acrylamide gel. A highly active substance was isolated by ion-exchange chromatography on a 5 % cross-linked polymer of acrylamide, *NN'*-methylene-bisacrylamide and diethylaminoethyl methacrylate.

2. The initial gel-filtration experiments were carried out under a wide variety of conditions and resulted in the same yield of high-molecular-weight and low-molecular-weight fractions with inotropic activity.

3. It was concluded that it was unlikely that the low-molecular-weight fraction with inotropic activity was an artifact produced from the plasma proteins by the method of isolation.

4. The inotropic activity of the isolated substance was destroyed by incubation with protease.

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Formation and Breakdown of Collagen and Elastin in the Human Uterus during Pregnancy and Post-Partum Involution

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The post-partum involuting uterus presents one of the most favourable systems for an investigation of the mechanisms of protein catabolism in the mammalian organism. Involution involves an extremely rapid breakdown of muscle protein and of the connective-tissue framework of the uterus. Harkness & Moralee (1956) found that the half-life of uterine collagen was only 24 hr. during involution in the rat. The possible mechanism of collagen breakdown in the rat uterus was studied by Woessner (1962a), and it was postulated that the breakdown, which apparently proceeded to the free amino acid level, was due to the action of pro-

teolytic enzymes. Difficulties in obtaining sufficient tissue for enzyme studies led us to investigate the human uterus.

The rate and extent of collagen changes in the human uterus during pregnancy and post-partum involution have been studied by Montfort & Pérez-Tamayo (1961) and Morrión & Seifter (1962). We have extended these studies to show that elastin, as well as collagen, undergoes a marked increase in pregnancy and a rapid decrease during involution. Moreover, there is a striking effect of successive pregnancies on the uterus and its connective tissue. The proteolytic enzyme activity of the uterus and the nature of uterine collagen in both normal and involutionary states have been examined in a search for clues to the mechanism of collagen breakdown.

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EXPERIMENTAL

Uteri. Human uteri were obtained at surgery and placed immediately in the refrigerator at 4°. Processing of the uterus commenced within 1–48 hr. after surgery. The majority of the uteri were removed because of cervical diseases such as carcinoma *in situ*. Others were removed because of prolapse, rupture at delivery, ovarian cysts, mistaken diagnoses etc. The uterus was cut in half and both parts were weighed. One half was examined by a pathologist, and if a pathological condition was found in the body of the uterus the remaining half was discarded. If the body proved to be normal, the cervix was removed from the experimental half at the level of the internal os and was discarded. The remaining portion of the uterine body was weighed and used in these experiments. It has not been possible to demonstrate a correlation between any of the parameters measured in these studies and the pathological condition that occasioned the surgical removal.

Preparation of connective tissue. The uteri were cut into small pieces and 6 g. portions were homogenized in 60 ml. of 0.9% NaCl solution. A VirTis 40000 homogenizer (VirTis Co. Inc., Gardiner, N.Y., U.S.A.) was used at full speed for 2 min. while the sample was kept well chilled in ice. The homogenate was then centrifuged for 20 min. at 5000g at 2°. The supernatant layer was removed for assay of catheptic activity, and the foamy fibrous pellicle that formed on top was combined with the granular precipitate. This insoluble material was suspended in 10 vol. of 10% (w/v) NaCl by mixing briefly in the VirTis homogenizer. After standing for 2 hr., the suspension was again centrifuged as above. The pellet was taken up again in 10% (w/v) NaCl and extracted overnight in the cold. After a third centrifuging, the pellet was first washed twice with deionized water and then with several changes of acetone over a 24 hr. period. During the next 24 hr. it was washed with several changes of ether. The final preparation was spread out to dry in the air, then dried overnight in the vacuum oven at 100°. The dried product was weighed and stored in a desiccator over P₂O₅.

