ALTERATION IN THE COLLAGEN CONTENT OF THE HUMAN UTERUS DURING PREGNANCY AND POST PARTUM INVOLUTION*

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The physiological resorption of collagen during recovery from experimental cirrhosis was demonstrated in 1947 (1). Factors which influence the deposition and disappearance of collagen in experimental cirrhosis, as well as the phenomenon of the regression of scar tissue, were examined subsequently (2, 3). A striking resorption of collagen from the rat uterus following parturition was described by Harkness and Harkness in 1954 (4). Later these authors (5), and Harkness and Moralee (6), suggested that the post partum uterus is especially suitable for investigation of the physiological dissolution and catabolism of endogenous collagen. The present study was undertaken to determine the alterations in the nature and content of collagen occurring in the myometrium of the human uterus during pregnancy and the post partum involutionary period.

Materials and Methods

Specimens.—Human uteri were obtained from patients undergoing hysterectomy, caesarian hysterectomy, or at autopsy. Some specimens were analyzed immediately after removal and others were kept in the frozen state at −20°C and analyzed subsequently. The cervix was excluded from the present study. Scars from previous caesarian sections, when recognized in the gross, were excised and excluded from specimens chosen for analysis. Before being employed for preparation of enzyme extracts or for chemical study, uteri were trimmed free of endometrium, decidua, and blood clots.

Histological Examination.—Sections of uteri were prepared after fixation in formalin, formalin–mercury bichloride, and Zenker's solutions. Stains used were hematoxylin and eosin, Masson trichrome, van Gieson's, Laidlaw's reticulum, Schiff-McManus, Hales' colloidal iron, Sudan III, alcian blue–chlorantine red, and luxol fast blue–periodic acid-Schiff.

Fat-Free Dry Weight.—Aliquots of minced uterine tissue were extracted for two 24 hour periods with 100 volumes of acetone and then again by two similar treatments with ether. The materials were finally dried to constant weight in a vacuum desiccator.

Extractions for Collagen and Enzymes.—All procedures were carried out at 0 to 4°C. Trimmed uteri were minced in the cold with scissors and homogenized in a Lourdes homog-

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enizer while the containing vessel was immersed in an ice water bath. Centrifugation was performed in an International refrigerated centrifuge at 2°C at 12,000 RPM. or in a Lourdes centrifuge at cold room temperatures. Final preparations were dried from the frozen state and stored at −20°C. Soluble collagens were extracted by homogenization of minced uteri in 0.45 M NaCl according to the method of Gross (7) as well as by similar extractions utilizing water, 0.017 M acetic acid, or 0.1 M citrate buffer at pH 3.55. Extractions were carried out at 4°C with shaking overnight.

Preparation of Collagen for Hydroxyproline Analysis.—A modification of the method of Neuman and Logan (8) was employed. A portion of minced uterus weighing from 100 to 300 mg was ground in 10 to 15 ml of a 20 per cent solution of urea in water (w/v) using a glass tissue homogenizer. The specimen was allowed to stand at room temperature for 1 hour and centrifuged. The supernatant liquid was discarded. The residue was washed twice with distilled water, dispersed in 15 ml of 1.25 N NaOH, and placed in a boiling water bath for 8 hours. Aliquots of the hydrolyzed specimen were taken for determination of hydroxyproline.

Determination of Hydroxyproline.—The method of Stegemann (9) employing chloramine-T was used. The alkaline hydrolysates were neutralized with 5 ml of 3.75 N HCl before analysis. Salt concentration of the standards was made equal to that in the unknowns since this was found to be a factor in color development.

Total Nitrogen and Protein Analysis.—Total nitrogen was determined by micro-Kjeldahl analysis (10). Protein in extracts was determined by the method of Lowry and coworkers (11) employing a serum albumin standard of determined nitrogen content.

Viscometry.—Viscometric determinations were performed in a constant temperature bath held at 20.0°C. Viscometers were of the Ostwald-Fenske type and had flow times of 59 to 63 seconds with water or buffer.

Enzyme Determinations.—Proteinase activities were determined either viscometrically measuring the decrease in viscosity of standard solutions of gelatin or ichthyocol (12) or by increase in ninhydrin activity as measured by the method of Rosen (13). Prolidase was determined by a modification of the method described in Methods in Enzymology (14).

RESULTS

Quantitative Alterations of Uterine Collagen.—As shown in Table I and Fig. 1, the human uterus shows a gradual and progressive increase in content of collagen during pregnancy. The collagen content at term is about 800 per cent of the non-pregnant uterus value, whereas the *total uterine weight* is about 1000 per cent of the control value. Thus, although the increase in uterine collagen closely parallels the growth of the uterus, the actual percentage of collagen appears to be slightly lower during pregnancy and remains diminished as late as 5 weeks after parturition. The "transient" collagen formed during pregnancy, seemingly fated for resorption in the post partum period, totals approximately 53 gm.

