Changes in Histological Structure and Microvasculature of the Rat Tongue after Transection of the Hypoglossal Nerve

By

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Summary: This investigation was made on sequential changes in the tongue caused by unilateral transection of the hypoglossal nerve in the rat. These changes were examined on the basis of gross inspection of the lingual dorsal surface, histological aspects of the intrinsic muscle and interstitial tissues, morphological changes in the capillary loops of the filiform papillae utilizing plastic microcorrosion casts, and blood flux and number of erythrocytes in the lingual dorsum as determined by laser Doppler flowmetry. The period of examination following denervation of the hypoglossal nerve was divided into 3–5 days, 1, 2–3, 4–5, 6–7 and 66 weeks. The initial sign of histological change was an edematous change that later developed to atrophic change of the muscular element. This pathological change caused a change in volume on the lesion side of the tongue and finally formed a depression on the dorsal surface and a scallop-like lateral margin. Four to five weeks after denervation, the lesion half swelled, and its lingual apex elongated forward with a coving of the lingual median groove. The filiform papillae showed decreased keratinization, and interpapillary intervals were somewhat widened. Capillary loops in the papillae began to change, showing slight twistings at the tips of loops. Additionally, bulging, coiling and tortuosity were observed on both crura. The loops showed more complicated transformation, and little recovery was observed even 66 weeks after denervation. Blood flux in the dorsal mucosa decreased very slowly after a slight increase. This process seemed to be in proportion to the transformational complexity of the capillary loops in the filiform papillae. In conclusion, unilateral transection of the hypoglossal nerve, which is composed almost entirely of efferent neurons, caused edema and atrophic change in the intrinsic lingual muscle fibers with interstitial fibrosis in the lesion half of the tongue. Capillary loops in the filiform papillae were transformed markedly and successively by transection of the hypoglossal vasomotor neuron together with a slight decrease in blood flow in the dorsal mucosa. None of these changes recovered even at 66 weeks after transection.

Denervation caused by actual disconnection or blockade of axoplasmic transport has long been studied as an experimental procedure (Tower, 1939). Useful clinical signs after denervation are degenerative changes of the muscle element and interstitial tissue, both structurally and functionally (Albuquerque et al. 1970). This is a neurotrophic influence on muscle (Guth, 1968; Gutmann, 1976). Simultaneously, transection of vasomotor neurons passing in company with somatomotor neurons affects the microvascular architecture supplying the component tissue in an organ. This is revealed as morphological changes in the blood capillary system. Although the putative or actual mechanism of denervation is variable and occurs in sequence, few investigations have been carried out on changes in the microvasculature.

The present studies consist of detailed examination of sequential changes in the tongue after transection of the unilateral hypoglossal nerve in the rat. The unilateral half of the tongue is denervated essentially of all somatomotor neurons and almost all vasomotor neurons. This survey was carried on multiple sequential changes, that is, surface changes in the lingual dorsum on the denervated side, changes in the filiform papillae and blood capillary loop in each papilla, utilizing plastic microcorrosion casts by scanning electron microscopy, and the application of laser Doppler flowmetry, which can assess for a microcirculatory response on the denervated lingual dorsum.

Materials and Methods

1. Materials
A total of 122 healthy Wistar rats (body weight 270–340 gm) were used for this study. Eighty-three
rats of them were assigned to experimental groups and nine rats to a control group. Thirty rats were used for the laser Doppler flowmetry (LDF) examination during the experimental period. The rats in the experimental groups were assigned to the respective procedures shown in Table 1.

2. Transection of the hypoglossal nerve

A unilateral (left) denervation of the hypoglossal nerve was performed in 113 rats as follows: The rats were anesthetized intraperitoneally by pentobarbital sodium (Nembutal®; 40 mg/kg body weight), and an incision was made on the skin of the left retromandibular region. The left hypoglossal nerve was clearly exposed including the stylohyoideus, digastricus muscles and the medial border of the intermediate tendon of the digastricus muscle (Fig. 1). A nerve stimulator (49B-716 Model-180, Tokki, Japan) was used to confirm the hypoglossal nerve. Then the exposed nerve was isolated from surrounding tissues and a piece (3 mm in length) of the nerve was removed after ligation at both the proximal and distal ends. The incision was sutured routinely. The animals were fed using solid food for laboratory rats.

