THE HUMAN UTERINE CERVIX AND ELASTIN
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Introduction

In human pregnancy and birth the uterine cervix has two major physiological functions. First, it must remain closed during pregnancy to retain the fetus and to prevent infection. Second, it must dilate at birth to allow the infant to deliver. These studies were undertaken to begin to discover the role that elastin might play in these two functions of the cervix.

Materials and Methods

To demonstrate that elastin is a component of the matrix human cervical tissue was obtained from operative births at various stages of pregnancy and from surgical specimens of non-pregnant women. Tissues were studied utilizing a modified iron-hematoxylin stain that differentiates elastic fibers for light microscopy and by osmium tetroxide for ultrastructural observation. For biochemical studies cervical crude connective tissue was extracted with 1M NaCl, butanol and acetone. Elastin was isolated by the Lansing method. Both isolates were hydrolyzed and subjected to aminoacid analysis.

To determine the location and anatomical orientation of the elastic fibers the total uterine cervixes from seven surgical specimens were studied by histological methods utilizing serial frontal, cross and sagittal sections and by the development of reconstructions of the whole cervix. A clinical syndrome, the incompetent cervix was studied comparing tissue from women with this syndrome to the cervical tissue from normal pregnant and nonpregnant women. This tissue was analyzed by histological methods and when possible by radioimmunoas-
say for the elastin crosslink, desmosine. The radioactive antibodies were prepared from rabbit anti-desmosine serum. For the assay bovine alpha globulin was utilized as a carrier. To further study elastin in the uterine cervix, fibroblasts of human cervical cell origin were grown originally in Ham's F-12 with 20% fetal bovine serum. Later they were grown in MEM with 10% fetal bovine serum. Desmosine and other elastin crosslinks were recovered from the cellular media by two dimensional chromatography, followed by autoradiography of the electrophoretograms. Suc-(Ala)₃-Nan and Ala-Nan hydrolyzing activities have been found in conditioned media. Finally, cultures were incubated in the presence of interleukin-1, 0.5 to 1 ng., estradiol, 1x10⁻⁶, 1x10⁻⁷M and progesterone, 1x10⁻⁶, 1x 10⁻⁷M. Following these incubations the media was assayed for hydrolytic activity.

Results and Discussion

The human uterine cervix contains mature crosslinked elastin as demonstrated by the aminoacid analysis. (Table 1.) and was found to be 1.54-1.59% of the crude connective tissue fraction in early pregnant and nonpregnant samples.¹ At the end of pregnancy this percentage dropped. These determinations were based on both the desmosines present and the non-polar/polar ratio and not just solely on the desmosine content. Recently, studies by others have shown that in the uterus the desmosine formation and elastin synthesis is not coordinated.²,³ Not only are the physiological functions of the cervix opposite from each other in pregnancy and birth, but the physiological functioning of the uterus is opposite from that of the cervix. During pregnancy when the cervix is closed the uterus must stretch, hypertrophy and proliferate. At the completion of gestation when the cervix is undergoing connective tissue degrad-
ation the uterus must contract. Thus it is not surprising that the biochemistry of the matrix proteins in the uterus is the reverse of that in the cervix. Thus elastin increases in the uterus as pregnancy advances with the desmosine crosslinking increasing also, at a slower rate. At the same time elastin in the cervix decreases.

The reconstruction of the serial sections of the uterine cervix reveals that the elastic fibers, which contain elastin, run just below the mucosa, then course downward into the deeper layers of the stroma under the epithelium of the endocervical canal and portio vaginalis. The fibers run in a band from external os to the internal os. Of significance no elastic fibers were seen around or in blood vessels by either light or electron microscopy. This band of elastic fibers is then oriented in a manner that would allow retraction upward and inward during effacement. The elastic fibers would be directly under the presenting part so uterine contractions would push the fetus against the elastin causing it to stretch.

Biomechanical studies indicate that cervical dilation is a passive phenomenon, which occurs by a slippage-like mechanism of the connective tissue proteins.

The amount of the desmosine crosslinked elastin is decreased during pregnancy. The lowest amount being found in the incompetent cervix, a clinical syndrome in which the cervix opens at 16 to 24 weeks gestation without uterine contractions. The fact that the amount of desmosine correlates with clinical outcome is provocative.

In vitro, human cervical fibroblasts make cross-linked elastin as determined by the presence of desmosine. In addition, these fibroblasts demonstrate Suc-(Ala)₃-Nan and Ala-Nan hydrolyzing activities. The hydrolyzing
activity cleaving Suc-(Ala)₃-Nan is approximately 30 fold lower than that of Ala-Nan hydrolyzing activity. Initially, data suggested that estradiol-17-beta increased the Suc-(Ala)₃-Nan hydrolyzing activity. However, subsequently, this has not proved to be the case. At the present time, studies seem to indicate that interleukin-1 increases these activities.

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References

6. Cleary, T. Personal Communication
Table One

Amino Acid Composition of Purified Elastins

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<th>Residues per 1000</th>
<th>1 Pooled primate cervix</th>
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<th>3 Nonpregnant human cervix</th>
<th>4 16-week pregnant human cervix</th>
<th>5 20-week pregnant human cervix</th>
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Percentage of Elastin in Human Cervix

- Pregnant 40 weeks: 0.9%
- Nonpregnant premenopausal: 1.54%
- Pregnant 16 weeks: 1.54%
- Pregnant 30 weeks: 1.57%

Fig. 1

Amount of cross-linked elastin in human uterine cervix. Sample from a subject with pregnancy loss without incompetent cervix.

Fig. 2

Autoradiograph showing desmosines synthesized by human cervical fibroblasts. Cells were incubated for 24 hours with and without estradiol.

Crude Connective Tissue Fractions of Human Cervix