It is well known that the proteins and membranes are damaged by lipid peroxidation[10]. We observed that upon exposure of rabbit to high concentration of oxygen, lipid peroxides in the retina were increased with the decrease in electroretinogram and morphological change of the retina[11]. When chick embryo was exposed to high concentration of oxygen, lipid peroxides in the blood were increased and then those in the retina were increased accompanying with its morphological change[12]. These results indicate that the increase in lipid peroxides induces the degeneration of retinal cells. Considering these results, it was supposed that the mechanism of the presently observed deleterious effect of chinoform-ferric chelate on retinal neuroblasts would be due to the initiation of lipid peroxidation by iron transferred into the lipid layer of membranes of the cells by hydrophobic nature of chinoform. However, the fine mechanism should await further investigation, which is under progress in our laboratory.

There was remarkable difference in susceptibility to the toxicity of chinoform-ferric chelate between neuroblasts and epithelial cells. Our preliminary experiment showed that, when 50 μM of chinoform-ferric chelate was added to the culture medium of cerebrum, skeletal muscle and skin fibroblast cells, nerve cells and skeletal muscle cells degenerated but not other cells. Moreover, when linoleic acid hydroperoxide was directly added to the culture medium of neural retinal cells, neuroblasts were more sensitive to the hydroperoxide than epithelial cells. From these observations, it is suggested that the cells with excitable membrane such as nerve and muscle cells were more sensitive to the lipid peroxides than other non-excitable cells. Recently, Prasad et al.[13] reported that neuroblastoma cells were more sensitive to sodium ascorbate than glioma cells by the criterion of growth inhibition due to cell death and reduction of cell division. They suggested that sodium ascorbate led to the production of H_2O_2 which in turn induced lipid peroxidation. Accordingly, the difference in the susceptibility would be related to the mechanism of formation and elimination of lipid peroxides in these cells.

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Smooth muscle cells in 'venous patches' grafted into the rat common carotid artery. A structural study[1]

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Summary. In the 2nd week after surgery, well differentiated smooth muscle cells (SMC) were evident in the walls of venous patches in rat common carotid artery. Gap junctions were the only type of intercellular junction observed between SMC in the present study.

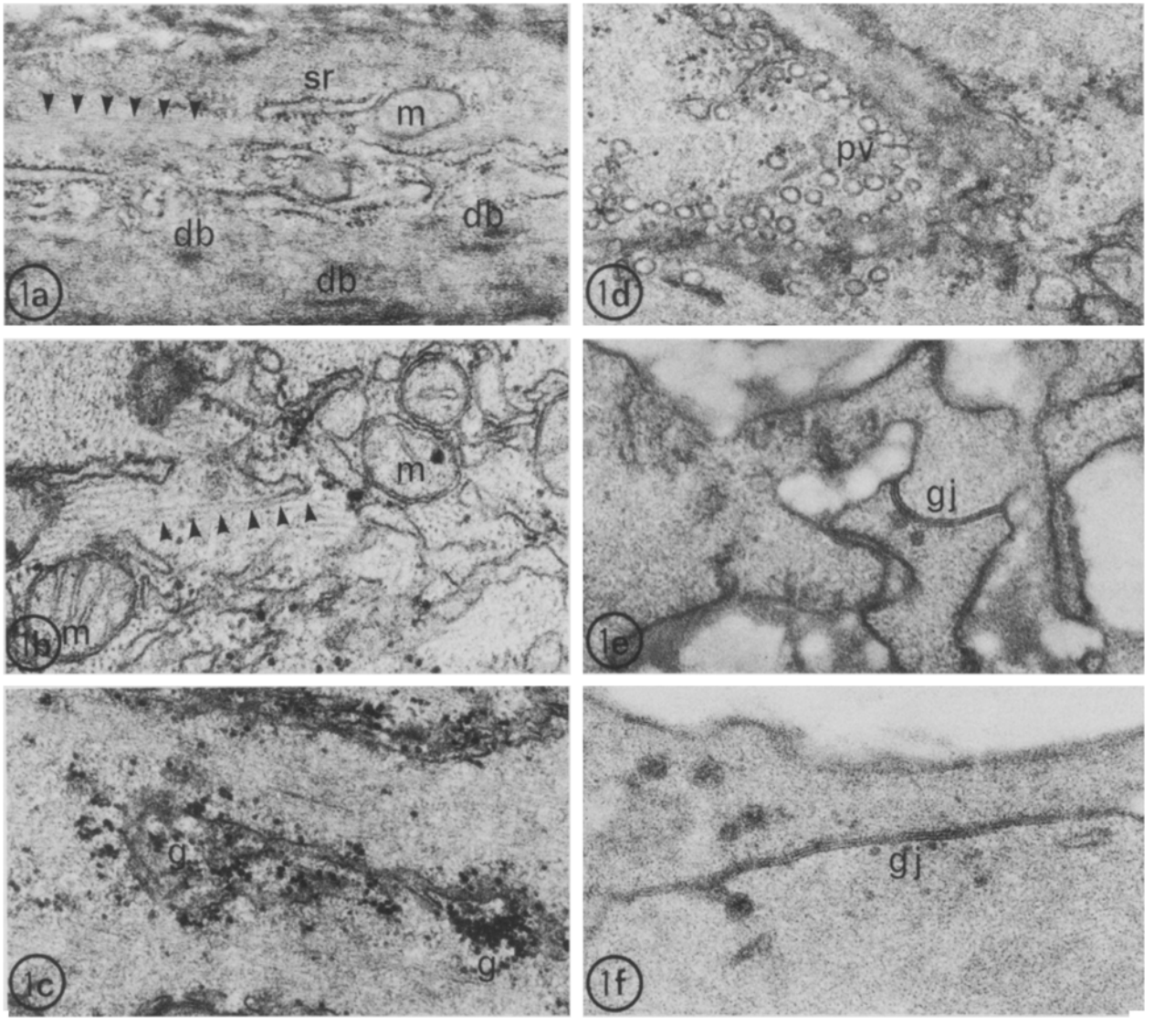
In the rat saphenous vein[4] a basal lamina separates the endothelium from the smooth muscle cells, which are distributed in 2 concentric layers perpendicular to the main axis of the vessel. Smooth muscle cells (SMC) are interconnected by gap junctions. Elastic fibers, parallel to the blood flow, are scattered between SMC. The most external part of the venous wall is formed by 3 fibroblast layers concentrically oriented towards the vascular lumen. Collagen fibers are present, running along the vein axis between fibroblasts. Inmediately following grafting of an autologous strip of saphenous vein (venous patch) into the common carotid artery in the rats, venous tissues undergo great alterations in response to ischemia and surgical trauma: the endothelium desappears by desquemation and the SMC and fibroblasts degenerate. After surgery, the wall of the venous patch is formed by cellular remains at different degenerating stages and a red thrombus.

