Uterine collagen content in young and senescent pregnant golden hamsters

Ali Rahima and A. L. Soderwall

Department of Biology, University of Oregon, Eugene, Oregon 97403, U.S.A.

The most prominent histological feature in ageing of the rodent uterus is the accumulation of collagen. There is a progressive increase in connective tissue between the two muscle layers, stroma, and around the blood vessels. Polyotocous laboratory mammals exhibit four characteristic phases during their reproductive lifetime: (1) a gradual increase in litter size, (2) a relatively constant litter size, (3) a decline in litter size, and (4) a failure to reproduce (Biggers, Finn & McLaren, 1962). In golden hamsters this decline begins at approximately 14 months of age (Soderwall, Kent, Turbyfill & Britenbaker, 1960), and is not caused by a depletion of oocytes, since some females continue to ovulate adequate numbers of eggs although the female fails to produce any offspring (Harman & Talbert, 1970). The ageing uterus is generally considered as the organ most responsible for a reduction of offspring (Finn, 1970). The amount of collagen increases in the uterus with age (Finn, Fitch & Harkness, 1963; Schaub, 1964; Maurer & Foote, 1972), and it has been suggested that increased deposition of collagen might impair uterine function, possibly by interfering with vascularization (Biggers *et al.*, 1962). We therefore investigated this in young and old golden hamsters.

Golden hamsters (*Mesocricetus auratus*) were housed singly under conditions of constant temperature (21–23°C) and lighting 05.00–17.00 hours). The young (2–10 months old) and ageing (14–22 months old) females were examined daily for the appearance of the post-oestrous discharge (Day 1) and only animals exhibiting a regular 4-day cycle were used. The females were mated on the evening of pro-oestrus and examined for a vaginal plug or spermatozoa in the vaginal smear. The animals were anesthetized with 30 mg sodium pentobarbitol/kg at 5 days of pregnancy and the entire uterus removed. Following the method of Woessner (1961), one horn was sealed with 5 ml 6 N-HCl in a test tube and hydrolysed for 3 h at 130°C. The tubes were then decanted into volumetric flasks and rinsed with water. The hydroxyproline content of the hydrolysate was determined by the method of Stegemann (1958) and the collagen content was calculated by multiplying the amount of hydroxyproline by 7.46 (Neuman & Logan, 1950).

The values of uterine collagen content of young and senescent females are presented in Table 1. There was a definite increment of uterine collagen content with age and the difference between the mean content in the two groups was highly significant ($P < 0.001$).

Table 1. Uterine collagen content ($\mu\text{g/ml}$) in pregnant young and senescent female golden hamsters

Young	No. of females	Age	Senescent	No. of females	Age
22-38	4	7 months	37-30	8	22 months
16-79	4	6 months	33-57	4	18 months
14-97	3	39 days	29-46	3	15 months
11-19	2	30 days	22-38	1	15 months
Mean $\pm$ S.D. 16.33 $\pm$ 4.65			30.68 $\pm$ 6.39		

Changes in the structure of collagen occur with increased age and the ability of collagenase to degrade collagen fibres is decreased (McGavack & Kao, 1963; Schaub, 1964). With age there is a progressive increase in connective tissue between the fibres of the circular muscle layer, a

collagenous, fibrous network is laid down in the stroma and the diameter of the entire uterus increases (Rolle & Charipper, 1949). Loeb, Suntzeff & Burns (1938) reported an increase in fibrillar connective tissue and deposition of hyaline materials in the stroma of the uterus, cervix and vagina of the senescent mouse. Similar histological studies were conducted in this laboratory and the results (unpublished) were in accord with those on the mouse. However, conclusions based on the appearance of sections of the uterus can be criticized on the grounds of lack of objectivity and specificity of staining and the impossibility of obtaining an accurate quantitative assessment of the amount of collagen present (Finn *et al.*, 1963). Chemical determination of the quantity of hydroxyproline present in a tissue gives an estimation of the collagen content which is not open to such criticism. The most commonly employed quantitative assay for this imino acid has been that of Neuman & Logan (1950) but the method has been criticized because of the interference of other amino acids (Hutterer & Singer, 1960).

The dependence of growing embryonic tissue for vascularity and its attendant nutritive supplies is obviously critical to a successful continuation of any pregnancy. Larson & Foote (1972) studied the uterine blood flow in young and aged female rabbits and found that the average uterine blood flow (ml/100 g tissue/min) was higher in young females. In rats, the collagen fibres become more strongly cross-linked in pregnancy (Schaub, 1964). This change in the collagen, together with the increase in amount of collagen, round the blood vessels could lead to occlusion of the vessels and poor circulation, bearing out the supposition of Biggers *et al.* (1962) that impairment of reproductive function in ageing animals might be caused by interference with vascularization.

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