PREGNANCY AND THE LOWER URINARY TRACT: PART I*

A STUDY OF HISTOLOGICAL CHANGES IN THE LOWER URINARY TRACT DURING PREGNANCY

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The common urological symptoms of pregnancy are of such early onset that they frequently constitute one of the primary clinical diagnostic criteria of the condition.

In the first trimester the symptoms are mainly represented by urinary frequency associated with a slight incidence of stress incontinence. In the second and third trimesters the incidence of stress incontinence rises sharply. Dysuria, pain and pyrexia constitute a minority group and occur mainly in the second and third trimesters.

Although the exact aetiology of the urinary symptoms is still a matter of conjecture, 3 categories of causative factors have been advanced: that is, those associated respectively with hormonal influences, with infective processes and with pressure effects.

In this first part of a series of 3 articles hormonal effects as shown by microscopical changes in the lower urinary tract will be evaluated.

The study of obstructive factors in pregnancy was not attempted, because of the difficulties associated with it. The only reliable methods are radiological, and the employment of advanced radiographic techniques such as cinematic voiding cysto-urethrography is prohibited by the known radiation hazards to the early conceptus.

HISTORICAL

In an extensive review of ureteral dilatation in pregnancy Fainstat[1] noted that the earliest observers of the condition were primarily concerned with infection, and as long ago as 1839 they attributed dilated ureters and urinary stasis to pressure effects by the enlarging uterus.[2]

Obstruction as a causative factor was the main preoccupation of most investigators who observed the condition in the following century. Significant histological observations of the lower urinary tract were first made in the late 19th and early 20th centuries. Sanger,[3] in 1892, noted hyperplasia of all pelvic structures, and Hofbauer,[4] in 1908, ascribed ureteric thickening to hyperplasia of the connective tissue. Küstner,[5] in 1925, made the noteworthy observation that the connective tissue and muscle fibres of the ureter were 'spread apart' and that they had undergone relaxation. Hundley *et al.*[6] reported on microscopical studies of the bladder, trigone, urethra and ureter. They noted varying degrees of oedema, hypertrophy of muscular layers and increased vascularity occurring simultaneously with similar changes in the genital tract during pregnancy. They reported marked hypertrophy of the connective-tissue sheath (Waldeyer's) of the lower ureter, and were sceptical about the ability of the pregnant uterus to exert enough pressure on an unaltered ureter to result in dilatation. They advanced the theory of hormonal influences acting on the ureter, specifically those brought about by 'estrin and gonadotrophins'.

As a result of the above and other observations it was generally accepted during the fourth decade of the present century that hormonal factors played an important part in the production of the common urological symptoms of pregnancy.

In a previous study of the pelvic girdle joints[7] histological changes were noted in the connective tissues of the ligaments of the symphyseal joint as a result of pregnancy.

In the non-pregnant Bantu female this structure, with ordinary haematoxylin and eosin staining techniques, showed, in the connective-tissue component, the characteristic long and thin collagenous connective-tissue fibres containing small, darkly-staining, pyknotic nuclei. Individual fibres were easily discernible and blood-vessels were small, thin-walled and sparsely distributed. The whole symphysis pubis of a pregnant Bantu who died in labour of intercurrent disease was obtained for comparative purposes.

It was immediately apparent that the changes which occurred in the ligamentous component were of a fundamental nature:

1. Both macroscopically and microscopically there was an increase in thickness of at least 100%.

2. There was a very noticeably increased vascularization of the purely connective-tissue part of the ligament. Blood-vessels were very much increased in size, filled with blood and thickly distributed throughout the connective tissue.

3. Individual fibres showed a marked hypertrophy. There were large, swollen and pale-staining nuclei contained in them, strikingly different from the small, dark, pyknotic nuclei previously seen. Individuality of the collagen fibres seemed to have been lost, to a large extent, the whole picture assuming much more of a homogeneous amorphous nature. In addition the characteristic thin and elongated appearance of the fibres was changed to a thick 'elastic-looking' whorly shape.

The presumably hormonal action on the very strong and rigid ligamentous component of the pelvic girdle joints is of such a profound nature as to allow for softening and relaxation of these ligaments to such an extent that movement in the joints is demonstrable.[7] The hormonal effect appears to be confined to the collagenous or connective tissues.

It is difficult to accept that such effect is confined only to the connective tissues of the pelvic joints. It seemed logical that all connective-tissue structures would be affected in pregnancy.