The rats in both groups were euthanized by an overdose of Nembutal® given intraperitoneally at 3 or 5 days, and every week from 1 to 7 weeks after the unilateral denervation. In the control group, the wound was sutured without any denervation.

3. Preparation of specimens

The materials obtained in the above nine periods were prepared as follows (Table 1):
(1) Macroscopic specimens of the lingual dorsum
Prior to sacrifice of the denervated rats, the lingual dorsum was carefully observed under a binocular microscope and photographed.
(2) Histological slides
Twenty-seven denervated rats were anesthetized and fixed by the perfusion of 10% formaldehyde via the ascending aorta. The tongue was dissected out carefully, postfixed in the same solution for 3 hrs, embedded in celloidin, sectioned serially at 15 μm in the frontal direction and stained with hematoxylin-eosin or Masson-Goldner’s trichrome.

(3) Specimens of the lingual dorsum for a scanning electron microscope
The denervated rats were anesthetized and fixed by perfusion of Karnovsky’s fixative via the ascending aorta. The whole tongue was dissected out and immersed in the same fixative for 72 hrs and washed with buffered phosphoric acid at pH 7.2 (0.1 M). Debris adherent to the mucous surface was washed away with 8N HCl for 15 minutes at room temperature, and specimens were postfixed with 2% osmic acid solution. The materials were freeze-dried with 2-methyl-2-propanol and coated with gold by ion-sputter (JEOL-1100 model) and examined under a JOEL JSM-T300 SEM operated at an accelerating voltage of 5 KV.

(4) Microcorrosion casts
Twenty-nine denervated rats were anesthetized by a method similar to that mentioned above and perfused with heparinized saline solution (300 IU, Green Cross®, APAM) via the femoral vein prior to bleeding to death from the femoral arteries. Acryl plastic was injected into the carotid system via cannulae inserted into the common carotid arteries by the plastic injection method (Ohta et al. 1990). After the injected plastic had been polymerized by keeping it at 50°C for an hour, the whole tongue was treated with 20% NaOCl to obtain microcorrosion casts of the lingual vasculature. The casts were coated with gold for SEM examination.

(5) Blood flow evaluated by laser Doppler
Thirty rats at 1, 3, 5 and 7 weeks after denervation, respectively, were examined with a laser Doppler flowmeter (MBF3D-dual channel model, Moor, England). Under anesthesia similar to that

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Table 1. Numbers of rats used for preparing specimens in control and experimental groups

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LM: light microscopy SEM: Scanning electron microscopy LDF: Laser Doppler flowmetry casts: Microcorrosion casts of microvasculature (): Use for LM and SEM in experimental animal groups *: Successive use for LDF study in each period up to 7 weeks

Total 122
mentioned above, they were laid in the supine position with the mouth open, at room temperature (28°C) and 60% humidity. The head was fixed with a plane standpost that provided less movement of the tongue, and permitted the approach of laser probes on the lingual dorsum, on which a probe with a tongue, and permitted the approach of laser probes position with the mouth open, at room temperature mentioned above, they were laid in the supine values obtained by LDF.

Histology and Microvasculature of the Rat Tongue after Hypoglossal Nerve Transection

Results

1. Control group

Typical aspect of the rat lingual dorsum was symmetrical about the median line in shape, color, moisture and size. Coving of the lingual median groove was not observed (Fig. 4a). Typical microvasculature of the lingual dorsum in the rat as reported by Kuramae (1989) was found. Filiform (conical) papillae were arranged regularly, maintaining 20–30μm intervals between each other with an arc line around the base (Fig. 4b). Lingual dorsal branches of the lingual artery finally ramified to the capillary loops in the lamina propria of the lingual dorsum (Shimizu, 1986). Capillary loops in the filiform papilla were composed of ascending and descending crura (afferent and efferent), which formed the intercrural interval (Fig. 4c, 4d). Each crus was around 30μm in diameter and 70–100μm in height (Fig. 4d, 4e). A symmetrical arrangement in the intrinsic muscle fibers and filiform papillae was seen on frontal sections of the tongue (Fig. 4f).

