Connective Tissue. XVIII. Age Differences in Protocollagen Hydroxylase of Porcine Uterine Homogenate (33587)

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Recently, we reported the presence of a highly active protocollagen (PC) hydroxylase in porcine uterine homogenates (1). The level of this enzyme activity varied with the phase of estrus cycle (2). The highest enzymic activity was associated with the secretory phase and lowest activity present in the early proliferative phase. Earlier reports from this laboratory (3, 4) demonstrated that compared with other tissues studied, the uterus had the highest capacity for synthesizing collagen and that this capacity decreased with increasing age of rat. It was shown by Mussini *et al.* (5) that PC hydroxylase activity is high in lung, skin, liver, and carcass of rat. In their studies, the enzymic activity decreased sharply from the third to the fifth days after birth. After the fifth day, they found no further change for the entire average span of life. The present study deals with variations of PC hydroxylase activity of porcine uterine homogenate in relation to age of the donor and weight of its uterus.

Methods. Porcine uteri from 6–8month (body wt. of pig 225–250 lb) and 1–4-year-old animals were used. Uterine weights in the 6–8-month-old group varied from 38–680 g and in the 1–4-year-old group from 200–1096 g. The uteri of the 6–8-month-old animals were further divided into 2 subunits: those with uterine weight less than 100 g (sexually immature) and those with uterine weight over 100 g (sexually mature).

Standard conditions for the preparation of protocollagen substrate, enzyme extract and cofactors; hydroxylation reaction; extraction of collagen and calculation of percentage of hydroxylation are described in the previous publication (1). Data are expressed as percentage of hydroxylation (i.e., disintegrations per minute = dpm in hydroxyproline × 100/total dpm in proline plus hydroxyproline) and further evaluated on the basis of unit weight of protein (i.e., percentage of hydroxylation per mg of protein).

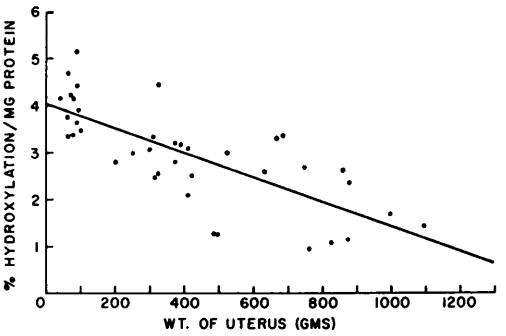

Fig. 1. Relation of uterine weight to protocollagen hydroxylase activity.

Results and Discussion. Differences in the PC hydroxylase activity of porcine uterine homogenate from donors of several ages are shown in Table I. Under the conditions of the experiment, there is, with increasing age, a decrease in the dpm in the hydroxyproline and in the total percentage of hydroxylation achieved. On a unit protein basis, there is a significant decrease in the percentage of hydroxylation per milligram of protein with increasing age. In the 6–8-month-old group, lower PC hydroxylase activity was observed in the uteri of sexually mature than in the uteri of sexually immature animals.

The relation of uterine weight to the activity of the PC hydroxylase is shown in Fig. 1. Data have been fitted by the method of least squares (7). A straight line has been fitted to the data $Y = 4.01 - 0.00261X$, where $Y =$ % hydroxylation/mg of protein and $X =$ wet wt. of the porcine uterus. A decrease in PC hydroxylase activity occurs with increasing uterine weight.

The present data indicate that uterine tissue behaves differently from other tissues in

TABLE I. Age Differences in the Activity of Protocollagen Hydroxylase of Porcine Uterine Homogenate.[a]

	Age of pig	No. of samples	(dpm) Hydroxyproline	(dpm) Proline	Hydroxylation (%) Total	Hydroxylation (%) Per mg of protein	p[d]
A	6–8 mos. (immature)	12[b]	4480 ± 456[c]	15,800 ± 360	23.64 ± 1.16	4.02 ± 0.18	A∼C <0.001 A∼B <0.01
B	6–8 mos. (mature)	10	3260 ± 391	15,700 ± 303	19.71 ± 1.68	2.97 ± 0.25	B∼C <0.02
C	1–4 yrs.	17	2400 ± 189	15,900 ± 340	15.84 ± 1.31	2.19 ± 0.19	

[a] The incubation mixture consists of 2 ml of PC substrate, 0.2 ml of crude enzymic extract, 1 ml of cofactor and made up to 10 ml. The mixture was incubated at 37°, air for 1 hr (1).

[b] Duplicates were done on each sample. Variations between duplicates were within 10%.

[c] Mean ± SE (6).

[d] Comparison of differences between the means only done for the last column.

the level of PC hydroxylase (5), since the PC hydroxylase activity did not decline rapidly in the newborn animal, but gradually during the whole life span.

Summary. Uterine protocollagen (PC) hydroxylase activity was studied in pigs of two age groups—those 6–8 months and those 1–4 years old. Lower PC hydroxylase activity was observed in uteri from pigs 1–4 years of age than in pigs 6–8 months of age. With increase in uterine weight, there is a decline in the level of PC hydroxylase.

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