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The intramuscular ganglia of the cat's tongue

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INTRODUCTION

The existence of nerve ganglia within the musculature of the mammalian tongue has been known for many years (see Barker, 1899). Okamura (1936) stated that they were attached to branches of the main nerves supplying the tongue. Ernyei (1937) noted a resemblance (in man and dog) between the component nerve cells and those of the submandibular ganglion, and considered the intramuscular ganglia to be distally displaced submandibular elements. Gairns & Garvan (1952) found ganglia along deeply placed nerve bundles in the tongue of the cat, rabbit, rat and hedgehog; some of them were located close to the terminal motor sprays of the hypoglossal nerve. Cooper (1953) found scattered groups of nerve cells in the tongue musculature of man, monkey, cat and lamb. Although the source of these small cell collections could not be determined, she also observed larger ganglia on the chorda-lingual and glossopharyngeal nerves close to their points of entry into the tongue. El-Rakhawy & Bourne (1961) used copper thiocholine technique for cholinesterase enzymes and observed a positive reaction in nerve cells near the intramuscular nerve trunks in the human tongue; processes were traced from the cells to minor salivary glands embedded in the musculature, and to arteries in the submucous layer. The types of enzyme present were not investigated. Wetzig (1962, 1963) compared the intramuscular nerve cells of several mammals with those of ganglia elsewhere, and showed that the former resembled autonomic rather than sensory cell types. Kubota (1966) has seen small ganglia in the musculature near the tip of the tongue of the Japanese Pika (a lagomorph). Because of their poor affinity for silver, their nature could not be determined.

The present work was undertaken in order to determine the distribution, the nature, and the connexions of the intramuscular ganglia in the tongue of the cat. The cat has proven especially suitable because the lingual salivary glands of this animal are confined to the posterior part of the tongue; study of the remainder of the organ was not impeded, as it is in many mammals, by the autonomic nerve supply to glands embedded in the anterior musculature.

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MATERIALS AND METHODS

(1) *Prenatal material.* The specimens, which are listed in Table 1, were freshly fixed in Bouin. Following paraffin embedding, serial 10 μm sections were processed by the double-impregnation silver technique (FitzGerald, 1964).

(2) *Postnatal animals.* Pieces of fresh adult tongue, together with superior cervical ganglia, inferior vagal ganglia, and small intestine were fixed in neutral formol saline at 4° C for 4 h, and sectioned in a cryostat at 40–60 μm. Acetyl and butyryl thiocholine substrates were prepared at pH 5·0 according to the method of Coupland & Holmes (1957). Inhibitors were incorporated in similar substrates, as follows: BW 284 [1,5-bis(4-allyl dimethylammonium phenyl) pentan-3-one dibromide] at a concentration of 3×10^{-5} M, to inhibit true cholinesterase; 10^{-7} M DFP (diisopropyl phosphofluoridate) to inhibit pseudocholinesterase; the above inhibitors together at their original final concentrations; and 3×10^{-5} M eserine salicylate to inhibit both types of enzyme (Koelle & Gromadzki, 1966). Where inhibitors were to be used the sections were first immersed in 0·9 % saline containing the inhibitors at the same concentrations for 30 min at room temperature. Sections were incubated for periods of 2, 6 and 18 h, washed in distilled water and treated with 2 % ammonium sulphide.

Table 1. *Data on the cat embryos and foetuses*

No. used	C.R. length (mm)	Age (d)*	Tissues studied	Plane(s) of section†
4	11·0	22	Whole embryo	SS, HS
2	13·0	23	Whole embryo	SS, HS
6	30·0	28	Whole head	SS, HS, FS
1	60·0	38	Half head	SS
3	105·0	50	Half head (1)	SS
			Tongue (2)	SS, FS

* Estimated from the data of Scott (1967). The gestation period in the cat is 60 d.
† FS, Frontal section; HS, horizontal section; SS, sagittal section.

Table 2. *Degeneration experiments in 31 cats*

Nerve sectioned	Survival (d)
Hypoglossal (11)	2–28
Chorda-lingual (6)	2–28
Hypoglossal and chorda-lingual (6)	7–28
Glossopharyngeal (6)	7–14
Sympathetic (2)	7–14

The left halves of three adult tongues (designated A, B and C), each 50 mm long, were fixed in ammoniacal formaldehyde–sucrose for 7–10 d. The volumes of specimens B and C were noted at the moment of fixation, and immediately prior to sectioning. No change in volume was detected. A cryostat was used to take serial frontal sections of each entire half tongue. Tongue A, cut at a mean thickness of 167 μm, yielded 300 sections; tongue B was cut at 51 μm and yielded 980 sections; tongue C, cut at 47 μm, yielded 1060. From each tongue every tenth section was processed in the

acetyl thiocholine substrate. The sections from tongue A were dehydrated, and mounted in balsam. The remainder were placed on warm slides and mounted in glycerin–gelatin. The position and number of the intralingual ganglia (excluding any seen in the dorsal mucous membrane) were recorded for each section. In tongues B and C the area of each section, and the observed mean diameter of the ganglia, were recorded. The actual diameter of the ganglia was obtained by means of Schwartz's (1934) correction and estimates of total numbers were then derived from the formula of Elias & Henning (1967):

$$N_v = \frac{{}^nA}{A(D+T)},$$

where N_v is the number of ganglia per section; ${}^nA$ is the number of sections, of total area A; D is the corrected ganglionic diameter; and T is the section thickness.