Determination of collagen and elastin. Portions (250 mg.) of the vacuum-dried connective tissue were placed in 40 ml. centrifuge tubes with 25 ml. of deionized water. These were autoclaved for 3 hr. at a pressure of 30 lb./in.² to extract collagen as gelatin. After a brief centrifuging, the supernatant was decanted and a second extraction was made under the same conditions. The pellet was then washed with water and the combined extracts were dried on a boiling-water bath. Both the autoclaved pellets and the dried extracts were hydrolysed in 6N-HCl in sealed tubes for 18 hr. at 110°. The tubes were opened and the contents washed out and neutralized as described by Woessner (1961). Suitably diluted hydrolysates were assayed for hydroxyproline (Woessner, 1961) and proline (Troll & Lindsley, 1955). The hydroxyproline content of the supernatant was multiplied by 7.46 (Neuman & Logan, 1950) to obtain the collagen equivalent. The hydroxyproline content of the pellet was multiplied by 62.5 (Partridge & Davis, 1955) to obtain the elastin equivalent. In this method of elastin assay care must be taken to remove all collagen. As a check on this, the ratio of proline to hydroxyproline was measured on the pellet. In all cases the ratio exceeded 8, the value expected for elastin, indicating the absence of appreciable amounts of collagen (in which the ratio is 1:12).

As a further check, the elastin content of ten uteri was determined by the gravimetric method of Lowry, Gilligan & Katersky (1941). The elastin values obtained by hydroxyproline assay were consistently lower by about 15% than those obtained gravimetrically, indicating that the gravimetric assay may be measuring a small amount of non-elastin material or that uterine elastin may have a lower hydroxyproline content than bovine elastin. All values are expressed on the basis of the total content in the body of the uterus.

Soluble fractions. Portions of involuting uterus were homogenized as described above. Then, instead of centrifuging immediately, the homogenates were shaken overnight at 2° to prepare a neutral-soluble fraction. The extracts were then centrifuged at 42000g for 30 min. The presence of non-diffusible, diffusible peptide-bound and free hydroxyproline was determined by the fractionation scheme described for rat uterus by Woessner (1962a). In further experiments, the pellet remaining after removing the 0.9% NaCl-soluble material was extracted with 0.5N-acetic acid (4 ml./g. original wt. of tissue) by shaking overnight at 2°. This was repeated four times, with centrifuging after each step. Finally, the extracts were dried, hydrolysed and converted into collagen equivalent by multiplying the hydroxyproline content by 7.46.

Protease effect on uterine collagen. Portions (25 mg.) of dried uterine connective tissue from involuting uteri were incubated with 5 mg. of protease (trypsin, chymotrypsin, elastase or papain) in 5 ml. of tris-HCl buffer, pH 7.6. Cysteine was added when papain was used. The tubes were shaken horizontally at 37° for 16 hr. They were then centrifuged at 42000g for 30 min. Hydroxyproline was determined on the hydrolysed supernatant and pellet to determine the percentage of collagen digested.

Catheptic activity. The supernatants of the original homogenates of involuting uteri were tested for their haemoglobin-digesting activity at pH 3.5 by the method of Anson (1938). A portion (1 ml.) of supernatant was incubated with 4 ml. of 1.5% (w/v) denatured bovine haemoglobin in 0.05M-sodium citrate buffer, pH 3.5, for 30 min. at 37°. The reaction was stopped by the addition of 5 ml. of 5% (w/v) trichloroacetic acid. Then 1 ml. of filtrate was mixed with 2 ml. of 0.5N-NaOH and 0.6 ml. of dilute Folin-Ciocalteu reagent, and, 10 min. after mixing, the extinction of the solution was measured at 660 mμ (1.0 cm. light-path). Blanks were prepared by adding trichloroacetic acid and enzyme preparations to incubated haemoglobin solutions. Autodigestion of the homogenates made a negligible contribution to the measured values for haemoglobin digestion. In the study of activity as a function of pH, tris-HCl and citrate buffers were employed, and the pH was measured before and after incubation.

Catheptic activity against collagen was measured by using the white fibrous pellicle that formed above the supernatant when homogenates of uterus were centrifuged. This material was composed almost entirely of collagen and comprised 60–90% of the total uterine collagen. It was resuspended in 0.9% NaCl and distributed into celluloid centrifuge tubes. A brief centrifuging precipitated the collagenous material and the saline supernatant was discarded. Portions of the uterine supernatant were adjusted to appropriate pH values and added to the pellets of collagen. Buffer was added to give a final concentration of 0.04M-citrate, pH 3–6, or 0.04M-tris-HCl, pH 7–9. Thymol

was also added and the tubes were stoppered and shaken horizontally for 16 hr. at 37°. The contents were then centrifuged at 42000g for 30 min. Determination of hydroxyproline on the hydrolysed supernatants and pellets gave a measure of collagen digestion. Pellets incubated with buffer in place of supernatant or at 2° instead of 37° served as blanks. These blanks provided a correction for non-enzymic solubilization and for the small hydroxyproline contribution from the homogenate supernatant.