Laser Doppler flowmetry (LDF) values: blood flux $326.8 \pm 89.3$ units/mm²/sec, concentration of RBC $226.7 \pm 68.5$ unit/mm³ and average RBC speed $15.2 \pm 6.8$ mm/sec on the left side and $319.2 \pm 98.8$ units/mm²/sec on the right side (Figs. 2, 3).

2. Experimental animal groups

Results are described for the experimental periods of 3 days through 7 weeks after unilateral transection of the hypoglossal nerve according to the following periods: 1) 3 to 5 days, 2) 1 week, 3) 2 to 3 weeks, 4) 4 to 5 weeks and 5) 6 to 7 weeks for the following items: gross inspection of the lingual dorsum, filiform papillae, capillary loops in the papillae on microcorrosion casts examined by SEM, general aspect of histological frontal sections of the lingual apex and values obtained by LDF.

1) Three to five days after denervation

Initially, slight edema and smoothing of the dorsal mucosa were observed, forming a slight swelling on the lesion half of the tongue (Figs. 5a, 5b). About 3 days after denervation, these changes and an involuntary, fasciculation-like movement of the lesion side were found. Five days after denervation, a depression was seen on the mucosa (Fig. 5b) as well as on its microcorrosion cast (Fig. 5c). Capillary loops in the filiform papillae revealed the beginning of twistings at the tips of the loops (Figs. 5d, 5e). The twistings became stronger, sometimes forming a pellet-chain-like crus (Fig. 5f), and finally became more complicated in some cases (Figs. 5e, 5g, 5h). On frontal sections of the tongue, intrinsic structures were not disturbed yet on the lesion side, but a slight interstitial edema appeared (Fig. 5h). Blood flux on the lesion side was $382.4 \pm 79.2$ units/mm²/sec, concentration of RBC $269.2 \pm 51.3$ units/mm³ and average RBC speed $16.8 \pm 9.3$ mm/sec (Figs. 2, 3), with no differences between days 3 and 5. Flux increased compared with the previous period.

2) One week after denervation

Swelling of the lingual mucosa caused by edema had disappeared, but in some cases a shallow depression on the mucosa and a scallop-like edge on the lingual lateral margin were found (Fig. 6a). In other cases an involuntary, fasciculation-like movement was seen on the lesion side. Keratinization at the bases of the filiform papillae had decreased (Fig. 6b), but their distribution was not influenced. Capillary loops in the filiform papillae showed twistings and taperings at the tips of loops and tortuosities at the crura (Fig. 6c). In some cases, bridgings were found between crura (Figs. 6d, 6e). Degenerative changes were observed as atrophy in the intrinsic lingual muscle fibers, which had partly disappeared (Fig. 6f). The lingual dorsum swelled in a round surface (Fig. 6f). Blood flux on the lesion side was $352 \pm 68.7$ units/mm²/sec, concentration of RBC $196.7 \pm 56.8$ units/mm³ and average RBC speed $16.0 \pm 7.3$ mm/sec (Figs. 2, 3). Flux diminished slightly compared with the previous period.

3) Two to three weeks after denervation

Depression on the lingual dorsum and scallop-like edge on the lateral margin became more marked (Figs. 7a, 7b). On frontal sections of the lingual aspect, the atrophic change on the lesion side did not seem to advance from the previous period. Filiform papillae showed a slight irregular arrangement, and keratinization around their bases decreased slightly. A circular line around the papillary base became an arc line (Fig. 7d). Transformation of the capillary loops had become so severe that the initial form and height typical of loops had disappeared (Fig. 7c). Especially, duplication and strong tortuosities of
the crura themselves were observed, and the injected plastic sometimes leaked from them (Figs. 7e, 7f). On frontal sections of the lingual apex, the lesion side did not swell but protruded more laterally in some cases (Fig. 7g). However, the lesion half still showed round swelling (Fig. 7h). Namely, the edema had almost disappeared and muscular fibers were arranged irregularly with interstitial fibrosis (Figs. 7g, 7h). Blood flux on the lesion side was $316.5 \pm 65.9$ units/mm$^2$/sec, concentration of RBC $184.5 \pm 59.7$ units/mm$^3$, and average RBC speed $14.7 \pm 6.2$ mm/sec (Figs. 2, 3). All these values were the lowest in the experimental period.

