This is to certify that the

thesis entitled

THE MECHANICAL PROPERTIES OF THE
RABBIT CERVIX THROUGHOUT PREGNANCY

presented by

ANDREA J. DANAS

has been accepted towards fulfillment
of the requirements for

Masters degree in Mechanics

Robert Wm Little
Major professor

Date July 10, 1981

THE MECHANICAL PROPERTIES OF THE RABBIT CERVIX THROUGHOUT PREGNANCY

By

Andrea Joyce Danas

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Metallurgy, Mechanics and Materials Science

1981

ABSTRACT

THE MECHANICAL PROPERTIES OF THE
RABBIT CERVIX THROUGHOUT PREGNANCY

By

Andrea Joyce Danas

The purpose of this research was to study the mechanical properties of the rabbit cervix throughout various stages of pregnancy and to gain a better understanding of similar properties in the human. White New Zealand Albino Rabbits were impregnated and their cervixes removed at designated stages of pregnancy. Three types of mechanical tests were employed on the excised tissue. The first set of tests were loading and unloading cycles to a specified strain level at various constant strain rates. The second series of tests were sinusoidal strain tests conducted at various frequencies. And finally, the third test was a stress-relaxation test held for a specified period of time. Histological examinations were performed on representative tissues at different stages of pregnancy to monitor the changes in the connective tissue composition and structure.

ACKNOWLEDGMENTS

The author expresses her deepest appreciation to Dr. Robert Wm. Little for his continual guidance and support throughout this project. I especially thank him for his friendship throughout my career.

I would also like to thank Dr. Robert Hubbard and Mr. Robert Schaffer for their technical assistance with the equipment needed for this project.

I sincerely thank Mrs. Jane Walsh for her preparation of the histological examinations for this study.

I would like to thank Mr. Robert Konopactz for his preparation of the electron microscopy studies.

I would like to thank the Departments of Biomechanics and Metallurgy, Mechanics and Materials Science for their support and assistance.

I especially thank all of my friends and family without whose continued support and encouragement this would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The definition of the cervix as an independent organ has been the subject of much debate. The classical description of the cervix defines it as the lowest segment of the uterus but recently, it has been considered an organ in its own right aiding in the birth process.

The cervix undergoes remarkable changes throughout pregnancy from a highly collagenous undistensible structure capable of resisting pressure and gravity, and holding the nondeveloped fetus, to a soft highly distensible structure permitting passage of the fetus at term. In order for this to occur, the cervix must undergo major changes in its connective tissue properties, the nature of which are not completely understood. It is believed that there is a breakdown or dissociation in the collagenous framework enabling the cervix to become more easily dilated, but the exact nature of this breakdown is unknown.

Several scientists have attempted to determine the amount of dilatation the cervix can undergo at various stages of pregnancy without incuring damage. They have attempted to measure tensile strength and the elastic modulus throughout pregnancy to help quantify these changes. Other scientists have attempted mathematical and geometrical models of the cervix in hopes of more fully understanding it's mechanical characteristics.

This research examined the mechanical properties and histological changes of the cervix throughout the various stages of pregnancy as well as the nonpregnant and post partum cervix. In order to do so, it was necessary to understand the composition of the cervix. The anatomical discussion of the cervix is included in Appendix A.

The function of the cervix during pregnancy is to help retain the conceptus by contributing to the closing of the uterine cavity. Its function during labor is to transmit increased pressure and tension resulting in increased dilatation in response to contractions of the corpus, thereby permitting the delivery of the fetus. (1,2)

Until the eighth week of pregnancy, the configuration of the human uterus is little changed from the nonpregnant uterus. From eight to twelve weeks of pregnancy, hypertrophy occurs with a lengthening and thickening of the isthmus. After twelve weeks, the conceptus fills the uterine cavity and further growth causes a gradual unfolding of the isthmus until the isthmus reaches the fibromuscular junction. At this time, further growth of the conceptus causes a thinning of the uterine walls (2). During this unfolding of the isthmus, the muscle fibers at the isthmocervical junction rearrange into a circular configuration which has no sphincterlike activity but does transmit a pull on the upper segment. During the sixth month of pregnancy, the isthmus and lower part of the corpus are drawn upward and the lower uterine segment is formed. This lower uterine segment is a cone of muscle which is continuous with the inner interlacing layer of muscle in the corpus, with an apex two-thirds of the way down the isthmus. The muscle fibers of the upper and lower uterine segments contract and relax drawing the lower uterine segment and the unfolding cervical canal upward, causing the canal to progressively shorten, bringing about effacement. The muscle cone works with the cervix to prevent dilatation. The cone dilates and is drawn up in late pregnancy allowing the fetal head to descend into the lower uterus and the conceptus to be supported by the cervix. The cervix becomes softer preparing for dilatation and is more responsive to the stronger contractions of the corpus. Cervical dilatation is controlled by uterine activity and mobility as well as the resistance of the lower uterine segment and the circular musculature in the lower part of the corpus.

Accoring to Philpott (2), Friedman analyzed the rate of dilatation of the cervix and compared the time rate of change of dilatation in the cervix with fetal head descent. Dilatation of the cervix is completed in the first stage of labor, which is divided into two phases, the latent and active phases. The latent phase is the time from the onset of labor when contractions become regular until the time when there is a noticeable increase in the rate of progress of cervical dilatation. The active phase then continues through cervical dilatation until it is completely dilated. The active phase is further subdivided into three stages, the acceleration phase, the middle phase where most of the dilatation takes place, and the deceleration phase.

There is only minimal head descent during the latent and acceleration phases. During the middle phase of cervical dilatation, fetal head descent begins. Maximal rate of head descent occurs during the deceleration phase and is completed during the second stage of labor (2).

During uterine contractions, the increase in amniotic fluid pressure causes the fetus to be pressed towards the cervix at which

point dilatation begins. Lingren (1) shows that friction occurs between the descending head and the uterine wall with a coefficient of 0.2. Maximum resistance from the cervix occurs at the base of the head. He also determined that uterine contractions when the cervix has effaced occur with a frequency of 11 - 12 per hour with a pressure of 25 - 30 mm Hg. Amniotic fluid pressure changes also from 30 initially to 45 mm Hg when the cervix is fully dilated.

Studies have been made of the microscopic changes which take place during cervical effacement. Since the cervix is mainly connective tissue, the changes must be explained in terms of connective tissue. Although there are changes in all connective tissue structures in the body during pregnancy, the cervix has the most pronounced of these changes. The cervix becomes softer and increasingly more dilatable. During labor, the cervix dilates to a diameter of 10 cm (3). One week after delivery it begins to regain its former contours and within one month to six weeks, the cervix returns to normal.

In the nonpregnant cervix, collagen appears as dense tightly woven bundles of interlacing fibers running parallel to one another (3), whereas in the pregnant cervix, the collagen fibers dissociated into fine fibrillar components which form branches (3). Fibrillar dissociation is the change that permits the fibers to loosen and slide upon one another consequently allowing the cervix to soften and dilate without injury. There is an increase in the dissociation of collagen with the increase of cervical ripening. In late pregnancy collagen fibers are similar to those of the nonpregnant cervix except for the intorduction of spaces between the bundles (3). Danforth, Buckingham, and Roddick (4) observed that the loosening of connective tissue

occurs during the last stage of pregnancy. The fibrous dissociation of collagen is completed during labor, when the fibers separate and branch in many directions rather than running parallel to each other (3). Collagen concentrations in the cervix decline during pregnancy, however the maximum drop accompanied with an increase in water content of the cervix occurs prior to labor and remains the same throughout labor.

Ichijo, et al (5) describes five grades of collagen dissociation. First, the fibers tend to lie straight, but many fibers are slightly wavy; second, the fibers show a more wavy course, and begin to dissociate; third, the fibers branch into fibrillar components; fourth, the dissociation process progresses further and results in many fine branchings; and finally all the fibers are branched with an increase in dissociation with cervical ripening. He claims that during effacement, the collagen fibers become immature as they dissociate. Consequently, either collagen fibers transform into reticular fibers or collagen fibers depolymerize and reticular fibers appear in their place. The basis for this idea lies with the fact that with persistant edema or inflammation, collagen fibers change to reticular fibers. Also, in the embryonic development process, collagen fibers evolve from reticular fibers, making it conceivable for this mutual transformation to take place in the cervix. An increase in the acid mucopolysaccharides in the cervical ground substance in late pregnancy may provide the conditions necessary for the transformation.

Prior to Ichijo, Danforth and Buckingham (6) questioned whether the ground substance was a factor in collagen dissociation or whether the trauma of labor could produce this loosening of the fibers. They noted that during and after pregnancy, the characteristic banding period of the collagen (640 A) is similar to the nonpregnant cervix, except the fibers may be further apart. They subjected a nonpregnant cervix to the most "violent mastication in the colloid mill" to see if the trauma of labor could produce loosening of the fibers. Although the fibers were broken, they found that they remained tightly held together. Therefore a possible cause could in fact be a change in the ground substance. (7,8)

The ground substance mass increases significantly during pregnancy, reaching a maximum at term and immediately after delivery, as evidenced by the change in water concentrations (3). The accumulation of water during pregnancy amounts to an increase of $7^{o}/o$ maximum, indicating that it seems inadequate to constitute a total explanation for the loosening and separation of the collagen fibrils. However, there is also an increase in the mucopolysaccharides in the ground substance which may affect the collagen bonds. According to Danforth and Buckingham (6) the metabolism of mucopolysaccharides is rapid compared to other macromolecules; therefore, this change in the acid mucopolysaccharide concentration may secondarily affect the changes in the collagen of the cervix.

Another aspect Danforth and Buckingham (6) investigated was collagen solubilities. Since old collagen is almost insoluble and newly formed collagen is readily soluble in a neutral salt or dilute citric or acidic acid, testing to see if there is any difference in the amount of soluble or insoluble collagen should indicate an increase in new or old collagen respectively. He found that in the fraction of collagen which is soluble in a dilute acid, the identity of the fibrils is lost and the banding period vanishes. Essentially, collagen has gone into solution. However, neutralization of the acid reconstituted the fibrils making them indistinguishable from the originals. The question arises as to whether this phonomena happens after labor.

More possibilities for causes of cervical effacement have been considered. Danforth, et al. (13) isolated an enzyme, collagenase, which specifically attacks collagen. It is the only enzyme isolated thus far that causes the degradation of collagen. According to Danforth, et al., Silbert(9) proposed that the hormonal activation of collagenases had caused fibrillar dissociation. Also in 1973, Groschel-Stewart, Herman and Schwalm(10) isolated a ground substance protein resembling anyloid from the nonpregnant cervix. They suggested that it may have some physiologic changes during pregnancy and labor.

Some changes between the collagen of the cervix and the collagen of the body were noted by Danforth and Buckingham (8). Amino acid contents in collagen in nonpregnant cervixes were similar to those in standard human collagen with a few exceptions. Hydroxyproline values were only 82^{0}/o of those in the standard; therefore, collagen represents only 82^{0}/o of the total protein in the nonpregnant cervix. Also, two acidic acids, asparic acid and glutomic acid, were increased by 117^{0}/o and 127^{0}/o respectively of there initial nonpregnant values.

Exploring the changes in concentrations of amino acids during pregnancy, Danforth and Buckingham discovered an absolute decline in collagen and an increase in noncollagenous protein. The increase in water content during pregnancy may result in a corresponding increase in proteoglycan content. Other changes in the collagen included a drop of hydroxyproline to 52^{0}/o of the standard when complete dilatation of the cervix was reached. There was also a considerable increase in glycosaminoglycans and acidic amino acids.

Danforth and Buckingham indicate that glycan constitutes about one-third of the collagen, therefore the drop in this amino acid confirms that hydroxyproline changes. They also found high levels of tyrosine and phenylaline which are normally present in small amounts suggesting the presence of some other protein. They were unable to identify the 18^{0}/o of the proteins present in the nonpregnant and 47^{0}/o present in the post partum cervix. It is known that they are noncollagenous and do not originate from either muscle or serum. Danforth and Buckingham proposed two possible solutions. The first is that it is a constituent of the ground substance in the form of proteoglycan core protein evidenced by the increase in this amino acid which composes a major part of the ground substance. The second possibility is that the new protein represents an increase in the acidic structural protein of Timpl, Wolff, and Weiser.

The nature of the bond which holds the collagen fibrils together in dense tissues or allows them to separate in looser tissues is not known. One solution is that the molecular configuration may shift to produce or prevent aggregation of the fibrils (6). A second idea postulated by Hughesdon is that the hormone relaxin may be responsible for the cementing of collagen together (11). Finally, another solution, and the one most pursued, is the influence of the ground substance in causing collagen fibers to bind together or slide apart (8). The ground substance may affect the intermolecular reactions causing either cohesion or dissociation.

Other changes in the cervix during pregnancy should also be noted. Changes occur in the reticulin fibers during pregnancy as well. In the nonpregnant cervix, the reticular fibers appear as short segments irregularly dispersed throughout collagen bundles (3). In the pregnant cervix, the reticular fibers are more robust and luxuriant and can be traced in thin wavelike branchings which tend to parallel the collagen fibers. The structure of the reticular fibers post partum appears to be much the same as in the cervix at term except they are more widely separated and somewhat thinner.

Secondly, some changes in the cervix can be accounted for by consideration of the cervical musculature. According to Danforth (4), Hughesdon claims that the degree of dissociation of collagen decreases as it approaches the internal os. The connective tissue surrounding the intrinsic muscle bundles does not dissociate during pregnancy as do the collagen fibers intermixed within them. Since there is more muscle higher up in the cervix, softening of the cervix is relatively less.