RESULTS

Magnitude of involution changes. Table 1 presents a comparison of 65 uteri from non-pregnant women with nine uteri removed at the time of parturition (term). The difference between the two sets of values for wet weight, collagen and elastin is a measure of the increase that occurs during pregnancy and also of the decrease that takes place during the post-partum involution or resorption process. This measure is only an approximation (as shown below), since each successive pregnancy alters the base-line value for the non-pregnant or 'normal' state. Pregnancy results in an 11-fold increase in wet weight, a 6.8-fold increase in collagen and a 5.6-fold increase in elastin. Since there is a proportionally greater increase in wet weight than in connective tissue, collagen and elastin constitute a smaller proportion of the total uterine mass at term than they do in the non-gravid uterus.

An indication of the rate of involution is provided by the values for the four post-partum uteri (8–11 days). The wet weight has completed 70 % of the involution needed to restore it to the base-line value; the removal of collagen is 77 % completed and that of elastin 86 % completed. The four post-partum uteri had an average of eight previous pregnancies each, so in actual fact they should be compared with the base-line values given in Table 2 for uteri with more than six pregnancies. When this comparison is made, it is seen that involution is even closer to completion than indicated in Table 1.

Failure of involution to restore the uterus to its original state. The effect of repeated pregnancies on the connective tissue of the uterus is shown in Table 2. Owing to the effects of age on the uterus (cf. Woessner, 1962*b*) only uteri between the ages of 30 and 49 years are included in the Table. Further, premature delivery and abortions alter the measurements, so the Table includes only those uteri that had experienced full-term pregnancies. Table 2 shows that multiple pregnancies increase the size of the uterus and the amount of connective tissue. By the time six or more full-term pregnancies have occurred, the uterus has more than doubled in weight. Collagen has also doubled and elastin has increased by a factor of five. At first glance it would seem that involution was an incomplete

Table 1. *Uterine connective-tissue changes related to pregnancy and post-partum involution*

Experimental details are given in the text. These results relate only to the body of the uterus without the cervix.

State of uterus	No. of uteri	Wet weight of uterus (g.)	Collagen (g./uterus)	Elastin (g./uterus)
Non-gravid	65	77	4.9	0.97
Term	9	878	33.4	5.39
Post partum (8, 9, 9 and 11 days)	4	314	11.3	1.58
Post partum (2 months)*	1	62	2.0	0.55
Post partum (4 months)†	1	80	2.3	0.67
Ratio: term : non-gravid	—	11.4	6.8	5.6

* Third full pregnancy.

† Fifth full pregnancy.

Table 2. *Effect of parity on uterine connective tissue*

Experimental details are given in the text. Only uteri from women 30–49 years of age are included in the Table. The parity number indicates the number of full-term pregnancies in the history of each uterus. Uteri were not included if they had experienced one or more pregnancies which terminated in abortion or premature delivery.

Parity	No. of uteri	Wet weight of uterus (g.)	Collagen (g./uterus)	Elastin (g./uterus)
0	4	44 ± 25	3.30 ± 1.18	0.32 ± 0.09
1	6	53 ± 18	3.89 ± 1.57	0.66 ± 0.32
2	7	88 ± 37	5.39 ± 2.20	0.92 ± 0.32
3	10	77 ± 24	4.59 ± 1.72	0.99 ± 0.30
4	3	90 ± 20	4.67 ± 1.24	1.32 ± 0.30
5	4	112 ± 33	6.93 ± 3.90	1.42 ± 0.63
6 or more*	6	118 ± 44	6.56 ± 2.28	1.76 ± 0.43

* Average of 10 pregnancies/uterus, at least 6 of which were full term.

process, resulting in successive increases in weight and connective tissue with each pregnancy. However, when two uteri were examined at 2 and 4 months *post partum* (Table 1), the values were well below average when compared with the appropriate parity group (2-month, cf. three pregnancies, Table 2; 4-month, cf. five pregnancies, Table 2). A similar observation was made by Morrione & Seifter (1962) on a single uterus. These results, although admittedly scanty, suggest that there is probably a superinvolution of the uterus in which weight, collagen and elastin are all decreased to values below normal, with a subsequent rebuilding of the uterus to the 'resting' condition. If this is true, it must be the rebuilding process which overshoots the base-line and leads to increased amounts of collagen and elastin.