4) Four to five weeks after denervation

Upon a gross inspection of the lingual dorsum, the mucosa showed pale and edematous change. The lesion half swelled markedly in volume, and its apex elongated forward beyond the level of the nonlesion half. The lingual median groove coved beyond the level of the original median line (Figs. 8a, 8b). The dorson of the lesion half was different from the nonlesion half in form and size (Fig. 8b).

Bases of the filiform papillae had thinned slightly due to decreased keratinization. The papillae appeared as thin pyramids without an arc line around the base (Fig. 8c). The capillary loops showed more complicated aspects since all the previous transformation had advanced, i.e., strong tortuosity, coiling and bulging. The original form of the capillary loop had disappeared entirely (Fig. 8d). Transformation of the loop in this period was represented by changes in capillary caliber, irregularly duplicated crura, widening of the intercrural intervals and leakage of the injected plastic (Figs. 8e, 8f). Swelling of the lesion half formed a forward protrusion owing to the increasing edema. Interstitial fibrosis was found between atrophic muscle fibers arranged irregularly (Figs. 8g, 8h). Blood flux on the lesion side was $324.6 \pm 71.5$ units/mm$^2$/sec, concentration of RBC $191.6 \pm 61.4$ units/mm$^3$, and average RBC speed $15.1 \pm 8.4$ mm/sec (Figs. 2, 3). Flux recovered almost to the control level.

5) Six to seven weeks and 66 weeks after denervation

Elongation or protrusion of the lingual apex on the lesion side and a scallop-like edge on the lateral margin were still observed, similar to the previous week (Figs. 9a, 9b). Depressions on the lingual dorsum increased in number (Fig. 9a). In a few cases, slight ulceration had formed on the lateral margin (Fig. 9b). Filiform papillae were decreased in height owing to abrasions of the papillae and decreased keratinization at their bases (Fig. 9c). Capillary loops in the filiform papillae became more complicated, appearing as cluster-like or glomerular in form as a result of coilings and bulgings (Fig. 9e) in many cases, and twisting and intercrural bridging (Figs. 9d, 9e, 9f). On frontal sections of the lingual apex, there was no large-scale edema, but interstitial fibrosis was observed between irregularly arranged muscle fibers inside the aponeurosis (Figs. 9g, 9h). Blood flux on the lesion side was $336.3 \pm 82.6$ units/mm$^2$/sec, concentration of RBC $210.7 \pm 73.5$ units/mm$^3$, and average RBC speed $15.8 \pm 10.3$ mm/sec (Figs. 2, 3). Flux increased very slightly.

Only two rats bred for 66 weeks after denervation still showed the characteristics (Figs. 10a, 10b) similar to those at 6–7 weeks (see above).

Discussion

Unilateral transection of the hypoglossal nerve in the present experiment was performed immediately at the entrance into the tongue, that is, the somatomotor neurons which control the intrinsic lingual muscles and the autonomic neurons, especially vasomotor neurons, were cut off. Accordingly, they influenced peripheral blood capillaries and blood stream in the dorsal lingual mucosa, the muscles and the dorsal mucosa itself.

