Total, Latent and Active Collagenase during the Course of Post-Partum Involution of the Rat Uterus

EFFECT OF OESTRADIOL

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(Received 27 September 1978)

1. Total, active and latent collagenase activities were determined by direct assay of tissue homogenates. 2. The rate of collagen breakdown during post-partum involution of the rat uterus is correlated with the total activity of collagenase. Both are low at parturition, reach a maximum within 24 h and fall slowly to low values by 5 days *post partum*. This temporal correlation strongly supports the hypothesis that collagenase participates in collagen breakdown *in vivo*. 3. Further support for this hypothesis is provided by the finding that oestradiol-17 β (100 μ g/day, intraperitoneally injected), which inhibits the breakdown of collagen by 36% during the first 4 days of involution, produces a closely corresponding decrease in total collagenase activity. 3. The effect of oestradiol in lowering collagenase activity is not due to alterations in collagen substrate, collagenase kinetic behaviour or latent-to-active enzyme conversion. 4. Of the total assayable collagenase, about 35% is fully active and 65% is in a latent form. 5. About 70% of this latent form can be activated by a serine proteinase found, together with collagenase, in the insoluble fraction of uterine homogenates.

The physiological role of collagenase in the breakdown of collagen has been established largely by indirect evidence (Gross, 1976). Tissues in which collagen resorption is occurring are excised from the animal and placed in tissue culture. After several days the culture medium can be demonstrated to contain collagenase activity. A general correlation is seen, such that tissues undergoing rapid resorption will often produce more enzyme than tissues that are quiescent. Thus rheumatoid synovial membrane produces more collagenase than does non-pathological tissue (Evanson *et al.*, 1967); bone stimulated *in vivo* by parathyroid hormone produces more collagenase in culture than does control bone (Walker *et al.*, 1964). However, it is often the case that tissues that do not appear to be the site of rapid collagen degradation may produce large amounts of collagenase in culture, for example, bovine gingiva (Birkedal-Hansen *et al.*, 1976) or human skin (Eisen *et al.*, 1968). It seems likely that cells that *in vivo* were producing little or no enzyme may suddenly be activated by the conditions in culture and begin to produce large amounts of enzyme. This leaves open the question of how much enzyme was actually present in the tissue *in vivo*.

The development of methods for assaying collagenase in tissue homogenates (Ryan & Woessner, 1971) and for extracting the enzyme from tissues (Weeks *et al.*, 1976) has opened the way for an

examination of how collagenase activity changes during processes of collagen resorption. I have selected the involuting rat uterus for such studies because of the extreme rapidity of collagen breakdown during uterine resorption and because of the non-pathological nature of the process. At the time of parturition, collagen breakdown is negligible. The breakdown mechanism is turned on within the first 24 h, attains a maximum rate by 1–2 days, and is almost complete by 4 days *post partum*. During this brief period 90% of the collagen in the uterus is degraded and lost from the organ (Woessner, 1962). A further feature of interest is the possibility of retarding the loss of collagen by treatment of the animal with oestradiol (Woessner, 1969).

If collagenase is implicated in collagen breakdown, we would expect to see a rise in enzyme activity shortly after parturition, and then a decline by 4 days *post partum*. Oestradiol should depress this rise in activity in proportion to the retardation of collagen breakdown. An earlier study (Ryan & Woessner, 1974) did, in fact, support this hypothesis, but there were several shortcomings in that study. The enzyme was not extracted from the tissues, so that it was not possible to offer a complete proof that it was a true collagenase. Also, it was not recognized that part of the enzyme was in a latent form, so the total enzymic content of the tissue was not determined. This left open the question of whether oestradiol did indeed

decrease the amount of collagenase, or whether it acted indirectly by blocking activation processes or raising inhibitor concentrations.

These issues have been more fully explored in the present study. It is concluded that total collagenase activity correlates closely with the rate of collagen breakdown, and that oestradiol treatment decreases the activity of active and latent collagenase in the tissue.