At three to four months of pregnancy, the intrinsic muscle fibers of the cervix are more prominent, making the cervix appear more muscular (11). However, the distribution of muscle is variable with more bundles of muscle in the central portions of the cervix. Since they are heavily interspersed with fibrous tissue, sphincteric effects are an impossibility. Therefore, changes in the cervix due to pregnancy are not due to muscular alterations.

During pregnancy there are connective tissue changes throughout the body. Cervical collagen is qualitatively similar to that of the anterior rectus sheath, where marked changes occur enabling the abdomen to accomodate the enlarging uterus without tenseness (3). Other changes in connective tissue occur in the skin where there is a loss of tensile strength during pregnancy (12).

Since there is a marked increase in blood volume, vascular changes occur in the body as well. The circulatory system may cause some of the changes the body undergoes during pregnancy. An active pancreatic enzyme, elastase, has been isolated which causes the breakdown of elastin and quantities of an elastase inhibitor have been discovered in late pregnancy two to three times the normal values (6). The significance of the increase is unknown but, it may tend to limit some vascular changes which are considered part of normal pregnancy. Other vascular changes include an increase in vericose veins, changes in both the aorta and peripheral vasculature. Further changes include the loosening of the joints or the pelvis and lower back, the mobility of the teeth and eye refraction. All of these are results of changes in connective tissue and may be caused by some factor carried by the circulation during pregnancy (3).

At this point, a brief mention should be made about cervical incompetency. In a study by Hafez (13), it was discovered that the percentage of muscle was higher in a group of patients with cervical incompetency than in a similar control group immediately following labor. Therefore, they believed that excessive amounts of muscle may be a factor in the production of cervical incompetency. Since several cervixes did not conform to this idea, cervical incompetency must be a result of many factors rather than just the deviation in cervical composition.

In order to determine the mechanical properties of the cervix correlating with the histological changes, several researchers have worked in various areas of mechanical testing of the cervix. One such area was to design an apparatus which dilated the cervix and recorded the amount of dilatation the cervix could undergo without damage to it.

T. Bakke (14) developed an instrument which measured the fibroelasticity or consistency of the cervix. The instrument measures, in vivo, the consistency of the cervix by measuring the depth and shape of an impression while recording the force needed to make this impression. He defined cervical consistency as the angle between the walls of the depressed area divided by the applied force. The instrument consisted of a wing shaped end which would conform to the shape of the cervix, attached to a sliding tube which would audibly register when the desired force is applied. Thus the angle between the walls of the cervix is measured by the spread of the wings, the force being set as desired , with the ratio of these two quantities being the fibroelasticity. This instrument can give quantitative measurement of the amount of cervical dilatation present in vivo.

Three studies have attempted mathematical models of the cervix. The first, Lui et al. (15) attempted to model the cervix as a thick-walled cylinder. They subjected, in vitro, the cervixes from multi-parous women to a uniform axial force by using a specially designed force measuring dilator and formulated a stress vs. strain relationship. From these curves, they were able to obtain a quasi-static moduli for the different degrees of dilatation. They also obtained theoretical distributions of the radial and circumferential stress across the cervical wall and found a yielding of cervical tissue at 9-11 mm dilation. The stress-strain curve for the cervix is characteristic of a viscoelastic tissue. The quasi-static modulus, a measure of the gradiant of a stress-strain relationship, increased with each stage of dilation.

Lui, et al. assumed the human cervix to be an elastic, isotropic, incompressible, homogeneous cylinder with a diameter and length of 25 mm. They also accounted for frictional resistance and the unknown recovery rate after stretching. They employed the Lame equations for a thick walled cylinder to obtain the quasi-elastic modulus for each dilatation. Values of radial and circumferential stresses were calculated for a range of dilations at increasing radii across the cervical wall. They found that both radial and circumferential stress decayed rapidly and nonlinearly towards the outer boundary of the cervical wall. For radial stress, the limiting value was zero.

In $95^{0}/o$ of the experimental tests, a radial tear beginning from the inside of the cervical wall was observed at dilatations of 13-14 mm diameter after an initial microscopic yielding at dilatations of 9-11 mm diameter. Therefore, they concluded that to avoid tissue damage 10 mm is a limitimg value of tissue compliance.

Rice and Yang (16,17) did two studies in which they mathematically modeled the cervix. In their first study, the cervix was modeled as an axisymmetric ring located at the external os, for which they qualitatively related intrauterine pressure to cervical dilatation during the first stage of labor. In the second study they developed a more mathematically detailed finite element nonlinear viscoelastic axisymmetric membrane model for the human cervix.

Two theories have been proposed to explain cervical dilatation. One concerned radial forces from the lower uterine ligaments. The other, the one which Rice and Yang supported, concerned forces caused by the wedging action of the fetal head driven by intrauterine pressure. Rice and Yang refuted the theory that uterine ligaments took part in dilatation
because the relative movement of these ligaments was so small.

Several assumptions for the two models were made: 1)the system consisting of the uterus, cervix and fetal head were axisymmetric and coaxial; 2) functional and inertial forces were negligible; 3) the cervix was passive; and 4) intrauterine force caused by uterine contractions was the only driving force.

Rice and Yang (16,17) had many reasons for their assumptions. The fetus excluding its head was completely passive and pliable so that the uterine contents were regarded as a homogeneous mass through which hydrostatic pressure was transmitted. During uterine contraction, head-to-cervix pressure in the axial direction summed over the cervix balances the axial thrust of the intrauterine pressure on the fetal head. Rice and Yang treated the uterus, uterine contents and cervix as an isolated mechanical system.

Frictional effects were ignored for two reasons. Firstly, there is a lubrication between the fetus and the structural system in the form of vernix caseosa, amniotic fluid, blood and other secretions. This lubrication significantly lowers friction effects. Secondly, a plot of intrauterine pressure, which is proportional to the axial force, versus dilatation was used as a rough indicator of relative motion or force since a direct measure of friction was impractical. The behavior of the curve indicated a lack of friction. Since the acceleration of mass for inertial forces was insignificant, inertial forces were also neglected.

In their first model, Rice and Yang (16) depicted the fetal skull as an ellipsoid and the cervix as an axisymmetric ring, the cervical wall being tangent to the fetal head at the equator of the head. The model, they attempted to design was one that quantitatively related cervical dilatation to intrauterine pressure using a model based only on the birth system geometry and tissue mechanisms, and a hydraulic mechanism neglecting friction and inertial effects.

Briefly, Rice and Yang developed their model using force balances and geometric relationships. In their formulation they determined whether the cervical tissue was linearly or exponentially elastic. The data of the cervix best fit an exponentially elastic curve, which was also shown to be linearly viscous. Linear regression formulae were used to determine various parameters.

The model was found to correspond well with the data gathered, therefore Rice and Yang concluded that the hypothesis of a hydraulic mechanism causing cervical dilatation was accurate.

In their second study, that of the axisymmetric membrane model (19), Rice and Yang also assumed that the cervix followed the following conditions: 1)the membrane was orthotropic with zero stress across its thickness; 2)there was no shear strain along the tissue axes; and 3)the tissue was incompressible (ν =0.5).

The results obtained were similar to those of the ring model, except they were more specific; 1)the cervix did dilate after every contraction showing elastic stretch and some viscous strain during each contraction. This was attributed to the viscoelastic properties of the cervix; 2) it was observed that dilatation was proportional to the strength of contraction, implying a monotonic relation which the model shows; 3)the larger the total dilatation, the larger the elastic contraction at any given strength; 4)the time delay between contraction and subsequent dialtation decreased as the inlatation increased (the model however, does not indicate this result); and 5)oxytocin was found to cause greater contractions and correspondingly faster dilatation. Greater pressure also resulted in faster dilatation, which the model did show.

The work presented in this dissertation parallels some of the work done by Harkness who performed several studies on rats to determine the changes mechanically and histologically that the cervix undergoes during and after pregnancy. In one such study, Harkness and Moralee (18) attempted to determine the route loss of collagen from the rat's uterus post partum. They wanted to determine whether the disappearance of collagen from the uterus after parturition was a result of internal reabsorption of the material, or, whether collagen was shed into the lumen of the uterus and lost through the vagina.

In another study, Harkness and Harkness (19) attempted to determine if physical properties of the cervix change after parturition. Stretching the cervix between two parallel steel rods, one through each canal, the tissues were initially loaded with a 50 g weight for 25 minutes. The load was then increased 25 g every 15 seconds until it ruptured. It is important to note that these tests applied load across the junction between the two canals and histological studies show that fibers run circumferentially around the canals and only the outer fibers surround both canals.

Harkness (20) has determined that the changes after parturition fall into two phases. In the first phase immediately after parturition, the reduction in circumference, was due to the contraction of the smooth muscle followed by a consolidation of the tissue due to the changes in the collagenous framework. He found that the circumference of the cervix dropped rapidly in the first 8 hours to about 1/3 of its initial value at parturition and believed that the rapidity of the change make it unlikely that there is a removal of collagen and replacement with a "newly formed framework of a smaller size" but, involves slip between collagen fibrils or fibers.

Harkness found that there was a decrease in weight of the cervix after parturition with a smaller decrease in collagen resulting in an overall rise in the concentration of collagen. After 24 hours, a slower decline in circumference occured and was accompanied with a decrease in weight and total collagen content. By the eighth day after parturition, the circumference of the cervix droped from 1/3 its initial value to 1/8 that of parturition taking until the 16th day to fall to its normal size.

Collagen, on the other hand, did not begin to be lost after parturition for about 24-48 hours after which there was an exponential decay in collagen content for approximately two days at which time it began to slow down but decreased to a below normal value for the nonpregnant cervix. Harkness concluded that collagen was lost by reabsorption which was in keeping with the observation that the epithelial layer of the endometrium almost completely covered the inside of the uterus within 24 hours of parturition. He noted that this change in collagen content resembled the contraction of wound tissue. The load required to break the tissues rose after parturition and then fell. Harkness believed this was due to the fact that the decline in circumference of the cervix proceeded faster than the loss of tissue at first, so the walls became thicker needing a greater breaking load. Later there was a loss of tissue , the walls becoming thinner, consequently less load was needed from a breaking tension. Breaking load per unit cross-sectional area of collagen varied little through the whole period after parturition (20).

Harkness and Harkness (21) performed three different experiments using rats to examine the physical properties of the cervix during pregnancy. One consisted of applying a constant load of 50g for 30 minutes at 22°C at various stages of pregnancy. The second involved subjecting the tissues to a load until they ruptured at both 22°C and 37°C. Finally, in the third experiment, the tissues were subjected to various constant loads for periods longer than 50 minutes.

In the nonpregnant rat, the cervix was only slightly distensible even by relatively large forces acting for long periods. At the end of pregnancy, however, the cervix was very viscous distending slowly and progressively under low forces. The change in the physical properties of the cervix did not begin until the 11 - 12th day of pregnancy after which progressive changes took place resulting in a decrease in the amount of smooth muscle present and a fall in the concentration of collagen.

In their experiments, Harkness and Harkness found that temperature had no effect on the tissues. The circumference of the cervixes from 21-day-pregnant rats was 4-5 times larger than the earlier stages but differed very little otherwise. They related load to change in circumference and found rupture strengths of 0.5 MPa for non-pregnant animals to 0.3 MPa at term with the rupture strength higher at midpregnancy. The stiffness of the cervix was found to be 120 MPa for nonpregnant decreasing to 40 MPa at term. These values are higher by a factor of 100 than the values found in this research.

When Harkness and Harkness (19) loaded the tissue for a prolonged period of time, they found that in the nonpregnant and 12-day animals, the creep rate of the cervix diminished continuously until the end of the experiment. They found the creep to be approximately a linear function of the natural logarithm of time. On the other hand, with cervixes from 21-day-pregnant rats the creep rate did not diminish continuously with time but became a constant. Therefore, the two major differences they found between the pregnant and nonpregnant cervix were; firstly, the tissue had a larger circumference, and secondly, the pregnant cervix behaved in a purely viscous manner.

Harkness and Harkness tried to model the response of the cervix by using a rheological model with a single viscous and elastic element in parallel, combined in series with a viscous element. They found however, that this model did not fit the curves well.

Other mathematical models of the cervix should be briefly mentioned. Kriewall (22) did a study on the uterine work in parturition. He related work expended by the uterus to the intrauterine pressure and cervical dilatation. Since contractions during labor dilate the cervix through the mechanics of retraction, to accomplish this work must be expended by the uterus. Considering cervical contractions to be a quasi-equilibrium process, the work of dilating the cervix can be calculated using the integral of pressure-volume, PdV, where pressure was measured and the change in volume was calculated from the change in cervical dilatation. Some of the energy of contractions which was not used to dilate the cervix was lost to hysteresis effects, in rearranging the molecular structure and to the nonelastic characteristics of the cervical tissue. He concluded that the net amount of work expended by the uterus to dilate the cervix equals zero because the elastic forces of the cervix exerted the same amount of work to restore the uterine volume as was needed to decrease the volume.

HISTOLOGY OF THE RABBIT CERVIX

The rabbit cervix is similar to the human cervix except that it is a double cervix. It is composed of glandular connective tissue and muscle fibers with epithelium lining the canals. The nonpregnant rabbit cervix will be considered followed by a look at the changes the cervix undergoes throughout pregnancy. Figures 1-3 are cross-sectional slides of the nonpregnant cervix at magnifications of 10X, 50X, and 100X respectively. The tissues were stained with a Hexachrome stain; the connective tissue staining yellow-green, the muscle fibers light pink, and the epithelium dark pink.

