Connective Tissue: I. Age and Sex Influence on Protein Composition of Rat Tissues. (24864)

KUNG-YING TANG KAO AND THOMAS H. MCGAVACK
Geriatrics Research Labs., Vet. Admin. Center, Martinsburg, W. Va., and Dept. of Medicine, George Washington University School of Medicine, Washington, D.C.

The aging process as currently understood is intimately connected with formation of new collagen(1,2,3,4), and modification of existing elastic tissue(5,6,7,8,9). However, the studies have involved principally aorta and skin; little is known about above processes of other tissues. In previous work on connective tissue obtained by implantation of polyvinyl sponges (Ivalon)*, collagen content/g of sponge implanted, increased with age of tissue(10). Collagen formed rapidly during first 2 or 3 weeks after implantation of sponge and then much more slowly. The present study was undertaken to determine in various tissues of rat, the relation of age and sex to content of soluble protein, soluble collagen, insoluble collagen and elastin. Such data would show whether tissues other than aorta and skin exhibit comparable changes with age.

Methods. Male and female rats, Wistar strain, of 3-5 weeks, 8 months and 2 years of age were used. Samples (0.1-1.0 g) of tail tendon, aorta, skin, uterus, lung, muscle, heart, liver, kidney and spleen were homogenized in 10 ml saline in Virtis "23" homogenizer for 5 minutes. Each homogenate was centrifuged at 20,000 x g for ½ hr and supernatant (saline soluble protein) was decanted. The residue was suspended with occasional stirring, in 20 ml 0.1 N NaOH and remained at 25° over night. The resulting mixture was centrifuged at 30,000 x g for ½ hr and the supernatant removed. The residue was extracted a second time with 10 ml 0.1 N NaOH for 2 hr at 25° and centrifuged as above. The 2 extracts, which contained the alkali-soluble protein (containing soluble collagen) were then combined. The above saline soluble and alkali-soluble protein fractions were together designated "soluble protein" (Fraction I). The residue was treated with 10 ml 0.1 N NaOH at 100° for 15 min, the mixture centrifuged at 20,000 x g for ½ hr and supernatant removed. This extraction was repeated once for all tissues and twice for skin. The hot alkali extracts of all samples were combined and designated as "insoluble collagen" (Fraction II); the residue from these extractions contained "elastin" (Fraction III). All protein extracts were concentrated to 5 ml and then hydrolyzed in 6 N HCl in sealed tubes at 110° for 16 hr. The hydrolysates were filtered and made to convenient volume. Nitrogen was determined by Conway microdiffusion method(11) and protein calculated as 6.25 x nitrogen. Hydroxyproline in the soluble protein fraction was determined by the method of Neuman and Logan(12). Soluble collagen was calculated by multiplying amount of hydroxyproline by 7.46(13). Total collagen represented the sum of soluble and insoluble collagen values. By "total noncollagen scleroprotein" is meant the difference between soluble protein and soluble collagen. Detailed data for male rats has been omitted from Tables except where significant sex differences were observed. All of these have been combined and recorded in Table VI.

Results. Soluble protein (Tables I and VI). Values for soluble protein were constant and independent of age and sex in the following tissues: voluntary muscle, kidney, lung, aorta, liver and spleen. For both sexes, a decrease in soluble protein with age was observed in tendinous, cardiac and dermal structures. Similar decreases were seen in uterine musculature. At 3-5 wks of age, the tail tendon of male contained more soluble protein than that of female rat. More soluble protein was found in connective tissue of skin of 8 mo female rats than in that of males (Table VI).

Insoluble collagen (Table II). Neither age nor sex appeared to have a consistent influence upon insoluble collagen content of connective tissue of upper leg muscle, kidney,

* Ivalon Surgical Sponge, Clay Adams Corp., N. Y.

TABLE I. Influence of Age on Content of Soluble Protein (g of Soluble Protein/100 g of Tissue) in Female Rat.