Experimental

Pregnant rats were obtained on the 17th day of pregnancy from Sprague-Dawley, Madison, WI, U.S.A. They were maintained in an interior room with controlled light and fed on Purina rat chow and water *ad libitum*. The time of parturition was noted, and the young were allowed to nurse. Oestradiol-17 β (Mann Research Laboratories, New York, NY, U.S.A.) was administered intraperitoneally as a suspension of the hormone in 0.9% NaCl (100 μ g in 0.1 ml) shortly after delivery was complete and on each successive day. It was shown previously that injection of the vehicle alone produced no significant effects on uterine collagen (Woessner, 1969). Rats were killed at appropriate intervals and their uteri were dissected free of mesentery and of nodules at the implantation sites. The uteri were immediately frozen in sealed containers until used (within 1-8 days).

Collagenase activity was measured by the method of Ryan & Woessner (1971). Uteri were thawed at room temperature, weighed, minced and homogenized in 10 vol. of ice-cold 0.01 M-CaCl₂ containing 0.25% Triton X-100. The homogenates were centrifuged at 2°C for 20 min to obtain a 6000g pellet. This pellet was resuspended to the original volume in 0.04 M-Tris/HCl/0.15 M-NaCl, pH 7.4, containing penicillin (200 units/ml) and streptomycin (250 μ g/ml); 2 ml of this suspension was distributed to each of five assay tubes. Tubes 1 and 2 were treated at 37°C for 3 min with 100 μ g of trypsin (bovine, twice crystallized; Worthington Biochemical Corp., Freehold, NJ, U.S.A.) to activate collagenase, followed by the addition of 360 μ g of soya-bean trypsin inhibitor (Worthington). Tube 3 received no additions. Soya-bean trypsin inhibitor was added to tube 4 to block activation of latent collagenase by an endogenous proteinase. CaCl₂ was added to tubes 1, 3 and 4 to a final concentration of 0.01 M. EDTA was added (0.01 M) to tubes 2 and 5 to provide blanks. A separate blank (tube 2) was required for tube 1 because trypsin released small amounts of hydroxyproline during the 3 min incubation. The tubes were all incubated for 18 h at 37°C with constant shaking. Soluble hydroxyproline-containing peptides were released by collagenase from the uterine collagen present in the pellets. These products were separated from un-

digested insoluble collagen by centrifugation of the digests at 4°C for 30 min at 30000g. The resulting supernatants and pellets were separately hydrolysed in 6 M-HCl and assayed for hydroxyproline (Woessner, 1961). The hydroxyproline released in tube 1, after subtraction of the blank value in tube 2, measures the total collagenase, both active and latent. Tubes 3 minus 5 measure collagenase that is already active plus that fraction that is activated by endogenous proteinase during the incubation. Tubes 4 minus 5 measure only that collagenase already active before incubation. To determine total hydroxyproline content of the uterus, the hydroxyproline of the supernatants and pellets were first added together. The resultant totals for each of the five assay tubes were then averaged to provide total hydroxyproline/200 mg wet wt. This value was then converted into hydroxyproline/uterus on the basis of the wet weight of the uterus.

Results

Fig. 1 illustrates the loss of uterine wet weight and uterine collagen during post-partum involution in control and oestradiol-treated rats. Similar information has been published previously, but, owing to the biological variability noted in Sprague-Dawley rats from year to year, it is necessary to establish these parameters for the particular uteri in which collagenase is measured. The results for control animals agree fairly closely with previous data (Woessner, 1962, 1969). Wet weight and collagen are lost at a rapid rate; by 4 days *post partum* there has been an 84-88% decrease in both parameters.

The effects of oestrogen treatment are quite clear and striking. The wet weight and collagen content of the uteri from oestradiol-treated rats are significantly ($P < 0.001$) greater than those of the corresponding controls at every time interval examined. By the 4th day *post partum*, the uterus has lost 84% of its weight, whereas the oestrogen-treated uterus has lost only 71%. In terms of the total weight loss expected during this period, oestradiol has caused a retardation or inhibition of about 16%. A greater effect is noted in the case of collagen: in controls, 88% of the collagen is lost by day 4, and in treated animals, only 56% has been lost. This corresponds to an inhibition of 36% of the total collagen loss expected during this time period.