Following parturition, the uterine collagen diminishes by a process which is apparent even at 14 hours. By the 8th day post partum, the collagen content is only 28 per cent of that seen in uteri at term.

Of interest is the increase in water content of the uterus during the phase of collagen resorption (Table I). Approximately 76 per cent of the weight of the non-pregnant uterus is water, and this percentage is constant throughout pregnancy. However, uteri 14 hours post partum already show an increase of

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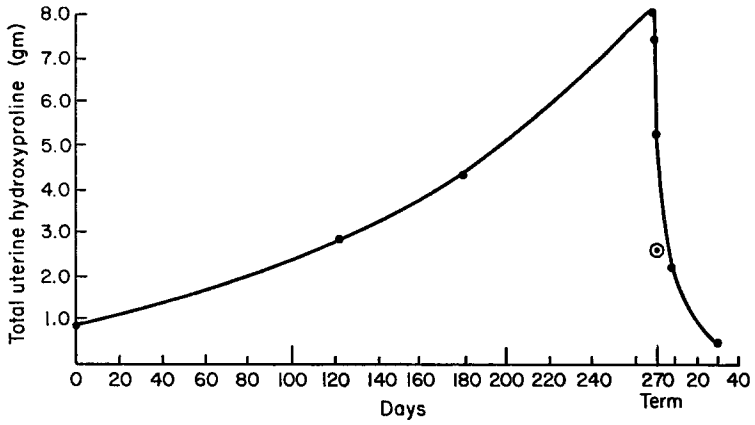

FIG. 1. Changes in the hydroxyproline content of human uteri during pregnancy and post partum involution.

TABLE I

Collagen (Hydroxyproline) Contents of Human Uteri

Nature of specimen	Wet weight of uterus	Non-fat solids	Hydroxy-proline	Total hydroxyproline	Total collagen*
	gm	*per cent tissue*	*per cent non-fat solids*	*gm*	*gm*
Non-pregnant (patient, age 39)	130	24.92	3.08	0.99	7.48
Non-pregnant (patient, age 43)	125	23.29	3.26	0.95	7.10
Pregnant, 126 days	490	24.12	2.46	2.91	21.82
Pregnant, 182 days	1010	23.21	1.98	4.62	34.65
Term, 270 days	1313	23.90	2.65	8.30	62.25
Term, 270 days	1290	22.67	2.62	7.66	57.45
Post partum, 9 hrs	1180	24.87	2.55	7.46	55.95
Post partum, 14 hrs	1020	19.56	2.63	5.25	39.37
Post partum, 2 days	502	—	2.58	—	
Post partum, 8 days	400	19.48	2.87	2.23	16.72
Post partum, 4 wks	98	19.07	2.78	0.52	3.90
Post partum, 5 wks	—	19.15	2.47	—	

* Calculated collagen = hydroxyproline × 7.5.

water content to 80 per cent, and this elevation is observed as late as 5 weeks post partum. No significant alterations in lipid content were revealed by histological examination of uteri during these periods.

Solubility Characteristics of Uterine Collagen.—In view of the decline of

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collagen content of uteri following parturition, the solubility characteristics of the collagens of pregnant and post partum uteri were investigated. Results obtained with a term uterus are shown in Table II and those obtained with a a 9 hour post partum specimen are shown in Table III. In both instances less than 3 per cent of the hydroxyproline (presumably, therefore, of the collagen) was extractable by any of the procedures used. Best extractability was achieved with 0.017 M acetic acid; neutral salts were less effective, as was acidic citrate buffer. Incorporation of sodium periodate into the acetic acid–extracting medium in an attempt to disrupt possible carbohydrate linkages did not increase

TABLE II

*Solubility Characteristics of Uterine Collagens of a Specimen Obtained at Term**

Extracting medium	Hydroxyproline extracted
	per cent total hydroxyproline
0.017 M acetic acid	2.28
0.1 M citrate, pH 3.55	0.72
0.1 M KCl	1.46
0.1 M $CaCl_2$	0.52
0.5 M KCl	0.32
1.66 M acetic acid containing 0.02 M sodium periodate (2 hrs. extraction period)	1.62
1.66 M acetic acid containing 0.02 M sodium periodate (4 days extraction period)	1.95

* Uterus previously extracted with 0.5 M KCl to remove myosin, then dried from frozen state before extraction with solvents listed here. Samples of 100 to 500 mg were used for extractions given in the table. For each 100 mg of dried material, 35 ml of extracting medium was employed. Each 100 mg of the dried specimen contained 5.37 mg of hydroxyproline.

