MORPHOLOGICAL AND CERTAIN HISTOCHEMICAL CHANGES IN STRIATED MUSCLE IN VARIOUS FORMS OF DENERVATION

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The condition of striated muscle following denervation has already received considerable attention [2, 5-7, 10]. However, despite the new information which has been obtained in this field, many aspects of the reaction of skeletal muscle to denervation remain unclear; these include the submicroscopical changes which occur in denervated muscle fibers, the composition of the enzymes systems, etc. In the majority of experiments, denervation of the muscle has been achieved by cutting across a mixed nerve, but this technique does not permit any assessment to be made of the part played by each component in the development of denervation changes.

In the present work we have attempted to determine the character of the changes in striated muscle as a result of sensory, motor, and sympathetic denervation.

EXPERIMENTAL METHODS

Adult domestic cats were used as experimental animals. The object of investigation was the tissue of the tongue. We carried out 3 groups of experiments: 1) sensory denervation of the tongue by means of the resection of the glossal and glossopharyngeal nerves; 2) motor denervation by means of resection of the hypoglossal nerve; 3) sympathetic denervation by means of resection of the superior cervical sympathetic nodes.

The material was examined over a period of 2 to 60 days. The tissue of the tongue was fixed in 12% neutral formalin solution, Zinker-formol solution, Carnoy's fixative, or neutral Shabadash mixture and was embedded in paraffin wax. General histological staining techniques were used, together with recorcin-fuchsin for demonstrating elastin fibers and the PAS reaction for glycogen [before and after treatment of the section with ptyalin (salivary amylase)]. Nucleic acids were demonstrated by methyl green-pyronin staining using Brachet's method and by means of the Feulgen reaction. Polysaccharides were investigated with the help of the PAS reaction and toluidine blue staining. In order to differentiate between certain groups of polysaccharides control sections were incubated in a solution of testicular hyaluronidase before staining. The Hale positive granules within cells were distinguished from granules of hemosiderin by means of the Perle's reaction. The presence of fat was demonstrated by staining frozen sections with Sudan III. The results of research into 75 animals is included in this article.

EXPERIMENTAL RESULTS

Sensory denervation of the cat tongue led to the development of inflammation in the deafferentiated region in the first few days of the experiment. There was a definite expansion of vessels accompanying the tissue edema and an infiltration of the tissues by neutrophil leucocytes. This was followed by the onset of tissue disorganization and death of a portion of the histological elements. In the epithelium, this process led to the formation of shallow, diffuse ulcers (Fig. 1) on the 10th-14th day. The epithelial cells around the ulcer showed an increased RNA content and glycogen and mucopolysaccharides appeared. The number of mitoses increased considerably. The floor of the ulcer was formed from its own layer of mucous membrane. The main substance of the connective tissue in this region showed an intense metachromatic coloration with toluidine blue and stained with Hale's colloidal green. Both staining reactions were suppressed completely by prior treatment of the sections with hyaluronidase. The cellular material at this point was very heterogeneous.

Fig. 1. Epithelium of ulcer after resection of glossal and glossopharyngeal nerves (14th day of observations). Ocular, 10×, objective 3×.

