UTERINE COLLAGENASE AND COLLAGEN IN YOUNG AND AGEING RABBITS

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(Received 12th January 1972, accepted 17th March 1972)

Summary. Collagenase activity was measured 66 to 75 hr after parturition in does at 34, 167 and 204 weeks of age. Uteri of ageing does contained less collagenase than those of young does. Uterine collagen content tended to increase with age. At Day 12 of pregnancy, uterine acid-soluble collagen was higher in does 174 weeks old than in does at 38 weeks of age.

Tissue collagen tends to increase with age. Both an increase and a change in the configuration of uterine collagen have been reported in the ageing rat (Burack, Wolfe, Lansing & Wright, 1941; Kao & McGavack, 1959; Finn, Fitch & Harkness, 1963; Schaub, 1964/65). A decrease in the activity of collagenase and other proteases capable of degrading collagen was also reported (McGavack & Kao, 1963; Schaub, 1964, 1964/65, 1965).

This investigation was conducted to examine changes in uterine collagen and collagenase activity in ageing female rabbits with declining reproductive ability.

Fifteen parous does were divided into three groups of five, averaging 34, 167 and 204 weeks of age and averaging one, nine and twenty-four previous litters, respectively. The does were killed and exsanguinated between 66 and 75 hr after parturition. In preliminary trials, this was found to be the time of highest collagenase activity. Woessner (1962) found the peak of collagen degradation in the involuting rat uterus to be between 48 and 72 hr after parturition. The uteri were removed and excess fluids were squeezed out before weighing. To measure collagenase content, each horn was cut longitudinally along the mesenteric and antimesenteric region and a 5-g (wet weight) sample was removed, minced and homogenized for 10 min with 30 ml 0.01 M-calcium acetate (according to Kit 08001-8300, Mann Research Laboratories). The homogenate was centrifuged at 15,000 *g* for 15 min and the supernatant was decanted and filtered into a measuring cylinder. Collagenase activity was determined according to the method of Wunsch & Heidrich (1963) using a synthetic chromophore, acyl-pentapeptide, 4-phenyl-azo-benzoyloxycarbonyl-L-prolyl-L-leucyl-glycyl-L-prolyl-D-arginine dihydrate (Mann Research Laboratories). The assay was conducted at 37° C and a pH of 7.1. These conditions were chosen

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to represent the optimum based upon preliminary enzyme activity studies conducted over a temperature range of 27 to 75° C and with pH varying from 3.5 to 10.7. The importance of Ca^{++} was shown by the fact that the addition of 1 M-ethylenediamine tetraacetic acid (EDTA), which binds Ca^{++} ions, reduced the activity of the uterine extract tenfold. Mandl, MacLennan & Howes (1953) have reported that Ca^{++} ions are necessary for collagenase activity. Cysteine, at 10^{-3} M, stimulated activity slightly whereas a 1-M concentration had no effect. These properties are similar to those reported for collagenase in rat uteri by Jeffrey & Gross (1970) and Ryan & Woessner (1971) and in *Cl. histolyticum* (Mandl *et al.*, 1953). In addition, our uterine homogenates were found to have collagenase activity when collagen from bovine Achilles tendon was used as the

TABLE 1
COLLAGENASE ACTIVITY OF DIFFERENT AGED DOES AFTER
PARTURITION

Age (weeks)	No. of previous litters	Uterine collagenase		
		Specific activity ($\mu\text{mol}/\text{min}/\text{mg}$ protein, $\times 10^{-3}$)	Wet weight ($\mu\text{mol}/\text{g}$ uterine tissue)	Dry weight ($\mu\text{mol}/\text{g}$ uterine tissue)
34	1 $\pm$ 0	2.8 $\pm$ 0.2	1.6 $\pm$ 0.2	9.8 $\pm$ 1.1
167	9 $\pm$ 2	2.6 $\pm$ 0.4	1.7 $\pm$ 0.3	10.4 $\pm$ 0.9
204	24 $\pm$ 4	2.2 $\pm$ 0.2*	1.4 $\pm$ 0.1	8.0 $\pm$ 0.2

The animals in the three age groups (average of five does/group) were killed 72, 66 and 75 hr after parturition.

Values expressed as means $\pm$ S.E.

* Differs significantly from other means in the same column ($P < 0.05$).

substrate (Mandl *et al.*, 1953). The protein content of the uterine extracts was determined according to the technique of Lowry, Rosebrough, Farr & Randall (1951).

