treated by laser therapy, the fact that irreversible changes follow the use of doses of irradiation exceeding 20.34 J/cm2 must be taken into consideration.

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HISTOCHEMICAL STUDY OF CHOLINERGIC STRUCTURES OF THE FROG TONGUE DURING CHRONIC ETHANOL ADMINISTRATION

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Investigators studying the effect of ethanol on cholinergic structures of the gastrointestinal tract have concentrated their attention mainly on organs such as the stomach and small and large intestine [8]. Meanwhile, the proximal part of the gastrointestinal tract, namely the mouth and tongue, have not yet been studied from this standpoint. Yet histochemical studies have shown that the tongue in man and various vertebrates has a rich cholinergic innercation [1-4, 7] which, in the opinion of the authors cited, plays an important role in the regulation of vascular tone, of gland function, and of activity of the taste receptor system, and which evidently is involved in the perception and transmission of tactile stimuli [1]. It has also been shown that cholinergic fibers maintain nervous connections between unimodal (gustatory) and heteromodal (gustatory and tactile) receptors of the tongue, and combine them into receptive fields [1].

The aim of this investigation was to study the chronic effect of ethanol on cholinergic structures of the frog tongue.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (*Rana temporaria*), kept under laboratory conditions at 10°C. Ethanol was administered to the frogs through the skin, i.e., by the natural way in which fluids enter these animals [5]. In this way the direct damaging action of ethanol on the tongue was excluded. The experimental animals (48 frogs) were kept away from water for 14-90 days, but were kept for 2 h daily in a crystallizing tank with 3% ethanol solution. Animals of the control group (20 frogs) were kept in water for the same time.

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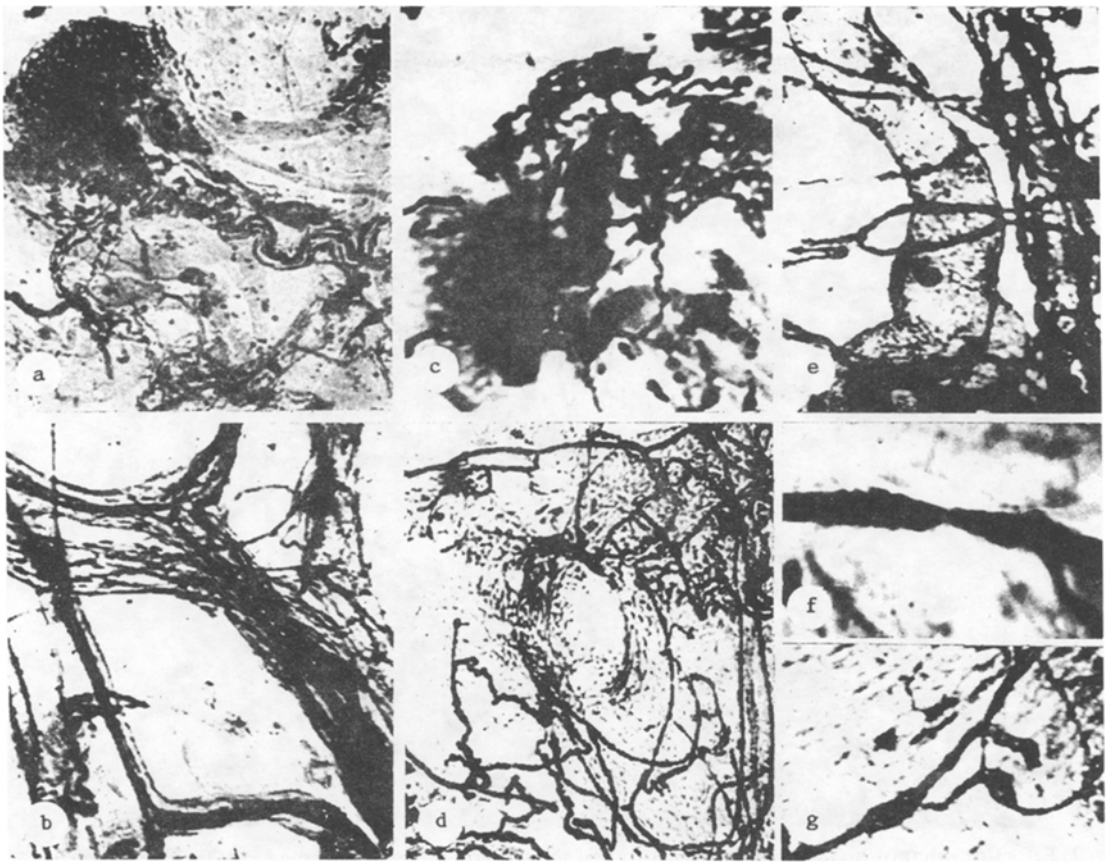

