They were immature, intact, virgin females.

The second animal chosen for this particular study was the golden Syrian hamster. These animals were adult females. No information was available regarding how many times these animals had borne litters of young, but it was assumed that they all had been bred previously.

All the mice were maintained on a stock diet which consisted of Purina Pellets and water ad libitum. The hamsters were maintained on the standard diet of the rat colony at the University of Arizona. This diet was in the form of a powder and was used because it has been previously shown (48) to produce periodontal lesions in hamsters because
of lack of mechanical cleansing of the teeth due to the lack of hard material or fiber.

All animals were housed in the animal colony of the Department of Agricultural Biochemistry in which the temperature is kept at a fairly constant value between 70 and 80° F. Standard cages were used in which 6 to 8 animals could be kept without unnecessary crowding.

In a pilot procedure to ascertain injection procedures and animal response in general, six adult female mice were chosen at random from the stock colony. No information was available as to their age nor how many litters of young each had borne. They were separated into two groups and the injection schedules worked out. The experimental animals were given, by means of subcutaneous injection, 1.1 mg. of estrogen in oil on each of 5 succeeding days. Then on three following days they were given estrogen in combination with relaxin in a vehicle of beeswax and sesame oil. The dose of relaxin was 1 mg. (150 G. P. U.) per day. No microscopic sections were made from these animals.

Twenty-eight animals were then divided into three groups; ten animals as controls, ten animals in group A, and eight animals in group B.

The standard assay procedure previously reported (21) was used with some variation in time sequence and dosage of drugs.
The 10 animals in group A were given estradiol benzoate on days 1, 2, 3, 4, 5, and 6. Then this was followed by relaxin only in 1 mg. (150 G. P. U.) amounts on days 7, 8, 9, and 10. On day eleven the animals were sacrificed (Table II).

Eight animals in group B were given the drugs as follows: estradiol benzoate in oil, 1 mg. on days 1, 2, and 3. This was followed by a mixture of estradiol benzoate and relaxin .66 mg. (100 G. P. U.) on days 4, 5, 6, and 7. The animals were sacrificed on day 8.

The control animals were sacrificed on day 4 of the injection routine.

In hamsters a periodontal disease has been observed that very closely resembles that found in humans (47, 48). As there seemed to be some possibility that the previous existence of periodontal disease may have some effect on the response of the animal tissues to relaxin, it was thought that the hamster would fit the experimental conditions.

No investigations could be found in the literature in which the hamster had been used as an experimental animal in connection with the use of relaxin.

For the experimental procedures 23 animals were separated into three groups; seven control animals and two groups (A and B) of eight animals each.
The animals in group A were injected subcutaneously with 1.66 mg. estradiol benzoate in oil on days 1, 2, 3, 4, 5, 6, and 7. This was followed by injection in the same manner 1,000 G. P. U. relaxin (Cervilaxin) on days 4, 5, 6, and 7.

The animals in group B were injected with relaxin only in 5 mg. doses (1/4 cc. of Cervilaxin or 1,000 G. P. U.) on days 1, 2, 3, and 4.

All animals were sacrificed at the end of their injection schedule, the heads prepared as described for the mice, embedded and histologically prepared and stained also in the same manner as for the mice.

The hormone relaxin is prepared and marketed commercially under at least two trade names, Releasin and Cervilaxin. Samples of both of these products were used during the course of this study.

The most suitable vehicle for the relaxin has been shown to be a beeswax in oil preparation (41). This was used in this study for the vehicle when administering Releasin.

---

1 Releasin - Warner Chilcott Co. No. W1165A, lot 49 1mg = 1 mg. standard (150 G. P. U.).

2 Cervilaxin - National Drug Co. Aqueous solution 20 mg./cc. (4,000 G. P. U.).

3 5% Beeswax in Sesame Oil Warner Chilcott Co. No. W1164-30P Lot 2.
The estrogen used to "prime" the animals in this study was in the form of Estradiol Benzoate in oil.\(^1\)

All drugs were given subcutaneously by injection using 2cc and 5cc Luer-Lok syringes and 20, 22, and 24 gauge stainless steel needles. In the cases where Releasin and estrogen were given on the same day they were mixed together and given with a single injection. When Cervilaxin was used two injections were necessitated because it is an aqueous preparation.

