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Age-Related Changes in Serum Proteins of the Spontaneously Hypertensive Rat (42764)

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Abstract. Urinary protein excretion and composition in spontaneously hypertensive rats (SHR) change dramatically with age and sex. In this study, serum proteins were analyzed by electrophoresis in male and female SHR and Wistar–Kyoto (WKY) normotensive controls aged 5 to 80 weeks. Serum albumin concentrations of SHR were significantly higher than those of WKY at 5 (4.02 ± 0.24 vs 3.60 ± 0.25 g/dl) and 20 weeks (4.30 ± 0.30 vs 3.77 ± 0.31 g/dl) and significantly lower at 73–80 weeks (2.73 ± 0.33 vs 3.45 ± 0.34 g/dl). In addition, male SHR had significantly lower albumin levels than female SHR after 40 weeks of age. These differences may contribute to the development of hypertension and reflect the appearance of pathologic proteinuria in SHR. In spite of their differences in albumin concentrations, the fractional composition of serum protein from SHR and WKY were undistinguishable. All animals, regardless of strain or sex, manifested a significant decline in the relative amounts of albumin and low molecular weight protein and a significant increase in the relative amount of high molecular weight protein with increasing age. The etiology and significance of these age related changes in the fractional composition of serum protein are unknown, but they differ from the normal developmental pattern in humans.

The spontaneously hypertensive rat (SHR) is generally considered an appropriate model for studying the pathophysiologic effects of sustained high blood pressure and is frequently used as an analogue of human essential hypertension. Previous work from this laboratory has defined the natural history of urinary protein excretion in male and female SHR using genetically matched normotensive Wistar–Kyoto rats (WKY) as controls (1–3). SHR of both sexes exhibit a characteristic pattern of increasing proteinuria with age which reflects progressive renal injury. We now complement this work with additional information regarding serum proteins in these animals.

Materials and Methods. Eight males and eight females of SHR and WKY strains (total $N = 32$) were obtained from Laboratory Supply Co. (Indianapolis, IN). The animals were allowed at least 1 week to adapt to our facility before any procedures were performed. All animals had free access to food and water. No differences in intake were observed among the groups. Body weights, blood pressure measurements, and tail vein blood for serum protein analysis were obtained at 5, 20, 40, 60, 73 (male SHR only) and 80 (female SHR, all WKY) weeks. Hematocrits were measured in all animals at 5 and 20 weeks of age. Body weight was measured to the nearest gram using a triple-beam balance (Ohaus Scale Corp., Union, NJ). Indirect systolic blood pressure was measured by a tail-cuff method (Narco Biosystems) without anesthesia. The average of three readings taken in a quiescent state was recorded. Blood was obtained by warming the animal, snipping the end of the tail and collecting freely flowing blood into a capillary tube. Serum total protein and albumin concentrations and fractional composition of serum protein were determined on diluted serum (1:51) by microcontinuous gradient gel electrophoresis as previously described (1). Proteins migrating slower than albumin were considered high molecular weight (HMW) proteins and those migrating faster, low molecular weight (LMW) proteins.

All values were expressed as means ± standard deviation. Student's t test was used to compare means; a P value less than 0.01 was considered significant. Because male SHR normally die before 80 weeks of age, measurements from this group at 73 weeks were compared to those from other groups at 80 weeks of age. We also measured the fractional composition of serum protein using

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tail blood from three different groups of female Lewis rats; one group at age 8 weeks (N = 15), another group at 20–24 weeks (N = 14), and a third group at 40–80 weeks (N = 18).

Results. *Body weights.* All animal weights were similar at 5 weeks of age. After 5 weeks, males of either strain were significantly heavier than their female counterparts. After 20 weeks, female WKY weighed significantly more than female SHR. Male WKY and SHR of the same age had similar weights, except at 20 weeks when male SHR were significantly heavier than male WKY (347 ± 14 vs 286 ± 21 g). All animals continued to gain weight during the study.

Blood pressure measurements. Systolic blood pressure remained normal through 80 weeks in WKY males and females (mean value of all measurements, 122.6 ± 5.2 mm Hg). There was no significant difference between these two groups. Male SHR became hypertensive (>150 mm Hg) after 5 weeks of age and from 20 weeks on their blood pressure was 199 ± 8.1 mm Hg. Female SHR blood pressures reached hypertensive levels at 20 weeks, but remained significantly lower than male SHR pressures until 60 weeks when they became identical to male values.