Collagen breakdown. Because collagen is normally relatively stable, particular attention was devoted to its rapid resorption during involution. Previous studies of the involuting rat uterus (Woessner, 1962a) gave evidence that collagen first became solubilized and then was broken down to the free amino acid level. To see if a similar mechanism might be operative in the human uterus, a study of soluble forms of hydroxyproline was conducted on three involuting uteri (Table 3). About 1% of the total hydroxyproline was present as soluble non-diffusible hydroxyproline (probably soluble collagens). Only slight amounts of diffusible peptide forms were present, but about 1% of the total hydroxyproline was present in the free form. These results resemble those found in the involuting rat uterus at a corresponding stage (3 days *post partum*), except that the concentration of free hydroxyproline is about one-half of that found in

the rat. However, it was not possible to determine if the course of breakdown parallels that found in the rat, since the observations were limited to a single period in the involutionary process (8–11 days *post partum*).

Not only was there no exceptional amount of neutral-soluble collagen, but acid-soluble collagen was also present only in small amount. When samples of involuting uteri were successively extracted with four portions of 0.5N-acetic acid, only 3–4% of the total collagen was brought into solution. Morrione & Seifter (1962) obtained similar results. Thus the collagen of the involuting uterus appears to possess the solubility properties of normal collagen. A test was also conducted to see if the uterine collagen was particularly susceptible to digestion by proteolytic enzymes that normally have little action on collagen. When collagen from four different term and involuting uteri was incubated overnight with considerable concentrations of various proteinases as described in the Experimental section, only small digestions were observed. The average percentages of the collagen digested were: by trypsin, 12.0%; by chymotrypsin, 11.6%; by elastase, 16.2%; by papain, 9.8%. Even these small digestions are probably to be attributed, at least in part, to a slight denaturation of the substrate during the vigorous homogenization procedure. It must be concluded thus far that the rapid breakdown of uterine collagen is not attributable to a high degree of solubility or to an unusual sensitivity of uterine collagen to proteolytic breakdown.

Cathepsin activity in the involuting uterus. A possible mechanism for the breakdown of muscle and connective-tissue proteins in involution might be an enzymic proteolysis. In the involuting rat uterus it was found (Woessner, 1962a) that both haemoglobin and uterine collagen were extensively attacked *in vitro* by a catheptic activity that was optimum at pH 3.5. This type of experiment was repeated with human uteri 8–11 days *post partum*. Fig. 1 shows the digestion of haemoglobin *in vitro* by uterine extracts as a function of pH. The digestion reached a peak at about pH 3.4 and then fell gradually to a minimum above pH 6. A slight activity was also found in the alkaline region, but this was not investigated further.

A similar picture was seen (Fig. 2) when the digestion of uterine collagen was tested. Again the peak activity occurred at pH 3.4; however, the enzyme activity dropped off rapidly on either side of the maximum, and negligible activity was found in the range pH 4–10. It can be shown in several ways that the effect produced by the extracts involves enzymic action on the collagen and is not due to a mere acid-solubilization. First, the blanks show that the digestion of collagen is highly tem-

Table 3. *Hydroxyproline-containing fractions of the post-partum uterus*

Extraction of the homogenized uterus with 0.9% NaCl yielded a soluble fraction. This was dialysed against water to obtain a diffusible and a non-diffusible fraction. Hydroxyproline was determined on hydrolysates of these two fractions. Free hydroxyproline was determined on the diffusible fraction before hydrolysis. The increase of hydroxyproline in this fraction after hydrolysis was attributed to peptide-bound hydroxyproline. The sum of the soluble forms plus the insoluble fraction gave the total hydroxyproline. The results are average values for three uteri removed at 9, 9 and 11 days *post partum*.

Fraction	Hydroxyproline ($\mu\text{g./g. wet wt. of uterus}$)
Total hydroxyproline	4590
Soluble non-diffusible hydroxyproline	51
Diffusible peptide-bound hydroxyproline	6
Free hydroxyproline	44

perature-dependent, with negligible (3%) action at 2°. Secondly, the solubilization is small in the buffer system at 37° when no enzyme is added. Thirdly, the sharp pH-dependence also argues against a physical process. Finally, when the digestion products are examined, about 25% of the hydroxyproline is found to be diffusible, indicating breakdown to small peptides.