In order to measure the length of the pubic ligament at the time of sacrifice the pubic area was dissected clear of surrounding tissue, the ligament visualized and measured in length by means of a Boley gauge (a caliper type of instrument with a Vernier scale in the metric system).

At the time of sacrifice the jaws were removed and fixed in a 10% formalin solution. Later they were decalcified in 5% nitric acid. After decalcification all tissues were processed in an Autotechnicon in preparation for embedding, embedded in a paraffin block and sectioned at 8 to 10 by means of a microtome. The sections were mounted on slides in preparation for staining.

\(^1\) Progynon - Schering Corp. Supplied in multiple dose vials in the strength of 3.33 mg./cc. Each vial contained 10 cc. of the aqueuous solution.
The sections were cut from the jaws of the animals in three ways:

(a) Coronal plane - to show the teeth in a buccolingual cross section.

(b) Sagittal plane - to show the molar teeth in cross section mesio-distally and the incisor in an incisal-apical direction.

(c) Orbital plane - to show a cross section of the roots of the teeth apical to the cemento-enamel line.

The sections were stained using two different types of stain:

(a) The standard histological staining procedure was carried out using haemotoxylin with a counterstain of eosin.

Approximately ten representative sections were made on each of 24 mice and 12 hamsters.

(b) Von Gieson connective tissue stain was used on 4 sections from 9 mice and 6 hamsters.

Usually only one tissue section was mounted on each slide, but occasionally two sections were mounted. Serial sections were made on one mouse head for the purposes of orientation.

A total of 580 slides were examined.
RESULTS

At the time of sacrifice the adult mice used in the pilot study had a ragged look and their fur appeared as if they were completely wet. Some of the drug preparation may have leaked out through the puncture hole caused by the injection, but it did not seem possible that so much would escape that it would cause the entire animal to have a wet appearance and still enough remain in the injection site to produce a maximal effect as was measured by the increase in the length of the pubic ligament. The reason for this appearance is not clear.

Also, the animal's skin took on a somewhat translucent look, it seemed as if it were almost possible to see the internal organs through the abdominal wall. The younger mice did not give this appearance, although they did show the same matting effect on their coats.

One of the mice showed such a replacement of the pubic ligament with loose fibrous tissue that the slightest pull caused it to disintegrate and no measurement could be made.

Figures 3 and 4 show a comparison of the length of the pubic ligament in a mouse used as a control animal and a mouse which had been given estrogen and relaxin. Figures 5 and 6 show a similar
comparison in a hamster used as a control and one which had been given estrogen and relaxin.

Tables I and II show the comparative lengths of the pubic ligaments in mice used as controls and mice which had been given estrogen and relaxin. Table III shows similar data for the hamsters used as controls and those given estrogen and relaxin. Tables II and III also show the comparative weights of the groups of animals used as controls and those given estrogen and relaxin.

In the normal mouse the periodontal ligament was composed of dense fibrous connective tissue with very few elastic fibers interwoven within it. The fibers were collagenous in nature and arose from the fibroblasts contained within the ligament. In the case of the incisor tooth the ligament averaged about 8-10 cells in thickness, the nucleii were round to ovoid in shape and very numerous. They were basophilic and stained well with haematoxylin. The collagenous fibers were acidophilic and thus stained strongly with the counterstain, eosin (Figure 7).

The socket of the tooth exhibited active bone resorption and deposition, which would be expected in the light of the continuous eruption of the tooth with subsequent removal and replacement of Sharpey's fibers. The socket itself was composed of a dense cortical type of bone, which was pierced by many openings where the blood vessels enter and exit. The surrounding bone was of cancellous type with the
TABLE I

Experimental results on pilot procedure using Swiss albino mice, showing the increase in length of the pubic ligament.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal Number</th>
<th>Length of Public Ligament (in millimeters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.7</td>
</tr>
<tr>
<td>Estradiol Benzoate on Days 1, 2, 3, 4, 5</td>
<td>4</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7.3</td>
</tr>
<tr>
<td>Relaxin on Days 6, 7, 8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE II