Unlike the human cervix with its longitudinally orientated muscle fibers, there are two distinct orientations of the muscle fiber bundles in the rabbit cervix. These can be seen in the light pink areas of Figures 1-3. The outermost muscle fiber bundles are longitudinal in orientation running from the uterus to the vagina, parallel to the cervical canal, similar to that of the human cervix. Just inside this ring of muscle is a second ring of muscle fibers circumferentially orientated. The muscle fiber bundles are surrounded with loose connective tissue which is more dense around the muscle bundles, becoming looser in the cervical folds. This is in accordance with Danforth and Hughesdon's descriptions of the human cervix. The cervical folds are devoid of all muscle.

Like the human cervix, the connective tissue of the rabbit cervix consists mostly of loosely intermixed collagen fibers having no apparent preferred orientation. Figures 1-3 show the yellow-green collagen fibers intermixed among the muscle bundles as well as in the cervical folds.

The dark elastic fibers are scarce, found mainly between muscle bundles and in the walls of blood vessels. Reticular fibers are also present in small quantities. They are found in association with muscle bundles, particularly around the boundaries between the connective tissue and other tissue types. They are continuous with the collagen fibers, with a gradual transition from one to the other.

Electron microscopy studies were also performed on the rabbit cervixes to examine tissue morphology. All specimens were fixed in a modified Karnofsky's fixative medium of 2.5^{o}/o Paraformaldehyde, 3^{o}/o glutanaldehyde in a 0.1 M phosphate buffer rinse and then put through a 10 step dehydration series from 10^{o}/o to 100^{o}/o Ethanol; freeze fracturing for some samples was done at this time. After Critical Point Drying, the samples were gold coated and examined with a ISI Super III Scanning Electron Microscope.

Figure 4 is a cross-section of the nonpregnant cervix at a magnification of 23.4X. The cervical canal is located at the lower right with the orientation of the canal running into the micrograph. Three rings appear in the photograph; the inner ring including the cervical folds consists of connective tissue and an epithelium lining; the second middle ring consists of circumferentially orientated muscle bundles intermixed with collagen fibers; and finally there is an outer most ring, in the upper left hand corner, consisting of longitudinal muscle fiber bundles intermixed with collagen fibers. Figures 5-8 are

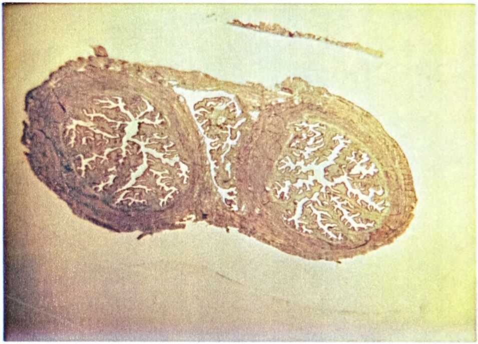

Figure 1. Hexacrome stained cross-section of the nonpregnant cervix (10X)

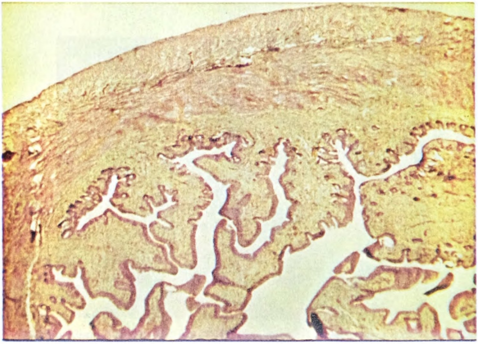

Figure 2. Hexacrome stained cross-section of the nonpregnant cervix (50X)

Figure 3. Hexacrome stained cross-section of the nonpregnant cervix (100X)

Figure 4. Electron microscopy of the nonpregnant cervix (23.4X)

magnifications of these rings.

Figures 5 and 6 show a cervical fold from the inner ring. The randomness in orientation of the collagen fibers is apparent. Figure 7 shows the middle ring. Again the randomness of the collagen fibers is apparent. It is difficult to distinguish between the collagen and muscle fibers in these photomicrographs. They can be distinguished readily, however, on the histological photographs. Figure 8 shows the outermost ring with similar randomness of collagen fibers.

According to many scientists, during pregnancy the cervix becomes increasingly edemic. The collagen fibers dissociate, branching into smaller intermixed fibers. With the dissociation of collagen, the amount of muscle appears to increase although in fact, the quantity of muscle doesn't change. The next set of figures shows the cervix at different stages of pregnancy.

Figure 9 is a slide from a rabbit cervix 23 days pregnant. It is a hematoxylin-eosin stain. The mucus secreting epithelium stained dark brownish-black and is seen around the edges of the cervical folds, lining the cervical canal. The increase in edema can be seen in the increased yellow spaces between the brownish collagen fibers. The muscle bundles are stained reddish-brown. Circumferential bundles can be seen in the upper right-hand and lower left-hand corners.

Figure 10 is a cross-sectional slide of a rabbit cervix at 33 days (term) pregnant. The collagen fibers are very dissociated, with very few muscle bundles present.

The next series of figures is taken from a 1 hour post partum cervix. One horn of the cervix was pregnant and the other was not enabling us to make a visual comparison of the effects of pregnancy.

Figure 5. Photomicrograph of a cervical fold from the inner ring.(78X)

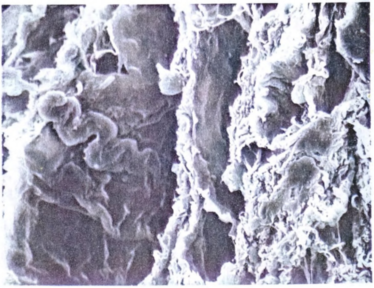

Figure 6. Photomicrograph of a cervical fold from the inner ring.(546X)

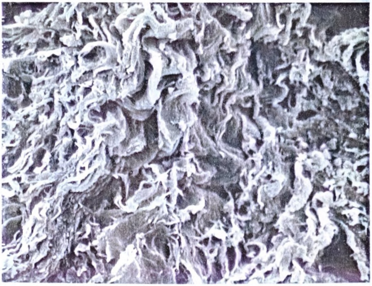

Figure 7. Photomicrograph of the middle ring. (780X)

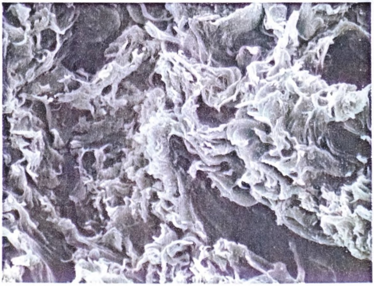

Figure 8. Photomicrograph of the outter ring. (700X)

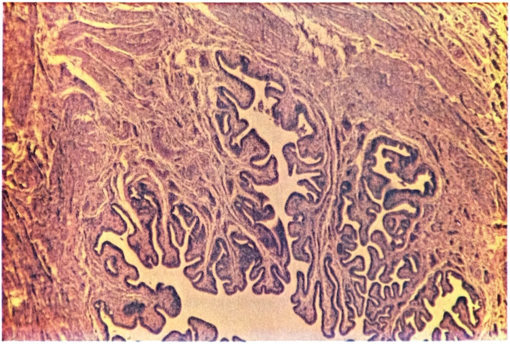

Figure 9. Hematoxylin-eosin stained 23 day pregnant rabbit cervix.

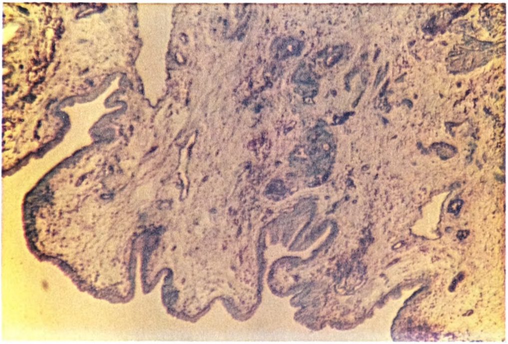

Figure 10. Cross-section of a 33 day (term) cervix.

Figure 11 is a hematoxylin-eosin stain showing the two horns; the nonpregnant horn is on the right. The difference in size between the pregnant and nonpregnant horn is obvious, as well as the dissociation of the collagen fibers. Figure 12 shows the degree of dissociation of collagen fibers in the pregnant horn. Figure 13 is a section from the nonpregnant horn; the bright green collagen fibers are much denser.

Figure 14 is a slide taken from a rabbit cervix 48 hours post partum. The collagen fibers have undergone considerable reconstruction.

Although the rabbit cervix structure is not quite the same as the human cervix, it undergoes the same changes throughout a term of pregnancy. From a very compact nonpregnant state, through a very dissociated pregnant state, and then back to a compact post partum state, the cervix is able to support and deliver without undergoing massive destruction.

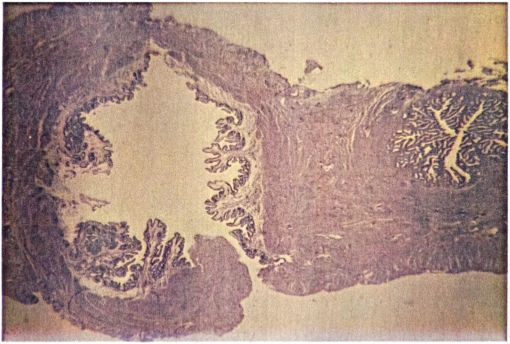

Figure 11. Hematoxylin-eosin stained pregnant/nonpregnant rabbit cervix. Left horn 1 hour post partum; right horn nonpregnant.

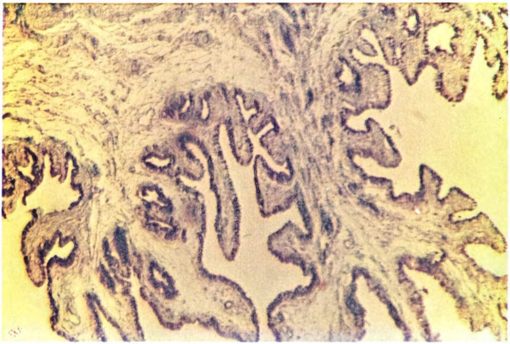

Figure 12. 1 Hour post partum left horn of the rabbit cervix.

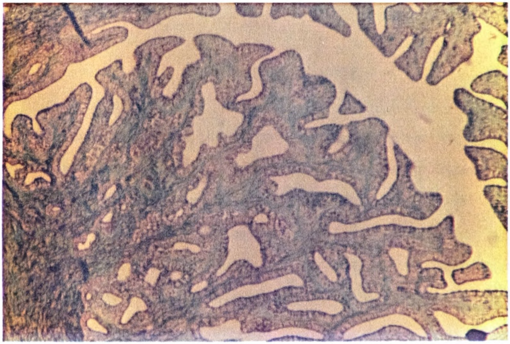

Figure 13. Nonpregnant right horn of the rabbit cervix.

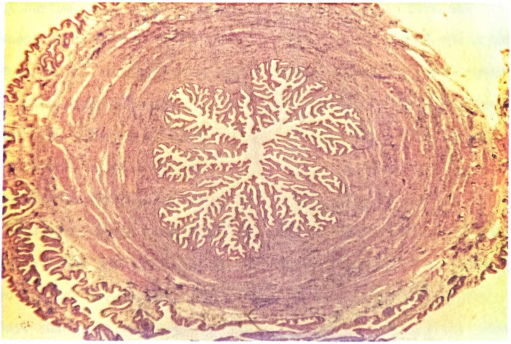

Figure 14. Hematoxylin-eosin stained 48 hour post partum rabbit cervix.

EXPERIMENTAL PROTOCOL

In order to determine the mechanical properties for the rabbit cervix, the specimens were subjected to three different types of tests. Relaxation tests were conducted at various levels of deformation. Loading and unloading the specimen at constant deformation rates from 0.5 to 100 $^{o}/o$ strain/second was used to examine hysteresis and strain rate effects. Finally, the responses of the cervix to sinusoidal extension tests were measured.

Female New Zealand rabbits from six months to a year-old were used. Cervixes were tested from nonpregnant rabbits, pregnant - 20 days, pregnant - 24 days, and pregnant-33 days (term), and 48 hours post partum. The rabbits were sacrificed with chloroform and dissection performed immediately. The rabbit was shaved and a lower abdominal incision used for entry. The cervix was located, removed at the isthmus and all extra tissue surrounding the cervix was dissected away. The tests were run on one side of the cervix with the other side intact. A drip of $0.9^{o}/o$ saline solution was used to keep the tissues moist throughout testing.

Two yoke-shaped grips were used to mount the cervix in the testing machine. Two semi-cylindrical pins, 0.048 inches in diameter were slid through the cervical canal and mounted in the slots of the grips. Care was taken not to damage the tissue and the grips were positioned so that there was no initial circumferential extension of the cervical canal. The initial dimensions of the cervix were measured with a micrometer before it was mounted in the testing fixture and the initial length was measured when a 0.1 N load was put on the tissue.

An Instron Materials Testing Machine was used for the tests with an Interface Model SSM-25 load cell. Specimen displacement was measured with a Linear Variable Differential Transformer within the hydraulic actuator of the Instron. Both load and displacement transducer signals were conditioned by amplifiers in the Instron instrumentation. The load and displacement of the specimens were recorded on a Nicolet 2090 Explorer Digital Oscilloscope, and load vs. time, displacement vs. time and load vs. displacement cervis were plotted using a Varian F-80 X-Y recorder.