The intralingual ganglia were displayed by means of haematoxylin and eosin, iron haematoxylin, or gallocyanin; and by silver methods for neurofibrils (Fitz-Gerald, 1963, 1964). Tongue blocks from Rasmussen's fixative were stained for terminal boutons either by his silver gelatin method (1957) or with protargol-S. Methylene blue (0·03 % in 0·9 % saline, or 0·03 % in 0·2 M phosphate buffer at pH 8·0) was used in four adult cats to display the innervation pattern of the tongue. The procedures included immersion of freshly cut sections, and perfusion of the carotid or lingual arteries.

From five kittens 12–13 d old, tissue blocks were fixed in osmium tetroxide buffered with s-collidine (Bennett & Luft, 1959). The blocks were dehydrated and were embedded in Epon 812. Sections of 18 intramuscular ganglia were taken at 1–2 μm and stained for light microscopy. Thin sections of three ganglia were mounted on carbon-coated copper grids, doubly stained with uranyl acetate and lead acetate, and examined in an RCA 2C electron microscope.

(3) *Degeneration experiments.* The distribution of the main nerves within the tongue was followed by means of nerve sections in 31 animals (kittens and young adults), as listed in Table 2. The operations were performed on the right side in each case, under Nembutal anaesthesia. The hypoglossal nerve was approached through an incision medial to the angle of the mandible; the submandibular gland was retracted and the posterior fibres of the mylohyoid incised to expose the nerve. The chorda-lingual nerve was approached through a similar incision, the mylohyoid being divided farther forward. The glossopharyngeal nerve was approached through an incision posterior to the angle of the mandible; the styloid musculature was displaced and the nerve exposed on the inferior surface of the tympanic bulla. In these three procedures a 2–3 mm segment of the respective nerve trunk was excised. Sympathetic neurectomy was performed by removal of the superior cervical sympathetic ganglion.

A sham operation was carried out with respect to each of the operations listed, the appropriate nerve being exposed but not transected. The tissue blocks taken later included both sides of the tongue. They were sectioned in frontal or horizontal planes, and impregnated with silver. The exceptions were two tongues taken 4 d after glossopharyngeal nerve section; here the material from each side was processed separately, in the oblique plane of passage of the lingual branch of the glossopharyngeal.

OBSERVATIONS

In 11–13 mm embryos the primordium of the tongue is beginning to separate from the mandibular mesoderm, and is permeated by premuscle cells. The first- and second-order branches of the chorda-lingual, glossopharyngeal and hypoglossal nerves can be identified. The first two are remarkable in being invested and infiltrated by elongated cells with argyrophil cytoplasm (Figs. 1, 3). The argyrophil cells are restricted to the intralingual parts of the nerves. Mitotic figures are found in a small proportion (about 1 %). The light microscope does not show any cellular infiltration in the hypoglossal nerve (Fig. 2). Discrete ganglia cannot be identified in the tongue.

In 30 mm embryos the tongue rudiment is 1·2 mm long (paraffin sections) and the lingual musculature has reached the early myotube stage of differentiation. It is resolving into the chief extrinsic and intrinsic muscle groups. The lingual salivary gland anlagen are revealed as irregular cell clusters connected to the dorsal epithelium of the posterior part of the tongue. The intermuscular spaces are now occupied by thin-walled vessels, and by loose mesenchyme in which connective tissue fibres are being laid down. The spaces are occupied also by well-defined cell groups which are 15–50 μm in diameter and are closely related to nerve bundles (Figs. 4, 5). The individual cells are rounded, with strongly argyrophil cytoplasm; their nuclei are larger than those of adjacent mesenchymal cells and their nucleoli are prominent. On the evidence, they are very probably neurones. They extend throughout the tongue but are more numerous on the external surface of the genioglossus muscle. Many of the related nerve bundles can be traced proximally into either the chorda-lingual or hypoglossal trunks; however, they cannot be assigned to one or other because of the complex connexions between the branches of these nerves.

At 60 mm the tongue is 6·5 mm long and the muscle groups are well defined. The lingual-hypoglossal nerve plexus occupies the middle third of the tongue, and small ganglia are attached to it. The larger ganglia are embedded in the ventral musculature adjacent to the points of entry of the nerve trunks and of the lingual artery. The minority are clearly related to the chorda-lingual nerve trunk and by means of graphic reconstructions the remainder could be assigned (*a*) principally to branches of the chorda-lingual nerve, (*b*) to the lingual-hypoglossal plexus, and (*c*) occasionally to branches of the hypoglossal nerve (Fig. 11).