Experimental results with the Swiss albino mouse, showing the increase in length of the pubic ligament and the gain in body weight.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal Number</th>
<th>Weight (grams)</th>
<th>Length of Pubic Ligament (millimeters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>average</td>
<td>17.55 ± 0.23</td>
<td>0.96 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>18</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>18.5</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
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<td>0.8</td>
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<td></td>
<td>6</td>
<td>17.5</td>
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<td>1.1</td>
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<td>8</td>
<td>17</td>
<td>0.8</td>
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<td></td>
<td>9</td>
<td>16.5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>20.2 ± 0.27</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>Estradiol on days 1, 2, 3, 4, 5, 6</td>
<td>11</td>
<td>23</td>
<td>3.0</td>
</tr>
<tr>
<td>Benzoate on days 7, 8, 9, 10</td>
<td>12</td>
<td>19 t=3.1</td>
<td>1.4 t=5.1</td>
</tr>
<tr>
<td>Relaxin on days 13</td>
<td>13</td>
<td>20</td>
<td>4.5</td>
</tr>
<tr>
<td>14</td>
<td>24.5</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>18</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Relarin on days 17</td>
<td>17</td>
<td>16.5</td>
<td>1.9</td>
</tr>
<tr>
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<td>20</td>
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<td>2.0</td>
<td></td>
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<tr>
<td>average</td>
<td>20.93 ± 0.21</td>
<td>3.14 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Estradiol on days 21</td>
<td>21</td>
<td>19</td>
<td>3.2</td>
</tr>
<tr>
<td>Benzoate on days 22</td>
<td>22</td>
<td>23 t=3.5</td>
<td>2.8 t=12.1</td>
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<tr>
<td>23</td>
<td>22.5</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Mixture of Estradiol Benzoate on days 24</td>
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<td>3.7</td>
</tr>
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<td>and Relaxin on days 25</td>
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<td>2.5</td>
</tr>
<tr>
<td>26</td>
<td>19</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>22</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>19.5</td>
<td></td>
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</tr>
</tbody>
</table>

*Standard error of the mean.
TABLE III

Experimental results with the Syrian hamster, showing the increase in the length of the pubic ligament and the variation in body weight.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal Number</th>
<th>Weight (grams)</th>
<th>Length of Pubic Ligament (millimeters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>113.1 ± 3.9</td>
<td>2.27 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>108.5</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>132</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>110</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>100.5</td>
<td>2.0</td>
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<tr>
<td></td>
<td>5</td>
<td>119</td>
<td>2.2</td>
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<tr>
<td></td>
<td>6</td>
<td>117</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>105</td>
<td>2.1</td>
</tr>
<tr>
<td>Estradiol Benzoate on</td>
<td>average</td>
<td>115.9 ± 2.5</td>
<td>4.04 ± 0.22</td>
</tr>
<tr>
<td>days 1, 2, 3, 4, 5, 6, 7</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>106 t=.01</td>
<td>3.7 t=.32</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>127</td>
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<td></td>
<td>16</td>
<td>120</td>
<td>2.0</td>
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<td>Relaxin only on days 1, 2, 3, 4</td>
<td>17</td>
<td>108. t=.002</td>
<td>4.2 t=.6</td>
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<td></td>
<td>18</td>
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<td>4.3</td>
</tr>
<tr>
<td></td>
<td>21</td>
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<td>3.8</td>
</tr>
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<td>118</td>
<td>3.6</td>
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<tr>
<td></td>
<td>24</td>
<td>109</td>
<td>4.1</td>
</tr>
</tbody>
</table>

* Standard error of the mean.
Figure 7. A photomicrograph showing the epithelium of the gingiva and the transseptal fibers of the periodontal ligament of a Swiss albino mouse which was used as a control animal. Note the tooth structure on each side and its relationship to the ligament and the supporting alveolar bone. In the lower portion of the photograph a cross section of a molar root is evident. (Magnification - 20X)
trabeculae arranged in a random manner. In some sections active hemopoietic tissue was evident in the cancellous spaces of the bone. In much of this bone clear reversal lines were evident showing a constant growth and recontouring process. Because of the age of these animals there was very little active resorption without subsequent deposition in evidence. In many of the sections the odontoblasts and ameloblasts could be seen actively participating in the formation of new tooth structure. This was true of the incisor tooth but not of any of the molar teeth.