MATERIALS AND METHODS

Histology

The status of the connective tissues of the lower urinary tract, in both the pregnant and the non-pregnant state, was investigated. Previously and to date, interest had centred mostly on the ureters, and the bladder itself as well as the urethra had seemingly received only passing attention.

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The bladder, especially in the region of the urethrovesical junction, is regarded as of prime importance in the production of the urinary symptoms of pregnancy.

Histologically the ureters fuse into the bladder. There is a continuation of the transitional epithelium of the ureter into the bladder; also of the 3 muscular layers of the lower end of the ureter, the fibres of which interlace and anastomose, but which can still be distinctly differentiated into an inner longitudinal, middle circular and outer longitudinal layer.

Supporting the mucosa and the muscular layers in both the ureter and the bladder there is a stroma of delicate fibrous tissue containing few elastic fibres and rich in cells.[8] The outer aspect of the ureter and bladder is covered by a fibrosa layer or investing fascia (relatively thicker over the bladder) which fuses with the supporting stroma of the muscularis. The investing fascia attaches the bladder to the surrounding structures.

Anteriorly these attachments thicken and condense in the region of the urethrovesical junction to form a so-called suspensory ligament connecting the urethrovesical junction and the urethra to the posterior aspect of the symphysis pubis, and on each side to the periosteum of the pubic rami. (In extraperitoneal dissection of the bladder neck these structures can be very clearly demonstrated *in vivo*.)

Biopsies

With the above anatomy in mind, biopsy specimens of the following structures were collected from both pregnant and non-pregnant patients.

(*a*) The symphysis pubis — the actual site was the posterior symphyseal ligament.

(*b*) The urethral suspensory ligament.

(*c*) The investing fascia of the bladder.

(*d*) In one non-pregnant case only, a section through the whole bladder wall.

The specimens were taken from patients who came to surgery. Five non-pregnant White females who were submitted to total abdominal hysterectomy were biopsied, and 5 pregnant White patients who required caesarean section were sampled. It will be readily appreciated that there are many practical difficulties in obtaining the specimens.

In addition to haematoxylin and eosin stains the various sections were selectively stained for collagen with Mallory's trichrome stain. This stain shows the connective-tissue layers and indicates that the hormonal effect appears to be mainly localized to the collagenous layers. Nuclear changes are more readily studied by means of the haematoxylin and eosin stains.

RESULTS

The results of the earlier investigation were in the main confirmed, although increased vascularization was not such a prominent feature. Hypertrophy of the individual fibres was very noticeable, especially in the thin urethral suspensory ligament where they are normally more sparsely distributed. This structure provided the best opportunity of comparing individual fibres under very high power. The fibres from the pregnancy specimens were markedly swollen and curly, and there were definite nuclear changes. The latter were paler-staining, larger and vesicular in appearance, apparently indicating an intracellular change. In some instances the fibres from the pregnancy cases appeared to be 3 or 4 times larger than those from the non-pregnant cases.

The thick concentration of connective tissue in the symphyseal ligament makes it difficult to isolate individual

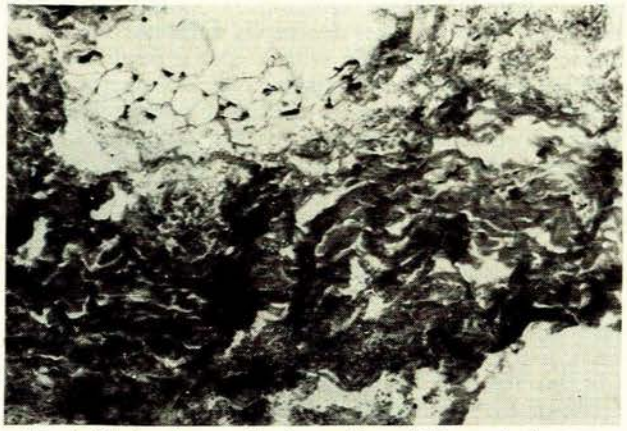

Fig. 1. Photomicrograph of a section of the posterior symphyseal ligament in a non-pregnant subject (× 50).