These collagen changes in the oestradiol group differ slightly from those in previous groups. The bifurcation of the curves occurs before 1 day *post partum*, where previously it branched at about 1 day (Ryan & Woessner, 1974). The content of collagen at 4 days *post partum* is similar to that in the group measured in 1974, but is lower than that in two earlier groups, which had about 5.6 mg/uterus (Woessner, 1969, 1976).

Fig. 1. Changes in (a) the wet weight of the rat uterus as a function of the time after parturition and (b) total collagen of the uterus as estimated by the hydroxyproline content ●, Control animals; ○, animals receiving 100 µg of oestradiol per day. The vertical bars represent the s.e.m. Where no bars are shown, the s.e.m. lies inside the symbol. There are 11–14 animals for each point on days 0, 1 and 2, and 5–7 animals for days 3, 4 and 5. The difference between points for oestradiol-treated and control groups at each time period is significant at the level $P < 0.001$, measured by Student's t test.

Fig. 1 provides the basic information on involution and collagen losses with which the data on collagenase activity may be compared. However, before such comparisons are made, several aspects of the collagenase assay method must be considered. The first point is to establish that the assay remains linear during the time of incubation. This had previously been established (Woessner, 1977) for control uteri at 1 day post partum. In the present study, it is shown (Fig. 2) that the assay is linear over 24 h for both control and oestradiol-treated uteri. Oestradiol does not affect enzyme activity by causing changes in the stability of the enzyme or otherwise altering the linear kinetic behaviour. Another important problem in the pellet assay is that the enzyme and substrate ratios cannot be varied. If the enzyme activity is changing owing to physiological or hormonal influences, then

one would like the substrate concentration to remain constant. Fig. 3 shows the actual substrate concentrations as mg of hydroxyproline/g wet wt. Fortunately, the oestrogen-treated samples show no significant change from day 1 to 5. However, there is a decrease during this time span in the case of the controls. (Note: the ordinate does not include 0.)

Fig. 2. Linearity of the pellet assay method with time Uteri were removed from control and oestradiol-treated rats at 2 days post partum. A series of uniform pellets was prepared, activated with trypsin and incubated at 37°C. Samples were removed for hydroxyproline assay at the time intervals shown. ●, Control; ○, oestradiol-treated.

Fig. 3. Concentration of collagen substrate available in the pellets used for collagenase assay The data are expressed as mg of hydroxyproline/g wet wt. of uterine tissue. The symbols and bars indicate means $\pm$ s.e.m.; ●, controls, ○, oestradiol-treated. Numbers of animals were as in Fig. 1.

In general, enzymes display hyperbolic dependence on substrate concentrations, $[S]$. At one extreme, an asymptotic rate is approached and the assay is independent of $[S]$. At the other extreme the rate is directly proportional to $[S]$. Both of these possibilities have been taken into account in analysis of the data from the collagenase assays.

Fig. 4 illustrates how this may be done for the control samples in the upper two curves. The left ordinate shows the release of hydroxyproline per g wet wt. of tissue by the total collagenase activity in that amount of tissue. The right ordinate shows the percentage digestion, that is $[(\text{hydroxyproline released/g wet wt.}) \times 100]/(\text{total hydroxyproline/g wet wt.})$. The two scales have been adjusted so that the two curves coincide exactly at 1 day *post partum*. Beyond this point the two curves separate, and proportionality is not maintained. The difference is due to the declining concentration of substrate (hydroxyproline/g wet wt.). The lower curve (Δ , hydroxyproline released/g wet wt.) corresponds to the limiting case in which the rate is independent of $[S]$. The upper curve ($\bullet$, per-

Fig. 4. Changes in the concentration of total collagenase activity during uterine involution

The left-hand scale and the symbol Δ (controls) show the mg of hydroxyproline released during the pellet assay from 1 g of wet uterine tissue. The right-hand scale and the symbol $\bullet$ show the percentage of hydroxyproline in the pellet that was released by collagenase digestion. In the case of oestradiol-treated animals, the two curves coincided within the width of the symbols, and are therefore represented by a single curve ($\circ$). The points and bars represent means $\pm$ S.E.M.; numbers of animals were as in Fig. 1. The control and oestradiol-treated groups were significantly different ($P < 0.001$) at every time point except day 5.

centage digestion) corresponds to the limiting case in which the rate is proportional to $[S]$, since each enzyme value is divided by its corresponding substrate concentration. As the substrate concentrations decline, the rates are increased in proportion. The two curves form an envelope that encloses the true curve of collagenase activity somewhere within its limits.