The epithelium of the oral mucosa was usually from 8 to 12 or 15 cells in thickness with very few rete pegs extending into the underlying connective tissue. That epithelium which covered the palate had a thick border of completely keratinized cells. This keratinized layer extended up to the gingival crest but not down into the gingival sulcus. In general, the epithelial attachment to the incisor tooth had a configuration which would lend support to the concept that the epithelium is attached directly to the enamel of the tooth rather than being of the "epithelial cuff" variety. The epithelium was attached to the tooth for a distance of about 10 cells and then, rather than peel away from the tooth in its full thickness, the cells themselves seemed to separate leaving a thin layer still attached to the tooth and extending incisally
along the surface for a short distance. It is this finding that led Weski (60) to propose his theory of epithelial attachment.

In the molar region the interdental fibers of the periodontal ligament were clearly in evidence, were quite dense in nature, and not as many nucleii seemed to be present as in the incisor region. The vascular network near the bone was clearly evident. The overlying epithelium was essentially the same as that found in the incisor region, but no evidence of a similar type of attachment to the tooth surface could be found. There was no evidence of inflammation in the gingivae of any of the sections examined.

In the experimental animals which were given relaxin, some histologic changes were found. These were confined for the most part to the connective tissues of the periodontal ligament. As far as could be determined by the methods of this experiment there was no change in the epithelium, bone, muscle, glandular tissue, or other structures of the mouse head present in the sections.

In the periodontal ligament, changes were evident which ranged in extent from very mild to very marked. They consisted primarily of a relative decrease in the number of fibroblast nucleii per unit volume of tissue, and separation of the fibers as is characterized by edema of the connective tissues. The nucleii of the fibroblasts
seemed to be somewhat pyknotic and seemingly compressed. This
gave the appearance that the tissue had been put under severe stretch
and the nucleii pulled out long and thin along their long axes.

These changes were most evident in the transseptal fibers of
the periodontal ligament between the molar teeth. In the incisor
region it was more evident in some areas than others along the axis
of the incisor tooth.

The overlying epithelium of the gingiva remained unchanged
and there was no evidence of inflammation in the experimental
animals.

The width of the periodontal ligament space seemed to be
unaltered and there was no evidence of increased bone resorption or
deposition.

Where it could be observed the connective tissue of the palate
in the animals which had been given relaxin seemed to show the same
changes as were seen in the periodontal ligament. It was consider-
ably looser in character than the same tissue in the control animals.

See Figures 8, 9, 10, 11, 12, 13 and 14 for comparisons between
the histologic structures in mice receiving estrogen and relaxin and
those used as controls.
Figure 8. A photomicrograph showing the interdental area of the molars of a Swiss albino mouse which had been given estrogen and relaxin. The overlying epithelium appears normal but at this magnification the tissue spaces caused by the edema in the periodontal ligament can be seen.

(Magnification - 20X)
Figure 11. A photomicrograph showing the transseptal fibers of the periodontal ligament of a mouse which had been used as a control animal. A small portion of the crest of the alveolar process can be seen in the upper left portion of the picture. This view shows the typical round to ovoid appearance of the nucleii of the fibroblasts with the fibers of the connective tissue between them.

(Magnification 270X)

Figure 12. A photomicrograph showing the transseptal fibers of the periodontal ligament of a mouse which had been given estrogen and relaxin. A portion of the overlying epithelium can be seen in the lower left portion of the picture. This clearly shows the large spaces between the fibers of the ligament and the disturbance in the morphology of the nucleii.

(Magnification 270X)
Figure 13. A photomicrograph of a cross section of the roots of the molar teeth of a mouse which had been used as a control animal. The periodontal ligament appears dense and the nuclei are fairly uniform in size and distribution.