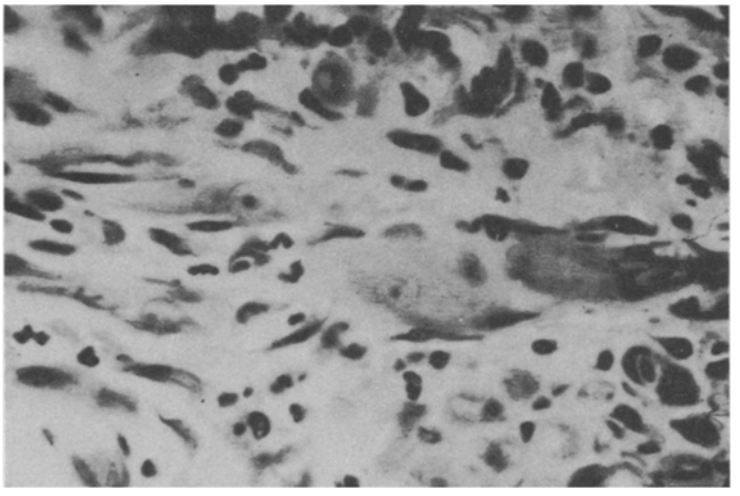

Fig. 2. Dissolution of muscle fibers in zone of leucocyte infiltration after resection of glossal and glossopharyngeal nerves (10th day of observations). Ocular 10×, objective 45×.

In addition to the congregation and disintegration of neutrophils, many macrophages, fibroblasts, and fat cells occurred in this region. The fibroblasts were characterized by having enlarged nucleoli and cytoplasmic granules positive for Hale's reaction but differing in their staining properties from granules of hemosiderin. The Hale positive granules of the fibroblasts were no longer apparent after the section had been incubated in hyaluronidase solution, a finding which suggests they are mucopolysaccharide in nature.

The number of nuclei in the striated muscle fibers of this zone increased as a result of their amitotic division. The nuclei first became rounded and migrated to the center of the fiber and the nucleoli then increased in size. The myofibrils and RNA-rich sarcoplasm disappeared from those parts of the fibers near to such nuclei. The glycogen granules of the fiber arranged themselves in a disorderly fashion. The RNA-rich fragments of the fiber may be isolated from each other as myosymplastiform structures [Fig. 2) in which nuclear division continues. Part of the muscle fibers are destroyed by the neutrophils; their sarcoplasm undergoes lysis and the fiber loses its identity. In those parts of the tongue farthest from the ulcer, the changes following afferent denervation are less marked, but their nature is as described above. There is a considerable increase in the number of fat cells both in the mucous membrane and in the muscle layers. Some of the blood vessels suffer a certain amount of destruction of their walls:

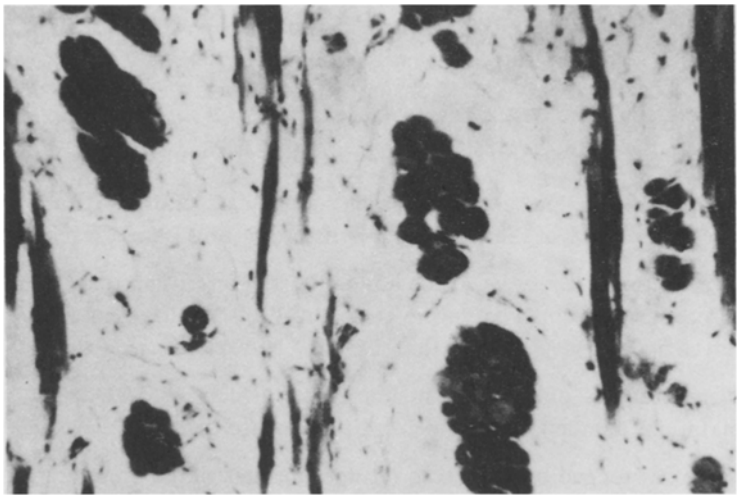

Fig. 3. Atrophy of muscles and development of adipose tissue in their place after resection of the hypoglossal nerve (45th day of observations). Ocular 10× , objective 10× .

the innermost layer of the blood vessel becomes thicker as a result of the increase in number of its cells, some of which become vacuolated and die. There is also some vacuolation of the smooth muscle layer, the nuclei of which stain less intensely than usual and later disappear. The internal elastic membrane suffers dissolution. A process of cell proliferation is resumed around the affected vessels. This leads to pericellular infiltration manifested by the accumulation of small cells with dense, rounded nuclei and RNA-rich cytoplasm. Fat and plasmatic cells constitute part of these infiltrates.