The lowest curve of Fig. 4 illustrates the total collagenase activity of the uteri from oestradiol-treated animals. Since there was no significant change in $[S]$ with time, the two curves merge into one, which may be read from either scale.

It can be seen from the foregoing that Fig. 4 represents the concentration of total collagenase in the tissue, i.e. the enzyme units per g wet wt. This concentration is very low at the time of parturition, in fact it was undetectable in four out of 13 uteri. This is similar to the activity in non-gravid uteri (1.06%, mean for six animals). The concentration of total collagenase activity rises rapidly to a peak at 1 day *post partum* in both groups, declines slightly by day 2, and falls to low values by day 5. The enzyme activities in the oestrogen-treated group are significantly lower than in the controls at each time point ($P < 0.001$) regardless of which curve is used. The exception is at day 5, at which point the activity in both groups falls to the same low level.

Fig. 5(a) combines the enzyme concentration data of Fig. 4 with data on the wet weight of the uterus to provide a measure of the content of total collagenase activity in the entire organ. As in Fig. 4 the data for the controls are shown as two curves allowing for the two possibilities as to the dependence of rate on $[S]$. In this case, the limits are quite close together. The whole-organ content of total collagenase in the controls is significantly higher than in the oestrogen-treated group only at day 1 ($P < 0.001$) and day 2 ($P < 0.01$).

There is a general correlation between total collagenase activity (Fig. 5a) and the rate of loss of collagen (slope of curves in Fig. 1b). This correlation is best seen if one takes as the slope the difference between 2 adjacent days and relates this 24 h breakdown to the collagen breakdown obtained in 18 h by incubating the uterine pellet removed on the first of these 2 days, e.g. total collagenase activity on day 2 related to the net loss of collagen between day 2 and 3. These differences have been plotted in Fig. 5(b) to facilitate comparison. The ratio of actual breakdown *in vivo* to the assayed breakdown *in vitro* ranges from 2 to 4, i.e. there seems to be only enough enzyme to degrade about half of the collagen that would be expected to be degraded in this time period. This is discussed below.

From Fig. 1(b) it can be estimated that oestradiol treatment retards collagen breakdown by about 36% by day 4. As a first approximation, the two control

curves in Fig. 5(a) have been compared with the corresponding curves for the oestradiol-treated group by simple planimetry of the area under the curves between days 0 and 4. The differences between the two control curves and the 'oestradiol' curve in Fig. 5(a) would correspond to 35–40% inhibition of breakdown. This is in good agreement with the 36% inhibi-

Fig. 5. Changes in the whole-organ content of total collagenase activity during uterine involution (a) and rate of collagen breakdown in vivo (b)

(a) Collagenase activity, determined after trypsin activation, is given as mg of hydroxyproline released from the entire uterus during the assay. Closed symbols, controls; open symbols, oestradiol-treated. Δ , $\blacktriangle$, Digestion as measured in the pellet assay; $\circ$, $\bullet$, the same data corrected for the continuously declining concentration of collagen substrate as shown by Fig. 3. The concentration at day 1 of 4.53 mg of hydroxyproline/g wet wt. of tissue was taken as the reference point and the subsequent values of digestion at each day were corrected by multiplying by the factor $(4.53/[\text{hydroxyproline}])$ at that day. This correction was applied to the data for each animal before determining the mean $\pm$ s.e.m. In the case of the oestradiol-treated group the corrected curve fell almost exactly on the uncorrected curve, so only a single curve is shown. Numbers of animals as in Fig. 1. (b) Values are derived from Fig. 1(b) by calculating the loss of hydroxyproline for each 24 h period commencing at day 1. $\bullet$, Controls; $\circ$, oestradiol-treated.

tion of collagen breakdown produced *in vivo* by oestradiol.