The following test protocol was followed. Initially the cervix was 'preconditioned' using a loading and unloading triangle function at a rate of 0.5 ^{o}/ostrain/second. Three such cycles resulted in a repeatable response in the tissue. After preconditioning, the specimens were loaded and unloaded using the single cycle triangle wave function at the same level of deformation varying the rates from 0.5 to 100 ^{o}/o strain/second to study the rate dependence of the specimen. The second type of test, sinusoidal extension, was performed at the same level of deformation for rates from 0.01 to 1.0 Hz. The last test was a relaxation test in which the specimen was loaded to the same level of deformation at 100 ^{o}/ostrain/second and held for 100 minutes.

RESULTS AND DISCUSSION

A typical stress-strain curve is shown in Figure 15. This curve may be divided into three regions. The first, is where the low slope of the stress-strain curve represents straightening and alignment of fibers. The second region is the transition region before the third region where the response is linear with a higher tangent modulus.

The effect of strain rate on the shape and magnitude of the curves is shown in Figures 16 and 17. As the strain rate increases, the toe region decreases and the curves shift towards the stress axis. Viscoelastic effects account for this phonomena; at the slower strain rates, the tissue stress levels have a chance to relax resulting in a longer toe region and lower peak stress values.

A computer routine to emperically fit the stress-strain response curves in the form $\sigma = A\varepsilon^2$ was used. The constant A was determined for each varying strain rates throughout pregnancy. Table 1 shows a comparison of the average values of A at different strain rates for each stage of pregnancy. Since the tissue was preconditioned at $0.5^{o}/o$ strain/sec for three cycles, the first three columns indicate the A values for the three preconditioning cycles. Two trends exist; the first involves the rate dependency of the tissue. With the increase in strain rates from $0.5^{o}/o$ strain/sec to $100^{o}/o$ strain/sec there is an increase in the parameter A for all stages of

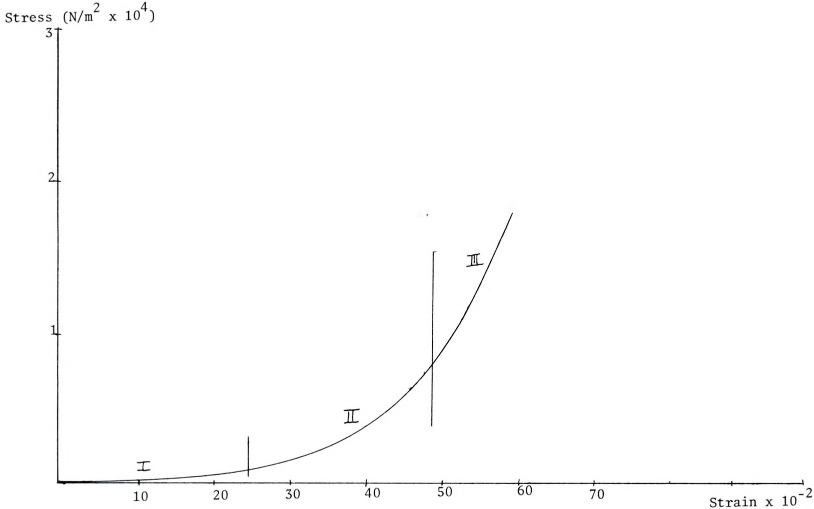

Figure 15. A typical stress-strain curve.

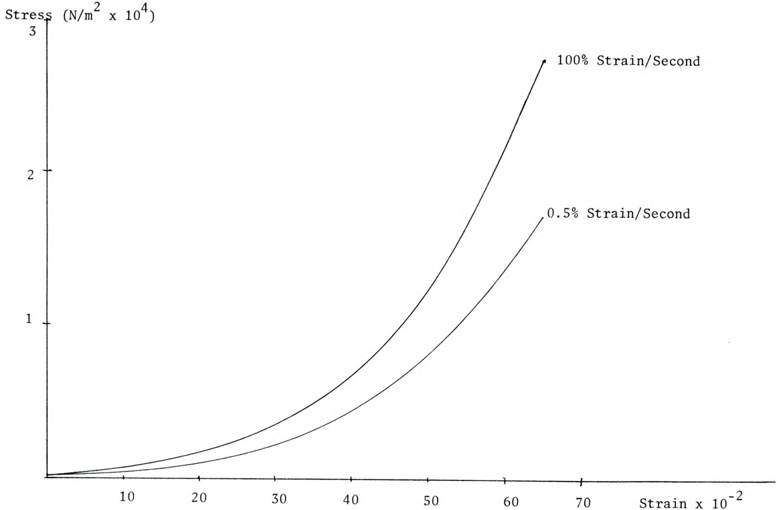

Figure 16. Strain rate effects on the stress strain curve.

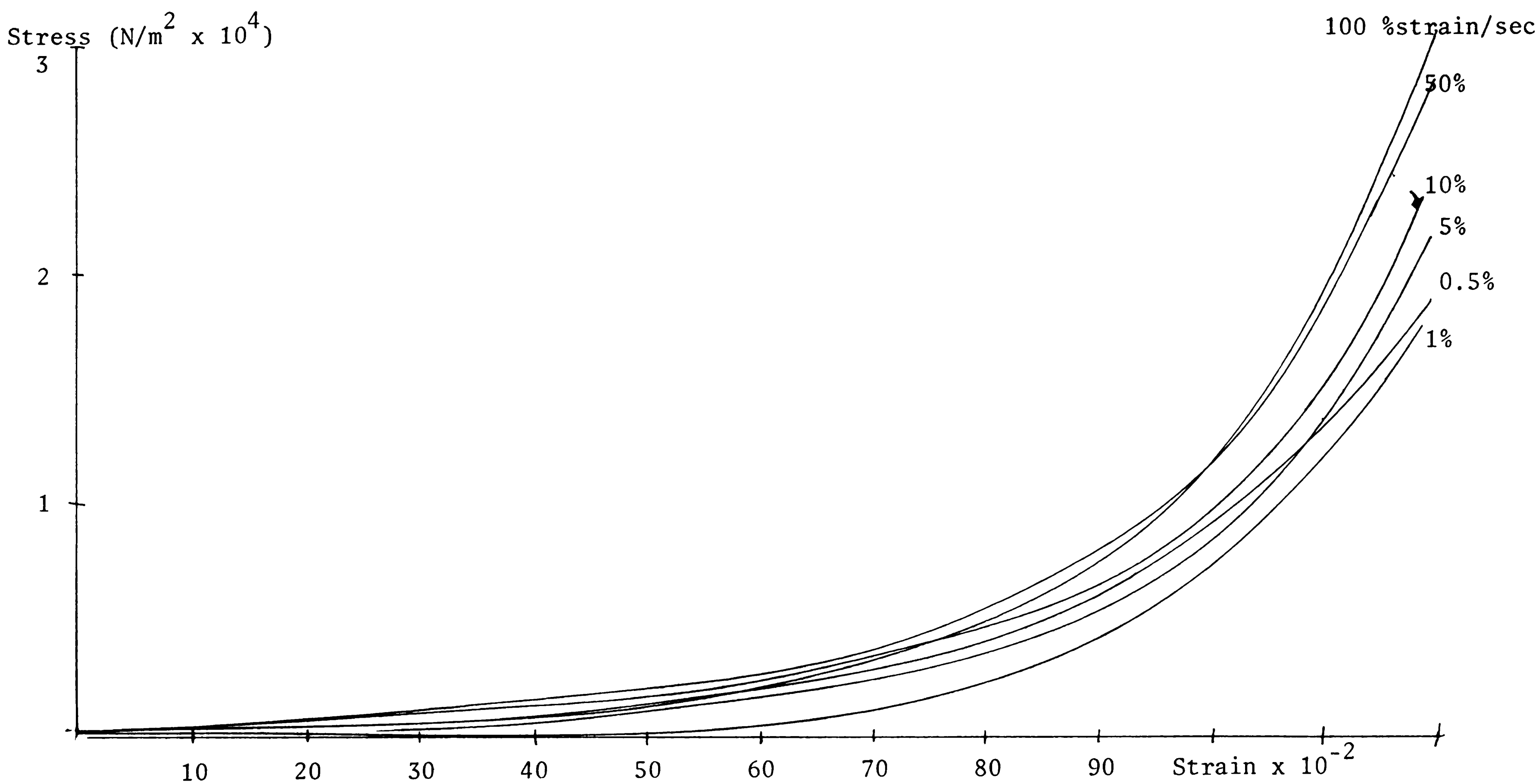

Figure 17. Strain rate effects on the stress-strain curves.

pregnancy, indicating that the strain rate at which the tissue is loaded affects the loading characteristics of the tissue. The cervix has a much stiffer response at the faster strain rates. The second trend in the data corresponds to the changes in the value of A with the change in the stage of pregnancy. As the rabbit nears term, approximately 30-33 days of its pregnancy, the value of A decreases indicating a loss in the stiffness of the cervix. However, 48 hours post partum the cervix regains some of its initial nonpregnant qualities, increasing its abiblty to carry higher loads at lower levels of strain.

Table 1: Average A values ($N/m^2 \times 10^4$)

Stage of pregnancy	0.5	0.5	0.5	1.0	2.0	10.0	50.0	100.0
	$^o/_o$Strain/Second							
Non	1.90	1.80	1.75	2.15	2.80	2.60	3.45	4.00
24 days	1.63	1.20	1.10	1.23	1.43	1.45	1.58	1.75
30 days(term)	1.35	0.95	0.85	1.05	1.50	1.25	1.25	1.35
48 hours post partum	2.15	1.90	1.75	1.75	2.07	1.20	2.50	3.25

To further investigate this change, terminal slopes were evaluated on the loading cycle of the stress-strain curve. Table 2 shows a comparison of these values for each stage of pregnancy at varying strain rates including the preconditioning cycles. At the same strain rate, there is a decrease in the value of the terminal slope with the increase in pregnancy followed by a recovery of the slope in the post partum cervix. The slope values at 1.0 $^o/_o$ strain/sec decrease from 11.11×10^4 N/m^2 in the nonpregnant rabbit to 1.9×10^4 N/m^2 in the cervix at term, with a recovery in the post partum cervix to 4.33×10^4 N/m^2. These are strong indications that pregnancy results in a loss of stiffness in the cervix. Harkness and Harkness (21) reported a similar loss but their stiffness values are much higher. This may be due in part to the manner in which the loading was applied across the two canals.

Table 2: Terminal Slope values ($N/m^2 \times 10^4$)

Stage of pregnancy	°/oStrain/Second 0.5	0.5	0.5	1.0	2.0	10.0	50.0	100.0
Non	16.67	14.00	22.50	11.11	15.00	16.43	21.00	37.50
20 days	15.38	13.89	12.06	10.83	17.33	19.55	22.73	----
24 days	3.93	2.07	2.98	2.11	3.34	2.59	3.78	5.07
33 days(term)	4.00	2.62	2.23	1.90	1.59	4.59	2.14	2.00
48 hours post partum	2.67	3.54	2.38	4.33	5.00	6.67	7.50	10.00

Table 3: Hysteresis values ($N/m^2 \times 10^2$)
B= energy absorbed during loading C=energy dissapated in hyst. loop

Stage of pregnancy		°/oStrain/Second 0.5	0.5	0.5	1.0	2.0	10.0	50.0	100.0
Non	B	14.82	8.23	7.65	7.53	9.40	13.29	12.35	14.23
	C	26.94	20.69	13.53	12.00	16.68	21.17	22.34	22.35
20 days	B	5.36	9.65	4.12	10.00	4.82	4.88	6.35	8.18
	C	12.07	17.54	8.94	4.65	10.88	11.99	13.82	18.06
24 days	B	2.06	1.77	1.83	3.06	2.77	2.35	3.18	5.71
	C	5.86	4.30	6.89	5.89	6.06	6.34	6.76	7.83
30 days	B	1.77	.826	2.47	3.12	2.82	3.06	4.24	4.38
(term)	C	4.18	4.01	6.96	5.60	7.74	8.24	5.06	6.96
48 hours	B	13.06	6.47	9.18	5.52	9.76	7.88	8.59	14.59
post partum	C	22.59	11.29	17.65	9.16	16.11	16.11	18.24	18.24

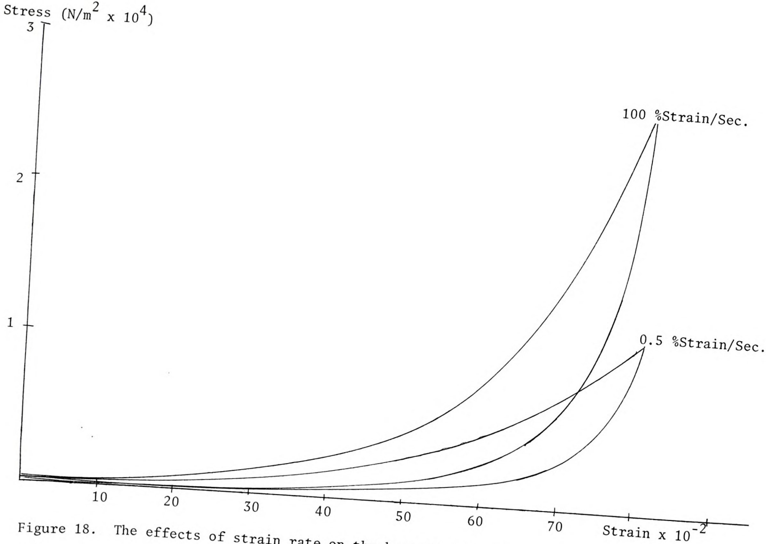

Figure 18. The effects of strain rate on the hysteresis loops.