Fig. 1. Reactive changes in cholinergic structures of the tongue in an intact frog after application of tasty solutions. a, b) Appearance of sensory cholinergic fibers and nerve trunks before taste stimulation of the tongue; c, d) swollen cholinergic fibers after taste stimulation of the tongue; e) swelling and change in optical properties of perineurium; f) single swollen fiber in region of Ranvier node; g) neurons of submucous plexus; magnification: a-e) objective 10, ocular 7, adapter 1.6; f) objective 20, ocular 7, adapter 2.5; g) objective 20, ocular 7, adapter 1.6.

The cholinergic innervation of the frogs' tongue was studied by the method in [6], 14, 30, 60, and 90 days after the beginning of ethanol administration. To detect acetylcholinesterase (AChE), acetylthiocholine iodide was used as the substrate. The presence of the enzyme was judged by the formation of a brown precipitate of copper thiosulfate — the end product of the histochemical reaction — in the structures. To detect disturbances of function in the cholinergic structures of the tongue which could arise under the influence of ethanol, immediately before material was taken, preliminary functional activation of these structures was carried out. For this purpose, tasty solutions were supplied to the receptor surface of the tongue of control and experimental frogs, immobilized by destruction of the brain and spinal cord: 0.5 M NaCl, 0.5 M glucose, 0.25 M sucrose, and 0.3 mM quinine chloride; the substances were rinsed away with Ringer's solution. Next, stretched preparations were obtained from the epithelium of the frog's lingual mucous membrane. The method of obtaining stretch preparations and of their subsequent processing was described previously [1]. The mounted preparations were examined in a BIOLAR light microscope. The experiments were carried out in the winter and spring.

EXPERIMENTAL RESULTS

Application of various taste stimuli to the tongue of the control animals was accompanied by reactive changes in the AChe-positive structures, manifested as swelling. For instance, most of the cholinergic nerve fibers innervating the taste buds were subjected to various degrees of swelling; this process affected both the main trunks of these fibers as well as their preterminal divisions, forming the basal plexus (Fig. 1c). As a result, an increase in the diameter of these nerve fibers was observed