Fig. 1. Coronal section of tongue primordium of 11 mm embryo. *CN*, Chorda-lingual nerve; *HN*, hypoglossal nerve; *LA*, lingual artery. Double silver impregnation.

Fig. 2. Enlargement of the hypoglossal nerve from Fig. 1.

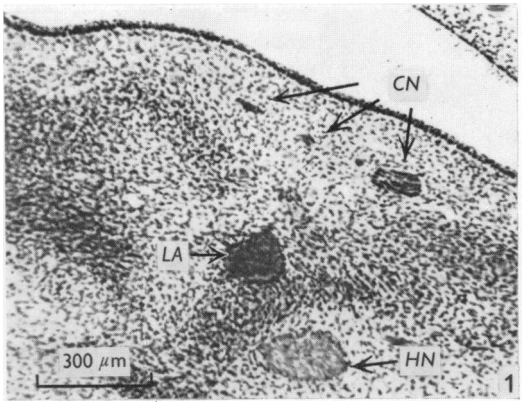
Fig. 3. Enlargement of the major chorda-lingual nerve branch from Fig. 1, for comparison with Fig. 2.

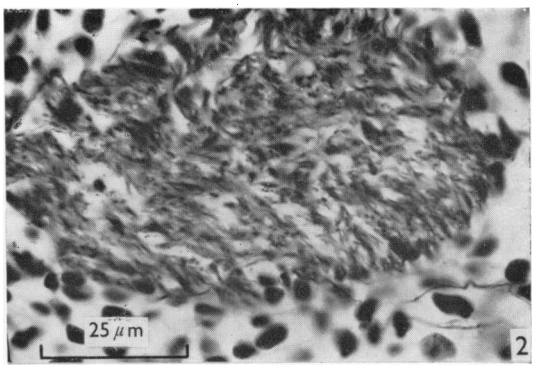
Fig. 4. Horizontal section of 30 mm embryo. *E*, Epiglottis; *M*, Meckel's cartilage. Ganglia are scattered among the developing muscles on both sides of the tongue. Double silver impregnation.

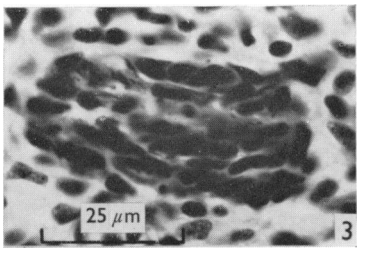
Fig. 5. Enlargement of the rectangular area from Fig. 4 to show three ganglia. Arrows indicate fine nerve bundles.

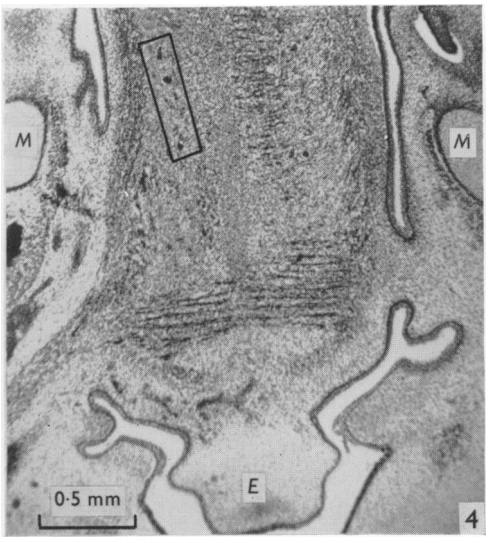
Fig. 6. Relatively large ganglion in the musculature close to the inferior surface of the tongue of a 60 mm embryo. Double silver impregnation.

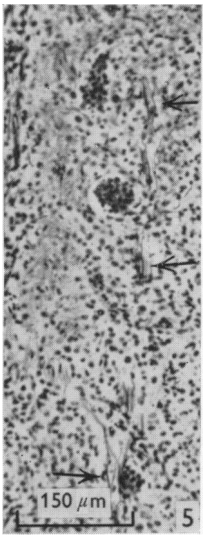
Figs. 7–10. Sections of the superior cervical sympathetic, vestibular, trigeminal, and sphenopalatine ganglia, respectively, in fields adjacent to Fig. 6.