The collagen content of the uteri of the same fifteen does which were used for collagenase studies and of the uteri from six additional 12-day pregnant does was determined. Each uterine sample was weighed (wet weight), freeze-dried, desiccated, reweighed (dry weight) and separated into alkaline- and acid-soluble collagen. The alkaline-soluble fraction was extracted according to the method of Kao & McGavack (1959), and the acid-soluble fraction according to the method of Fitch, Harkness & Harkness (1955). Each extract was analysed for hydroxyproline content by the method of Prockop & Udenfriend (1960). The hydroxyproline content was multiplied by 7.46 to give the collagen content (Neuman & Logan, 1950).

The uterine collagenase activity in does of different age groups is shown in Table 1. The oldest does had the lowest uterine collagenase, with a significant difference ($P < 0.05$) in specific activity. This lower uterine collagenase could result in less of the collagen being broken down. The enzymes responsible for uterine involution are activated by progesterone withdrawal (Goodall, 1965, 1966). Maurer & Foote (1972) found less progesterone in the ovarian venous blood of old pregnant does. This, in turn, might affect the activation of proteolytic enzymes. Oestrogens also can inhibit uterine involution but the effect

appears to be one of inhibiting collagen breakdown rather than a direct one on the enzyme concentration (Woessner, 1969).

The alkaline- and acid-soluble collagen contents of the uteri in *post-partum* and 12-day pregnant does of differing ages are presented in Table 2. In the *post-partum* animals, the old does had significantly more alkaline-soluble collagen ($P < 0.05$). Mean acid-soluble and total collagen values were highest for the oldest does also, but animal variability was very great in this age group.

The 12-day pregnant ageing does (Table 2) had a higher ($P < 0.05$) acid-soluble collagen content than the young females. Other comparisons were not statistically significant ($P > 0.10$). The tendency for acid-soluble and total collagen content to increase in the ageing female rabbit is in agreement with earlier findings in the mouse and rat (Kao & McGavack, 1959; Finn *et al.*, 1963; Schaub, 1964/65).

TABLE 2

ALKALINE- AND ACID-SOLUBLE UTERINE COLLAGEN CONTENT OF POST-PARTUM AND PREGNANT DOES AT DIFFERENT AGES

Does	Age (weeks)	Uterine collagen (based on wet weight)			Uterine dry weight (%)	Total collagen (% of uterine dry weight)
		Total uterine collagen (g)	Alkaline-soluble collagen (mg/g)	Acid-soluble collagen (mg/g)		
<i>Post-partum</i> †	34	14.5 ± 1.8	2.5 ± 0.8 (12)	17.9 ± 2.9 (88)	16.9 ± 0.5	12.1 ± 2.0
	167	22.5 ± 2.5	1.8 ± 0.2 (11)	15.2 ± 0.8 (89)	16.4 ± 0.3	10.7 ± 2.0
	204	11.0 ± 1.8	6.5 ± 1.8* (20)	26.4 ± 6.4 (80)	17.3 ± 0.4	22.9 ± 16.3
12-day pregnant‡	38	14.4 ± 1.1	1.7 ± 0.1 (13)	11.4 ± 1.6 (87)	17.5 ± 0.6	7.6 ± 1.2
	174	13.3 ± 5.0	1.7 ± 0.2 (9)	16.7 ± 1.6* (91)	18.0 ± 1.8	11.1 ± 2.2

Values expressed as means ± S.E. Numbers in parentheses are percentages of total collagen.

* Differs significantly from other means of *post-partum* does in the same column and group ($P < 0.05$).

† Five does/group.

‡ Three does/group.

Since the dry-weight fraction of the uterus did not change with age, none of the differences in uterine collagen concentration associated with age appear to be due to changes in water content of the uterus.

Collagen structural changes occur with increased age; this reduces the ability of collagenase to degrade collagen fibres (McGavack & Kao, 1963; Schaub, 1964, 1964/65, 1965). This structural change in collagen together with the reduction in collagenase could contribute to the higher uterine collagen content of old does. The higher uterine collagen along with lower ovarian progesterone output in old does reported previously (Maurer & Foote, 1972) may reduce the efficiency of the uterine environment sufficiently to be partly responsible for the increase in embryonic deaths in old does (Maurer & Foote, 1971).

This investigation was supported in part by Grant HD 03471 from the National Institute of Child Health and Human Development, USPHS. The authors are grateful to Mr Bruce Hakes and Mr Michael Simkin for their assistance in collecting the data.

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