With the decrease during pregnancy in stiffness in the loading curves, there is also an effect on the hysteresis response of the tissue. Hysteresis is affected by both strain rate and pregnancy. Figure 18 shows a variation between two hysteresis curves. Table 3 compares the energy absorbed during loading, B, and the absolute energy dissapated in the hysteresis loop, C, at varying strain rates and the different stages of pregnancy. Comparing the different strain rates for each stage of pregnancy further indicates the rate dependency of the tissue. Pregnancy as well as strain rate affects the response of the tissue. There is a decrease in both the input energy and the absolute energy dissapated in the cervix with increasing stages of pregnancy. The decrease in stored energy is consistant with the loss of stiffness in the tissue with pregnancy. The post partum cervix indicates a gain in both energies B and C consistent with an increase in stiffness of the tissue. The ratio of C to B remained constant at approximately 50^{o}/o for different stages of pregnancy and different strain rates.

Tables 1,2 and 3 are indicative of the trends in the cervix as a result of pregnancy and strain rate. Values have been averaged between rabbits at a particular stage of pregnancy to show trends in the data. Variations in the data are within experimental scatter in biological tissues.

Relaxation tests have been evaluated for the cervix at various stages of pregnancy. A typical relaxation plot of normalized stress vs. log. of time is shown in Figure 19. Stress values after 6 seconds of relaxation and the slope of the relaxation over 100 minutes. $(\Delta\sigma/\sigma_{max})/\Delta \log t$ were measured for all stages of pregnancy. Variations in this parameter between stages of pregnancy were within scatter of

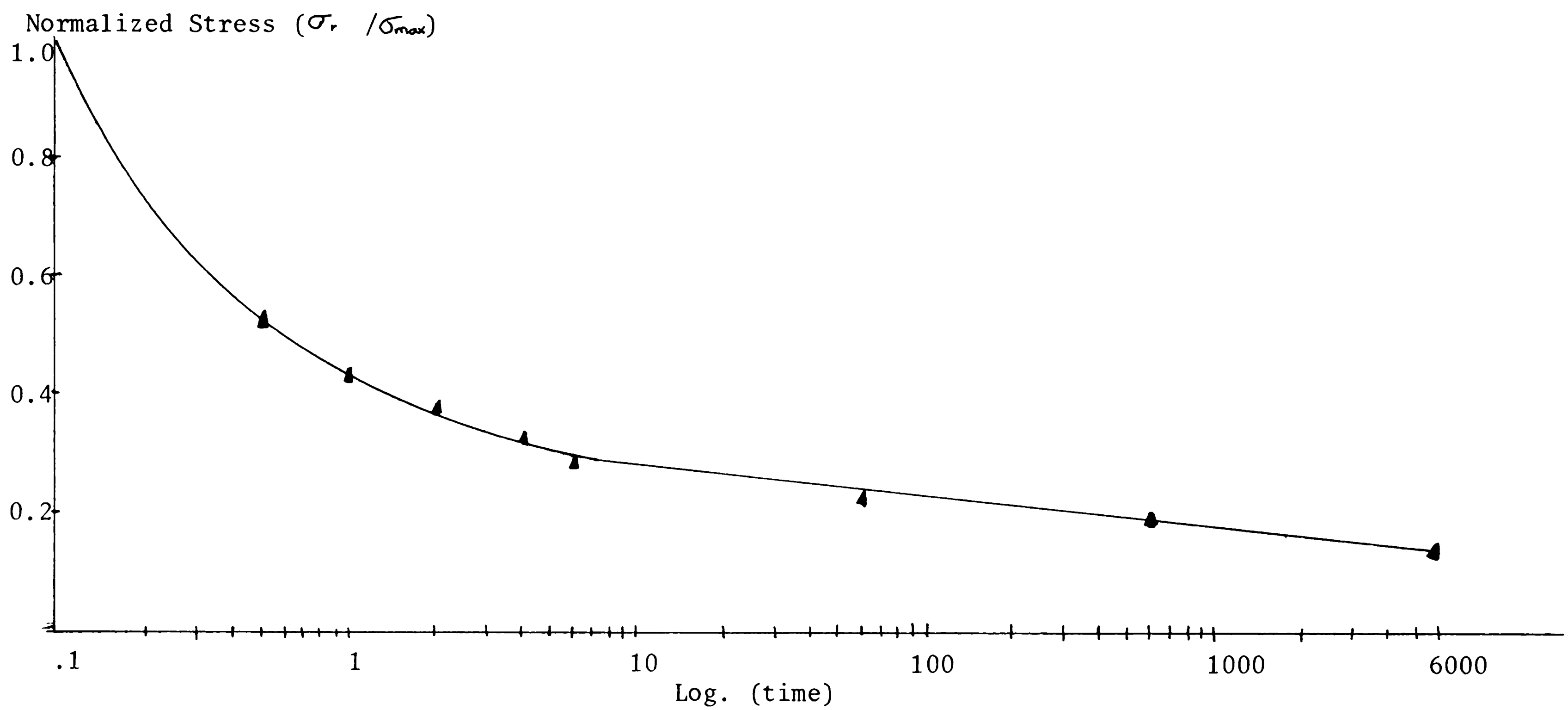

Figure 19. A typical relaxation plot of normalized stress vs. log. t.

the data and therefore not meaningful. An average value of the slope of the relaxation curve was -2.8×10^4/log sec . Changes in this viscous parameter as a result of pregnancy were not significant.

Cyclic data shows definite trends with pregnancy. In Figures 20 - 27, average peak stress for each cycle versus number of cycles was plotted keeping frequency and stage of pregnancy constant. In figures 20 -24 a comparison of the frequency is made for each stage of pregnancy. There is a more rapid decrease in peak stress for each cycle in the lower frequency tests. This is not necessarily an indication of frequency or strain rate dependence. If the response of the cervix is a time phonomena and these curves were compared on an absolute time basis instead of number of cycles, the higher frequency data would collapse on the lower frequency data. The rate of decay of the peak stress in the high frequency data is incorporated in the beginning cycles of the lower frequency data.

In Figures 25-27, each stage of pregnancy is compared at a constant frequency. There is a decrease in the stress amplitude with the number of cycles. At later stages of pregnancy this decrease is greater than in the earlier stages of pregnancy and in nonpregnant rabbits under cyclic deformation. The peak stress decay in the post partum cervix resembles that of the nonpregnant cervix indicating a recovery from the response of the pregnant cervix.

Using this test protocol, changes in the cervix due to pregnancy were determined. Constant strain rate loading and unloading tests showed a strain rate dependency of the tissue at each stage of pregnancy. Terminal slopes of the loading curves indicated a decrease in stiffness of the cervix as it neared term followed by a recovery in stiffness post partum. Hysteresis area was also found to decrease with

Figure 20. °/o of peak stress vs. number of cycles for the non pregnant rabbit cervix at frequencies of 1, 0.1 and 0.01 Hz

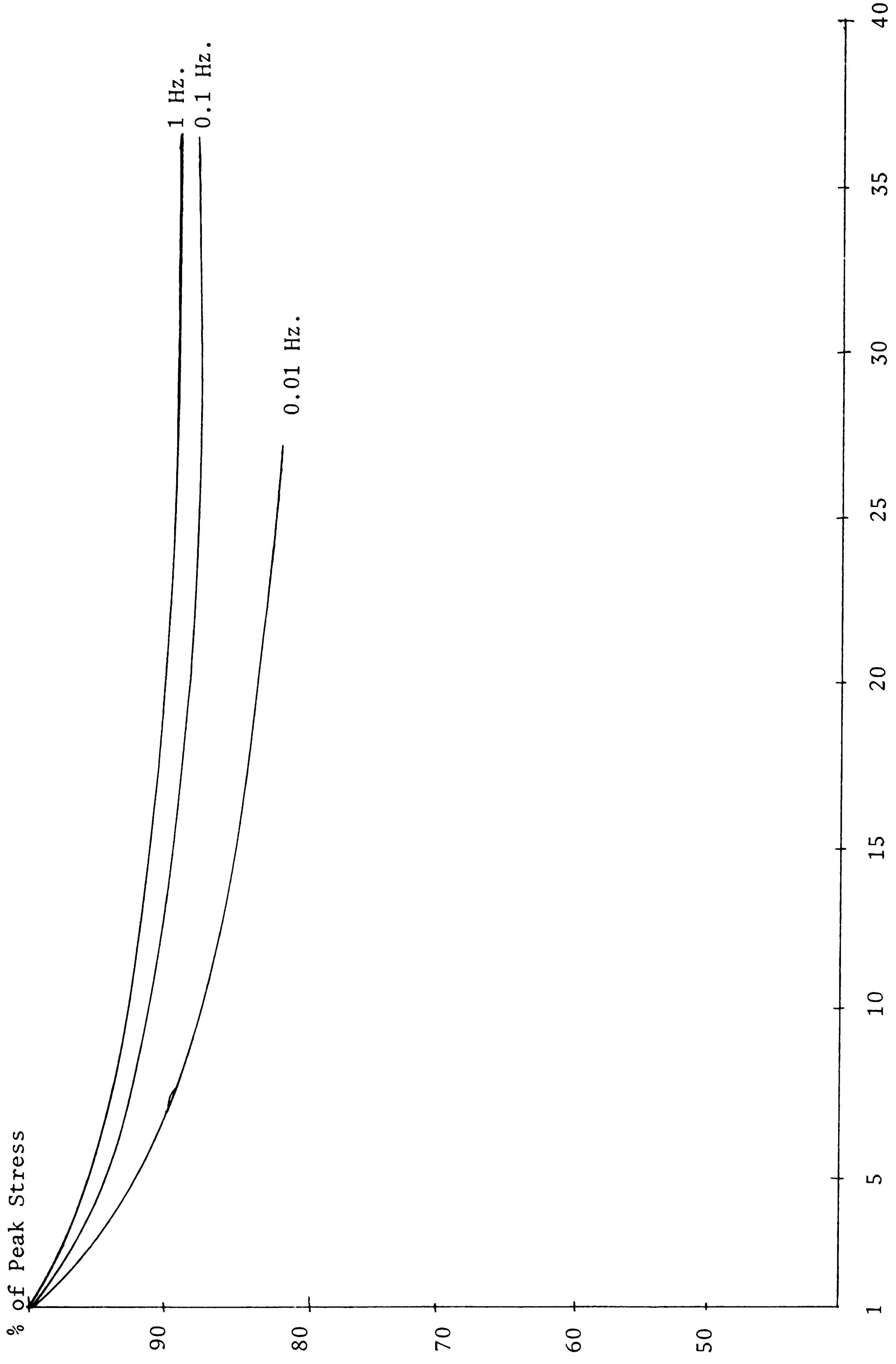
% of Peak Stress
90
80
70
60
50
1
5
10
15
20
25
30
35
40
1 Hz.
0.1 Hz.
0.01 Hz.
Number of Cycles

Figure 20.

Figure 21. °/o of peak stress vs. number of cycles for the 20 day pregnant rabbit at frequencies of 1, 0.1, and 0.01 Hz.

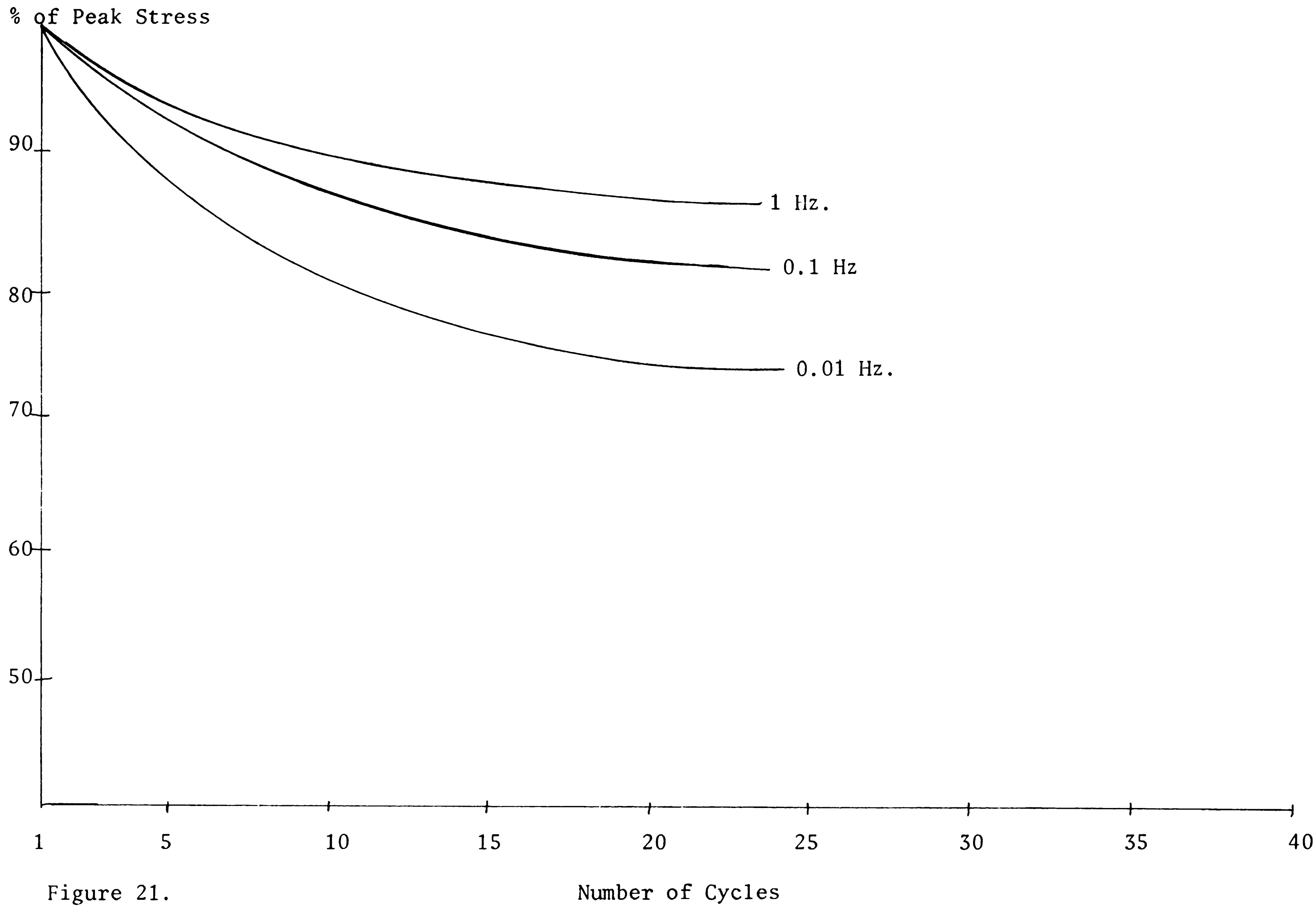
% of Peak Stress
90
80
70
60
50
1
5
10
15
20
25
30
35
40
Number of Cycles
1 Hz.
0.1 Hz
0.01 Hz.