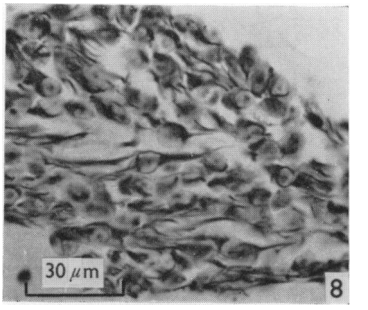

CN
LA
HN
300 μm
1

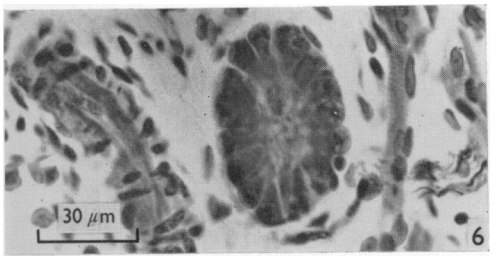

25 μm
2

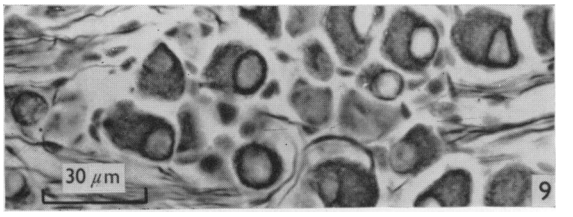

25 μm
3

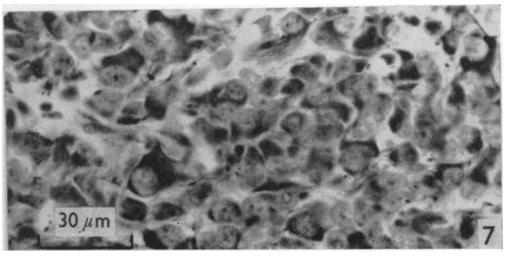

M
M
0·5 mm
E
4

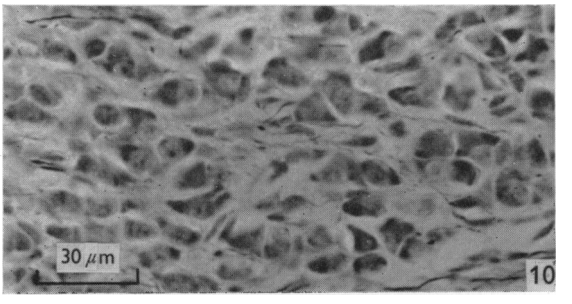

150 μm
5


30 μm
8


30 μm
6


30 μm
9


30 μm
7


30 μm
10

The lingual branch of the glossopharyngeal displays a ganglion, 120 μm in diameter, directly above the hyoid bone; several smaller ganglia follow the nerve within the longitudinalis superior muscle; these have a close topographic relationship to the salivary gland anlagen in this part of the tongue.

The cells of the intralingual ganglia measure 8–10 μm in diameter. Their cytoplasm is moderately argyrophil. The cells in the largest ones show rosette formation (Fig. 6), their nuclei are peripheral, and their piriform cytoplasm tapers toward the centre of the ganglion, which is occupied by very fine axons (0·3 μm or less). The axons of the related nerve trunk appear relatively much larger (up to 2 μm). In respect of size, configuration, and reaction to silver, the intralingual ganglion cells resemble those

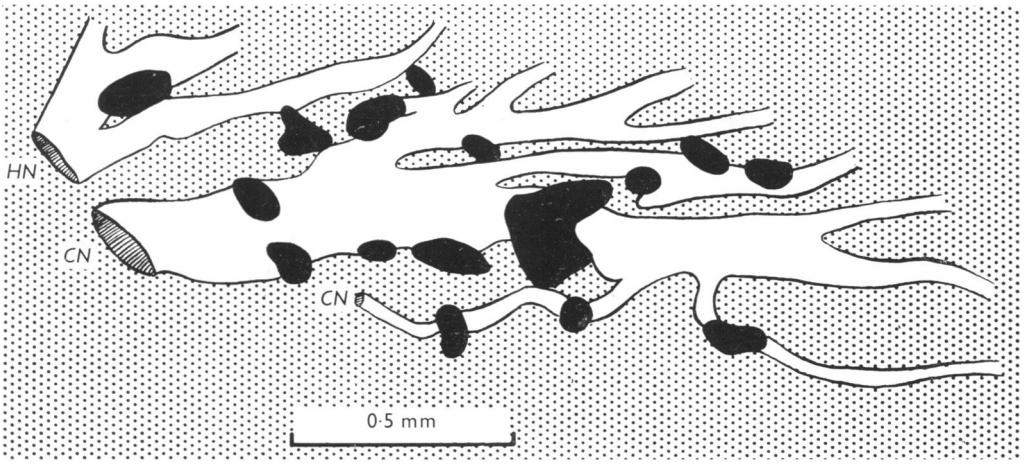


Fig. 11. Graphic reconstruction in sagittal plane from a 60 mm embryo (36 sections at 10 μm). The intramuscular trunks (labelled *CN* and *HN*) could be traced proximally into the chorda-lingual and hypoglossal nerves, respectively. Because of minute interconnexions between the various intramuscular nerve branches, the degree of admixture within the labelled trunks is unknown.

of the submandibular ganglion. They differ from those of the sphenopalatine (Fig. 10), ciliary, and otic ganglia only in being more compactly arranged. Many of the cells of the superior cervical ganglion, in contrast, are darker staining and are already

Fig. 12. Ganglion, *G*, from which a fibre bundle passes to the wall of a blood vessel, *V*; *ME*, motor end-plates. Cholinesterase technique (acetyl thiocholine substrate), 18 h incubation at pH 5·0.