The preliminary extraction with 0.5 M KCl resulted in removal, by solubilization, of 0.54 per cent of the total hydroxyproline.

the solubility of the collagen. Thus, approximately 97 per cent of the collagen in the term and involuting uteri was found to be "insoluble."

Preparation of Soluble Collagen.—Attempts were made to isolate and purify the small amounts of "soluble" collagen of the pregnant uterus. The tissue was first repeatedly extracted with 0.5 M KCl to remove the myosin in order to facilitate the solution of collagen. Thus, in one preparation uterine tissue was homogenized with cold 0.5 M KCl and extracted for 4 successive days with that reagent. A residue was obtained containing 3.8 per cent hydroxyproline. Microscopic examination of the preparation showed remnants of muscle cells, probably of arterial origin and apparently not easily extracted. The residue was then treated with cold 1 M calcium chloride solution at neutral pH for 18 hours and centrifuged for 4 hours at 12,000 RPM. The suspension was then filtered

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through fine sintered glass and dialyzed against cold 0.02 M dibasic sodium phosphate. The fibers which formed were dried from the frozen state. The total yield of typical collagen fibers was 18 mg from 110 gm of myometrium. The hydroxyproline content of the fibers was 6.21 per cent, but amino acid analysis using the automatic analyzer of Spackman, Stein, and Moore showed significant amounts of tyrosine and cystine, indicating that the preparation was no more than 80 per cent pure collagen. Further, these fibers were difficult to solubilize, even in 0.1 M calcium chloride solution.

Uterine Proteases and Prolidase.—Preliminary attempts were made to extract and assay protease and prolidase activities of uteri in order to determine whether such enzymes were associated with the process of involution and disappearance of collagen. Previous reports of the existence of uterine pepti-

TABLE III

*Solubility Characteristics of Uterine Collagens of a Specimen Obtained 9 Hours Post Partum**

Extracting medium	Weight of uterus extracted	Volume of extracting medium	Hydroxyproline extracted
	gm	*ml*	*per cent total hydroxyproline*
0.017 M acetic acid	28.74	332	2.17
0.1 M citrate, pH 3.55	50.78	234	1.21
0.45 M NaCl	56.57	245	<0.1‡
Water	25.00	400	<0.1‡

* In this instance the extracting medium was applied directly to the homogenized wet tissue without prior extraction to remove myosin.

‡ The amount of hydroxyproline was too small to measure accurately by the method used; a maximum figure is therefore given.

dases (15) and proteinase (16) are extant. In the present studies no success was achieved in finding enzymes in post partum or pregnant uteri which could degrade undenatured calf skin–soluble collagen or ichthyocol; however, proteolytic enzymes active against gelatin were extractable by water and dilute acetic acid from homogenized post partum uteri. Thus from a 2 day post partum uterus an enzyme was obtained which slowly degraded gelatin at pH 3.85 but was virtually inactive at pH 7.0. As yet non-pregnant uteri have not been examined for presence of this enzyme, but Woessner has described a proteinase in non-pregnant uteri which is active at acid pH (16).

Prolidase, which conceivably would be present in involuting uteri acting further on glycylproline peptides resulting from proteolytic degradation of collagen, was sought in extracts of non-pregnant, pregnant, and post partum uteri. Extracts of uteri were made by homogenization with 0.2 M tris buffer of pH 8.0 and tested in that buffer, fortified with manganous chloride, against glycyl-L-proline at 40°C. These results are presented in Table IV. It is seen

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that prolidase activity in selected specimens showed no increase during pregnancy as compared with the non-pregnant state, but was elevated to the extent of 75 per cent in a 2 day as well as in an 8 day post partum uterus. A progressive diminution of prolidase activity was observed in uteri which were 4 and 5 weeks post partum. Tris buffer was found to be more effective than water or 0.15 M NaCl in extracting prolidase from uterine homogenates. In the series shown in Table IV only the specimen obtained 14 hours post partum did not follow the trend described.

Histological Changes.—Sections of uterine myometrium were studied with a view toward correlating histological findings with the chemical data. The post

TABLE IV

Hydrolysis of Glycyl-L-Proline by Extracts of Human Uteri

Nature of specimen	Protein per milliliter of extract assayed	Substrate ($\times 10^{-3}$) hydrolyzed per mg of tissue protein
	mg	*μg*
Non-pregnant	8.48	3.64
Non-pregnant	10.67	3.54
Non-pregnant	12.20	5.00
6.5 months gestational	9.50	5.24
Term	11.11	5.26
14 hrs. post partum	10.51	4.17
2 days post partum	6.69	7.21
8 days post partum	7.84	7.01
4 wks. post partum	11.10	6.20
5 wks. post partum	13.51	5.47

partum uteri showed three interesting findings: interstitial edema, cellular infiltration, and altered reticular pattern.