Figure 21.

Figure 22. $^{0}/_{0}$ of peak stress vs. number of cycles for the 24 day pregnant rabbit at frequencies of 1, 0.1, and 0.01 Hz.

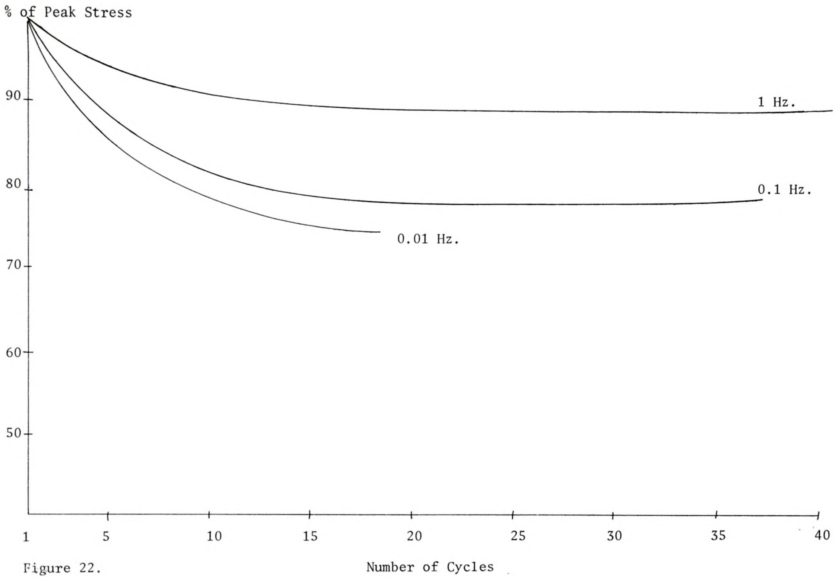
% of Peak Stress
90
80
70
60
50
1 Hz.
0.1 Hz.
0.01 Hz.
1
5
10
15
20
25
30
35
40
Number of Cycles

Figure 22.

Figure 23. $^{0}/_{0}$ of peak stress vs. number of cycles for the 33 day (term) rabbit at frequencies of 1, 0.1 and 0.01 Hz.

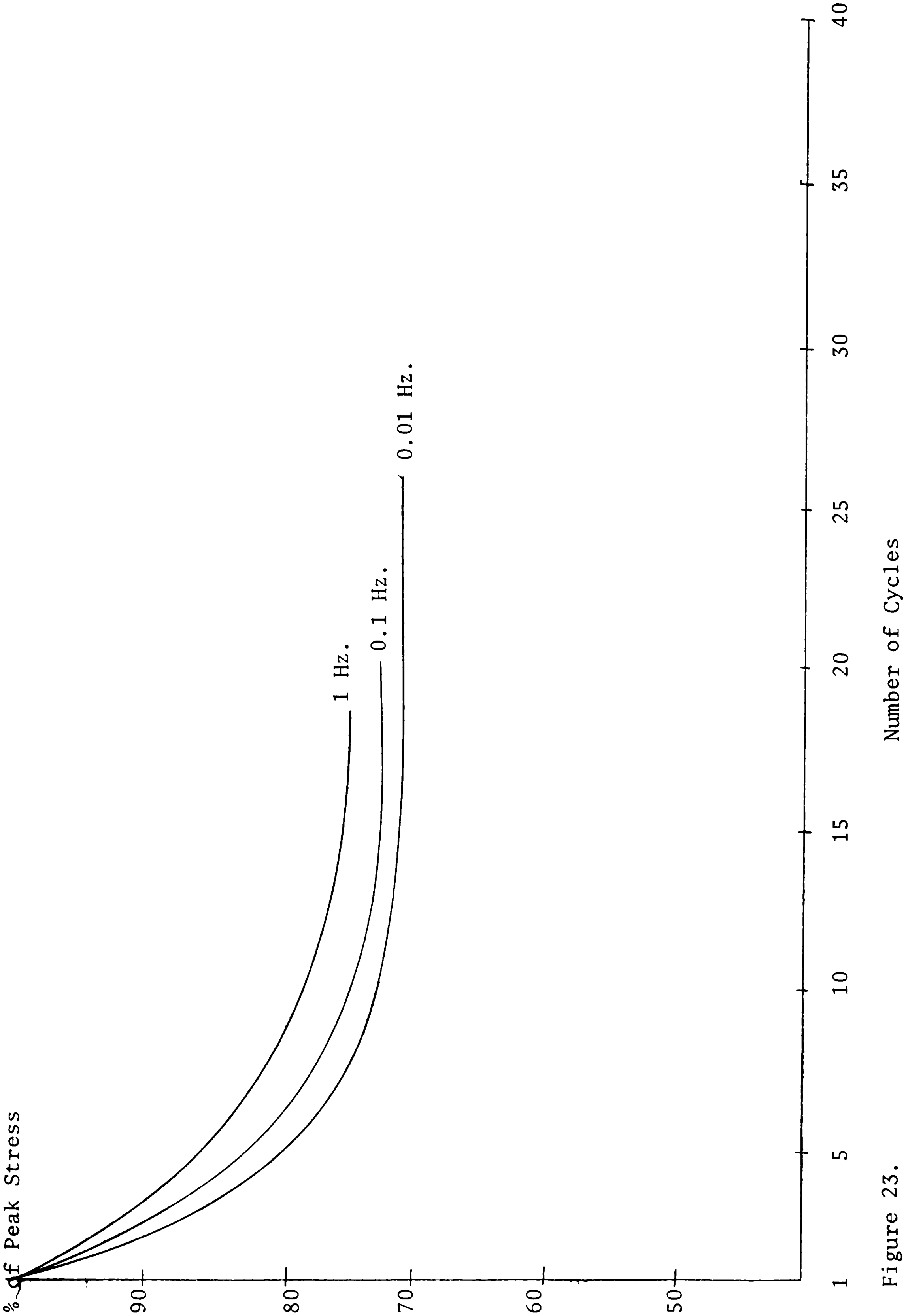
% of Peak Stress
90
80
70
60
50
1 Hz.
0.1 Hz.
0.01 Hz.
1
5
10
15
20
25
30
35
40
Number of Cycles

Figure 23.

Figure 24. $^{o}/o$ of peak stress vs. number of cycles for the 48 hour post partum rabbit at frequencies of 1, 0.1 and 0.01 Hz.

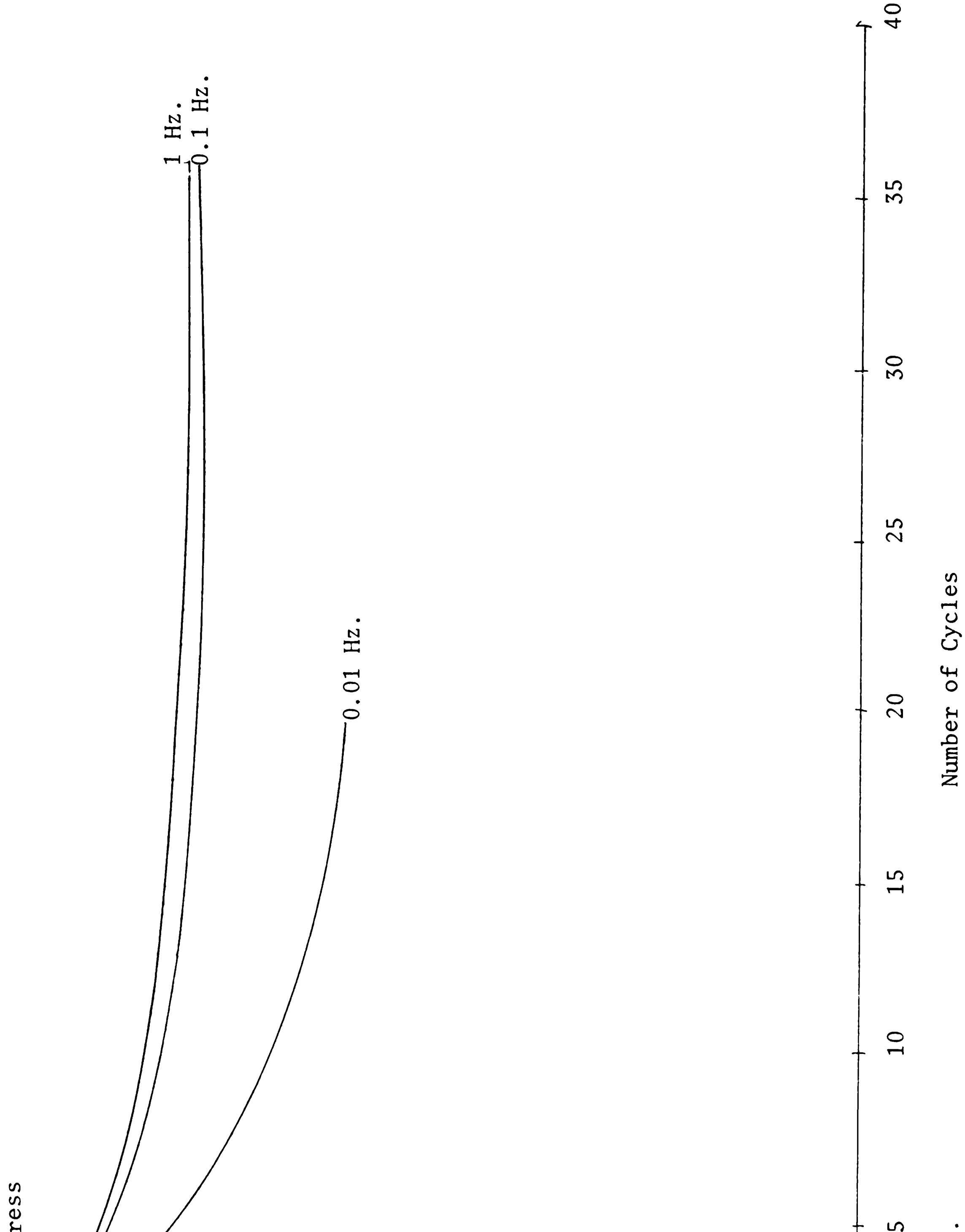
% of Peak Stress
90
80
70
60
50
1 Hz.
0.1 Hz.
0.01 Hz.
1
5
10
15
20
25
30
35
40
Number of Cycles

Figure 24.

Figure 25. °/o of peak stress vs. number of cycles for a frequency of 0.01 Hz. for all stages of pregnancy.