Fig. 5(a) was selected for the preceding comparisons because I wanted to compare the whole-organ content of total enzyme with the changes in total collagen. Fig. 4 exaggerated the differences in collagenase activity because oestradiol retards the loss of wet weight; as a result, the concentration of enzyme is decreased to a greater extent than is the content of enzyme per organ.

Fig. 6 illustrates the distribution of total collagenase activity among the various forms of active and latent enzyme. The total of all assayable forms of collagenase

Fig. 6. Distribution of total collagenase activity among latent and active forms during the course of uterine involution

The total collagenase activity at each time is taken as 100%; this is the activity revealed when the pellets are fully activated by trypsin. $\bullet$ and $\circ$, Activity measured when trypsin is not added. $\blacktriangle$ and Δ , Activity measured when soya-bean trypsin inhibitor (STI) is added before assay. Closed symbols, controls; open symbols, oestradiol-treated. Points represent means, bars are s.e.m.; numbers of animals were as in Fig. 1 except that the day-1 oestradiol-treated group had only seven animals. Reliable data were not obtained for the oestradiol-treated samples treated with soya-bean trypsin inhibitor at 1 and 4 days. Differences between control and oestradiol-treated were significant for the 'no addition' values at 1 and 2 days ($P < 0.001$ and 0.01) and for the 'STI' values at 2 and 3 days ($P < 0.01$ and 0.05).

is set equal to 100% for each time point. This value includes active enzyme and all latent forms that can be activated with trypsin. The lower curves, with soya-bean trypsin inhibitor present, represent collagenase that is already fully active at the beginning of the assay incubation period. This is typically 20–40% of the total. The upper curves, with no addition, represent the active enzyme plus collagenase that is initially in a latent form, but becomes activated during the assay (within about 4h) by the action of a serine proteinase present in the uterine pellets (Woessner, 1977). If soya-bean trypsin inhibitor is present, this serine proteinase is blocked, and only the active collagenase is detected. The difference between the activity with no additions and the full value of 100% represents latent enzyme that is not activated by the uterine proteinase but is activated by trypsin. This fraction, in the control group, constitutes 10–20% of the total. It is not known whether the tissue proteinase is incapable of activating this fraction, or whether it is too dilute or denatures too rapidly to complete the job.

The effects of oestradiol, according to Fig. 6, are a decrease in active enzyme and a diminished conversion of latent into active enzyme by the tissue proteinase. A simple explanation of this would be that oestradiol treatment has interfered with the conversion of latent into active collagenase *in vivo* and *in vitro*. But it must be borne in mind that total collagenase and latent collagenase activities are severely depressed by oestradiol (Figs. 4 and 5). If we think of latent collagenase as being decreased in concentration, then normal concentrations of the tissue's activating proteinase would be expected to act more slowly on this diminished concentration of substrate.

Although the results to this point indicate strongly that oestradiol decreased the total amount of collagenase in the uterus, there are alternative explanations to consider. The assay was performed on the pellet of oestrogen-treated tissue. Perhaps this treatment altered the collagenous substrate, increased the concentration of some inhibitory material, or otherwise produced some change that interfered with the assay. To test these possibilities, uteri were removed at 2 days *post partum*, and collagenase was extracted by the method of Weeks *et al.* (1976), including the method for estimating the recovery of enzyme. The activities of extracted collagenase from control and oestradiol-treated uteri were directly proportional to the corresponding activities measured by the pellet assay. Moreover, recovery in both cases was 60–65%. Therefore, when collagenase was extracted from the uterus and assayed on a different collagen substrate, the effects of oestradiol remained the same. Table 1 explores the possibility that oestrogen treatment caused a shift in the collagenase from an insoluble form to a soluble form. Recoveries of soluble forms of collagenase in the supernatant were the same in both control and oestrogen-treated groups.