(Magnification 60X)

Figure 14. A photomicrograph showing a cross section of the root of a molar tooth in a mouse which had been given estrogen and relaxin. The periodontal ligament has some small edematous spaces and appears somewhat more friable than that of Figure 13.

(Magnification 60X)
In the normal hamster the periodontal ligament of the incisor averaged about 12-15 cells in thickness being somewhat thicker toward the apical end where active tooth formation was taking place. In the molar region the ligament was also about 12-15 cells in thickness, but on the average somewhat less than in the incisor region.

The overlying epithelium was about 15-20 cells in thickness and in all areas except that portion which forms the epithelial attachment to the tooth it was covered by a thick layer of completely keratinized cells.

The vascular network within the periodontal ligament space was very rich and as in the case of the mouse was relatively more prominent in the apical region and in the gingival region. The fibers of the ligament could be seen running into the bone for a considerable distance as Sharpey's fibers.

The bone of the hamster jaws was very dense and where cancellous bone was evident the trabeculae seemed proportionately quite thick. There was evidence in many places of active resorption and deposition. This was most marked in the fundus of the sockets of the teeth and the resorption was most marked along the mesial borders of the roots of the teeth with the deposition being most in evidence along the distal borders of the teeth. Although this has not been
reported in hamsters, it is a common finding in humans and is caused by the mesial drift of the teeth.

No attempt will be made to describe any of the other tissues that appeared in the sections as they appeared histologically normal in both the control animals and those injected with the drugs.

In the groups of experimental animals that were given relaxin definite changes were seen in the connective tissue but no changes could be evaluated in any of the other tissues by the techniques employed in this study.

Essentially, the changes were similar to that described in the mouse and consisted of changes in the periodontal ligament connective tissues which ranged from very slight to very marked (Figures 15, 16, 17 and 18).

In general, the periodontal ligament of the animals injected with relaxin showed a relative increase of the fibers over the number of nucleii, a separation of the fibers as might be seen in edematous tissue, and the nucleii of the fibroblasts appeared to be stretched along the long axis of the cell until they appeared to be short straight dark lines instead of the normal contour of rounded to ovoid nucleii.

This was most evident near the alveolar crest and in the trans-septal fibers between the molar teeth. In the periodontal ligament of
Figure 15. A photomicrograph showing portions of two molar teeth, interseptal bone, and the transseptal fibers of the periodontal ligament of a hamster that was used as a control animal.

(Magnification 20X)

Figure 16. A photomicrograph showing portions of two molar teeth, interseptal bone, and the transseptal fibers of the periodontal ligament of a hamster that had been given estrogen and relaxin. Note a small piece of dental calculus in a bed of inflammatory cells at the crest of the interdental papilla.

(Magnification 20X)
Figure 17. A photomicrograph showing the transseptal fibers of the periodontal membrane of a hamster that was used as a control animal. A small portion of the overlying epithelium is evident at the top of the picture and the crest of the interdental septum can be seen at the lower edge. Note the round to ovoid appearance and even staining qualities of the nucleii of the fibroblasts.

(Magnification 60X)

Figure 18. A photomicrograph showing the transseptal fibers of the periodontal ligament of a hamster that had been given estrogen and relaxin. The epithelium can be seen at the top and the interdental septum at the bottom. The fibers can be seen attached to a portion of tooth structure in the upper left portion of the picture. Note the edematous spaces and the drawn, pyknotic appearance of the fibroblast nucleii.

(Magnification 60X)
the incisors the changes varied along the length of the tooth as it varied from animal to animal, i.e., from very slight to more marked. In general, the greatest degree of edema was evident near the coronal end of the attachment and just coronal to the fundus of the socket.

The intervening tissues appeared for the most part essentially normal.

Particles of dental calculus and portions of the plaque described by Mitchell (48) were evident in the majority of the sections of the molar teeth. This most often occurred at the crest of the interdental papilla. Where this occurred there was a localized abscess formation within the upper border of the epithelium which did not extend through the full thickness of the epithelium into the underlying connective tissue. In none of the sections studied were any inflammatory cells seen other than in the superficial layers of the interdental papillae (Figure 19).

