18) Morphological Study of Sarcolemmal Permeability caused by Lipid Peroxidation in Hypertrophied SHR Myocardium. Mikinori Torii, Hiroyuki Ito and Tsuneyuki Suzuki. Department of Pathology, Kinki University School of Medicine, Osaka-fu 589.

We have been examining hypertrophied SHR myocardium in order to elucidate the pathogenesis of myocardial vulnerability and have previously reported that the anti-oxidative potential of SHR myocardium might possibly be insufficient, thereby resulting in a potential for membrane system lipid peroxidation both in vitro and in vivo. In order to morphologically elucidate membrane abnormality in SHR myocardium, tracers were used to examine membrane permeability after administration of doxorubicin and the results were compared to those for normotensive WKY.

Twenty-four 16-week-old male SHR and WKY, divided into 3 groups respectively were used. Doxorubicin(DOX, 20 mg/kg BW) was given intravenously to two groups at a rate of 5 ml/min and saline was given the third group as control. On the 1st and 4th day following DOX administration, tracers of various molecular weights were given under pentobarbital sodium anesthesia and the hearts were examined as follows: 1) FITC-dextran(10 mg/kg BW, MW 150,000, Sigma) was given i.v. and the hearts excised two hours later. The left ventricles were sliced horizontally at the middle level and 7 μ m frozen cross sections were obtained and observed under a Nikon fluorescence microscope. 2) Horseradish peroxidase(HRP) was given i.v.(10 mg/100g BW, type II, Sigma) under anesthesia. After 10 min, the hearts were fixed by retrograde perfusion with Krebs-Henseleit buffer(pH7.4, 37°C) and with successive Karnovsky fixative at an infusion rate of 8.0 ml/min through thoracic aorta using a Perista mini-pump R. 7 μ m cross sections were immersed in a 1% 3,3'-diaminobenzene tetrachloride solution for 10 min as described by Younge et al.(Am J Pathol 134:171,1989) and routinely processed for light microscopy. Some parts of the left ventricular free walls were postfixed in 1% OsO₄ and embedded in Epon 812. Ultrathin sections were stained with lead citrate and observed under a JEM-100 CX electron microscope.

Dextran fluorescence was detected in the papillary muscle and subendocardial myocytes only in SHR myocardium at day 4. In comparison, no fluorescence was found in the myocardium of the saline groups, of SHR at day 1 or of WKY at day 1 or day 4. On the other hand, HRP were found focally in the papillary muscle and in a part of the subendocardial myocytes of SHR myocardium on day 1. In SHR myocardium, cells containing HRP increased in number and were widely distributed in the ventricular wall on day 4, in comparison to day 1. In some of these cells, obvious microscopic examination revealed osmophilic and granular HRP in the extracellular space both in SHR and in WKY in the control group. In SHR myocardium, HRP was also found in the sarcoplasmic reticulum and in intercalated discs on day 1, then were seen much more frequently on day 4. Moreover, ample HRP were seen throughout cytoplasm of severely injured myocytes of SHR at day 4. Although HRP were seen in the extracellular space in WKY myocardium, no granules were observed within the myocyte at either day 1 or day 4.

These results clearly indicate a marked increase in membrane permeability in SHR myocardium. This could be due to progressive membrane structure changes which, in turn, might be caused by lipid peroxidation following doxorubicin administration. Morphological findings in the present experiment support our previous biochemical data showing the membrane vulnerability of SHR myocardium.