To test the distribution of catheptic activity the uterus was cut into sections corresponding to the outer, middle and inner thirds of the myometrium. The endometrium was cut off and discarded. When enzyme preparations from these different regions were tested against collagen from the whole uterus, the proportions of collagen digestion were 44:66:70 for the outer:middle:inner zones. Thus the activity is distributed throughout the uterus.

Cathepsin activity in normal uterus. To determine whether the involuting uterus contains a unique cathepsin or a unique form of collagen, comparisons were made between it and uteri in the non-gravid state. Table 4 compares enzyme and collagen preparations from both types of uteri in all possible combinations. It is immediately apparent that both kinds of collagen are extensively attacked by both enzyme preparations, although in this particular experiment the enzyme from involuting uterus was slightly less active than that from the non-gravid uterus. The pH-activity curve (not shown) for the digestion of haemoglobin by normal uterus cathepsin corresponds exactly to that of Fig. 1.

Fig. 1. pH-dependence of haemoglobin digestion by extracts of involuting human uterus (9 days *post partum*). Enzyme preparation (1 ml., corresponding to 100 mg. wet wt. of tissue) was incubated with 4 ml. of haemoglobin at 37° for 30 min. An equal volume of 5% (w/v) trichloroacetic acid was added. After filtration, 1 ml. of filtrate, 2 ml. of 0.5N-NaOH and 0.6 ml. of Folin-Ciocalteu reagent were combined and the extinction at 660 mμ was determined at 10 min. in a cuvette with a 1 cm. light-path.

The cathepsin from non-gravid uterus has been partially purified and characterized (Woessner & Brewer, 1960). Thus far no difference between the cathepsins from uteri in the two different physiological states has been found. Preliminary studies indicate, however, that the collagen from involuting uterus is attacked more rapidly by bacterial collagenase than is collagen from non-gravid uterus. Since collagen from old uteri is attacked by collagenase more slowly than that from young uteri (Woessner, 1962*b*), it is likely that the greater rate of digestion of collagen from involuting uterus is related to its young age (less than 9 months old).

Haemoglobin digestion was used as a measure of catheptic activity for the purposes of comparing the different stages of pregnancy. The results are

Fig. 2. pH-dependence of collagen solubilization produced by extracts of involuting human uterus. Uteri removed 9–11 days *post partum* were homogenized and centrifuged. The supernatant containing catheptic activity was incubated with washed collagen fibres from the same uterus for 16 hr. at 37°. After centrifuging at 42000g, the supernatant and pellet were hydrolysed. The percentage of collagen solubilized was estimated from the hydroxyproline in the two fractions and corrected by appropriate blanks obtained in the absence of enzymic activity.

Table 4. Comparison of collagen and enzyme preparations from non-gravid and involuting uterus

	Collagen solubilized (%)	
	Collagen from non-gravid uterus	Collagen from involuting uterus
Enzyme preparation from non-gravid uterus	80	79
Enzyme preparation from involuting uterus	71	73

Table 5. *Catheptic activity of uteri in different physiological states*

Experimental details are given in the text. The cathepsin units are in terms of extinction values obtained by the haemoglobin assay described in Fig. 1.

State of uterus	No. of uteri	Cathepsin activity (units/100 mg. wet wt. of tissue)
Non-gravid	42	0.259
Pregnant, 3 months	1	0.127
Term	3	0.121
Post partum (8-11 days)	4	0.349
Post partum (2 months)	1	0.274

presented in Table 5. During pregnancy and at term the cathepsin activity/g. of tissue is depressed by about 50 %. During the involutionary period, the activity is about 50 % above normal, and by 2 months *post partum* it has returned to normal (only one sample). The interpretation of these results is difficult because of the accompanying changes in wet weight. Although the cathepsin concentration is decreased by 50 % at term, the uterus is 11 times larger than normal and in absolute amounts the total cathepsin in the uterus has increased almost sixfold. Similarly, during involution, when the cathepsin concentration is elevated, wet weight is decreasing at a rapid rate. The catheptic activity is not actually increasing in total amount/uterus during involution, and the concentration increase might be due merely to a more rapid loss of other components of the uterus. On the other hand, the involutionary phase is characterized by the rapid breakdown of proteins, and the cathepsin may also be one of the proteins undergoing rapid breakdown. In this case there would have to be an increased synthesis of cathepsin during the involution period to maintain the elevated levels of enzyme activity.