Tissue \ Age Group	3-5 wk A	8 mo B	2 yr C
Upper leg muscle	17.6 ± 1.1 (4)	19.6 ± 1.6 (7)	19.4 ± 2.3 (3)
Lower " "	20.7 ± 2.4 "	19.9 ± 1.4 "	17.2 ± 1.6 "
Abdominal "	18.6 ± 3.6 (3)	17.9 ± 1.0 "	20.0 ± 1.1 (2)
Kidney	18.6 ± 2.3 (4)	15.8 ± 2.2 "	15.7 ± .5 (3)
Lung	17.3 ± 1.8 (3)	15.5 ± 1.7 "	16.1 ± .7 "
Liver	17.7 ± 3.4 (4)	20.7 ± 1.0 "	18.5 ± .5 "
Spleen	19.1 ± 2.0 (3)	19.3 ± .9 "	18.3 ± .8 "
Tendon	18.5 ± 1.2 "	16.7 ± 5.8 (4)	*6.5 ± 1.8 (4)*
Heart	19.0 ± 1.1 "	18.7 ± .9 "	*14.8 ± .3 (3)*
Aorta	7.7 ± 1.0 (4)*	7.3 ± 1.1 (6)	7.2 ± .8 (4)
Skin	13.5 ± 1.2 (3)	13.4 ± 2.0 (4)	*9.1 ± .9 (4)*
Uterus	14.5 ± 1.5 "	9.3 ± 2.5 (3)	11.6 ± 3.1 "

Values represent means with stand. dev. immediately following.

Figures in parentheses represent No. of animals and corresponding determinations unless otherwise specified.

Figures showing significant differences within the 5% level of confidence are italicized. Data in male rats are comparable to those in female, except as shown in Table VI, where only significant differences are recorded.

* Each determination consists of pooled sample of 5-6 aortas.

lung, liver or spleen. In both sexes, there was an increase with age in insoluble collagen of connective tissue of lower leg and abdominal muscles, tendon, aorta and skin. In the uterus a similar change occurred, which reached a plateau at 8 mo. In cardiac muscle insoluble collagen decreased with age. However, this decrement reached its maximum at 8 mo of age and remains unaltered at 2 yrs of age. The highest values for insoluble collagen at all ages were observed in the skin; at 3-5 wk, the descending order of magnitude in other tissues was as follows: tendon, aorta, uterus, abdominal muscle, leg muscle, heart, lung, spleen, liver and kidney.

More insoluble collagen was present in the abdominal muscle of females at 3-5 wks and at 8 mo than in males of corresponding ages. The insoluble collagen of tendon was higher at 3-5 wks in females than in males, but lower in females than in males at 8 mo. At 8 mo of age, more insoluble collagen was present in the male than in female aorta; at other ages studied no significant sex differences were observed. A significantly greater amount of insoluble collagen was present in skin of female as compared with male at 3-5 wk of age, while the reverse was true at 8 mo of age (Table VI).

Elastin (Table III). In relation to age

TABLE II. Influence of Age on Content of Insoluble Collagen (g of Insoluble Collagen/100 g of Tissue) in Female Rat.

Tissue \ Age Group	3-5 wk A	8 mo B	2 yr C
Upper leg muscle	1.0 ± .5 (4)	1.2 ± .3 (3)	1.0 ± .4 (3)
Lower " "	1.0 ± .8 "	1.4 ± .3 (4)	1.8 ± .8 "
Abdominal "	1.5 ± .2 (3)	1.7 ± .4 "	*2.2 ± .4* "
Kidney	.2 ± .1 (4)	.5 ± .1 (7)	.4 ± .1 "
Lung	.9 ± .2 "	1.4 ± .2 (5)	1.0 ± .1 "
Liver	.2 ± .0 (3)	.2 ± .1 (7)	.1 ± .0 "
Spleen	.8 ± .2 (4)	.5 ± .1 "	.6 ± .3 "
Tendon	4.5 ± .5 (3)	*10.3 ± 1.5 (4)*	*15.7 ± 2.0 (4)*
Heart	.9 ± .2 "	*.6 ± .1* "	.7 ± .2 (3)
Aorta	3.0 ± .4 (4)*	*6.6 ± .7 (6)*	*7.4 ± .1 (4)*
Skin	7.3 ± .8 (3)	*15.3 ± 1.8 (4)*	*20.8 ± 2.8* "
Uterus	.8 ± .1 (2)	*3.3 ± .9 (3)*	*3.2 ± .5* "

* See footnote, Table I.