Table 1. *Distribution of total collagenase activity between pellet and supernatant in homogenates of uteri removed at 1 day post partum*

Uteri were collected at 1 day *post partum* and homogenized as usual. Part of each homogenate was centrifuged to prepare a pellet for the normal assay; the remaining part was assayed directly without removal of the supernatant. In both cases it could be shown that treatment with 100 µg of trypsin was adequate to give maximum activation of latent forms. Each value is the mean $\pm$ S.D. for four samples.

Tissue	Hydroxyproline released (%)		Percentage of total in supernatant
	Pellet assay	Whole homogenate	
Control	15.6 $\pm$ 3.1	18.5 $\pm$ 4.8	19
Oestradiol-treated	10.0 $\pm$ 2.3	11.9 $\pm$ 3.0	20

Discussion

Relationship of total collagenase activity to collagen resorption

The involuting uterus of the rat provides an excellent example of a physiological process of resorption of collagen that can be correlated directly with the tissue activity of collagenase. At the time of parturition, the total collagenase activity is close to zero. It then rises rapidly within the first 24h, and falls gradually to a low level again by 5 days *post partum*. This activity parallels quite closely the rate of collagen breakdown. Further support for this correlation is provided by oestradiol treatment, which retards collagen breakdown and decreases total collagenase activity in similar proportion. These results constitute the first complete study of the tissue content of collagenase during the entire course of a process of collagen breakdown; they give strong support to the hypothesis that collagenase is directly involved in the resorption of collagen *in vivo*.

It may be asked whether such a study is necessary, since much the same information can be obtained by culturing tissue and measuring the collagenase released *in vitro*. In considering this question, the present results on the direct assay of tissue collagenase may be compared with the study of Jeffrey *et al.* (1971a) on collagenase production by cultured fragments of rat uterus. In that study, maximal release of enzyme into culture media occurred with uteri removed at 1 and 2 days *post partum*; uteri from days 3 and 4 produced no enzyme. The present study indicates that, although the uterus may have lost its potential for collagenase production in culture by 3–4 days *post partum*, there is still considerable activity

in the uterus *in vivo* and collagen is still being degraded in appreciable amounts. A further example is offered by the results obtained by Cartwright *et al.* (1977); uterine fragments from non-gravid rabbits, when cultured *in vitro*, produced amounts of collagenase in excess of the amounts produced by cultures of post-partum uterus. The enzyme is in a latent form, which led Cartwright *et al.* (1977) to postulate that previous failure to detect collagenase from non-gravid uteri was due to failure to unmask latent forms. However, in the present study, non-gravid uteri were examined by using trypsin activation, and the small digestion observed (1%) must be regarded as barely detectable. It seems likely that the tissue is changing under the conditions of culture to produce enzyme, whereas little enzyme was being produced *in vivo*. Other examples of collagenase production by cultures of tissues that are presumably quiescent *in vivo* were mentioned in the introduction and many more could be adduced.

The present results also contradict earlier findings on the immunofluorescent detection of collagenase in the uterus. Montfort & Pérez-Tamayo (1975) used a monospecific rabbit antibody against rat uterus collagenase to localize collagenase in the rat uterus. Considerable staining was found in the non-gravid uterus, localized to collagenous structures, and this staining diminished sharply after parturition. Similar preliminary findings have been reported by Dingle (1976) for the rabbit uterus. These results are diametrically opposed to the direct-assay results. The monospecificity of collagenase antisera is currently undergoing critical re-evaluation in several laboratories; it is possible that impurities may account for these discrepant results.

It is surprising that the measured activities of collagenase are so low; that is, there is only about half as much digestion in the pellet assay as would occur *in vivo* in the same amount of tissue. It may be argued that the assay system involves a 10-fold dilution of both enzyme and substrate. However, this argument is not valid because the enzyme and substrate are quite probably bound to one another. Lowering the dilution to 1:5 increases the rate only slightly, not 2–4-fold as predicted by kinetic theory. The homogenization may disrupt structural organization that exists in the tissue, lowering the rate of digestion *in vitro*. Another possibility is that collagenase does not account for the complete digestion of the uterine collagen. Indeed, there is good evidence that uterine collagen is extensively phagocytosed in the form of recognizable pieces of fibrils (Parakkal, 1969; Schwarz & Güldner, 1967; Tansey & Padykula, 1978). If this be the case, a great deal of the digestion of collagen may occur through the action of acid cathepsins within lysosomes. Collagenase would then be required in amounts just sufficient to make an initial attack on fibres or to digest that collagen not phagocytosed.

