37) Change in Collagen Types of The Rat Aorta with Aging and Hypertension. Akira Ooshima, Yasuo Nara, Masahiro Kihara, Ryoichi Horie*, and Yukio Yamori. Japan Stroke Prevention Center, Dept. of Pathology, and Neurosurgery*, Shimane Medical Univ., Izumo 693.

We have demonstrated that hypertension does induce an increased synthesis of cardiovascular collagen (Ooshima, A. et al.: Proc. Nat. Acad. Sci. USA 71, 3019-3023, 1974). In this report, we will describe change in aortic collagen types of rats associated with ageing and hypertension.

Material: Hypertension was induced in uninephrectomized, 8-week-old male Wistar rats by the twice-weekly subcutaneous injection of DOCA (5mg/rat) maintained on 1% saline. As a control, uninephrectomized Wistar rats with the same age and sex maintained on 1% saline were used. To determine age-related changes, male Wistar rats with various ages (1 to 64 weeks after birth) were sacrificed. All experimental animals were killed by decapitation. The aorta and skin were excised and washed overnight in physiological saline. Collagen was extracted in 0.5M acetic acid containing pepsin (1mg/ml) overnight with continuous stirring after homogenizing tissues. The homogenate was clarified by centrifugation (30,000 x g) at 4°C for 1hr. Pellets were then suspended and stirred overnight in the pepsin-acetic acid solution to re-extract collagen. The solution was centrifuged (30,000 x g) at 4°C for 1hr. Both supernatants obtained from the first and second extraction were combined. And collagenous material was precipitated by adding 4 M NaCl to a final concentration of 0.75 M. Collagen was separated by centrifugation (16,000 x g) at 4°C for 30 min and dissolved in 0.5 M acetic acid. After centrifuging collagenous solution (30,000 x g) at 4°C for 1 hr, collagen was precipitated again by adding 4 M NaCl as described above. Collagenous material was dissolved in 0.5M acetic acid, dialyzed exhaustively against 0.05 M acetic acid and finally lyophilized. Type of collagen was determined after denaturation of samples at 60°C for 30 min by the interrupted gel electrophoresis of Sykes, B. et al. (Biochem. Biophys. Res. Commun. 72, 1472-1480, 1976). To estimate the content of collagen in each sample, hydroxyproline assay by Kivirikko, K.I. et al. (Anal. Biochem. 19, 249-255, 1967) was used after hydrolysis in 6 N HCl for 15hr at 110°C. Stained gels were scanned by densitometer and the ratio of type III to type I collagen was calculated.

Results with Discussion: Aortic collagen taken from rats at 1 and 2 weeks of age showed a small ratio of type III to type I collagen (0.05 - 0.06). However, with advancing age the ratio started to increase from 4 weeks of age, yielding a maximum value of 0.23 at 8 weeks of age. Then the ratio tended to decrease gradually up to 64 weeks of age. On the other hand, the ratio of type III to type I collagen in dermal collagen showed a gradual decrease from the age of 1 week (0.18) to that of 64 weeks (0.09). This age-related decrease in the proportion of type III collagen is consistent with the previous report by Epstein, E.H.Jr. (J. Biol. Chem., 249, 3225-3231, 1974). The blood pressure of DOCA-salt treated rats reached to severely hypertensive level (210 mmHg) after 5 weeks of the DOCA-salt treatment, while the blood pressure of controls remained at the normotensive level (126 mmHg). The relative proportion of type III collagen was increased in the aorta of DOCA-salt hypertensive rats (0.27) as compared to normotensive controls (0.21). The age-related change observed in aortas of normotensive rats was unique and may reflect the developmental process of establishment of the circulatory system. The reason for the increased ratio of aortic type III collagen is unknown. However, it can be speculated that medial smooth muscle cells which are major collagen-producing cells in the blood vessels may be transformed during the process of arteriosclerosis induced by hypertension.