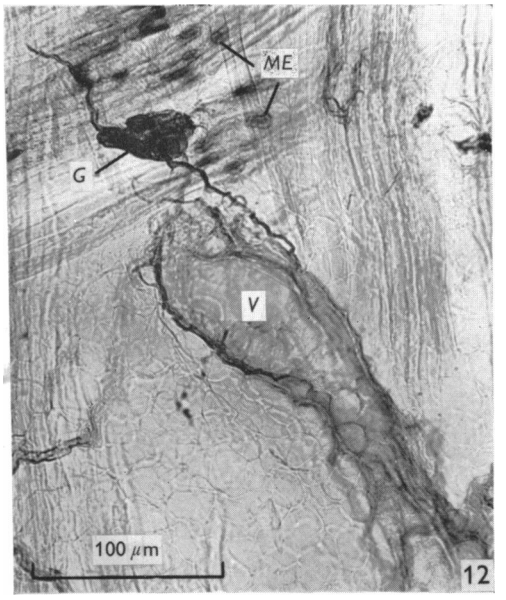
Fig. 13. Ganglion, adjacent to a small vein in the intermuscular connective tissue. *N*, Nerve cells. Myelinated nerve fibres are seen at each pole of the ganglion. Buffered osmium, methylene blue.

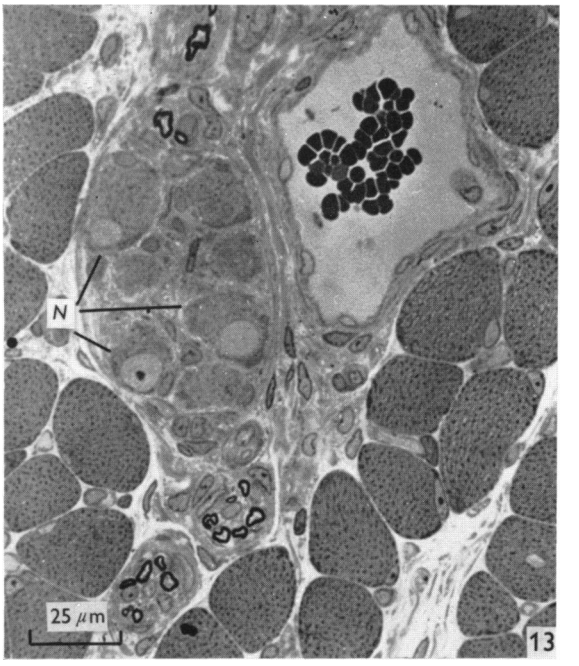
Fig. 14. Details from a small intramuscular ganglion. *C*, Capillary; *M*, myelinated nerve fibre; *N*, nerve cells. Buffered osmium, methylene blue.

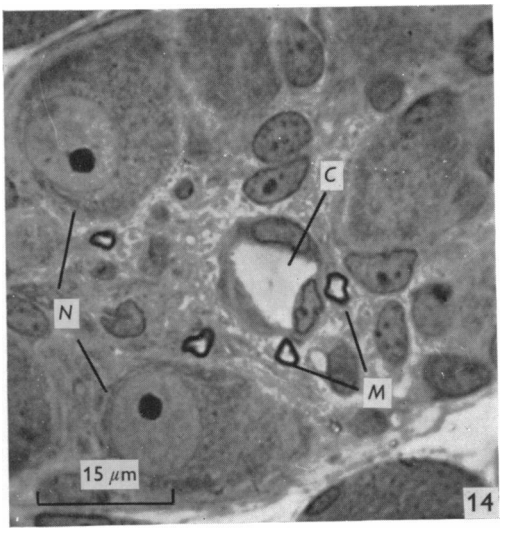
Fig. 15. A multipolar neurone adjacent to a pharyngeal mucous gland. Double silver impregnation.

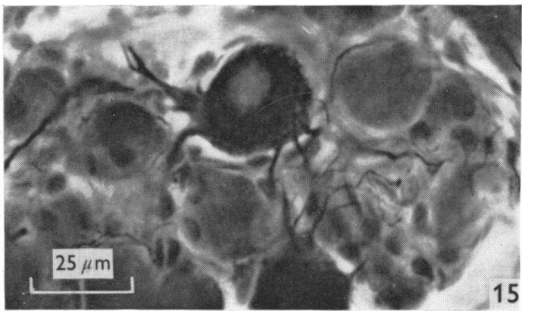
Fig. 16. Cholinesterase-reactive fibres surrounding a small intramuscular artery in the tongue. Technique as in Fig. 13.