DISCUSSION

The observed changes in the human-uterus connective-tissue proteins in pregnancy and involution agree with previous reports. Schwalm & Cretius (1958) were the first to point out that the connective-tissue proteins did not increase to the same extent as non-collagen protein during pregnancy, but their results did not indicate the total amounts of the various components. Montfort & Pérez-Tamayo (1961) and Morrione & Seifter (1962) observed 8-fold increases in collagen compared with the 6.8-fold increase found by us. This is probably due to the fact that our uteri had all experienced multiple pregnancies and hence had started from a higher base-line, as indicated by Table 2.

No previous quantitative studies have been made of the changes in elastin. The present study shows that uterine elastin is just as dynamic as uterine collagen; it increases markedly during pregnancy and is rapidly resorbed during involution. For these reasons, the gravid uterus should offer an excellent model system for a study of the poorly understood mechanisms of elastin synthesis and degradation.

Involution is about 75 % complete by 8-11 days *post partum*. The time required for all involution processes to be completed has not yet been determined. Montfort & Pérez-Tamayo (1961) found that the uterus was larger than normal at 22 days, and Morrione & Seifter (1962) found a superinvolution at 4 weeks. According to our results, this hyperinvolution persists beyond 4 months, but eventually it must be followed by a return to values higher than those found before the pregnancy (Table 2). If hyperinvolution is occurring, as is suggested by the scanty results, then the human uterus is behaving in the same fashion as the rat uterus, in which a clearly defined hyperinvolution is observed (Harkness & Harkness, 1954; Woessner, 1962a). The phenomenon of hyperinvolution may be caused by lactation, as indicated by studies of the cat (Dawson, 1946) and human (Mavromati, 1950). In our experiments the uterus 4 months *post partum* was from a lactating woman, but information is lacking for the specimen 2 months *post partum*.

One of the most striking results of the present study is the finding of large increases in both collagen and elastin after repeated pregnancies. Though the involution processes apparently remove not only all the new material but also part of the collagen and elastin that existed before pregnancy, these are later replaced in excess, leading to values above the base-line. The elastin increases are attributable chiefly to a process of replication of the elastic lamina of the larger vessels. According to Maher (1959) this occurs by a pinching-in of the vessel walls, followed by a fusion of the walls to form a vessel with a smaller lumen. The pinched-off sections then tend to persist, leading to the accumulation of elastin. An examination of the fragments of elastic lamina can usually lead to a fairly accurate prediction of the number of pregnancy cycles undergone by the uterus. Though the main features of this explanation are sound, and we have been able to confirm histologically the presence of extra elastic lamina about many of the uterine vessels, there still remains the problem of how the elastin content can fall to below normal in hyperinvolution and then return to above normal at the end of the process.

The mechanism of collagen breakdown in the human uterus is not elucidated by the present

study. There are similarities to the collagen involution of the rat uterus, but the limited number of involuting uteri makes it difficult to establish any general patterns. It appears likely that the rapid breakdown of uterine collagen is not due to intrinsic properties of the collagen, but depends on outside agents producing the breakdown. Thus, in agreement with Morrione & Seifter (1962), we could find no unusual solubility properties of the collagen which would suggest weakness of the fibre structure or unusual lability. Nor was the uterine collagen unusually susceptible to proteolytic activity. There may, however, be a difference in uterine collagen compared with other types of collagen in that it has a slightly lower shrinkage temperature and lacks fluorescent properties (Brown, Consden & Glynn, 1958).

As reported by Woessner & Brewer (1960), both normal and involuting uteri contain a cathepsin activity that can digest haemoglobin and uterine collagen at an optimum pH of 3.4. Morrione & Seifter (1962) found a catheptic activity in involuting uteri that digests gelatin at pH 3.85. This is probably the same activity as that reported here.

Because of the failure of a number of researchers to find any breakdown of collagen in homogenates of involuting uteri incubated at neutral pH, we favour the hypothesis that collagen and other proteins may be degraded by the action of acid cathepsins. In the present study it was found that the cathepsin activity against haemoglobin is depressed in pregnancy and at term, and is elevated during involution. But these changes in concentration of cathepsin would seem to be too small to explain the marked changes in protein and collagen metabolism accompanying pregnancy and involution. If such a cathepsin is involved, it is necessary to postulate that it is localized in some cellular structure such as the lysosome which controls its effective activity in the different physiological states and enables it to attain full activity only during involution. Such a postulate is not unreasonable in view of such findings as those of Frankland & Wynn (1962), showing that liver lysosomal cathepsin can digest collagen at acid pH, and of Dingle (1962), showing that resorption of chick-limb-bud cartilage in tissue culture under conditions of hypervitamin A is related to increased liberation of lysosomal cathepsin.