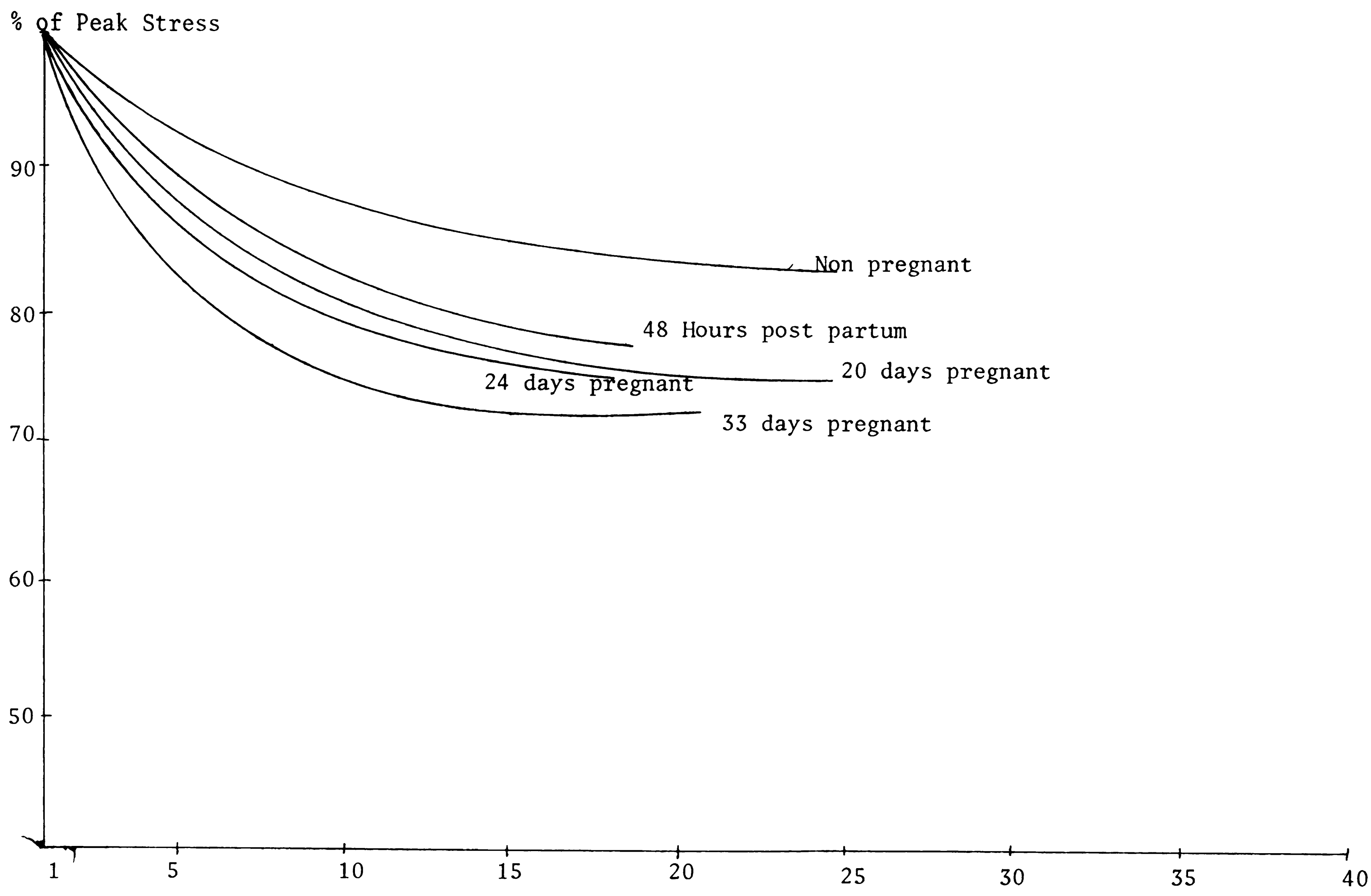
% of Peak Stress
90
80
70
60
50
1
5
10
15
20
25
30
35
40
Number of Cycles
Non pregnant
48 Hours post partum
20 days pregnant
24 days pregnant
33 days pregnant

Figure 25

Figure 26. $^{o}/o$ of peak stress vs. number of cycles for a frequency of 0.1 Hz for all stages of pregnancy.

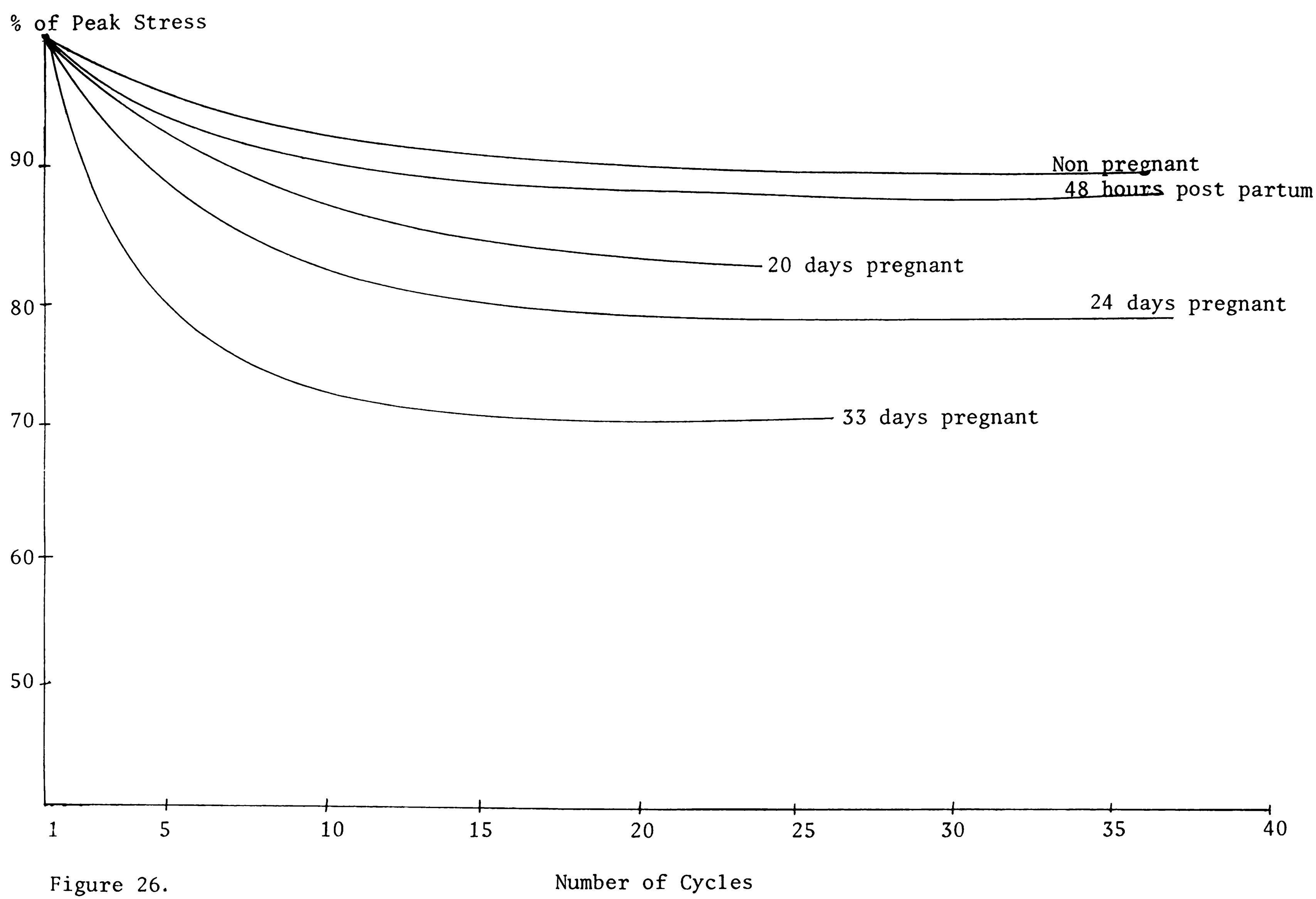
% of Peak Stress
90
80
70
60
50
Non pregnant
48 hours post partum
20 days pregnant
24 days pregnant
33 days pregnant
1
5
10
15
20
25
30
35
40
Number of Cycles

Figure 26.

Figure 27. $^{o}/o$ of peak stress vs. number of cycles for a frequency of 1 Hz for all stages of pregnancy.

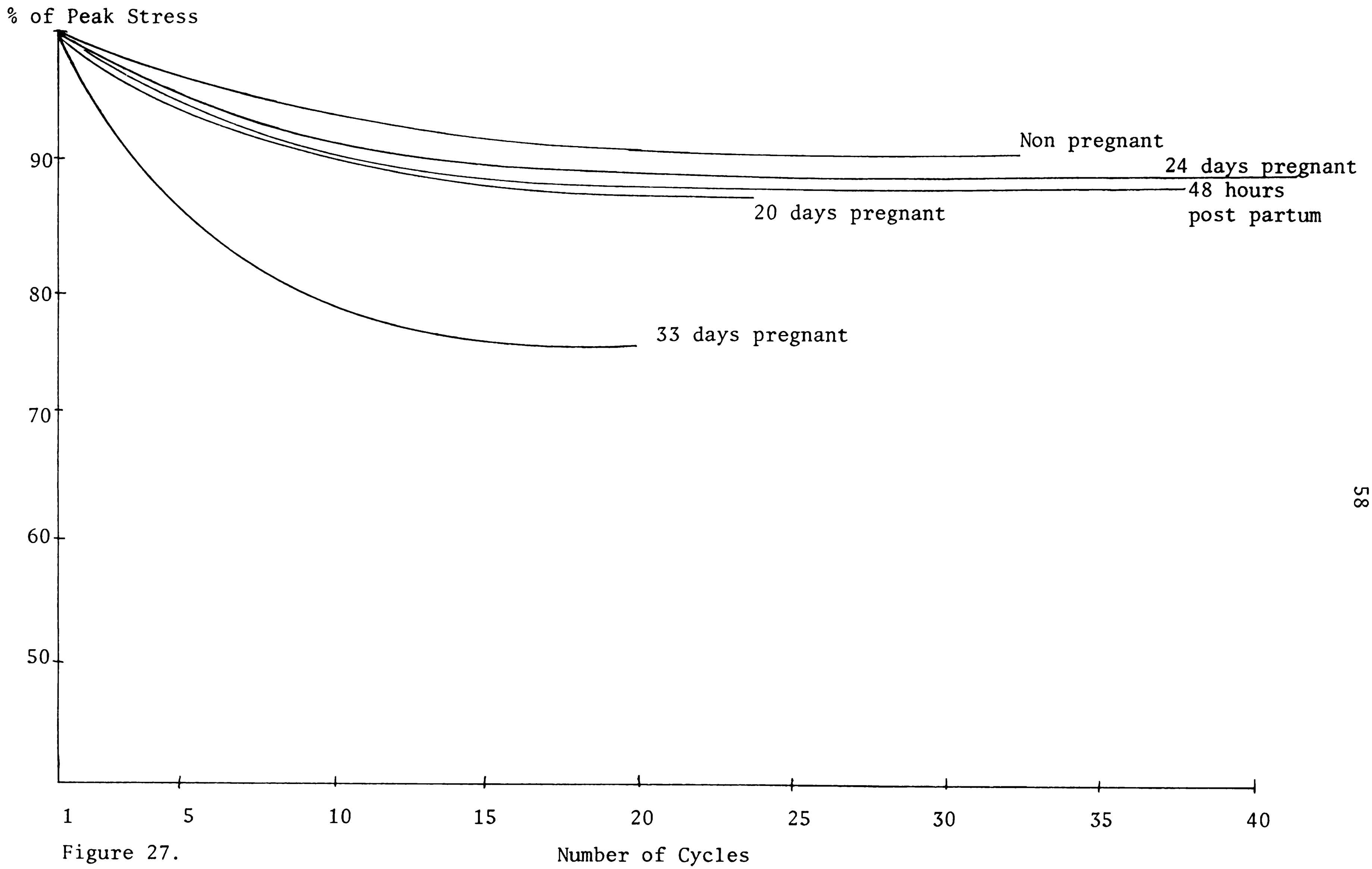
% of Peak Stress
90
80
70
60
50
Non pregnant
24 days pregnant
48 hours
post partum
20 days pregnant
33 days pregnant
1
5
10
15
20
25
30
35
40
Number of Cycles

Figure 27.

the increase in pregnancy and recover post partum. Relaxation tests, however, showed very little change due to pregnancy indicating that viscosity of the cervix would not change with pregnancy. Finally, cyclic deformation tests did not show a frequency dependence but very definitely showed a decrease in stress amplitude with the increase in pregnancy followed by a recovery with the post partum cervix.

Unlike Harkness and Harkness (18, 18, 19) who show an increase in the viscous parameters in the cervix, the relaxation data reported here does not indicate an increase in viscosity during pregnancy but cyclic loading does show increases in the viscoelastic response of the cervix. The maximum tangent modulus data indicates a loss in stiffness during pregnancy as discussed earlier. A creep test was not run and it is difficult to calculate reciprocal comparisons in nonlinear tissues between creep and relaxation tests. However, the lack of change due to pregnancy in the relaxation data would indicate that one might not see changes in creep response with pregnancy. The cyclic data were deformation cycles and not force cycles but if the tissue were subjected to force cycling, one would expect to see the deformation amplitude increase giving an increase in the compliance.

Several problems were encountered during this research which made the data not as conclusive as it could have been. A combination of five load cell set ups were used to measure very low loads in attempting to obtain an acceptable degree of accuracy. The sensitivity of the Instron Materials Testing Machine varied with different load cells and a comparison of data from different specimens was not always possible.

Another problem encountered was in the measurement of the initial length, l_0, of the specimen. There was a problem detecting initial load on the tissue and it was decided that l_o would be measured when a 0.1 Newton load was placed on the tissue. As the stage of pregnancy increased, so did l_o. Since the cervix behaves with a strongly viscous component, l_o had to be continually increased in order to hold the initial load. This variation in l_o could affect the initial linear range on a stress-strain curve possibly making a more compliant tissue appear stiffer than a less compliant tissue.

The amount and orientation of the components of the cervix contribute to the response of the cervix to mechanical testing. The rabbit cervix is mainly composed of connective tissue and muscle fibers. Collagen is thought to be the main load bearing component of the cervix. The muscle fibers are imbedded in the collagen fibers therefore probably do not serve as a significant load-bearing component in the cervix. Since elastin exists in such small quantities it is believed to have no roll in the mechanical response of the cervix both in the pregnant and nonpregnant rabbit. The contribution of the reticular fibers is neglibible compared to that of the collagen fibers.