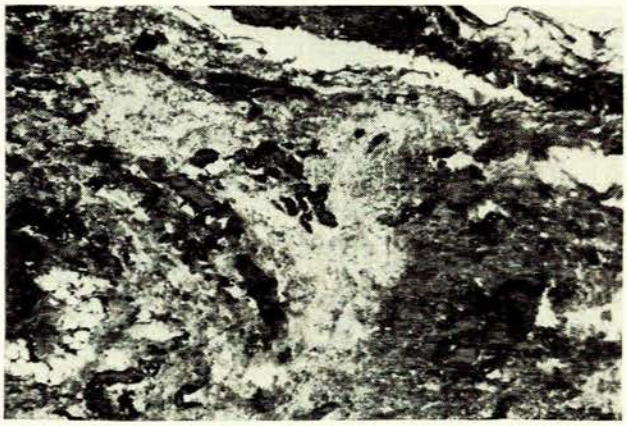

Fig. 2. Photomicrograph of a section of the posterior symphyseal ligament in a pregnant subject. Note the increased vascularization (× 50).

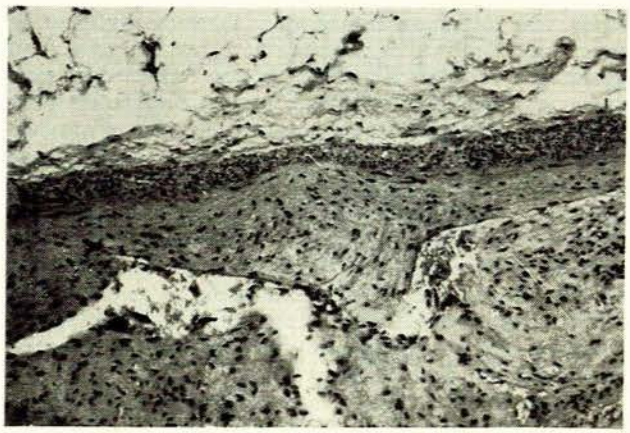

Fig 3. Photomicrograph of a section of the posterior symphyseal ligament in a non-pregnant subject (× 50).

fibres for separate study, but again the impression was gained that the whole structure had become amorphous, pliable and plastic in nature. The changes in the symphyseal ligaments bore a striking resemblance to those in the pregnant cervix as noted by Danforth and Buckingham.[9] There is also evidence that the connective-tissue changes are more marked in the denser structures, e.g. the ligaments of the symphysis pubis, than in those areas where

Fig. 4. Photomicrograph of a section of the posterior symphyseal ligament in a pregnant subject. Note the increased thickness of the connective-tissue fibres as compared with Fig. 3 (× 50).

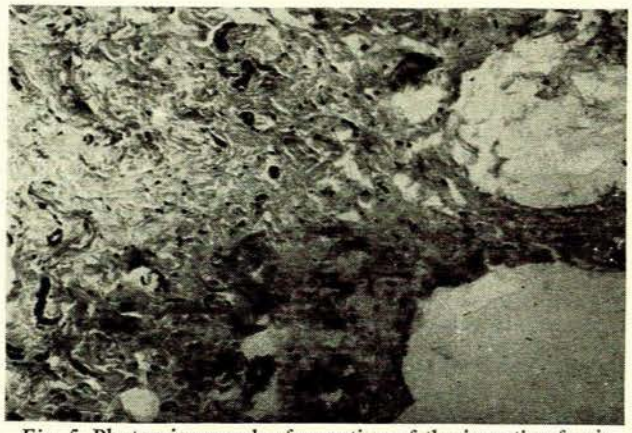

Fig. 5. Photomicrograph of a section of the investing fascia of the bladder in a non-pregnant subject (× 50).

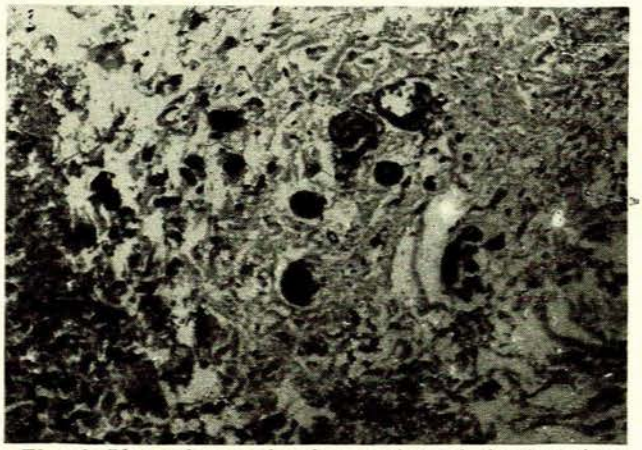

Fig. 6. Photomicrograph of a section of the investing fascia of the bladder in a pregnant subject (× 50).