The main mass of striated muscle fibers, situated at some distance from the ulcer, are characterized by an increase in number of their nuclei, by the rounded form of their nuclei, and by their large nucleoli. The transverse striation of these fibers is preserved. Those muscle fibers situated near to the rounded cell infiltrates lose their transverse striation and their sarcoplasm becomes rich in RNA.

Beginning from the 4th week of the experiment, in addition to the changes already described, that of the dissolution of vacuoles should be added. This process leads ultimately to the destruction of the whole fiber. It is impossible to stain the contents of the vacuoles and hence their appearance does not change throughout the course of the dissolution process. Fibers which have retained their normal structure may be encountered at all stages of the experiment.

The response of the cat tongue to motor denervation differs only in respect of changes in the striated muscles and culminates in their atrophy. This process becomes noticeable from the 2nd day of the experiment and is characterized by a progressive atrophification of the fibers, during which their striations are preserved. The nuclear apparatus undergoes no essential changes. The amount of glycogen in the atrophying fibers gradually decreases but the distribution of glycogen granules in relation to the transverse striations is usually preserved. During observations, after the experiment had proceeded for more than 4 weeks, it became apparent that there was a lack of correspondence between the diminution in amount of glycogen in the muscle fibers and the degree of their atrophy. The atrophying muscle becomes diluted with adipose or fibrous connective tissue [Fig. 3]. The final outcome of the process of atrophy is a complete disappearance of the muscle fibers. We have never observed any degeneration or fatty infiltration of muscle fibers following motor denervation.

Sympathetic denervation of the tongue of cats is characterized by hyperemia of all tissues throughout the whole period of observation. The vessels of the arterial system are the first to dilate, followed by the veins and capillaries. Commencing from the 2nd or 3rd week of the experiment attenuation of the smooth muscle cells in the arterial walls takes place. The nuclei of these cells undergo lysis. The interstitial collagen fibers lying between the smooth muscle cells increase. No changes in the epithelium or in the striated muscle fibers have been observed to take place as a result of sympathetic denervation.

Thus, our researches into the histological changes in the cat tongue (particular of its striated musculature) under conditions of various types of denervation, indicate that each kind of intervention brings about its own specific tissue reaction. Resection of the sensory nerves leads to changes in all tissues of the region deprived of its afferent nerve supply. The resultant inflammation is accompanied by an increase in the RNA content of cells and muscle fibers, an increase in mucopolysaccharide production, and more mitotic and amitotic activity of the tissues concerned.

All these activities testify to a reduction in the degree of maturity associated with the tissue components of the organ and reflect the severe disturbance of the histo-metabolism resulting from afferent denervation.

The literature contains reference to a change in the antigenic structure of proteins following afferent denervation [4] such that the organism responds by manufacturing antibodies [3]. We have noted an increase in the number of plasmatic cells in our experiments and it is possible that this may be related particularly to these processes.

All these things suggest the presence of a sensory neuron factor which provides for the structural integrity of of the organ concerned and maintains an adequate degree of differentiation among its tissues [1].

Afferent denervation of the cat tongue only leads to changes in the striated muscle. The latter develop atrophy, the outcome of which is the disappearance of the fiber and their conversion to connective tissue. This confirms the accepted point of view that the motor neuron exerts a trophic influence on the muscle fiber it innervates [5-9].

Sympathetic denervation has no effect on the morphology of striated muscle but brings about the dissolution of smooth muscle cells in the vascular system.

SUMMARY

A study was made on the cat tongue tissues, in particular the striated muscles, in various types of denervation. The material was examined by histological and some other histochemical methods in terms ranging from 2 to 60 days. The sensory denervation was characterized by the development in the deafferentated region of an inflammatory process accompanied by a decrease in the degree of maturity of highly differentiated tissue structures. The motor denervation caused atrophy of the striated muscles, their destruction and replacement by connective tissue. Desympathization had no effect on the structure of the straited muscles, but caused the destruction of the smooth muscles of the walls of certain vessels.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
