Changes in Cell Age Distribution of Erythrocytes and in Glutathione Peroxidase Activity along with Aging of SHRSP. Tetsuo Murakami*, Kumiko Takemori*, Hajime Yoshizumi* and Hiroyuki Ito**. *Department of Food Science and Nutrition, Faculty of Agriculture, Kinki University, Nara 631. **Research Institute of Hypertension, Kinki University, Osaka 589.

The tracing of glutathione peroxidase (GSH-Px) activity in erythrocytes has been shown to be useful as an index for judging progress of cerebral stroke biochemically (Murakami et al.; Biosci. Biotech. Biochem., 59, 1459, 1995). In the present experiments, for elucidation of the mechanism of fall of GSH-Px activity at cerebral stroke, erythrocytes were fractionated by density gradient centrifugal method by using Percoll solution, and changes of GSH-Px activity in erythrocyte fractions with different in densities reflecting degrees of aging were investigated.

Methods Male SHRSP at various ages raised in our laboratory were used throughout experiments, and were fed commercial pellet diet (Funabashi SP). The diet and drinking water were given ad libitum. The blood was collected from abdominal aorta of rat under anesthesia with ether. Blood samples were suspended in physiological saline, and centrifuged (3,000rpm, 5min, 4°C). By the modified method of Vettore et al., erythrocytes were fractionated by utilizing density difference by the density gradient centrifugal method with Percoll and 1.5M-NaCl. The density gradient system used in fractionation employed Percoll solution with a specific gravity of 1.212. For fractionation, 1.0ml of packed cells were carefully layered on this gradient system and centrifuged for 10min 12,000rpm and 4°C. After centrifugal separation, 3.5ml of upper layer of the round bottomed centrifuge tube was separated as the low density erythrocyte fraction (juvenile erythrocytes) and 3.5ml of lower layer as high density erythrocyte fraction (aged erythrocytes). Bone marrow was obtained from the femur and tibia of rat. To prepare crude enzyme solution from bone marrow, 50mM phosphate buffer solution (pH 7.0) was added, and the mixture was homogenized. The GSH-Px activity was assayed by the method of Whanger et al. Hemoglobin (Hb) was measured by using a hemoglobin measuring kit (Wako Pure Chemical Co.).

Results (1) The distribution rate of juvenile erythrocytes and aged erythrocytes in SHRSP was 4:1 at 5 weeks of age, and the rate of the aged erythrocytes increased along with aging. However, independently of the changing in distribution rate, there was no change in the GSH-Px activity between the 2 fractions. On the other hand, in 30 weeks-old SHRSP, the GSH-Px activity in the aged erythrocyte fraction was lowered, in proportion to the increase of the distribution rate of aged erythrocyte fraction. (2) In SHRSP having cerebral stroke, the rate of juvenile erythrocytes and aged erythrocytes was 1:9, showing that the juvenile erythrocyte fraction decreased and the aged erythrocyte fraction increased markedly. The GSH-Px activity in the aged erythrocyte fraction was 13.3 ± 1.0 units/mgHb which was hardly different from the value before fractionation (11.5 ± 2.5 units/mgHb). In the juvenile erythrocyte fraction, however, the activity was 18.8 ± 2.0 units/mgHb, which was higher than the activity before fractionation. (3) The GSH-Px activity in bone marrow was markedly low as compared with the activity in erythrocytes, but the change in GSH-Px activity along with aging was similar to that in erythrocytes. At cerebral stroke, there was no change in the activity in the bone marrow.

Discussion It is clarified in the present experiments that the fall of erythrocyte GSH-Px activity observed at cerebral stroke is due to decrease of juvenile erythrocytes and increase of aged erythrocytes the latter of which have lowered activity. However, which nothing definite is known about the relation between the fall of GSH-Px activity and the disorder in cerebral tissue, it appears that the activity fall causes at least functional and structural change in erythrocytes. Thus interfere with the delivery of oxygen to peripheral tissues may trigger oxidation stress in cerebral tissues.