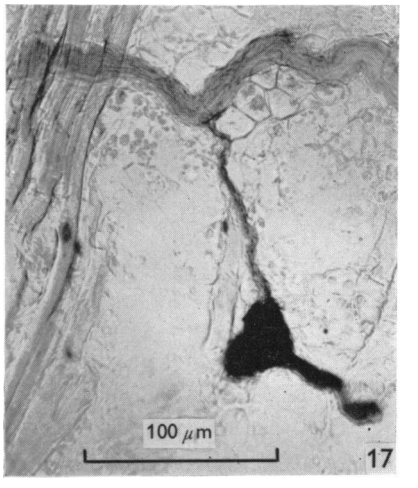
Fig. 17. Strongly reactive ganglion attached to a nerve bundle of the lingual-hypoglossal plexus. Technique as in Fig. 13.

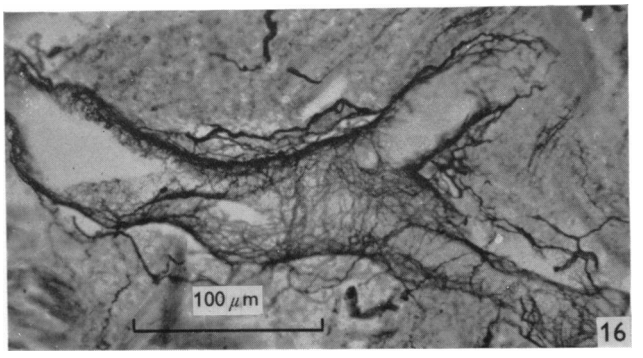

ME
G
V
100 μm
12


N
25 μm
13


C
N
M
15 μm
14


25 μm
15


100 μm
17


100 μm
16

assuming multipolar form (Fig. 7). Cells of the trigeminal and nodose ganglia are unipolar and measure 15-20 μm (Fig. 9). Vestibular and cochlear ganglion cells are bipolar and have large, pale nuclei (Fig. 8).

In the 105 mm foetuses the tongue is 16 mm long. The distribution of ganglia resembles that of the 60 mm material. In one specimen two ganglia could be seen within the epineurium of the largest branch of the chorda-lingual nerve. The more peripheral ganglia are related to the branches and interconnexions of the chorda-lingual and hypoglossal nerves. A relationship to the chorda-lingual is again predominant; in one graphic reconstruction (134 sections at 10 μm) all of the 26 ganglia seen were assigned to one of its branches.

The glossopharyngeal nerve is strewn with small ganglia from the level of the tonsil to the termination of its lingual branch among the minor salivary glands of the posterior tongue. The suprahyoid ganglion (lateropharyngeal ganglion of Wetzig, 1963) is 150 μm in diameter.

The similarity between intralingual, submandibular, sphenopalatine, and otic ganglia is again obvious.

In postnatal material the smallest ganglia consist merely of a chain of three or four cells lying among the fibres of a major intralingual nerve trunk. Larger ganglia are outside the epineurium of the related nerves, in a separate connective tissue capsule (Fig. 13). The nerve cells are directly subjacent to the capsule, are round or oval, and have eccentric nuclei with prominent nucleoli (Fig. 14). The cytoplasm is moderately argyrophil but is otherwise featureless in silver preparations. The poor uptake of silver does not seem to be the fault of technique, because pharyngeal autonomic ganglia in the same sections react strongly (Fig. 15). After gallocyanin, and in epoxy-embedded sections stained with toluidine blue, Nissl substance is prominent in the perikaryon. The neurones are invested by satellite cells, and these in turn are thinly coated with reticular connective tissue (Fig. 28).

The central zone of each ganglion is devoid of nerve cells and often contains capillaries. A bundle of coarse fibres enter through one pole (Fig. 25). It breaks up into finer branches which are lost to view among the nerve cells. One or more fascicles of extremely fine axons leave the ganglia but, except where they pass to the related nerve trunks, their immediate destination cannot be determined in paraffin sections. In the thicker, frozen-silver sections they can occasionally be traced to paravascular nerve plexuses surrounding small arteries in the intermuscular connective tissue septa.

Methylene blue has given no additional information. In carotid-perfused material, nerve staining in the tongue is largely confined to the mucous membrane. The relative pallor of the musculature indicates that the lingual artery has been by-passed by the perfusate. Direct perfusion of the lingual artery yields satisfactory staining of the intramuscular and paravascular nerve networks, but diffusion of the dye within the ganglia obscures the individual nerve cells.

Material taken from Rasmussen's fixative gives an equivocal reaction for terminal boutons. The reaction takes the form of very fine 'spotting' over the surfaces of the perikarya. Occasional 'spots' exceed 1 μm in diameter but most are barely visible under oil immersion. Differentiation is improved by the substitution of 1% protargol-S (for 1 h at 37° C) for silver gelatin, with development in hydroquinone sulphite.

The electron microscope shows the individual neurones to be invested, apparently

completely, by satellite cells. Tongues of satellite cytoplasm, often 0·5 μm or less in thickness, separate the perikarya from the surrounding connective tissue. The nerve cell nucleus is round and translucent and its chromatin is evenly dispersed (Fig. 18). The nucleolus, which is eccentric and single in random sections, is highly opaque.