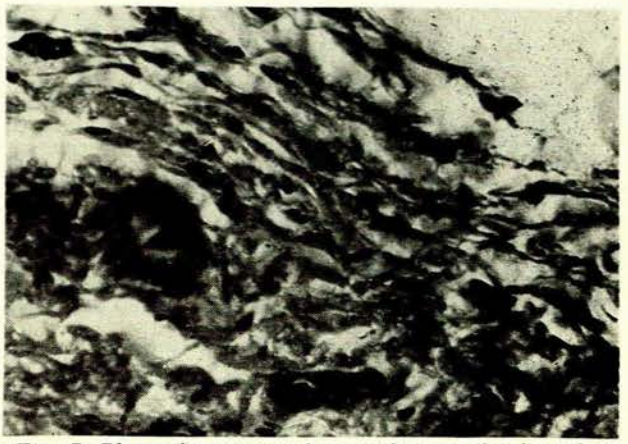

Fig. 7. Photomicrograph of a section of the investing fascia of the bladder in a non-pregnant subject (× 320).

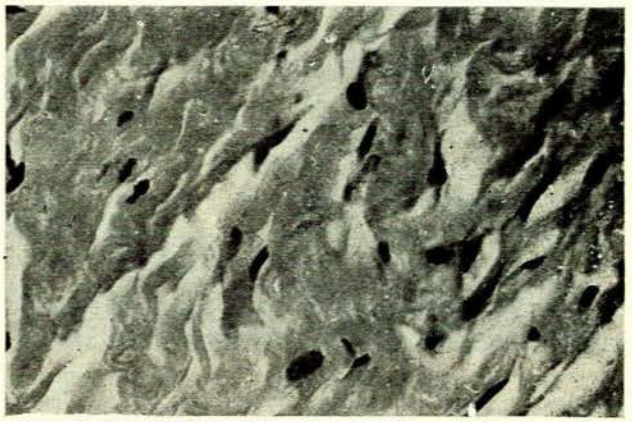

Fig. 8. Photomicrograph of a section of the investing fascia of the bladder in a pregnant subject. Note the marked hypertrophy of the connective-tissue fibres and also the more vesicular nuclei (× 320).

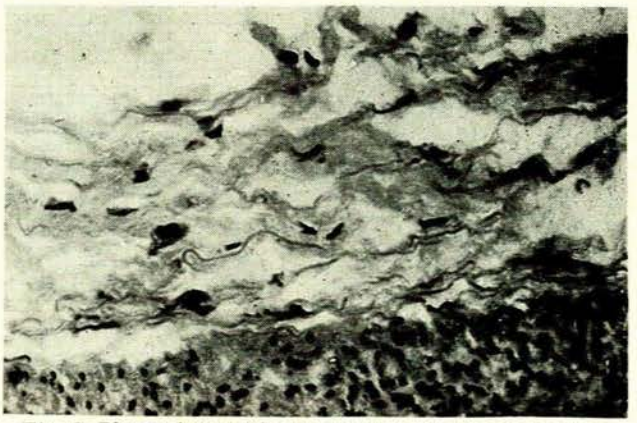

Fig. 9. Photomicrograph of a section of the urethral suspensory ligament in a non-pregnant subject. In this thin structure individual connective-tissue cells are discernible (× 320).

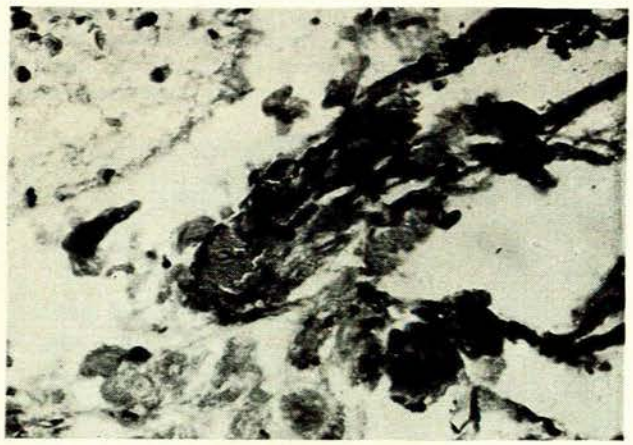

Fig. 10. Photomicrograph of a section of the urethral suspensory ligament in a pregnant subject. Note the marked hypertrophy of individual connective-tissue cells (× 320).