TABLE III. Influence of Age on Content of Elastin (g of Elastin/100 g of Tissue) in Female Rat.

Tissue \ Age Group	3-5 wk A	8 mo B	2 yr C
Upper leg muscle	.09 ± .05 (4)	.04 ± .02 (6)	.03 ± .02 (3)
Lower " "	.07 ± .04 (2)	.06 ± .03 "	.04 ± .03 "
Abdominal "	.06 ± .04 (4)	.05 ± .02 "	.03 ± .01 "
Kidney	.26 ± .25 "	.04 ± .01 (5)	.04 ± .02 "
Lung	.33 ± .20 "	.32 ± .12 (7)	.30 ± .03 "
Liver	.03 ± .02 "	.04 ± .01 (6)	.01 ± .01 "
Spleen	.16 ± .11 "	.11 ± .07 (7)	.15 ± .13 "
Tendon	.13 ± .03 (6)	.26 ± .11 "	*.30 ± .09 (9)*
Heart	.16 ± .11 (3)	.10 ± .08 (4)	.06 ± .01 (3)
Aorta	6.14 ± .36 (4)*	5.93 ± .56 (6)	6.14 ±1.15 (8)
Skin	1.31 ± .33 (5)	.60 ± .27 (10)	.41 ± .16 (7)
Uterus	.11 ± .03 (3)	.35 ± .35 (8)	.25 ± .16 (9)

* See footnote, Table I.

and sex, no change was observed in elastin content of upper leg, lower leg or abdominal musculature, or in kidney, liver, spleen and uterus. In tendinous tissue, elastin increased with age, with virtually the entire change taking place during first 8 mo of life. A consistent decrease in elastin occurred with age in connective tissue of hearts of both sexes. In female rats, there were no demonstrable changes with age in amount of elastin present in lungs. However, in the male, there was a decrease from 3-5 wk to 8 mo and no further decrement up to 2 yrs of age. More elastin was observed in male than in female aorta at 8 mo of age. More elastin was present in skin of females than in that of males at 3-5 wk of age (Table VI).

Non-scleroprotein (Table IV). Calculations for nonscleroprotein were made in 4 tissues only: tendon, aorta, skin and uterus. No significant changes with age or sex were observed in values of non-scleroprotein for aorta nor with age for uterus. In tendon and skin, non-scleroprotein decreased with age in both sexes and more dramatically in the former tissue.

Total collagen (Table IV). In both sexes, there was an increase in total collagen of tendon up to 8 mo of age. At all ages from fifth week onward, the amount of collagen present in male tendon was significantly greater than that in the female (Table VI). Total collagen content of *aorta* increased steadily with age in both female and male rats from third wk to eighth mo. No significant change occurred thereafter. There was an increase in total collagen with age in skin and uterus of all animals.

Percentage of soluble collagen in total collagen (Table V). In uterus and tail tendon,

TABLE IV. Influence of Age on Total Non-scleroprotein and Total Collagen (g of Protein/100 g of Tissue) in Female Rat.