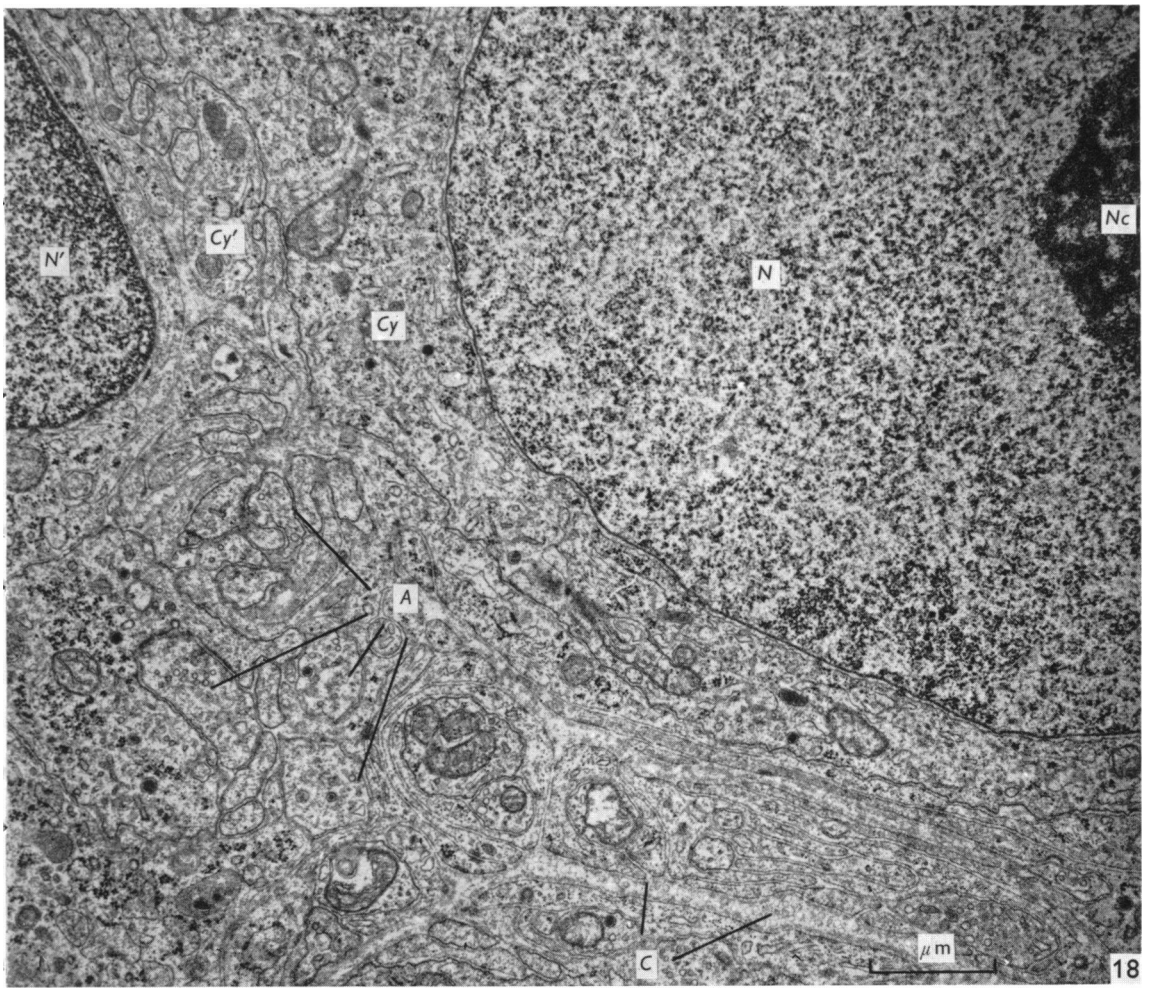
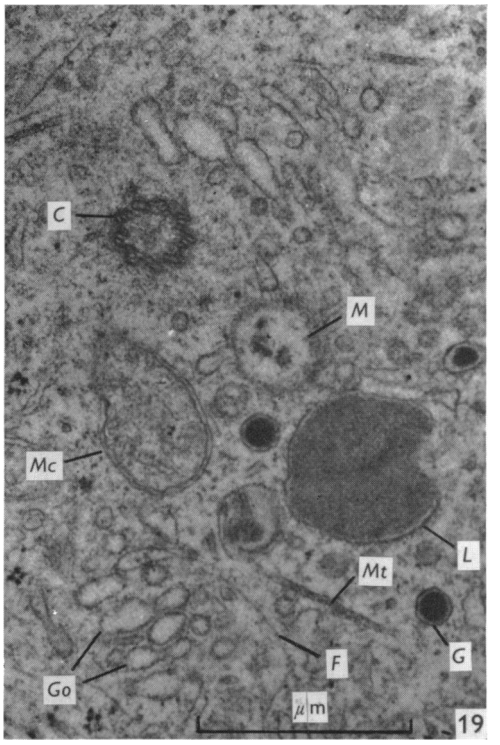


Fig. 18. Survey electron micrograph showing nucleolus (*Nc*), nucleus (*N*), and cytoplasm (*Cy*) of the nerve cell. The nucleus (*N'*) and cytoplasm (Cy') of a satellite cell are also seen. Many unmyelinated axons (*A*) occupy the lower part of the field. *C*, Connective tissue space. The scales marked only μm (without a number) in this and subsequent figures denote 1 μm.