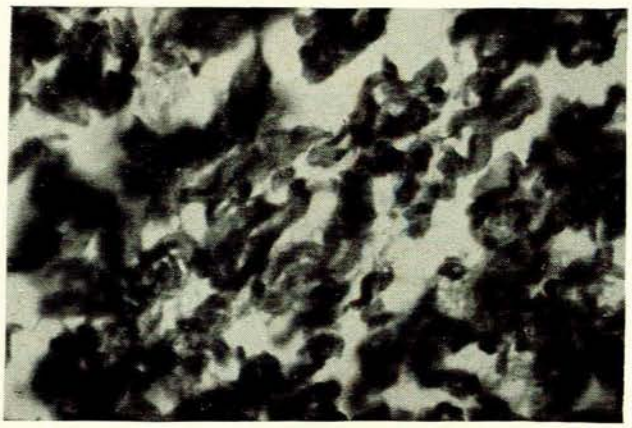

Fig. 11. Photomicrograph of a section of the urethral suspensory ligament in a non-pregnant subject (Mallory's trichrome stain × 600).

Fig. 12. Photomicrograph of a section of the urethral suspensory ligament in a pregnant subject, showing the very marked hypertrophy of individual collagen cells and also their thick curly appearance (Mallory's trichrome stain × 600).

the fibres are sparse and further separated, e.g. in the urethral suspensory ligament.

On the basis of these studies it is difficult to provide an answer to the question of hypertrophy and/or hyperplasia of the connective tissue. Nuclear counts per high-power field are subject to inaccuracies because of obvious variables such as the previously mentioned density of collagen fibres, but on reasonably comparable average counts, based on the principle of the number of nuclei per high-power field being in inverse proportion to the amount of hypertrophy, there appears to be no doubt that hypertrophy is a major feature, and would on a conservative estimate amount to about 100%.

Hyperplasia of the connective tissue cannot be excluded, but from these studies it would seem to be of relatively minor importance. The connective-tissue changes were uniformly noted in all the specimens of the investing fascia of the bladder taken from the pregnant subjects (Figs. 1 - 12).

DISCUSSION

Since the 1930s, when the importance of pregnancy hormonal influences on the urinary tract was first recognized, every ovarian and pregnancy hormone has in turn been implicated as the specific aetiological factor.

Fainstat,[1] in his review of ureteric dilatation in pregnancy, recounts in detail the findings of various workers who, by way of animal ureter *in vitro* and other animal experiments, have attempted to clarify the subject. In his concluding comment Fainstat states:

'Although the experimental results do not follow a clear-cut pattern to date, it appears that gonadotrophins and progesterone tend to induce hypomotility of the ureter. On the other hand, estrogens do not appear to reduce ureteral contractions *in vitro*. However, the estrogen-induced growth-promotive and extracellular fluid retention capabilities, so well-known with regard to the genital tissues, may be present to some degree in the ureter. Ureters, as well as uteri, may be less able to maintain their tone and motility after their microstructure has been modified during pregnancy. In this manner estrogens, as well as progesterone and gonadotrophins, may contribute to the dilatation of the ureter during pregnancy.'

From our own studies on the bladder it would seem that changes in the microstructure of the organ mainly originate from alterations in the connective-tissue layers. The hormone which without doubt seems to exert its action primarily and rapidly on connective tissue, namely relaxin, appears to have been overlooked in many investigations on the subject.

A great difficulty in assessing the role of each hormone appears to be the fact that their actions overlap to a certain extent. Oestrogen and progesterone, either alone or in combination, can cause relaxation of guinea-pig pelvic ligaments after days of administration, while relaxin obtains the same effect in a matter of hours.[10]

Recent work seems to indicate that while overlap of action undoubtedly exists, the major action of each ovarian hormone could be summarized as follows:

Progesterone

This substance causes reduction of motility of the myometrium, ureteric muscle or human smooth muscle. *In vitro* experiments indicate that this hormone can paralyse either spontaneous or oxytocin-induced contractions in the above structures.[11,12]

Oestrogen

This hormone causes the well-known growth promotive action with extracellular fluid retention capabilities.

Relaxin

Relaxin initiates a direct action on the collagen by a fibrillar dissociation of the fibres; a change which occurs rapidly.

