RESISTANCE OF THE BLOOD-NERVE BARRIER IN PERIPHERAL NERVE TO MECHANICAL COMPRESSION; CHANGES IN THE VASCULAR PERMEABILITY OF THE PERIPHERAL NERVE TRUNK, DORSAL ROOT GANGLIA AND NERVE ROOT

Introduction
Disturbance of blood flow and intraneural edema due to mechanical compression by the surrounding tissues appear to be closely involved in the appearance of neurological symptoms when entrapment neuropathy affects the peripheral nerves. Generally, the peripheral nerve has the perineurium as a diffusion barrier and the blood-nerve barrier is also existing in the endothelial cells of the endoneurial microvessels. These barriers of the peripheral nerve protect and maintain the nerve fibers in a constant environment. (5) We studied the integrity of the blood-nerve barrier in peripheral nerves using clips to compress the dorsal root ganglion containing the primary sensory neurons, the spinal nerve root through which the central branches of these neurons pass, and the peripheral nerve trunk through which the peripheral branches run.

Materials and Methods
Adult mongrel dogs were used in this study. The 7th lumbar nerve root was used for the dorsal root ganglion(N=20) and nerve root compression experiments(N=20), and the median nerve(N=20), which has of virtually the same diameter as the nerve root, was used for the peripheral nerve trunk compression experiment. Compression was achieved by applying vascular clips with a pressure of 7.5, 15, 30, or 60 gram force (gf) when opened to 2 mm. The uncompressed side was used as the control in all experiments. For median nerve compression, an incision was made in the skin of the lower one-third of the foreleg under general anesthesia, the median nerve was exposed, and a clip was applied for 1 hour. For compression of the dorsal root ganglion, laminectomy of the 7th lumbar vertebra was performed, and the dorsal root ganglion of the 7th lumbar nerve was compressed with a clip for 1 hour. For nerve root compression, laminectomy was performed by the same method, and the 7th lumbar nerve root was compressed midway between the dura mater and the dorsal root ganglion for 1 hour. To examine blood-nerve barrier function, Evans blue-albumin (EBA) was injected intravenously after removal of the clips, and the animals were killed 1 hour later. The nerves resected from each animal were fixed in formalin for 24 hours and cut into 20-μm sections with a cryostat. Then the nerves were embedded in glycerin and examine under a fluorescence microscope.

Results
On fluorescence microscopy of the control specimens without clipping, red fluorescent EBA was confined to the blood vessels in the median nerve and nerve root, but there was leakage of EBA into the endoneurial space in the dorsal root ganglion. In other words, there was a blood-nerve barrier in the blood vessels of the median nerve and nerve root, but no such barrier in the dorsal root ganglion vessels. After compression of the median nerve at 7.5 or 15 gf, EBA was still confined to the blood vessels. At 30 gf, congestion of blood flow was apparent, but the blood-nerve barrier remained intact. After compression at 60 gf, however, there was loss of the integrity of the blood-nerve barrier, resulting in extravascular leakage of EBA. After compression at 7.5 gf, the dorsal root ganglion exhibited no increase of extravascular EBA compared to the uncompressed side. However, extravascular leakage of EBA was greater than on the uncompressed side and neuronal separation due to edema was present in 3 out of 5 animals after compression at 15 gf and in all animals after compression at 30 and 60 gf. Compression of the nerve root for 1 hour at 30 gf or more caused, marked intraradicular edema in all animals. After compression at 15 gf, only some extravascular escape of EBA at the nerve root margin was observed in 1 out of 5 animals, and the blood-nerve barrier remained intact at 7.5 gf.

Discussion
In 1941, Manery and Bale reported the occurrence of blood-nerve barrier in the peripheral nerve similarly to the blood-brain barrier in the central nervous system.(4) There after, several worker have demonstrated deep involvement of breakage of this blood-nerve barrier in intraneural edema that induces neural dysfunction by basic studies. Capillaries are generally classified as continuous, fenestrated, or discontinuous, based on the morphology of the endothelium. (1,6) Continuous capillaries can be divided into brain capillaries, which have tight junctions between their cells, and muscle capillaries with gap junctions. Brain capillaries are found chiefly in nerves, and, unlike the continuous capillaries seen in muscle tissue, have a blood-nerve barrier that maintains a constant environment inside the nerve. Fenestrated capillaries have fenestrae approximately 700 in diameter in the endothelium, and are found in tissues with a high metabolism, such as endocrine organs, the kidneys, the intestinal mucosa, and the dorsal root ganglia. Discontinuous capillaries have interendothelial gaps several microns in size, and these are also found in the bone marrow and the spleen. In addition, each of these vessel types exhibits organ specificity. In peripheral nerves, the internal environment is kept constant by the blood-nerve barrier of the vascular endothelium, as well as by the perineurium, which acts as a diffusion barrier. Generally, intraneural edema caused by mechanical compression is a vasogenic edema induced by congesting venous blood flow and develops owing to accelerated permeability of capillaries in the endoneurial space, that is, leaking of water and macromolecular substances such as protein from blood vessels into endoneurial space. Then, leakage of edematous fluid into the endoneurial space elevates endoneurial pressure, and appears to induce demyelination that is deeply involved in onset of neuropathy. (3,7) The dorsal root ganglion still has a diffusion barrier, but there is no blood-nerve barrier. The nerve root, which passes through the subarachnoid space, has a blood-nerve barrier, but the root sheath is thin and weak at this site and does not function as a diffusion barrier, thereby allowing cerebrospinal fluid to readily flow in and out. (2) Compression of the median nerve at 60 gf, the nerve root at 30 gf or more, and the dorsal root ganglion at 15 gf or more resulted in marked leakage of EBA into the endoneurial space and appreciable edema. Structural differences in the nerve sheath and the vascular endothelial cells at each site are thought to have played a major role in these differences. The results indicate that the dorsal root ganglion is particularly susceptible to damage by mechanical compression in patients with spinal canal stenosis and disc herniation.

Conclusion
The blood-nerve barrier showed the greatest resistance to mechanical compression in the peripheral nerve trunk (median nerve), followed by the nerve root and then the dorsal root ganglion.

References