The changes the cervix experiences histologically as a result of pregnancy suggest reasons for changes in the mechanical properties of the cervix due to pregnancy. In the nonpregnant state, the cervical collagen fibers of the rabbit are coarse and intermixed having no preferred direction. In the pregnant cervix, however, they become fine and highly branched indicating that the amount of load or the stiffness the cervix withstands during pregnancy would diminish as the rabbit nears term due to this change in cross-linking within the individual collagen fibers. The increasing edema and glycosaminoglycans in the cervix with pregnancy also suggests an increase in the viscoelastic response of the cervix.

The three mechanical tests in this study indicate changes in the response of the tissue with pregnancy which are consistant with the microscopic changes in the cervix. Constant strain rate loading and unloading tests indicate both a strain rate dependence and a loss in stiffnes due to pregnacy followed by a recovery in the 48 hour post partum cervix. Terminal slopes also indicate both a strain rate dependence and a loss in stiffness due to pregnancy followed by a recovery in the 48 hour post partum cervix. Hysteresis indicates a strain rate dependence and a decrease in area with pregnancy,the area recovering in the 48 hour post partum cervix. The relaxation tests show very little change with pregnancy indicating little change in the viscous parameter in the tissue. Finally, the cyclic data shows no frequency dependence, a decrease in the stress amplitude of each cycle with pregnancy and an increase in the viscoelastic response of the cervix with pregnancy.

APPENDIX A

APPENDIX A

The classical description of the uterus divided it into several parts: the fundus being the uppermost portion; the body or corpus lying above the internal os; the isthmus lying between the internal os and the external (histological) os; and the cervix lying below the external os (see Figure A-1). The internal os is the constriction where the corpus meets the isthmus, and the external os is the transition between endocervical and endometrial epithelium. Danforth (23) eliminated the isthmus as a separate structure indicating that the corpus was chiefly the muscular part of the uterus and the cervix was the fibrous part of the uterus, the isthmus or internal os being the junction between the two.

The nulligravida cervix is a cylinder-shaped organ tapering at both ends about 2.5 - 3.0 cm in diameter. The uterus is superior to the cervix and is located in the pelvic cavity above the bladder and in front of the rectum. The cervix is positioned slightly downward and backward from the uterus. Since it is less freely moveable than the uterus, the long axis of the cervix is seldom in line with that of the corpus. At the transition between the corpus and cervix, the isthmus or internal os , the uterus has the form of a sphincter.

The cervical canal crosses the cervix and communicates between the uterine cavity above the internal os and the vaginal canal below the external os. It is broader in the middle than at either end. (12, 24, 25)

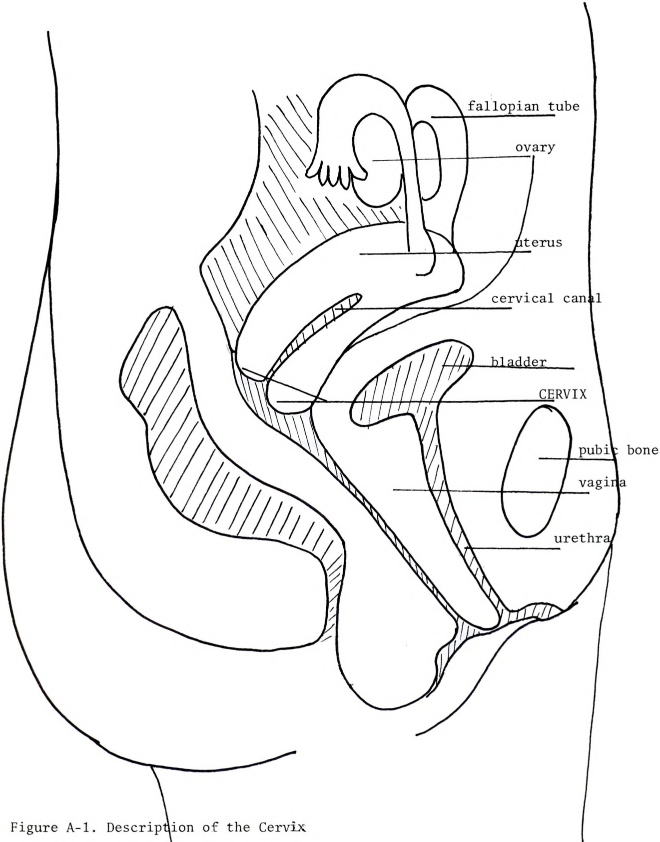

Figure A-1. Description of the Cervix

The human cervix, like the uterus, consists of an endometrium (mucosa) and myometrium; the mucosa rests upon the myometrium which is predominantly composed of fibrous connective tissue with smooth muscle present in small quantities and elastic tissue is virtually nonexistent. The corpus of the uterus is composed primarily of muscular tissue. There is only about a 5 - 10 mm space from which the fibrous cervix transcends from the muscular corpus. Smooth muscle comprises about $15^{o}/o$ of the cervix and elastic tissue is present only on the walls of blood vessels (23).

In the cervix there are two types of epithelium present which line the endometrium, stratified squamous and columnar. The epithelium lining the mucosa of the cervix differs according to the cavity it faces. The portio vaginalis is covered by stratified squamous epithelium whereas the mucosa of the endocervical canal is covered by a mucous membrane composed of tall columnar cells, some of which are ciliated. The cells are uniform, arranged in single rows and actively secrete mucin. The squamocolumnar junction, or external os, is the boundary between the squamous epithelium and columnar epithelium, occuring over a region of 1 - 10 mm. (12,24,25)

Glands, also present in the cervix, extend from the surface of the cervical canal to the underlying connective tissue. They too are lined with columnar epithelium. (12,24,25)

The major thickness of the cervix, the myometrium, consists of fibrous connective tissue with moderate amounts of smooth muscle interwoven. The arrangement of the smooth muscle and connective tissue is important in order to understand the mechanical response of the tissue. The amount and orientation of the smooth muscle in relation to the connective tissue varies throughout the cervix being a subject of much debate. The most distal portion of the cervix, the portio vaginalis, is devoid of muscle, consisting mainly of connective tissue; the middle portion contains the terminal fibers of central longitudinal uterine muscle; and the upper portion contains 50 - 60^{o}/o of the smooth muscle (23,12). Danforth (26) and Hughesdon (11) described strands of smooth muscle in the cervix that were continuous with the muscle of the corpus superiorly, and inferiorly with the walls of the vagina. They believed that the cervix consisted of an outer one-fourth which is mainly muscular and an inner three-fourths which is predominantly connective tissue. From saggital sections, Hughesdon (11) noted a high muscle content of immature muscle fibers in a submucous layer in which the intrinsic muscle is present in radial and longitudinal bundles which lie in a mass of dense collagen. Hughesdon concluded that the cervix consisted of an outer contractile layer of muscle embedded in a mass of collagen making contraction difficult, and an inner noncontractile mass. He also noted a mass of circular smooth muscle at the internal os arranged in a spiral pattern. It has not been established whether or not this mass of muscle has sphincteric capabilities.

Danforth (26) questioned the ability of the cervix to contract or whether the contractions were transmitted from the corpus or were otherwise extracervical. He tested the ability of the cervix to contract and relax by suspending excised strips of cervical tissue in a bath and recording their activity. He found the ability of the cervix to contract was negligible when compared to that of the corpus.

The major portion of the cervix involved in mechanical activity is connective tissue. The most dominant constituent of the connective tissue of the cervix is collagen. Collagen undergoes the major changes within the cervix during pregnancy and these changes will be considered later. Collagen fibers in the nonpregnant cervix appear as dense tightly woven bundles of interlacing fibers running parallel to one another (3).

Collagen posseses hydroxyproline, an amino acid which is unique to it alone. The hydroxyproline concentration in collagen is constant at 13 or 14^{0}/o dry weight regardless of the physical characteristics from which the collagen was extracted (6). The amount of hydroxyproline found in a tissue may be used to determine the amount of collagen present in the tissue. In the case of the collagen in the cervix, hydroxyproline can also be measured in the urine.

The second major fibrous component of connective tissue is elastin. Elastin fibrils are thinner than collagen fibrils, measuring only about 70 A in diameter (6). The amount of elastin in the cervix is insignificant, comprising a fraction of 1^{0}/o of the total fibrous tissue of the cervix. They are present, sparsely scattered randomly throughout the cervix, being predominantly found in the walls of larger blood vessels. Because of the insignificant amounts of elastic fibers, their contribution to the mechanical response of the cervix is negligible.

The other component of cervical connective tissue, the reticular fibers, are fine threadlike structures similar to immature collagen fibers which are found around the bundles of collagenous fibers and around the smooth muscle cells. Some studies have shown reticular fibers to be continuous with collagen fibers (6). In the nonpregnant cervix, the reticular fibers appear as short segments, very irregularly despersed or clumped in a haphazard relation to the collagen bundles. In the pregnant cervix, reticular fibers are more robust and, in general, more luxuriant and tend to parallel the collagen fibers (4).

LIST OF REFERENCES

LIST OF REFERENCES

1. Lingren, L. (1973) Biodynamics of the Cervix during Pregnancy and Labor. <u>The Biology of the Cervix.</u>, pg. 385-415.

2. Philpott, R.H. (1976) Biodynamics of the Cervix. <u>The Cervix.</u>, pg. 217-250.

3. Buckingham, J.C., Selden, R., and Danforth, D.N. (1962) Connective Tissue Changes in the Cervix during Pregnancy and Labor. Annals, New York Academy of Sciences, 97, pg 733-742.

4. Danforth, D.N., Buckingham, J.C., and Roddick, J.W. (1960) Connective Tissue Changes Incident to Cervical Effacement. Am. J. Obst. and Gynec., 80, 5, pg 939-945.

5. Ichijo, M., Shimizu, T. and Sasai, Y. (1976) Histological Aspects of Cervical Ripening. Tohoku J. Exp. Med., 118, pg 153-161.

6. Danforth, D.N. and Buckingham, J.C. (1963) Connective Tissue Mechanisms and their Relation to Pregnancy. Obst. a Gynec. Survey, pg 715-732.

7. Danforth, D.N., Veis, A., Breen, M., Weinstein, H.G., Buckingham, J.C. and Manalo, P. The effect of pregnancy and labor on the human cervix: Changes in collagen, glycoproteins, and glycosaminoglycans. Am. J. Obst. a Gynec., 120, 5, pg 641-651.

8. Danforth, D.N. and Buckingham, J.C. The Effects of Pregnancy and Labor on the Amino Acid Content of the Human Cervix. <u>The Biology of the Cervix</u>, pg 351.

9. Silbert, J.E., In Prenez-Tamayo, R. and Rojkind, M. editors: Molecular Pathology of Connective Tissues, New York 1973, Marcel Dekker, Inc. pg 391ff23.

10. Groschel - Stewart, U., Hermann, U. and Schalwm, H., Br. J. of Exp Pathol. 54: 192, 1973.

11. Hughesdon, P.E. (1958) The Fibromuscular Structure of the Cervix. J. Obst. and Gynec., 65, pg 763-766.

12. Singer, A. and Jordan, J.A. (1976) The Anatomy of the Cervix. The Cervix., pg. 13-35

13. Hafez, E.S.E. (1973) Comparative Anatomy of the Cervical Stroma. The Biology of the Cervix. pg. 40

14. Bakke, T. (1974) Cervical Consistency in Women of Fertile Age Measured with a New Mechanical Instrument, Acta. Obstet. Gynec. Scand. 53, pg 293-302.

15. Lui, D.T., et al. (1975) Dilatation of the Parous Non-Pregnant Cervix. BR. J. of Obst. and Gynec., 82, pg 246-251.

16. Rice, D.A., Yang, T.Y. and Stanley, P.E. (1975) A Simple Model of the Human Cervix during the First Stage of Labor. J. Biomechanics 9, pg 153-163.

17. Rice, D.A., Yang, T.Y. (1976) A Nonlinear Viscoelastic Membrane Model Applied to the Human Cervix. J. Biomechanics 9, pg 201-210.

18. Harkness, R.D. a Moralee, B.E. (1956) The Time Course and Route Loss of Collagen from the Rat's Uterus during Post-partum Involution. J. Physiol. 132, pg 502-508.

19. Harkness, M.L.R. a Harkness, R.D. (1958) Changes in the Distensibility of the Uterine Cervix of the Rat After Parturition. J. Physiol. 141, pg 34

20. Harkness, M.L.R. a Harkness, R.D. (1959a) Effect of enzymes on mechanical properties of tissues. Nature, Lond., 183, pg 1821

21. Harkness, M.L.R. a Harkness, R.D. (1959b) Changes in the physical properties of the uterine cervix of the rat during pregnancy. J. Physiol. 148, pg 524-547.

22. Kriewall, T.J. (1976) Uterine work in parturition. J. Applied Physiology, 41, 3, pg 316-322.

23. Danforth, D.N. (1947) The Fibrous Nature of the Human Cervix, and its Relation to the Isthmic Segment in Gravid and Nongravid Uteri. Am. J. Obst. and Gynec., 53, pg 541-559.

24. Kranz, K.E. (1973) The Anatomy Of the Human Cervix, Gross and Microscopic. The Biology of the Cervix., pg. 57-70.

25. Grays Anatomy. "The Cervix Uteri". pg. 1357

26. Danforth, D.N. (1954) The Distribution and Functional Activity of the Cervical Musculature. Am. J. Obst. and Gynec., 68, 5, pg 1261-1269.