1) Changes in the lingual tissue and dorsal surface

In the initial stage, that is, a few days after transection of the hypoglossal nerve, slight edema and involuntary, fasciculation-like movement are found on the lesion side. This movement usually disappears after one week (Salafsky et al. 1968), but it may occur with the increase in blood stream that comes with the edema and smoothing of the lingual dorsal mucosa at 3 to 5 days. However, in some cases the denervated rats show a slight atrophic change (Goldberg, 1969; Stewart et al. 1972) in the dorsum. One week after transection, edema on the lesion side expands widely in the connective tissue layer between the dorsal epithelium and aponeurosis and forms depressions on the dorsal mucosa and a scallop-like edge on the lingual lateral margin. Such edema may be caused by structural differences in location between areas superficial to and inside the lingual aponeurosis. Coving of the lingual median groove toward the nonlesion side and forward elongation of the lesion half occur with edematous swelling of the dorsum on the lesion half and constriction on the genioglossus muscle or the opposite, nonlesion side. Paling of the dorsum at 4 to 5 weeks after transection that was observed in all experimented rats may be caused by edema and a decrease in the blood stream. Even in two experimental rats unexpectedly bred for 66 weeks after transection, all the changes mentioned above remained without any signs of recovery.
(Figs. 10a, 10b). When a motor neuron innervating a muscle fiber is cut, both structures should fall into atrophic degeneration. Atrophy of a muscle fiber makes it thinner due to a severe decrease in its structural protein (Sugita and Osame, 1976), while fibrosis occurs in the interstitial tissue. A quantitative decrease in muscle protein is found 5 days to one month after transection of the motor neurons, and this levels off about one month after the lowest period of 10 to 15 days (Sugita and Osame, 1976). This process was observed as atrophy of the lingual muscle fibers and interstitial fibrosis on histological slides prepared during each stage after hypoglossal transection. In other words, the two changes are observed in two different structures: the intrinsic lingual muscle fibers inserted inside the lingual aponeurosis and on the outer surface adhering closely to the lamina propria and dense submucosal tissue. Accordingly, atrophic degeneration of the muscle fibers and interstitial fibrosis advance slowly inside the aponeurosis, and edematous change occurs superficial to, that is, outside the aponeurosis. Irregular undulations appearing on the lingual dorsum and lateral margin should be caused by structural differences between the inner and outer tissues of the tongue mentioned above. The two changes are physically in contrast to each other as it were.

2) Transformation of capillary loops in the filiform papillae

Generally, capillaries beneath the epithelium are revealed by reflective of the epithelium and its lamina propria. The characteristic form and arrangement of capillaries in the subepithelial layer of the squamous stratified epithelium appear quite different from those in other epithelia. It is very important to elucidate vasculogenesis and the restorative process after injury of the capillary loops in the lamina propria. The capillary loops in filiform papillae are similar to each other or more developed than beneath the usual squamous stratified epithelium, and show various trials in reaction to functions of the tongue. There have been studies on sequential changes during healing of the capillary loops after experimental injury of the lingual dorsal mucosa (Fang and Suwa, 1993). For this purpose, the most effective approach is to examine microcorrosion casts of a wide area by SEM. However, no studies have surveyed sequentially the healing process of capillary loops in an organ innervated by a motor nerve isolated from adjacent organs such as a tongue after unilateral transection of the hypoglossal nerve.

Transformation of the capillary loop in the present study was observed within a few days after transection of the hypoglossal nerve and continued in various complicated forms for 7 weeks or more. The present authors examined sequential changes in the caliber of the loop capillary and course of the crura in order to understand the fundamentals of morphological transformation. Capillaries lack uniformity in caliber owing to the impairment of vasomotor neurons innervating capillary or arteriolar walls. First, a tapering or partial thinning is found at the tip of loops at 1 to 2 weeks after transection. Simultaneously, the capillary wall collapses and shows irregular bulgings, a pellet-like chain or sometimes leakage of the injected plastic on corrosion casts. These findings indicate increasing fragility of the capillary wall. Twisting observed near the tips of loops with or without the tapering have also been reported as a recovering form of the capillary loop in the lingual mucosa (Fang and Suwa, 1993). However, in this study no essential collapse of the capillary loop itself was observed. Thus the initial twisting, including tapering, may be caused by other factors. Twisting resulted in an increased frequency of convolutions, and during two weeks extended to the loop crura, which appeared tortuous. This change has also been found during the restoration of experimentally damaged loops (Kawamura, 1989; Fang and Suwa, 1993). Twisting and tortuosity become stronger all through the crus or crura, develop to coiling or looping and finally create congregates or glomenui. These beginning changes are four basic patterns of transformation. During this transformation, a dense lamina propria and loose submucosal layer have been continuously swollen by edematous change and softened morbidly by the liquid contained in widened tissue spaces. Transformation of the loops in the edematous tissue are so complicated and irregular that it is difficult or impossible to find any formulation for the classification of these patterns. As normal patterns, irregular or underdeveloped loops in underdeveloped transverse palatine ridges and atrial folds in rats were reported by Ohta et al. (1992) and Sugio and Ike (1993). However, it is impossible to conclude that the transformation of loops observed in this study could be identified with the sinusoidal aspects found in some healthy or developmental stages. The full length of the capillary loop was finally elongated in the pattern of a complicated loop. In two rats bred for 66 weeks after transection, changes in the loop similar to those observed at 7 weeks were still seen. Transformation of the loop never recovered, but remained some patterns in this study. However, blood circulation is not terminated even if innervation of the vasomotor nerve is lost. On the contrary, transformed loops in other cases of injury have recovered to their initial form slowly but completely about 4 weeks or more after experimental injury. That is, the transforming loop is eventually recon-
structured as an independent loop (Fang and Suwa, 1993). Transformation of the loop in the filiform papilla after hypoglossal transection may carry on as uncontrolled patterns in the softened subepithelial layer since the vasomotor neuron was cut. In this study, since autonomic nerves accompanying the lingual artery and its ramifications were not transected, further study must be attempted on rats in which all branches communicating with the hypoglossal nerve would be free.