The possibility of collagen degradation by a lysosomal cathepsin and the attainment of the requisite acid pH values have been discussed in some detail in connexion with the involution of the rat uterus (Woessner, 1962*a*). A study has been made by Lobel & Deane (1962) which shows that the involuting rat uterus contains increased numbers of lysosomes as revealed by histochemical localization of acid phosphatase; and Weiss (1962)

has presented evidence that the pH at cell surfaces may normally be at an acid pH of about 5.

If the cathepsin of the uterus is involved in the catabolism of collagen and other proteins during involution, and if it is localized in the lysosome, what mechanisms control its function? One technique of rupturing the lysosomes is to decrease the oxygen pressure of the environment. It is possible that shortly after labour, when the uterus contracts and the vessels are collapsed, the condition of partial anoxia that then obtains might be sufficient to cause lysosomal rupture, as well as favouring the production of acid. However, a generalized uncontrolled rupture might not be of much significance because of the buffering capacity of the surrounding cytoplasm. A specific discharge of the lysosome contents into intracellular digestive vacuoles and into confined regions between cell surface and fibre surface would be more in keeping with the needs of the hypothesis and with the observation of increased numbers of lysosomes during involution. A controlled discharge of lysosomes might act as a stimulus to the cells to produce additional lysosomes and lysosomal enzymes. In addition to partial anoxia, the vast changes in hormonal balance occurring *post partum* might also play an important regulatory function with respect to lysosomal activity.

SUMMARY

1. During pregnancy the human uterus increases at least 11-fold in wet weight, 7-fold in collagen and 5- to 6-fold in elastin content.

2. After parturition there is a rapid involution which is 75 % complete by 8-11 days *post partum*. Collagen and elastin undergo rapid breakdown during this process.

3. When involution is complete, the uterus has not returned to its original condition but is enlarged and contains more collagen and elastin.

4. Successive pregnancies produce further enlargement of the uterus. In non-gravid uteri that have undergone six or more pregnancies, collagen and wet weight are double, and elastin is five times, the values found for nulliparous uteri.

5. It was not possible to demonstrate any unusual solubility or digestibility properties of uterine collagen which might explain its rapid breakdown.

6. A cathepsin activity that can digest collagen *in vitro* at acid pH was shown to be distributed throughout the uterine wall in non-gravid, gravid and involuting uteri.

7. This catheptic activity is depressed in pregnancy and elevated during involution. However, the magnitude of these changes cannot in itself account for the vast changes of uterine protein metabolism in pregnancy and puerperium.

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The Frequency of Errors in Protein Biosynthesis

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It is widely recognized that variations in the amino acid sequence of particular proteins within and between species reflect corresponding variations in the genetic material of the cell in which the proteins are synthesized. Thus the 'error' in which valine replaces glutamic acid in haemoglobin synthesis (Ingram, 1958) may be regarded as a 'genetic error'. On the other hand, even where the genetic message is perfect, there may be errors in the transmission of the message or in the execution of the genetic instructions owing to the finite ability of macromolecular surfaces to distinguish between closely related molecules. Mistakes of this sort might be termed 'non-genetic errors in protein biosynthesis' and are the subject of the present paper.

It is generally believed that protein synthesis proceeds from free amino acids through amino acyl adenylates and amino acyl 'transfer' ribonucleic acid (s-RNA) as shown in the following equations:

where AA_i represents a particular natural amino acid, E_i is the 'activating enzyme' that specifically converts AA_i into the mixed amino acyl adenylate ($AA_i \sim AMP$), s-RNA_i is the 'transfer' ribonucleic acid specific to AA_i , and $AA_i-AA_j-AA_k$ represents the growing polypeptide chain containing various amino acids. Reaction (1) was first demonstrated by Hoagland (1955), and enzymes of the type E_i were shown to be quite specific for their particular amino acid by Sharon & Lipmann (1957). Reaction (2) was identified by Hoagland, Zamecnik & Stephenson (1957) and by Hoagland, Stephenson, Scott, Hecht & Zamecnik (1958), who with others (Berg & Ofengand, 1958; Schweet, Bovard, Allen & Glassman, 1958) showed that natural amino acids did not compete with each other. From this latter observation it was inferred that there were 'transfer' ribonucleic acids unique

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