The histological changes in the pubic ligament of mice have been studied and reported by many investigators. So far, no report has appeared in the literature describing the changes or lack of them which relaxin will produce in the pubic ligaments of hamsters.

Grossly the bones of the pelvic arch appeared to be joined with a dense white-appearing tissue which is roughly H in shape with the central bar occupying the actual space between the bones and the arms
Figure 19. A photomicrograph showing the tip of the interdental papilla from a hamster which had been given estrogen and relaxin. A piece of dental calculus is present with a rather thick border of inflammatory cells immediately beneath it. A few inflammatory cells can be seen migrating upward through the epithelium but these are relatively few in number. (Magnification - 60X)
extending along the bony margins in all four directions (Figures 5 and 6). This tissue was dense and hard and had the gross appearance of cartilage. The muscles of the hind legs were attached to the bone right up to the cartilagenous tissue if not actually onto it.

The distance between the pelvic bones did vary somewhat between the control and the experimental animal but not to a statistically significant degree.

The microscopic sections (Figures 20, 22, and 24) showed normal cancellous bone on both sides of a central area of densely stained normal-appearing hyaline cartilage. The cartilage was covered with a thin layer of fibrous connective tissue to which a few small fragments of muscle could be seen to be attached. The bone was of the cancellous type with the spaces filled with hemopoietic tissue.

The sections from the animals receiving estrogen and relaxin showed that the cartilagenous portion of the pubic arch was completely replaced by a rather loose-appearing fibrous connective tissue (Figures 21, 23 and 25). All along its border the bone had the appearance that only the cartilage had been removed in toto and the surface was now somewhat roughened, although in quite a straight line and it showed evidence of active resorption. In several places large osteoclasts were evident in their lacunae. The replacement of the cartilage
Figure 20. A photomicrograph showing the interpubic area of a hamster that had been used as a control animal. The area between the bone tissue can be seen to be hyaline cartilage instead of fibrous ligament.
(Magnification 20X)

Figure 21. A photomicrograph showing the interpubic area of a hamster that had been given estrogen and relaxin. The cartilaginous portion which was evident in Figure 20 can be seen to have been completely replaced with a loose fibrous type of tissue.
(Magnification 20X)
Figure 22. A photomicrograph showing the interpubic area of a hamster that was used as a control. The area between the bone tissue is completely filled with hyaline cartilage and the periosteum can be seen to be continuous across the entire area. In the borders of the cartilaginous area calcification and replacement of the cartilage by bone is evident.
(Magnification 60X)

Figure 23. A photomicrograph showing the interpubic area of a hamster that had been given estrogen and relaxin. The complete replacement of the cartilage by a fibrous connective tissue is evident.
(Magnification 60X)
Figure 24. A photomicrograph showing the area of the pubic arch of a hamster used as a control animal in which the cartilage and bone join. The calcification of the cartilage and its subsequent removal and new bone formation can be seen.  
(Magnification 270X)

Figure 25. A photomicrograph showing the pubic arch of a hamster that had been given estrogen and relaxin. It can be seen that no cartilage remains but is completely replaced by fibrous connective tissue.  
(Magnification 270X)
seemed to have no effect on the attachment of the muscle fibers as they were still evident attached to the connective tissues in the edges of the area.

This amount of change took place in a matter of three days which again showed the speed of the adaptation caused by the hormone.
DISCUSSION AND CONCLUSIONS

The changes in the connective tissues of the periodontal ligament of both mice and hamsters was consistent with previously observed changes in connective tissue caused by relaxin (9, 37). The fibers seemed to be more loosely arranged with a much less dense appearance and during the staining procedures did not take the counterstain quite as well as did those in the control animals. The interfibrillar spaces were filled with fluid bearing out the findings of increased capillary permeability with subsequent leakage of fluid into the tissue spaces (56, 61).

The mucosal and gingival epithelium of both the mice and hamsters appeared to be unchanged and did not exhibit any of the characteristic features that have been described in pregnancy gingivitis. In all sections there appeared to be a normal maturation of the cells outward from the basement membrane and the proliferation of the rete pegs into the underlying connective tissue was not evident as has also been reported in human pregnancy gingivitis (32, 65).