Muscular hyperplasia is a constant phenomenon in the walls of small vessels submitted to different microsurgical techniques[3]. The origin of the smooth muscle cells (SMC) which are present in the fibromuscular hyperplasia of small vessels operated on with microsurgical techniques has not yet been explained, and the mechanism of muscular proliferation is also controversial. In grafted venous patches in the rat common carotid artery, a fibromuscular hyperplasia is evident at the end of the 1st week after surgery[4]. The purpose of the present paper was the ultrastructural study of SMC in venous patches grafted into the right common carotid artery of the rat during the period when fibromuscular hyperplasia is detectable with a light microscope.

Material and methods. In this study, 35 Wistar rats, averaging 200 g were used. With the help of an operating microscope, a 10–12 mm long segment of the saphenous vein was extirpated and longitudinally incised on a rectangular strip that was immediately placed in a saline solution. The right common carotid artery was then dissected and clamped as caudally and cranially as possible with Scoville clips, and a 5–8 mm longitudinal arteriotomy was performed. The autologous venous patch was grafted onto the edges of the carotid incision with interrupted sutures of 10-0 Nylon monofilament. 10–20 days after surgery, the animals were anaesthetized with i.p. Nembutal and fixed by

retrograde perfusion via the abdominal aorta according to a slightly modified version of the method of Forssmann et al.[5]. Several 0.5 × 1.0 mm cylindrical segments of the operated artery were cut with a razor blade under a stereomicroscope, rinsed overnight in 0.1 M Na cacodylate buffer and post-fixed with osmium tetroxide in an 0.1 M Na cacodylate buffer and dehydrated with ethanol and propylene oxide. These segments were embedded in Epon 812[6] using flat moulds. Ultrathin serial sections of venous patch were cut with an LKB IV ultramicrotome, stained with uranyl acetate and lead citrate[7] and examined under a Phillips EM 301 electron microscope.