Tissue \ Age Group	3 wk A	4 wk A-1	5 wk A-2	8 mo B	2 yr C
			Total non-scleroprotein tissue		
Tendon	10.8 ± 1.4 (4)	8.6 ± .1 (2)	10.2 ± 1.5 (3)	*5.0 ± 2.1 (4)*	*3.0 ± 1.2 (4)*
Aorta	5.7 ± 1.1 " *	6.9 ± 1.1 (3)	5.5 (1)	6.5 ± .7 (4)	6.5 ± .8 (4)
Skin	7.2 ± .7 (3)	6.2 ± .4 (4)	10.6 ± .6 (3)	*8.1 ± 1.3 (4)*	*5.7 ± .8 (4)*
Uterus	7.3 ± .0 (2)	6.0 ± 2.5 "	9.6 ± 5.4 "	7.9 ± 1.2 (3)	10.4 ± 3.0 (4)
			Total collagen		
Tendon	12.5 ± 1.4 (4)	14.3 ± 1.2 (2)	12.7 ± .6 "	*20.3 ± 1.1 (4)*	*19.2 ± 1.2 (4)*
Aorta	5.0 ± .4 " *	6.0 ± .9 (3)	5.5 (1)	*7.8 ± .8* "	*8.1 ± .9* "
Skin	7.3 ± 1.7 "	*10.1 ± 1.6 (4)*	*10.8 ± .9 (3)*	*20.6 ± 2.1* "	*24.6 ± 2.9* "
Uterus	2.3 ± .1 (2)	2.3 ± .9 (4)	2.2 ± 1.1 (3)	*4.5 ± 1.0 (3)*	*4.3 ± .6* "

* See footnote, Table I.

TABLE V. Influence of Age on Percentage of Soluble Collagen in Total Collagen (g of Soluble Collagen/100 g of Total Collagen) in Female Rat.

Age	3 wk	4 wk	5 wk	8 mo	2 yr
Group	A	A-1	A-2	B	C
Tissue					
Tendon	85.6	82.1	*68.0*	*48.8*	*18.6*
Aorta	37.4*	29.2	36.6	*17.6*	*9.0*
Skin	70.0	*45.1*	*32.0*	*25.7*	*14.0*
Uterus	65.8	*42.8*	26.8	*28.1*	*26.0*

* See footnote, Table I.

aorta and skin of both sexes, the percentage of soluble collagen in total collagen decreased with age. At 3 wk of age, more soluble collagen was present in tail tendon of female than of the male. At 8 mo a significantly higher percentage of soluble collagen in total collagen was found in the skin of females than of males (Table VI).

Comment. These data show that in both sexes tissues rich in scleroprotein (tendon and skin) undergo greater changes in protein composition than tissues low in scleroprotein. In general, in these tissues there is a decrease in non-scleroprotein with aging and a concomitant increase in scleroprotein. In other tissues, with a few exceptions, levels of protein fractions were relatively constant from 3 wk to 2 yr of age. With increasing age the percentage of soluble collagen in the scleroprotein fraction decreased and amount of insoluble collagen increased with age. The third component of the scleroprotein fraction, elastin, remained relatively constant in all tissues except tendon in which there was an increase with age. The principal differences between sexes were that in skin, tendon and aorta, the insoluble collagen was higher in young females while at 8 mo males had higher levels. After this time there were no differences between the sexes. The data indicate that the aging process in terms of changes in scleroprotein occurs principally in tendon, skin, uterus and aorta.

Summary. Tissues from tail tendon, aorta, skin, uterus, lung, muscle, heart, liver, kidney and spleen of male and female rats at 3-5 wk, 8 mo and 2 yr of age were fractionated into soluble protein, insoluble collagen and elastin. Amount of soluble collagen in the soluble protein fraction was calculated according to amount of hydroxyproline. Significant changes in these fractions with age occurred only in skin, tendon, uterus and aorta, with scleroprotein increased and non-scleroprotein decreased.

The authors gratefully acknowledge suggestions and advice of Dr. C. H. Treadwell and technical assistance of James Leslie, Sally A. Hendricks and William Hitt.

TABLE VI. Sex Differences* in Composition of Connective Tissue.