Latent and active collagenase

A further surprise is provided by the distribution of collagenase between active and latent forms (Fig. 6). Only 30–40% of the total collagenase is active at the moment homogenate pellets are incubated. It might have been expected that some enzyme would be latent, perhaps stored in the cells or not yet released after synthesis, but the large proportion of latent enzyme is puzzling. The nature of this latency is not fully understood. It was at first thought that uterine collagenase is produced as a proenzyme, which is activated by a serine proteinase of the uterus. It has not been possible to activate the enzyme by the many methods used for other latent collagenases: NaSCN treatment (Abe & Nagai, 1972), NaI (Shinkai *et al.*, 1977) and 4-chloromercuribenzoate (Werb & Burleigh, 1974). Recently, however, we (A. Sellers & J. F. Woessner, unpublished work) have been able to achieve 40–50% activation of extracted latent collagenase with 4-aminophenylmercuric acetate (Sellers *et al.*, 1977). This indicates that at least part of the latency is due to the formation of an inhibitor–enzyme complex. This complex either pre-exists in the tissue or forms during the homogenization and incubation for the pellet assay. The latter explanation would mean that there is actually more of the active form *in vivo* than suggested by the assay results. The serine proteinase could act either to activate proenzyme or to reverse inhibitor–enzyme complexes. In either event, its function would be to maintain a high activity of active collagenase at the fibre surface.

Oestradiol effects

The previously observed effects of oestradiol in decreasing the breakdown of collagen can now be understood more clearly in the light of the present results, namely total assayable collagenase is clearly decreased by the hormone treatment. This decrease is not to be attributed to changes in the substrate, the kinetics of assay, inhibitor increases or the lack of activation of latent forms.

It is not clear how oestradiol effects a decrease in collagenase activity. When oestradiol is added to cultures of involuting rat uterus, it has no effect at all on collagenase production (Jeffrey *et al.*, 1971b). This led us to suggest that the hormonal effect is not mediated by the uterus, but by some other receptor in the body (Ryan & Woessner, 1974). This idea has become increasingly attractive since the work of Tansey & Padykula (1978). They show that a large variety of cells, including monocyte macrophages, heterophils, eosinophils etc., infiltrate the uterus shortly after parturition in an inflammatory type of response. Oestradiol, at the same dosage as used here, suppressed the influx of macrophages, eosinophils and heterophils. If one or more of these cell types

served as the source of uterine collagenase, then oestradiol, in acting to block their entry to the uterus, would effectively decrease collagenase activity *in vivo*, but would show no effect on cultured uterus. The same observations would explain why the uterus at parturition contains almost no collagenase and has no potential for collagenase production in culture.

On the other hand, this explanation is not completely satisfactory. Cartwright *et al.* (1977) found complete inhibition of collagenase release from cultured uterine explants by oestradiol at concentrations of 50 μM . If rats are treated with oestradiol and subsequently their bones are removed to culture, the explants produce less collagenase (Cruess & Hong, 1976). These cases are unlikely to involve the blocking of cell ingress. Wahl (1977) has found that treating guinea pigs with oestradiol inhibits the ability of peritoneal macrophages removed from these animals to be stimulated in culture by endotoxin to produce collagenase. Direct addition of the hormone to the culture decreased collagenase production by macrophages by 53%. The observations by Schwarz & Gldner (1967) suggest that, in the rat uterus, there is an activation of tissue histiocytes to macrophages as well as an entering of macrophages from the circulation. If the conversion of resting cells into active cells were inhibited by oestradiol, or if their enzyme production were partially blocked, this could account for the effects noted in this paper. The question, then, is still left open as to how oestradiol decreases tissue collagenase activities. But the list of systems in which oestradiol diminishes collagen breakdown and collagenase production continues to grow.

This research was supported by Grant HD-06773 from the National Institutes of Health. I thank Ms. Carolyn Taplin for her excellent technical assistance.

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