The interstitial edema corresponded with the increased water content which was chemically demonstrated in post partum uteri. Macrophages, lymphocytes, mast cells, and eosinophiles were noted in significantly increased numbers within the edematous interstitial connective tissue of the myometrium of post partum uteri.

The involuting uteri showed a remarkable persistence of intact fibrillary collagen and reticulum as demonstrated by special stains with preservation of normal staining properties. The pattern of argyrophilic reticulum, however, showed alterations accompanying involution. In term uteri, the reticulum appeared as a tremendously abundant delicate and intricate plexus of argyrophilic fibers surrounding individual muscle cells. By contrast, the involuting uteri showed a marked diminution in the number and complexity of the more delicate fibers, frequently with a fragmented and disorganized pattern.

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DISCUSSION

A comparison of the observations of Harkness, Harkness, and Moralee (4–6) on the pregnant rat uterus with those reported in the present study for the human uterus discloses four interesting similarities: First, the increase in collagen in the rat uterus is approximately 600 per cent at term as compared with 800 per cent found in the human uterus in this study; second, in spite of the marked differences between length of gestational period in rat and in the human, the slopes of the curves depicting collagen alterations during pregnancy and the puerperium are virtually identical in the two species; third, in the human there is a decrease in the concentration of collagen in the uterus during the second half of pregnancy similar to that observed in the rat uterus (5, 6); and fourth, the collagen content of the uterus in both species at the end of involution appears to be below the normal non-pregnant content. The last phenomenon would not appear to represent a pathological hyperinvolution, but seems to be a limited continuation of the post partum physiological mechanism of resorption of collagen.

The relative insolubility of the major portion of the uterine collagen deposited during pregnancy is an unexpected finding, since one might have predicted that newly formed collagen, eventually to be resorbed, would be extractable in one of several media used for the *in vitro* dissolution of collagen. In a comparable study of the solubility characteristics of collagen in reversible cirrhosis in rats, a condition in which the collagen is also resorbed, Bazin and Delaunay (17) found that approximately 97 per cent of the hepatic collagen after 5 weeks of treatment with carbon tetrachloride was "insoluble." In comparison, 94 per cent of the collagen in normal rat liver is insoluble under similar conditions of extraction. The question of the significance of the extractable collagens has been recently discussed by Jackson and Bentley (18) who conclude that in developing connective tissues, at any given time, there is a continuous spectrum of collagen aggregates of varying ages, solubilities, and degrees of strength of cross-linkage.

Although no evidence for the presence of a "true" collagenase was found, it is of interest that prolidase activity was found to rise during the period of involution and to decline toward normal values of the non-pregnant uterus within 5 weeks after parturition. This enzyme would not be involved in the primary process of collagen breakdown but rather would be responsible for the further disposition of glycylproline peptides formed by a proteolytic process. Woessner (19) has found a proteinase present in involuting post partum rat uteri which is active at pH 3–4, and digests heat-denatured collagen. He also found appreciable levels of prolidase and prolinase, suggesting that collagen is completely degraded within the uterus. Smith has previously shown that non-pregnant human uterine tissue is rich in peptidases, particularly dipeptidases (15).

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SUMMARY AND CONCLUSIONS

Quantitative collagen determinations demonstrate an increase of approximately 800 per cent in the collagen content of the human uterus at term as compared with the non-pregnant state. Following parturition, there is a rapid resorption of collagen. The amount of collagen which disappears from the post partum uterus is approximately 53 gm.

By the 8th day post partum, the human uterus has lost approximately 72 per cent of the total collagen which was present at term. The slopes of curves depicting alterations in collagen content of the uterus of the rat and human are virtually identical, despite the marked differences in the length of the gestational periods of the two species.

The alterations in the collagen content of the human uterus during pregnancy and involution closely follow changes in total uterine weight. There is a slight decrease in per cent collagen content of the uterus during pregnancy, and an increase in the water content of the post partum uterus.

Approximately 97 per cent of the collagen which is present in the human uterus at term is of the so called "insoluble" type. The physiological resorbability of uterine collagen, as evidenced by its post partum dissolution, cannot therefore be correlated with its *in vitro* solubility characteristics.

No collagenase was demonstrable in the myometrium of post partum uteri. A 2 day post partum uterus was found to contain an enzyme which slowly degraded gelatin at pH 3.85 but was virtually inactive at pH 7.0.

There is an increase in myometrial prolidase activity of approximately 75 per cent, commencing 2 days post partum. Prolidase activity remains elevated until the 8th day post partum, and subsequently falls to almost normal levels by the 5th post partum week.

During the process of involution, the post partum uterus shows histological evidence of edema and partial destruction of its reticular framework.

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