		Tissue value (g/100 g)							
		Abdominal muscle		Tendon		Aorta		Skin	
Constituent	Age change observed	♂	♀	♂	♀	♂	♀	♂	♀
Soluble protein	3-5 wk			26.5	18.5				
	8 mo							8.3	13.4
Insoluble collagen	3-5 wk	.6	1.5	2.2	4.5			5.3	7.3
	8 mo	.5	1.7	22.9	10.3	8.2	6.6	22.0	15.3
Elastin	5 wk							.5	1.3
	8 mo					7.4	5.9		
Total collagen	5 wk			17.6	12.7				
	8 mo			27.0	20.3				
	2 yr			25.1	19.1				

* These are statistically significant differences within 5% level of confidence.

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Received March 5, 1959. P.S.E.B.M., 1959, v101.

Nature and Source of Appendical Secretion.* (24865)

JOHN F. PERRY, JR., MERLE K. LOKEN AND O. H. WANGENSTEEN
Dept. of Surgery, University of Minnesota, Minneapolis

Demonstration that the vermiform appendix of man(1), chimpanzee(2), and rabbit (3) secretes fluid at pressures approaching systolic blood pressure has provided a basis for understanding the pathogenesis of obstructive appendicitis in man. However, because of the simplicity of treatment of appendicitis by surgery, there has been little stimulus for study of the site or mechanism of this fluid production. The following experiments were undertaken to determine the cellular elements responsible for this process in the vermiform appendix. Normally the rabbit appendix secretes from 10 to 56 ml of a slightly cloudy, colorless liquid of specific gravity 1.001-1.006 and pH 8.1-8.5 in 6 hour period.

Method. Adult albino rabbits of both sexes were utilized. Measurement was made of volume of secretion and secretory pressure with appendix ligated at base and attached to a collecting balloon or manometer respectively. Several experiments were designed to modify or nullify participation by certain cellular elements: I. *Suppression of lymphoid elements.* a) Exposure doses of x-ray in range of 250-1000 R were delivered to a series of appendixes.† Volume and pressure measurements were made 2 to 21 days thereafter. b) Cortisone was given parenterally in dosage of 5 mg/day for 7 and 14 days and 12.5 mg/day for 14 days. This steroid causes dissolution of lymphocytes and actual decrease in weight of lymphoid elements(4). II. *Stimulation and exhaustion of mucous cells.* a) Pilocarpine(5) in 2.5 mg dosage was administered hourly for 4 hours prior to fluid collection and in another group of animals the drug in same dosage was given hourly during a 6-hour collection period. b) Mustard oil(6) in olive oil was instilled into appendical lumen for 6 hours and fluid collection and pressure measurements carried out thereafter. III. *Destruction of surface epithelium.* a) Silver nitrate in 2 and 5% concentrations was instilled into the appendix for 5 minutes prior to pressure and volume measurement. IV. To determine if a cellular process requiring oxygen utilization was actually involved, sodium cyanide M/600 in 2 ml amounts was placed in appendical lumen and pressure determined(7). Appropriate control volume and pressure measurements were made. Histologic examination was made of all appendixes studied. Specimens were fixed in formalin and stained with hematoxylin and eosin or fixed in absolute alcohol and stained by Mayer's mucicarmine technic.

Results. Volumes of fluid secreted by the appendix under the experimental conditions are shown in Table I. Following X-irradiation, there was significant decrease in volume of fluid produced, both in early post-irradiation period and after 3 weeks. Sections of irradiated appendixes uniformly demonstrated severe suppression of lymphoid elements; those appendixes showing great reduction in secretory volume uniformly demonstrated epithelial damage as well. Irradiation was quite

* Supported by the United States Public Health Service Grant RC-4780 (C2) and the Donald J. Cowling Fund for Surgical Research.

† For irradiation a G. E. Maximan x-ray machine was operated at 140 KV, 15 MA with no added filter (H.V.L. = 3.7 mmAl). Using 27 cm F.S.O. exposure dose rate was 232 roentgens/minute as measured with Victoreen r-meter.