another experiment (unpublished data), the RSSE serum did not neutralize Powassan virus by the IC route of inoculation in 21-day-old mice. Those data indicate that the relationship between 791A-52 and Powassan viruses and RSSE virus is nonreciprocal.

Conclusions. A virus closely related, if not identical, to Powassan virus has been isolated from *Dermacentor andersoni* from northern Colorado. A nonreciprocal relationship between RSSE virus and 791A-52 virus is indicated by the cross neutralization tests. The importance of *Dermacentor andersoni* in maintenance of this virus is unknown. Although thousands of this tick species collected in many areas in the western United States have been examined for viruses in this laboratory, this is the only isolation of this agent. Since these prior studies of ticks at the Rocky Mountain Laboratory were directed specifically toward study of the ecology of Colorado tick fever virus, the ecology of the 791A-52 virus is unknown. Field and laboratory studies on this subject are in progress.

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Since this paper was submitted, an article has appeared by J. Casals (*Can. Med. Assn. J.*, 1960, v82, 355) showing that Powassan virus is more closely related to the RSSE complex of viruses than to any other arbor virus and is a distinct virus.

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Connective Tissue III. Collagen and Hexosamine Content of Tissues of Rats of Different Ages.* (25837)

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Sobel and his co-workers have reported that hexosamine and collagen content of skin of rat, guinea pig, squab, rabbit and human, femur of rat and lung of rabbit(1,2,3,4) increase with age. They have also shown that rate of hexosamine deposition decreased with age more than that of collagen deposition; therefore, ratio of hexosamine to collagen decreased with age. Houck and Jacob(5) reported that concentration of hexosamine in rat skin was relatively unaffected by age of the animal, but ratio of hexosamine to nitrogen decreased with increases in body weight up to 330 g. Above this figure, however, the ratio varied directly with body weight. The present study is concerned with determination of the relation of age to: (1) total protein, (2) collagen and (3) hexosamine in aorta, tendon and skin of rat.

Methods. Male and female rats, Wistar strain, were used in this study. Rats from 4 weeks to 8 months of age were raised in our laboratory on Purina chow and water *ad lib.* Two-and-one-half-year-old rats were purchased from Research Supply Co., Philadelphia, Pa. One-tenth to 0.5 g of tissue were extracted with fat solvents(6). For total protein and collagen determinations the fat free tissues were hydrolyzed in 6N HCl at 110°C for 16 hours in a sealed tube. Nitrogen was determined by Conway microdiffusion method (7) and protein was calculated as 6.25 x nitrogen. Hydroxyproline was determined by the method of Neuman and Logan(8). Collagen in tendon and skin was calculated by multiplying the amount of hydroxyproline by

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7.46(9). Since rat aorta contains a constant level of 6% elastin(10) and rat elastin contains 2.3% hydroxyproline(9), amount of collagen in rat aorta was calculated as [total hydroxyproline—(6 x 2.3%)] x 7.46. For hexosamine determination, the fat-free tissues

TABLE I. Influence of Age on Content of Protein, Collagen and Hexosamine in Tissues of Rat (g/100 g of Tissue).

Tissue	Age		Protein	Collagen	Hexosamine	H/C ($\times 10^{-3}$)
Aorta*	4 wk	(6)	22.8	7.4	.18	24.3
	A		B <.01	B <.01 CD <.001 E <.05	B <.01 E <.001	
	8 wk	(4)	28.9	9.5	.23	24.2
	B		C <.01 E <.001			
	4-5 mo	(6)	23.0	10.4	.21	20.2
	C				E <.02	
	8 mo	(7)	25.1	10.4	.20	19.3
	D		E <.05		E <.01	
	2½ yr	(4)	21.0	9.0	.25	27.8
	E					
	Pooled S.D.		± 2.6	± 1.2	± .03	
Tendon	4 wk	(10)	32.2	23.1	.17	7.49
	A		B <.01 CD <.001 E <.02	BCDE <.001	C <.001 D <.02	
	8 wk	(11)	38.1	30.2	.18	5.92
	B				C <.001 D <.01 E <.05	
	4-5 mo	(15)	41.7	32.7	.13	3.95
	C					
	8 mo	(10)	40.5	31.7	.14	4.21
	D					
	2½ yr	(12)	37.9	30.4	.15	4.95
	E					
	Pooled S.D.		± 4.8	± 3.8	± .03	
Skin	4 wk	(15)	24.2	12.9	.14	10.97
	A		C <.01 D <.001 E <.05	BCDE <.001	C <.02 E <.01	
	8 wk	(10)	27.4	16.9	.16	9.36
	B			D <.001 E <.01	CE <.01 D <.02	
	4-5 mo	(14)	27.3	19.4	.12	6.48
	C			D <.05		
	8 mo	(12)	30.1	22.5	.13	5.60
	D					
	2½ yr	(12)	26.9	21.4	.12	5.86
	E					
	Pooled S.D.		± 3.4	± 3.2	± .02	

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

P value relating to age differences, when within 5% level of confidence, is listed beneath results of actual determination and represents comparison between that figure and that of age groups designated by letter or letters preceding the value.

* Each determination consists of a pooled sample from 2-3 rats.

were hydrolyzed in 4N HCl in sealed tubes at 100° for 5 hours. Hexosamine content of the hydrolysate was determined by the method of Rondle and Morgan(11).

Results. Changes in the contents of protein, collagen and hexosamine in tissues of rat with age are shown in Table I. In *aorta* the protein content increased between 4 weeks and 8 weeks, then decreased in older animals. Collagen increased between 4 and 8 weeks of age with no significant change thereafter. Hexosamine increased between 4 weeks and 2½ years of age. There was no constant regular effect of age upon H/C ratio. In *tendon* the content of protein and collagen increased between 4 and 8 weeks and maintained high levels thereafter. Content of hexosamine decreased between 4 and 8 weeks and 4-5 months of age. H/C ratio decreased between 4 weeks and 4-5 months of age. In *skin* the content of protein increased between 4 weeks and 4-5 months and was relatively constant thereafter. Content of collagen increased between 4 weeks and 8 months of age. Hexosamine content was constant between 4-8 weeks, then decreased at 4-5 months. There was a decrease of H/C ratio with age between 4 weeks and 4-5 months.

Discussion. These data show a gradual increase in collagen content of skin from 4 weeks up to 8 months of age, while in the aorta and tendon the increment occurs between 4th and 8th weeks. Hexosamine content of aorta increases with age as compared to tendon and skin in which there is a decrease. Therefore, no change in H/C ratio was observed in aorta. The decreased H/C ratios in tendon and skin are the net result of increases in collagen and decreases in hexosamine with age.

Summary. 1. The relation of age to collagen and hexosamine content of aorta, tendon and skin was studied in rats from 4 weeks to 2½ years of age. Collagen content increased with age between 4 and 8 weeks in aorta and tendon; between 4 weeks and 8 months in skin. Hexosamine increased with age in aorta and decreased with age in tendon and skin.

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