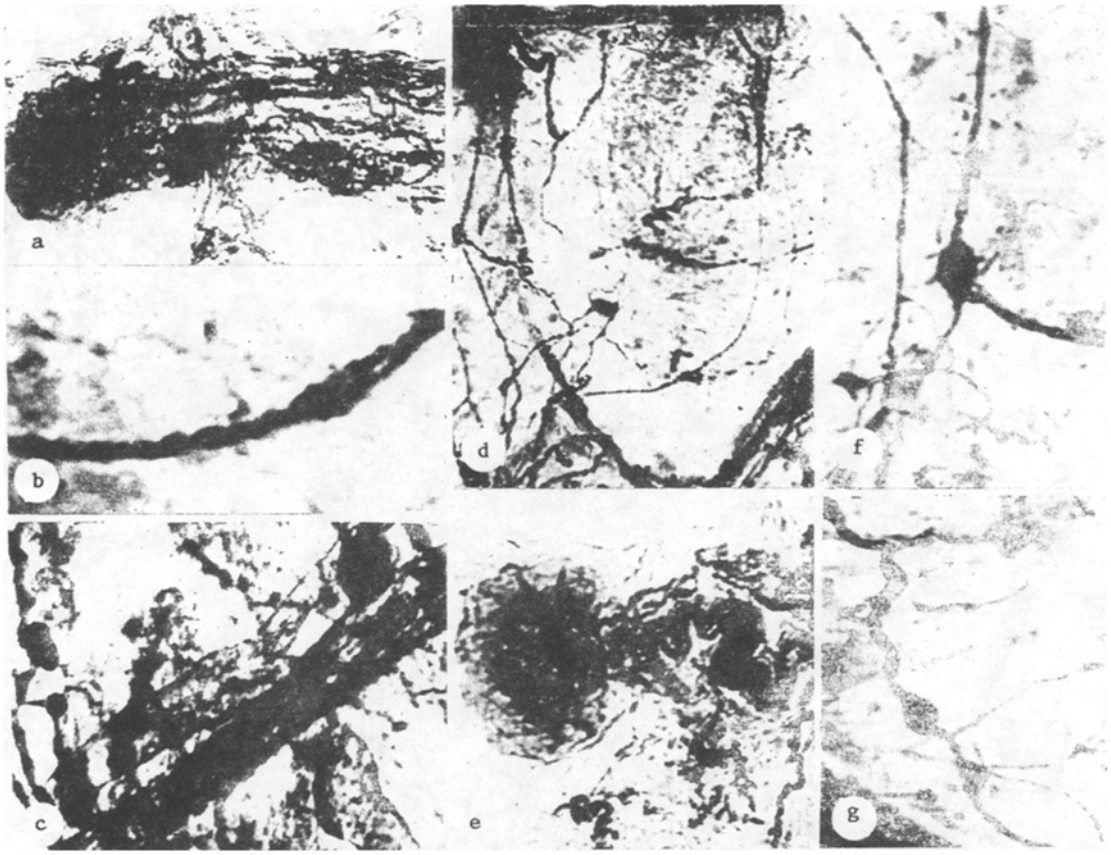

Fig. 2. Effect of ethanol on cholinergic structures of the tongue in the early stage (14-30 days) of alcoholization. a) Absence of reactive changes in sensory cholinergic fibers to taste stimulation of tongue after administration of ethanol for 14 days; b) single nerve fiber with signs of deformation and modified Ranvier node at same period of alcoholization; c) reduction of reactive response of perineurium to taste stimulation of tongue at same period of alcoholization; d) closed ducts of lingual glands at same time of alcoholization; e) recovery of structural lability of individual sensory cholinergic fibers after 30 days of chronic administration of ethanol; f, g) swollen neurons of submucous plexus at same period of alcoholization. Magnification: a, c, d, e) objective 10, ocular 7, adapter 1.6; b) objective 20, ocular 7, adapter 2.5; f, g) objective 20, ocular 7, adapter 1.6.

compared with functionally inactive fibers (Fig. 1a, b). Similar reactive changes were found in cholinergic fibers inervating the ducts of the lingual glands (Fig. 1d).

Besides swelling of the nerve fibers in response to taste stimulation of the tongue considerable swelling of the connective-tissue membrane covering bundles of nerve fibers also was observed, with changes in its optical properties. Whereas before stimulation of the tongue the perineurium appeared as a semitransparent contour, surrounding the nerve trunk and not preventing the clear visualization of the nerve fibers and of the neurons located in this part (Fig. 1b), after stimulation it became optically denser, as a result of which the 0 nerve fibers could not be examined (Fig. 1e). Meanwhile, during the reactive changes in single myelinated nerve fibers, their structural components, such as the Ranvier nodes, became clearly visible (Fig. 1f). At the same time, in response to stimulation of the tongue by tasty solutions no changes could be observed in the cholinergic neurons lying in the interpapillary tissue (Fig. 1g).