Results and discussion. At the beginning of the 2nd week, the venous patch grafted into the rat common carotid artery contains SMC in different stages of differentiation[4]. At the end of the 2nd week, the venous patch wall shows differentiated SMC. The cytoplasmic organelles are confined in the central part of sarcoplasm (fig., a and b). The remainder of the sarcosplasm is occupied by myofilaments which usually appear thin (fig. a). Characteristic dense bodies are distributed throughout the sarcoplasm (fig. a). The glycogen appears as β-particles distributed throughout the sarcoplasm mainly in its central part (fig. c). Caveolae intracellulares, surface vesicles, pynocytotic vesicles or plasmalemmal vesicles[8–16] are distributed in groups separated from each other by regions of smooth membrane (fig. d). The plasmalemmal vesicles are considered to be equivalent to the T-tubules of striated muscle[13,14,17] and together with sarcoplasmic reticulum and mitochondria, are supposed to provide a functional entity for ion transport[15,18,19]. Gap junctions are present in ultrathin sections of SMC in the venous patch (fig. e and f). The regions of specialized membrane exhibit the septilaminar appearance characteristic of gap junctions. These junctions vary in diameter from 0.30 (fig. e) to 1.5 µm (fig. f). Gap junctions are often present when a protrusion of one SMC comes into close contact with another (fig. e). Occasionally, a large surface area of 2 SMC sarcolemma is in close contact through a gap junction (fig. f). Tight junctions are not seen between muscle cells in venous patch. The vascular SMC are interconnected by gap



Electron micrographs from SMC ultrathin sections of venous patch wall in rats 12 days after surgery (a–d) and 20 days after surgery (e–f). *a* Longitudinal section of SMC; sr, sarcoplasmic reticulum; db, dense bodies; m, mitochondria; arrows: myofilaments, × 28,400. *b* m, mitochondria; arrows: myofilaments; × 57,500. *c* g, glycogen, × 28,400. *d* pynocytotic vesicles in sarcolemma of SMC, × 43,700. *e* and *f*, gj, gap junctions between 2 SMC, × 57,500.

junctions (communicating junction, nexus)[8,20-29]. Tight junctions between arterial SMC have been demonstrated with a freeze-etching study only by Tani et al.[16] in arteries of Willis' circle in adult dogs. Gap junctions are thought to be composed of intercellular channels that mediate electrotonic coupling and the movement of ions and small molecules between cells (for review, see Bennett and Goodenough[30]). Details of the number and arrangement of gap junctions in smooth muscle effector bundles in different organs and their relation to density of innervation have not yet been determined. In conclusion, at the moment when muscular hyperplasia begins, the venous patch wall shows SMC ultrastructurally similar to the SMC of the wall of the host common carotid artery. The origin of the SMC observed in the venous patch wall has to be explored further using thymidine-labeling experiments.

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Gap junctions in myo-endothelial bridges of rabbit carotid arteries[1]

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Summary. Myo-endothelial contacts have been studied in rabbit carotid arteries. Structures, featuring as gap junctions, have been depicted by means of lanthanum hydroxide.

Cytoplasmic bridges passing through fenestrae of internal elastic lamina and apparently connecting endothelium to smooth muscle cells were first detected in coronary arteries by Thoma[2]. Similar structures have been revealed by light and electron microscopy in arteries, arterioles, precapillary sphincters and capillaries[3-9]. Nevertheless, their exact nature and functional significance have not yet been established. It is not clear whether these myo-endothelial bridges form specific contacts between the 2 cytotypes[9] or represent an anchorage of endothelium to the underlying vascular wall. Since different types of myo-endothelial bridges have been detected in large elastic arteries[10,11] we have chosen to investigate the ultrastructure of the intercellular contacts they establish between smooth muscle and endothelial cells in rabbit carotids by means of lanthanum hydroxide. With this technique we have detected pentalaminar structures featuring as gap junctions at sites where opposing membranes, belonging to a smooth muscle and to an endothelial cell, are joined very closely. The morphological and functional significance of these structures will be briefly discussed.

Materials and methods. New Zealand rabbits of both sexes weighing 2-3 kg were anesthetized with pentobarbital. A longitudinal laparatomy was made and a polyethylene catheter inserted into the abdominal aorta up to its thoracic tract. Then a perfusion with oxygenated Krebs-Ringer bicarbonate was started and blood washed out through the severed jugular veins that had been previously exposed. After the blood had been completely eluted, carotids were fixed with 2% gluteraldehyde solution buffered at pH 7.2 with 0.1 cacodylate for 20 min. All solutions were perfused at controlled physiological pressure and temperature. The samples were washed overnight, then immersed for 2 h at room temperature in a solution containing 1% osmium tetroxide and 1% lanthanum hydroxide. Then the tissue was dehydrated in an ascending series of alcohols and lanthanum hydroxide was added to all fluids up to the absolute alcohol. Once embedded in Epon 812, specimens were cut with the aid of an LKB ultramicrotom. Series of 10-15 thin sections with or without lead citrate counterstaining were collected and observed under a Philips 301 electron microscope.

Results. By light micoroscopy, cytoplasmic bridges, passing through the fenestrae of internal elastic lamina, were seen frequently, connecting the endothelium to the first row of smooth muscle in the underlying media. In random sections