63) Effect of High Fat-cholesterol (HFC) Diet on the Microviscosity and Phospholipid/Cholesterol Ratio of Erythrocytes in Stroke-prone SHR (SHRSP). Yukio Yamori, Kiyoe Ohta*, Ryoichi Horie, Yasuo Nara, Michiya Ohtaka, and Akira Ooshima. Japan Stroke Prevention Center, Department of Pathology, Shimane Medical University, Izumo 693 and Department of Pathology, Faculty of Medicine, Kyoto University, Kyoto 606*.

INTRODUCTION Ion permeability of erythrocyte membrane was shown to be elevated in SHRSP as compared with that in SHRSR (Yamori et al.: Jap Heart J 18:604, 1977). This experimental system for an *in vitro* approach to membrane permeability seems to be the first step for investigating the essential difference between SHRSP and SHRSR at the cellular level. In the present study, the microviscosity of ghost erythrocytes and platelets was estimated in SHRSP either fed on a normal diet or a high fat cholesterol diet in comparison with Wistar-Kyoto (WK). The numbers and volume distributions of erythrocytes and platelets were also estimated in WK, SHRSR and SHRSP.

MATERIALS AND METHODS (1) Samples: (a) Platelet: Blood was collected from the femoral vein using sterile plastic syringes containing sodium citrate as an anticoagulant and centrifuged for 10 min at 1,050 rpm. The upper layer was washed 3 times by saline. (b) Red Blood cell ghost: The sediment was centrifuged at 3000 rpm for 10 min. The erythrocyte was washed with 20 mM 0s. phosphate buffer (pH 7.4) 3 times and each time centrifuged at 20,000 x g for 20 min at 5°C. (2) DPH dispersion for the labelling of cells: 0.1 mg of the 1, 6-diphenyl 1, 3, 5-hexatriene (DPH) stock solution (2×10^{-3} M in tetrahydrofuran) was injected into 100 ml saline, and then vigorously stirred for 10 min at room temperature. For the labelling of biological samples, 2 ml of DPH dispersion was mixed with 2 ml of samples and incubated for 30 min at room temperature. (3) The cells were washed once or twice by centrifugation, resuspend in saline at the original concentration and counted by a Coulter Counter. The same amount of the material diluted in the buffer without DPH was used as control for each sample. (4) The degree of fluorescence polarization of the labelled sample was measured at a given and constant temperature with an Elscint Microviscosimeter. (5) The volume distribution and the number of platelets and red blood cells were recorded by a Coulter Counter.

RESULTS WITH DISCUSSION The degree of fluorescence polarization in WK was higher as compared to SHRSR and SHRSP in both ghost erythrocytes and platelets. That is, the membrane of SHRSP and SHRSR showed a greater fluidity than that in WK. The ratio of cholesterol to phospholipid of surface membrane of platelets was greater in 6-month-old WK (1.08 ± 0.003) in comparison with SHRSR (1.03 ± 0.006) and SHRSP (1.02 ± 0.006) ($P < 0.05$). The same was true in ghost erythrocytes of WK (1.39 ± 0.03) as compared to SHRSR (1.24 ± 0.02) and SHRSP (1.17 ± 0.08). These results demonstrate a difference among SHRSP, SHRSR and WK in the microviscosity of ghost erythrocyte and platelet surface membrane lipid layer that is related directly to the difference in the level of their membrane cholesterol. Moreover, it was found that the membrane fluidity in platelets was decreased when fed on high cholesterol diet. As may be expected, the incorporation of cholesterol increases the microviscosity of the surface membrane lipid layer of SHRSR and SHRSP to a level that is similar to that of WK. This finding indicates that the incorporation of cholesterol into the surface membrane of ghost erythrocytes and platelets *in vivo* can be conducted, in principle, by lipoproteins, provided that the cholesterol level in the blood serum is high enough. Low level of endogenous serum cholesterol, which may be due to a metabolic defect, may be one of the factors that confine the influx of serum cholesterol. The analysis of the number and volume distribution of platelets demonstrated that platelets of SHR, especially SHRSP were larger than those of WK. This alteration in platelet volume may be related to the essential difference in membrane characteristics between SHRSR and SHRSP.