3) Blood flow evaluated by LDF

The present authors employed LDF to clarify serial changes in blood flux after hypoglossal transection. Data obtained in this study are discussed in terms of a time comparison. Values obtained through the mucous epithelium by LDF generally corresponded with blood stream and speed in about 1 mm². The capillary bed in the tongue is quite dense since its metabolic needs are great. After hypoglossal transection, the blood stream in the lingual dorsal mucosa diminishes owing to muscle atrophy and other factors. Judging from LDF values, the slight increase on the lesion side at 3-5 days after transection was in proportion to the slight edema in the mucosa that occurred with initial transformation of the capillary loops. Then, the blood stream decreased for 3 weeks and eventually recovered to the level of the nonlesion side. Three weeks after transection we observed the lowest value for the blood stream and complicated transformation of the capillary loops, since complicated forms of the loops probably impeded the blood stream (Figs. 2, 3). This diminishing may be caused by a decreasing RBC concentration without changes in the speed of the blood stream (Fig. 2). The blood volume was maintained or slightly increased. This process should influence slow restoration of the RBC concentration (Fig. 3). The present study suggests that there is a relatively close correlation between LDF values and the transformative patterns of the capillary loop.

Acknowledgments

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Literature cited

Plate I

Fig. 1. Exposure of the left hypoglossal nerve (arrowhead) via a ventromedial approach. The nerve was located along the medial border of the intermediate tendon (arrow) and anterior venter of the digastricus muscle. *: Stylohyoideus muscle.

Fig. 2. Changes in blood flux and concentration of RBC on lesion and nonlesion sides after unilateral transection of the hypoglossal nerve.

Fig. 3. Changes in the average speed of RBC on lesion and nonlesion sides after unilateral transection of the hypoglossal nerve.
Plate II

Figs. 4a—4f. Control group.

Fig. 4a. Lingual dorsum of the rat in the control group. Fig. 4b. Filiform papillae (arrowheads) showing clearly an arc line at each base and fungiform papillae (arrow). Fig. 4c. Cast of the microvasculature of the lingual dorsum. Figs. 4d and 4e. Capillary loops in the filiform papillae. Arrows: ascending crus, arrowheads: descending crus, *: venules. Fig. 4f. Frontal section of the lingual apex stained with trichrome. Symmetrical arrangement of the intrinsic muscle fiber bundles is observed.
Plate III

Figs. 5a–5h. Three to five days after denervation. Three days after denervation in Figs. 5a, 5d, 5h, and five days in Figs. 5b, 5c, 5e, 5f, 5g.

In Figs. 5a and 5b, slight edema is seen on the left lesion side of the lingual dorsum. In Figs. 5b and 5c, a depression (arrow) is seen on the mucosa and a cast of the lingual dorsum. Simple twisting at the tip of the loop in Fig. 5d, and a pellet-chain-like crus (arrowheads) in Fig. 5f are seen. More distinct twisting (arrowheads) of the loops in Fig. 5e and indefinitely complicated twisting in Figs. 5f and 5g are seen. Fig. 5h. No distinct histological changes are observed yet.
Plate IV

Figs. 6a–6f. One week after denervation.