Survival. One male WKY died just before 60 weeks of age. The remaining seven male WKY and all eight female WKY survived through 80 weeks and appeared healthy. All eight male SHR were deceased before 80 weeks of age. Four of eight female SHR lived through 80 weeks of age.

Hematocrit. No significant strain-related differences in hematocrit were detected. At 5 weeks of age, WKY hematocrit was 49.7 ± 2.6% and SHR 48.9 ± 2.3%. At 20 weeks, WKY hematocrit was 53.1 ± 3.1% and SHR 52.1 ± 2.5%.

Serum total protein and albumin concentrations. There were no significant age- or sex-related differences in serum total protein and albumin concentrations between male and female WKY throughout the study. At 5 weeks of age, the albumin levels of male and female WKY were 3.92 ± 0.26 and 3.56 ± 0.29 g/dl, respectively. At 80 weeks, these measurements were 3.39 ± 0.29 g/dl (male WKY) and 3.51 ± 0.38 g/dl (female WKY).

The serum albumin concentrations of male and female SHR were similar until 60 weeks of age when males had a significantly lower value than females (3.38 ± 0.33 vs 3.80 ± 0.35 g/dl). After 60 weeks, albumin concentrations of male and female SHR continued to decline, reaching values of 2.70 ± 0.38 g/dl in males at 73 weeks and 2.76 ± 0.34 g/dl in females at 80 weeks. These measurements were significantly lower than corresponding 5 week values. Serum total protein concentrations also tended to be lower in 60- and 73-week-old male SHR than in female SHR but the difference was not statistically significant.

The serum total protein and albumin concentrations of all WKY and SHR are shown in Table I. At both 5 and 20 weeks of age, SHR of both sexes had significantly higher values than WKY. While the albumin levels of WKY remained constant throughout the study, SHR values progressively fell after 20 weeks. At 80 weeks, the serum albumin concentration of SHR was significantly less than that of WKY. The total protein concentrations of both strains increased significantly from 5 to 20 weeks and then remained relatively constant thereafter.

Fractional composition of serum protein (Table I). The percentages or relative amounts of albumin, LMW, and HMW proteins were similar in WKY and SHR serum at every age studied. However, both strains demonstrated identical age related changes. From 5 to 80 weeks of age, the percentage of albumin declined and the percentage of HMW protein rose significantly in both WKY and SHR. In addition, the relative amount of LMW protein in both strains decreased significantly between 5 and 20 weeks (WKY 3.7 ± 0.7 vs 2.6 ± 0.5%; SHR 3.2 ± 0.8 vs 2.4 ± 0.5%), but remained constant thereafter.

These age-related changes in the fractional composition of serum protein are illustrated in Fig. 1 which displays the ratio of % albumin/% HMW protein (albumin/globulin ratio) versus age. From 5 to 20 weeks, % albumin/% HMW protein in WKY fell from 1.6 ± 0.2 to 1.1 ± 0.1 and in SHR from 1.7 ± 0.2 to 1.0 ± 0.1. This significant decline continued in both strains from 20 to 80 weeks of age (WKY 1.1 ± 0.1 to 0.8 ± 0.1; SHR 1.0 ± 0.1 to 0.6 ± 0.2). When males of both strains were compared to females, there was a significant difference in the ratio of %