After administration of ethanol for 2 weeks, reduction of the structural lability of the sensory nerve fibers was observed in the experimental animals (Fig. 2a), and this was manifested more clearly in the zone of their terminal ramifications, in the basal plexus, as a result of which the swelling of the nerve fibers in response to taste stimulation of the tongue was weaker than in the control. At the same time, individual nerve fibers with signs of deformation appeared. The swellings and nodules located along the course of these fibers gave them a bead-like appearance (Fig. 2b). A feature of these nerve fibers was the presence of modified Ranvier nodes. The medullary cones of these nodes were widened and rounded, as a result of which the intersegmental clefts appeared to be smaller than in the control (Fig. 2b). At these times of alcohol poisoning, inhibition of AChE activity also

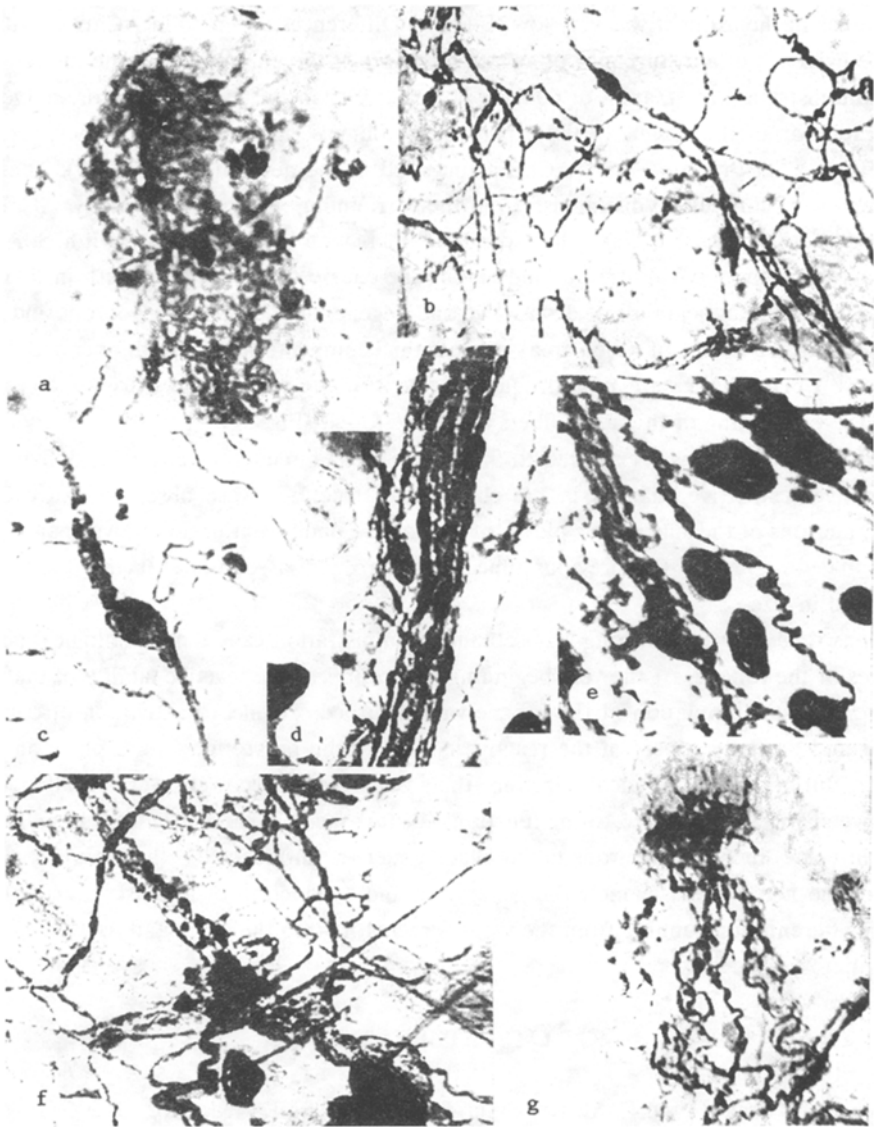

Fig. 3. Cholinergic structures of the tongue after chronic administration of ethanol for 60-90 days. a) Absence of reactive response of sensory fibers to taste stimulation of the tongue; b) lowering of AChE activity in nerve fibers; c) neuron of submucous plexus with marked degenerative changes; d-g) destructive and degenerative changes of cholinergic fibers. Magnification: a, b, d, f, g) objective 10, ocular 7, adapter 1.6; c) objective 20, ocular 7, adapter 1.6; e) objective 10, ocular 7, adapter 2.5.