Fig. 6a. A shallow depression (*) on the dorsum and scallop-like edge (arrowhead) on the lingual lateral margin of the lesion side. Fig. 6b. Keratinization (arrows) around the bases of filiform papillae and a fungiform papilla in the center. Fig. 6c. Twisting and tapering (arrows) of tips of the capillary loops. Fig. 6d. Twisting and bridging between crura (arrows). Fig. 6e. Bulging (arrow) at the tip of the capillary loop and tapering (arrowheads) on the ascending crus. Fig. 6f. Atrophic change (arrowhead) and edema (arrows) in a frontal section are observed in the lamina propria and interstitial tissue. Lesion half of the dorsal surface swells roundly.
Plate V

Figs. 7a–7h. Two to three weeks after denervation. Two weeks after denervation in Figs. 7a, 7c, 7e, 7g, and 3 weeks in Figs. 7b, 7d, 7f, 7h.

Figs. 7a and 7b. Depressions (*) on the dorsal mucosa and scallop-like edge on the lingual lateral margin have become somewhat more distinct compared with those in the previous period. The lingual median groove in Fig. 7b begins to cove toward the nonlesion side. Fig. 7c. Twisting and duplicated (arrow) capillary loop and leakage (arrowheads) of the injected plastic. Fig. 7d. Arrangement of the filliform papillae becomes slightly irregular. Keratinization around the bases is clearer but decreased. Figs. 7e and 7f. Duplication (arrows) and bulging (arrowheads) of the capillary and widened intervals between crura. Original form and height of the typical loop disappear. Fig. 7g. Lateral margin of the lesion half protrudes laterally on a frontal section stained with HE. Figs. 7g and 7h. Edema scarcely remains but interstitial fibrosis (arrowheads) is seen between irregularly arranged muscle fibers on a frontal section.
Plate VI

Figs. 8a–8h. Four to five weeks after denervation. Four weeks in Figs. 8a, 8d, 8e, 8g, and five weeks in Figs. 8b, 8c, 8f, 8h.

Fig. 8a. Two depressions (*) are seen on the lingual dorsum with a slightly paled mucosa with a lateral protrusion. Fig. 8b. Three depressions are seen and the lesion half swells markedly, and its apex elongates forward with paling and smoothing. The lingual median groove coves beyond its original location. Fig. 8c. Arc lines (arrows) around the base of the filiform papillae disappear and its tip descends slightly toward the base due to decreased keratinization. Fig. 8d. Original form of all capillary loops disappears as a result of indefinite transformation (arrows), such as strong tortuosity, coiling and bulging. Figs. 8e and 8f. Irregularly complicated forms of the capillary loops with double or triple crura (arrows) or a leakage of the injected plastic (arrowhead). Figs. 8g and 8h. A lateral protrusion of the lesion side on a frontal section becomes milder. Edema is not observed, but interstitial fibrosis is seen in dotted areas.
Plate VII

Figs. 9a–9h. Six to seven weeks after denervation. Six weeks in Figs. 9a, 9d, 9e, 9f, 9g, and seven weeks in Figs. 9b, 9c, 9f 9h.

Figs. 9a and 9b. Many depressions (arrows) on the dorsal surface, a scallop-like edge (arrowhead) on the lateral margin and elongation of apex on the lesion side are still observed from the last period. Fig. 9b. In some cases, a slight ulceration (arrow) is found near the lateral margin. Fig. 9c. Abrasive invasion (arrow) is found on the filiform papilla, the height of which becomes lower (*) owing to poor keratinization. Figs. 9d, 9e, 9f. Each loop appears in a cluster-like or congregate form (arrow). Fig. 9e. Both crura are connected with a bridging (arrow), and small bulging is seen on the tip. Fig. 9g. Slight edema is still seen between irregularly arranged muscle fibers in a dotted area on a frontal section. Fig. 9h. A progressive fibrosis is seen in a dotted area on a frontal section (stained with trichrome).
Figs. 10a and 10b. Sixty-six weeks after denervation. Severe twisting (arrow) and coiling (arrowheads) of various calibers are still observed in the capillary.