TABLE I. SERUM ALBUMIN AND TOTAL PROTEIN CONCENTRATIONS AND FRACTIONAL COMPOSITION OF SERUM PROTEIN IN WKY AND SHR

Age (weeks)	Strain	N	Albumin (g/dl)	% Albumin	% LMW protein	% HMW protein	Total protein (g/dl)
5	WKY	16	3.60 ± 0.25[a]	58.5 ± 3.4	3.7 ± 0.7	37.9 ± 3.2	6.11 ± 0.56[a]
	SHR	16	4.02 ± 0.24	59.6 ± 4.9	3.2 ± 0.6	37.1 ± 4.2	6.70 ± 0.51
20	WKY	16	3.77 ± 0.31[a]	49.7 ± 3.2[b]	2.6 ± 0.5[b]	47.7 ± 3.1[b]	7.68 ± 0.70[a,b]
	SHR	16	4.30 ± 0.30	49.5 ± 1.6[b]	2.4 ± 0.5[b]	48.1 ± 1.3[b]	8.73 ± 0.81[b]
40	WKY	16	3.68 ± 0.29	47.3 ± 2.4[b]	2.4 ± 0.5[b]	50.1 ± 2.1[b]	7.77 ± 0.49[b]
	SHR	16	3.77 ± 0.29	46.4 ± 1.8[b]	2.0 ± 0.5[b]	51.6 ± 2.9[b]	8.15 ± 0.75[b]
60	WKY	15	3.85 ± 0.30	45.7 ± 5.3[b]	2.3 ± 0.4[b]	51.8 ± 5.0[b]	8.47 ± 0.89[b]
	SHR	14	3.62 ± 0.36[c]	46.2 ± 4.4[b]	2.3 ± 0.7[b]	51.4 ± 4.0[b]	7.84 ± 0.64[b]
73–80	WKY	15	3.45 ± 0.34[a]	43.0 ± 4.3[b,c]	2.4 ± 0.7[b]	54.5 ± 4.1[b,c]	8.03 ± 0.72[b]
	SHR	9	2.73 ± 0.33[b,c]	37.4 ± 6.1[b,c]	2.8 ± 0.3	59.7 ± 6.2[b,c]	7.39 ± 0.50

[a] $P < 0.01$ vs SHR of same age.
[b] $P < 0.01$ vs 5-week rat of same strain.
[c] $P < 0.01$ vs 20-week rat of same strain.
[d] $P < 0.01$ vs 20-week SHR.

albumin/% HMW protein at 5 weeks of age (males 1.4 ± 0.2 vs females 1.7 ± 0.2). At this age, females had similar absolute amounts of albumin (3.78 ± 0.23 vs 3.82 ± 0.32 g/dl) and lower absolute amounts of HMW protein (2.16 ± 0.13 vs 2.66 ± 0.09 g/dl) in their serum than males. No other sex-related differences were found.

The fractional composition of female Lewis rat serum protein from age 8 to 48 weeks is shown in Table II. These rats demonstrated a significant decline in the relative amount of LMW protein and in the ratio of % albumin/% HMW protein with increasing age. This pattern was identical to that found in WKY and SHR during the same time interval.

Discussion. Several strain-, age-, and sex-related changes in serum proteins were detected during this study. SHR were distinguished from WKY only by differences in their serum albumin concentrations (Table I). Albumin levels of WKY did not vary with either age or sex. However, serum albumin

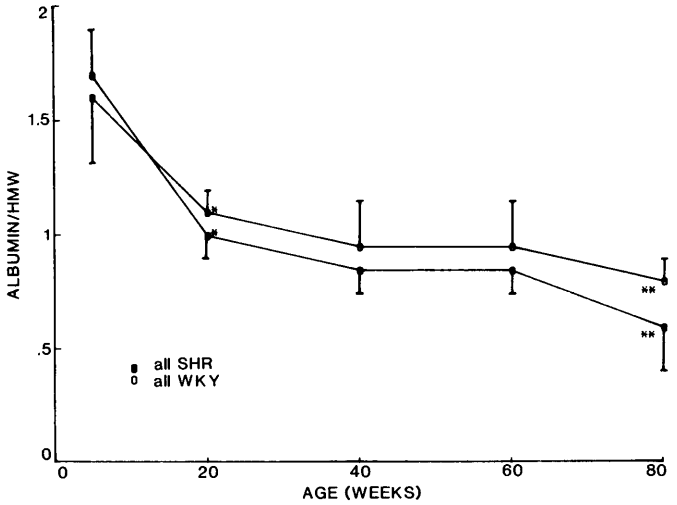

FIG. 1. Ratio of % albumin/% high molecular weight (HMW) protein in all WKY and SHR from age 5 to 80 weeks. *$P < 0.01$ vs corresponding 5-week value. **$P < 0.01$ vs corresponding 20-week value.