was observed in the nerve fibers, as shown by weakening of staining of the precipitate. However, not all fibers appeared to be changed. Some of them were indistinguishable in enzyme activity in their structures from the control. Under the influence of ethanol the reactive lability of the connective-tissue membrane of the nerve trunks was reduced appreciably (Fig. 2c). The ducts of the glands in nearly all the experimental animals, incidentally, were closed or had a small, often slit-like external aperture (Fig. 2d).

On the 30th day, despite continuing ethanol administration, partial recovery of the structural lability of some sensory fibers and nerve trunk membranes was observed. However, their reactive changes in response to stimulation of the tongue by tasty solutions were weaker than in the control animals (Fig. 2e). At the same time, sensory nerve fibers were often seen which did not react at all to stimulation, or did so only weakly. Processes of varicose deformation, now affecting the greater part of the nerve fibers, became widely distributed. At these times changes in the cholinergic neurons of the submucous plexus were clearly defined. The bodies of the neurons were enlarged, sometimes so much so that they were converted from spindle-shaped into round or spherical (Fig. 2f, g). Judging by the weakening of staining of the precipitate, AChE activity was depressed in most

neurons (Fig. 1f). In other neurons it was not detected equally everywhere. For instance, AChE activity of the neuron body remained very high, whereas in the axons it was very low (Fig. 2g). Differences observed in AChE activity in the body and axons of these neurons may be evidence of a disturbance of axonal transport of the enzyme under the influence of ethanol.

After chronic administration of ethanol for 60 days, no reactive changes were found in the nerve fibers and perineurium in response to taste stimulation of the tongue of the experimental animals, so that externally they were similar to functionally inactive nerve fibers (Fig. 3a). At the same time, AChE activity fell in the nerve fibers (Fig. 3b). These fibers lost their clear outlines, became pale, and were difficult to distinguish from the surrounding tissues. Incidentally, AChE activity did not fall at the same time in all fibers, but in one nerve trunk there could be fibers with low and others with high AChE activity. Moreover, this type of change in enzyme activity could also be found along the course of nerve fibers, both in their terminal divisions and far from them. At these times, the appearance of destructive and degenerative changes in neurons and their axons, and also of individual sensory fibers along the course of which areas of gross thickening alternate with thinner areas (Fig. 2d-g). In the distal parts of the sensory nerve fibers, these were most frequently fibers of the second accessory bundle, innervating a taste bud; marked tortuosity and uneven staining of the nerve fibers also were found (Fig. 2g).

If the period of alcoholization was extended to 90 days, a further reduction of AChE activity and an increase in the severity of the degenerative-destructive changes in the cholinergic structures were observed. An ever-increasing number of sensory nerve fibers and neurons of the submucous plexus of the tongue underwent degenerative changes. The character of these changes was similar to that described at the previous time. As before, just as earlier still, the ducts of the lingual glands remained closed and reduced in size.

Our investigations thus showed that long-term ethanol administration causes morphological and functional changes in the cholinergic structures of the tongue. At the very beginning of alcoholization, reactive lability of the sensory nerve fibers and perineurium in response to taste stimulation of the tongue was disturbed. Thanks to activation of compensatory and adaptive mechanisms, a transient and partial recovery of the reactive lability of the nerve fibers took place on the 30th day of chronic alcoholization. However, during continued alcoholization, their compensatory powers were in all probability exhausted, and dystrophic and destructive changes were added to the functional disturbances. It can be tentatively suggested that morphological and structural changes in nerve fibers and neurons taking place under the influence of ethanol are accompanied by disturbance of the basic functions of the nerve fibers, namely the generation and conduction of impulses, and ultimately this may lead to reduction of the sensory afferentation running from the various receptors into the CNS, and to disturbance of peripheral neural communication and regulatory processes.

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