As relaxin apparently directly affects the collagenous component of connective tissues, it is necessary to clarify the relationship between collagen and the connective tissues. The following summary is, in the main, extracted from the review of 'Connective tissue mechanisms and their relation to pregnancy' by Danforth and Buckingham.[9] Connective tissues consist of:

1. *Formed elements* which include: (*a*) fibrous constituents which are collagen, reticulin and elastin, and (*b*) cellular components which are principally fibroblasts, mast cells and wandering cells.

2. *Ground substance*: This is the gel-like mixture filling the spaces between the fibrous and cellular elements. It contains acid and neutral mucopolysaccharides, non-collagenous protein, water and other substances derived from the blood plasma.

Collagen is the principal fibrous element, and is the one on which most work has been done, especially by the leather chemists. It accounts for approximately 30% of the total body protein. Its local content varies from structure to structure; e.g. in tendons it forms 80 - 95% of the total protein, in the organic matrix of bones it forms 90 - 95%, in skin 50 - 75%, and in muscle only 1 - 6% of the total protein.

Old collagen fibrils are basically similar at molecular level, being long chains of amino-acid units that are arranged in the form of a left-handed helix, of which 3 link together to form the trihelical structure of the collagen fibril.

Collagen fibrils are themselves inelastic. Their tensile strength has been measured and found to be a little more than that of cast iron and a little less than that of steel. Individually they are submicroscopic, but aggregates of many fibrils are organized into a system of fibres which vary in diameter from 1 to 200 microns. It is not known what holds the fibrils together in denser tissues, or, on the other hand, what permits them to slide or separate in the looser tissues. It has been suggested that molecular configuration-changes control either their aggregation or dissociation and that the ground substance may have to do with the intermolecular reactions.[13]

Old collagen is almost insoluble, whereas embryonic or newly formed collagen is readily soluble in neutral salt or dilute citric or acetic acid. In the fraction of collagen soluble in dilute acid, the identity of the fibrils is lost; the banding period vanishes altogether and for practical purposes the material has gone into solution. The neutralization of the substrate instantly reconstitutes the fibrils, and they are as before.

The amino acids hydroxyproline and hydroxylysine are uniquely found in collagen. Hydroxyproline concentrations in collagen are remarkably constant, regardless of the tissues from which the collagen is extracted; consequently the demonstration of hydroxyproline in any tissue provides evidence of the presence of collagen. Hydroxyproline can be quantitated in the urine, and is found to be present in considerable amounts in the neonatal period, and to decline thereafter.

The ground substance may be of great importance in the action of relaxin on the collagen. The fibrillar dissociation of the collagen fibrils said to result from the action of this hormone may be evidence of direct action, but may be secondary to ground substance changes.

The gel-like consistency and water-binding capacity of the ground substance are due to the presence of acid mucopolysaccharides and their protein complexes.

Since the metabolism of mucopolysaccharides is rapid when compared with other macromolecules, the possibility immediately arises that physiological changes such as in the symphysis pubis, in the cervix and in the bladder may be caused secondarily to changes in the acid mucopolysaccharide concentrations.

The histological changes in the collagen fibres at ordinary microscopical levels appear to indicate a much more marked reaction in the structures where there is an abundant concentration of ground substance, e.g. the symphysis pubis as compared with the urethral suspensory ligament (Figs. 1 - 12).

SUMMARY AND CONCLUSIONS

Comparative microscopical studies were done on the bladder and the urethral suspensory and posterior symphyseal ligaments of 5 non-pregnant females and 5 pregnant subjects. These studies show that in the case of all the 5 pregnancy specimens there are noticeable histological changes as compared with the 5 non-pregnancy specimens. The histological changes all refer to the collagenous component of the structures examined and consist of hypertrophy and possibly hyperplasia of the connective-tissue elements. The hypertrophy may be associated with an intracellular change as evidenced by nuclear changes in the connective-tissue cells of the pregnancy specimens and an increased vascularization of the pregnancy structures.

The changes are more marked where there is reason to suspect the presence of increased amounts of ground substance, e.g. in the thick ligaments of the symphysis pubis as opposed to the thin urethral suspensory ligament.

It seems reasonable to conclude that these changes are brought about by hormonal influences during pregnancy. Evidence from the literature strongly supports this view. Although no specific hormone can at this stage be implicated as the aetiological agent, it appears likely that relaxin is of primary significance.

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