TABLE II. FRACTIONAL COMPOSITION OF FEMALE LEWIS RAT SERUM PROTEIN

Age (weeks)	N	% Albumin	% LMW protein	% HMW protein	% Albumin / % HMW protein
8	15	55.8 ± 1.2	3.6 ± 1.1	40.5 ± 1.7	1.4 ± 0.1
20–24	14	51.5 ± 3.3	2.0 ± 0.8[a]	46.3 ± 3.1[a]	1.1 ± 0.1[a]
40–48	18	47.6 ± 3.5[a]	1.9 ± 0.7[a]	50.3 ± 3.8[a]	0.9 ± 0.2[a]

[a] $P < 0.01$ vs corresponding 8-week value.

concentrations of SHR were significantly greater than those of WKY at 5 and 20 weeks and lower at 80 weeks of age. The decrease in albumin levels in 73- to 80-week SHR may reflect the pathologic albuminuria that develops in these animals with age. After 60 weeks of age, urinary albumin excretion is approximately 20 times higher in SHR than WKY (2).

The cause of elevated albumin levels in SHR during the maturational period of 5 to 20 weeks is unclear. Nutrition and growth do not appear to be involved. All animals had similar weights at 5 weeks of age and, although male SHR grew significantly faster than male WKY from 5 to 20 weeks, female WKY and SHR grew at comparable rates but still had significantly different serum albumin concentrations. In addition, WKY and SHR hematocrits were identical at both 5 and 20 weeks of age. Presumably, alterations in synthesis or metabolism account for higher SHR albumin levels during this time. Although its etiology is unclear, the elevation in SHR serum albumin may play a role in the development of renal fluid retention and hypertension in these animals. It is known that young SHR kidneys retain excessive amounts of salt and water compared with those of WKY (4). The combination of comparatively higher serum albumin concentration and increased filtration fraction present at this age could contribute to sodium retention by raising peritubular oncotic pressure and enhancing proximal tubule fluid absorption (5–7).

In spite of their differences in serum albumin concentrations, the fractional composition of serum proteins from SHR and WKY were indistinguishable (Table I). In both strains, the percentage of LMW protein decreased significantly from 5 to 20 weeks. Lewis rats demonstrated a similar decline (Table II), suggesting that this maturational phenomenon is normally present in other rat strains. It is known that the relative amount of urinary LMW protein increases significantly during maturation in male rats, suggesting a possible explanation for declining serum LMW protein during this time (8). However, female rats do not manifest a similar increase in LMW proteinuria, but still manifest a fall in the relative amount of serum LMW protein (8).

In addition, there was a progressive decline in the relative amount of albumin and a progressive increase in both the relative and absolute amounts of HMW protein (globulins) with age in WKY, SHR, and Lewis rats. These trends produced a reversal in the albumin/HMW protein or albumin/globulin ratio over time from much greater than one at 5 weeks to approximately one at 20 weeks and subsequently less than one by 80 weeks. In clinical practice, low albumin/globulin ratios have been associated with essential hypertension, congestive heart failure, and heavy albuminuria as well as various inflammatory and neoplastic disorders (9). The development of decreased ratios in SHR is therefore not unexpected. In contrast, at least in healthy humans, albumin/globulin ratios are consistently greater than one from birth to 80 years of age (10, 11). Although we did not specifically investigate WKY and Lewis rats for disease, they appeared in good health and had normal blood pressures and renal function. It is likely that a declining albumin/globulin ratio and an increasing absolute amount of serum globulin are characteristic of the aging laboratory rat. Coleman *et al.* noted similar trends in their aging studies of Fischer male rats (12).

Two sex-related changes in serum proteins

were found. First, SHR males had significantly lower serum albumin concentrations after 40 weeks than female SHR. This difference relates to the fact that pathologic albuminuria begins at an earlier age and is of greater magnitude in male SHR (2). Second, the ratio of % albumin/% HMW protein in 5-week WKY and SHR females was significantly greater than that of their male counterparts. Because serum albumin concentrations were similar in females and males at this age, this difference was caused by a greater absolute amount of HMW serum protein in 5-week-old male rats of both strains. The etiology of this difference is not known and it disappeared as the rats aged.

In summary, the major findings of this natural history study of SHR serum proteins were (1) Compared with those of WKY, serum albumin concentrations of SHR are higher early in life possibly contributing to the genesis of hypertension by promoting fluid retention, and lower later in life reflecting the development of pathologic albuminuria in SHR. (2) The fractional composition of rat serum protein appears to change with increasing age independent of strain, sex, or absolute serum albumin concentration. The relative amounts of albumin and LMW protein decrease and the relative and absolute amounts of HMW protein rise. The etiology and significance of these changes are unknown.

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