Where it was evident the connective tissue of the hard palate in the mice appeared much looser in character with a disorganization of the fibers. Again, in this area the epithelium appeared unchanged.
In the sections where evidence of dental calculus could be seen the resulting inflammation was confined to the superficial layers of the epithelium and in no instance could any evidence of appreciable inflammatory cell infiltration be seen in the underlying connective tissue. This lack of massive inflammation or progression of the epithelial attachment down the side of the root of the tooth, in spite of the presence of dental calculus, may have been due to the fact that these hamsters were still considered young adult animals.

It can be concluded that in this study the presence of inflammation did not have any conditioning effect on the response of the underlying connective tissues to the action of estrogen and relaxin.

On examining the weights of the mice receiving relaxin and comparing them with the weights of the control animals an appreciable gain was found in those animals receiving estrogen and relaxin. This was a statistically significant change and could be considered consistent with the previously reported actions of relaxin of causing urinary retention and capillary permeability (56, 61).

The weight of the hamsters showed no significant change which suggested that these animals are not so readily susceptible to that action of relaxin or that they are sufficiently larger in size that the dosages used did not produce as pronounced effect.
As no previous reports were available as to the changes that could be expected in the pubic area of the hamster, it was not known whether these changes could be used as a criterion for the effectiveness of the dosage of the drug used or not. Grossly the change in the length of the pubic ligament was not of sufficient magnitude to make the hamster a desirable animal for assay procedures. However, it could easily be demonstrated histologically that relaxin caused the breakdown of the hyaline cartilage between the pubic bones and its replacement with a rather loose fibrous connective tissue. Grossly the shape of the interpubic area could readily be observed to have been changed.

The results of this experiment showed that the production of the hormone relaxin during the course of pregnancy would tend to loosen the connective tissues supporting the teeth. This would tend to decrease the localized resistance of these tissues to the invasion of bacterial invaders with resulting inflammatory response.

It did not appear that the relaxin had any effect *per se* on the hyperreactivity of the gingivae to local irritation or enhanced in any gross way the often observed increase in the size of the gingivae. Also, it would probably have no effect *per se* on the production of the pregnancy tumor.
In the condition of pregnancy gingivitis the pregnancy can only be considered as a conditioning factor in its production. There are other factors which must be taken into consideration during pregnancy which would affect the state of the gingivae such as poor oral hygiene, change in dietary habits, nausea and vomiting of pregnancy, and lack of general well-being at times during the course of the pregnancy.

The presence of relaxin during the course of pregnancy could be classed as one of the systemic factors which allows a much more rapid progression and greater ease for bacterial invasion with subsequent aggravation of any pre-existent inflammation in the gingivae of the pregnant woman.
SUMMARY

1. A study was carried out to determine what effect the hormone, relaxin, might have on the periodontal tissues of the Swiss albino mouse and the golden Syrian hamster. These animals were first "primed" with estrogen in the form of estradiol benzoate followed by the injection of relaxin in both an aqueous and oily vehicle.

2. As a result of the administration of relaxin, the connective tissues of the periodontal ligament showed edematous changes which have been reported as occurring in other connective tissues of the body. The response to the administration of relaxin varied from animal to animal but was unmistakably present in most of the animals receiving it.

3. The animals that received the injections of relaxin gained an appreciable amount of weight during the experimental procedure. In the mice this gain was statistically significant, while in the hamsters it was not.

4. The previously reported changes in the pubic ligaments of mice was confirmed in this study.
5. Changes were found in the pubic area of hamsters. These changes consisted of the replacement of the cartilage joining the pubic bones with a loose fibrous connective tissue.

6. The hamster as used in this study would not be a suitable animal for assay procedures but might lend itself well to other types of experimental procedures.

7. The connective tissue of the palate of the animals receiving relaxin was somewhat looser in character than the tissue in the same location in the control animals. This was consistent with other reported effects of relaxin on connective tissue.

8. The presence of dental calculus with resulting inflammation had no effect on the changes observed in the connective tissue of the periodontal ligament of the hamster.
LITERATURE CITED


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