The satellite nuclei show condensations of chromatin, especially on the inner aspect of the nuclear membrane, and their nucleoli are centrally located. The perikaryon contains the organelles common to neurones in general. Groups of closely stacked lamellae of rough-surfaced endoplasmic reticulum correlate in position and size with the Nissl substance seen with the light microscope. Free ribosomes are also abundant.

The neuronal plasma membrane makes undulating contact with that of the satellite cells, the two being separated by a clear interval about 15 nm wide (Fig. 20). Dendritic processes can be observed, but they are rare. From a narrow (0·1–0·3 μm), stalk-like attachment to the cell, they form expansions of up to 5 μm in diameter (Fig. 21). Mitochondria and free ribosomes are abundant in dendrites and perikarya, together with multivesicular bodies and both granulated and agranular vesicles (Figs. 19, 21, 22). The granulated vesicles have an external diameter of 50–80 nm and enclose an electron-dense, spherical droplet which occupies 60–90% of the space. The agranular vesicles measure 10–50 nm.



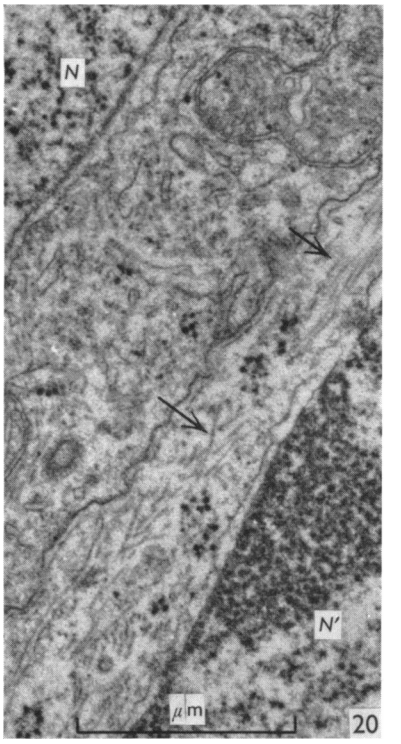
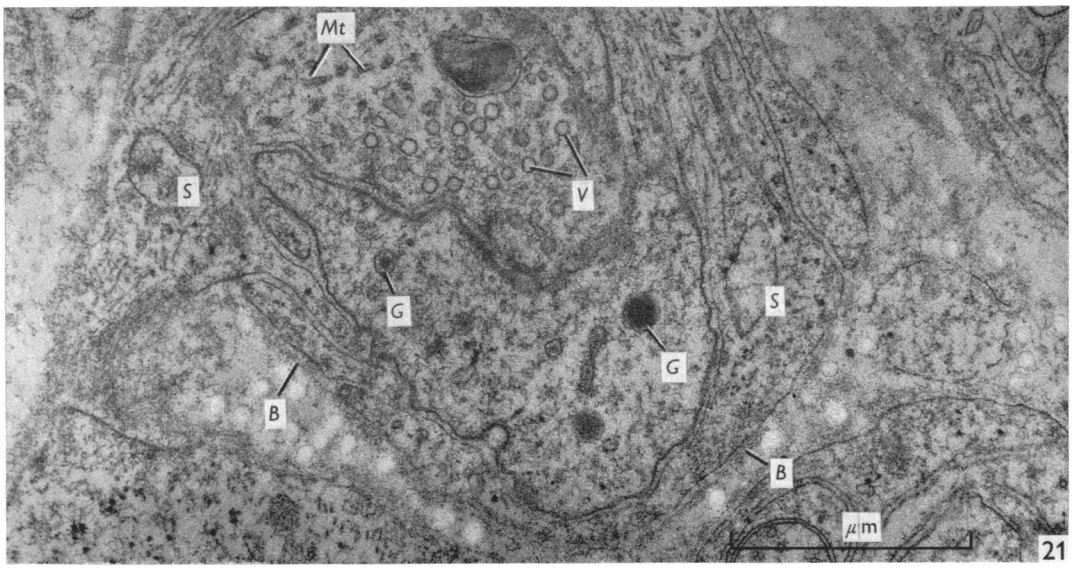


Fig. 19. Organelles in perikaryon. *C*, Centriole; *G*, granulated vesicle; *F*, neurofilament; *Go*, Golgi figures; *L*, lysosome; *M*, multivesicular body; *Mc*, mitochondrion; *Mt*, microtubule.

Fig. 20. Nucleus of nerve cell (*N*) and of satellite cell (*N'*). The satellite cytoplasm contains abundant filaments (arrows).

The neurones show remarkably few neurofilaments. They are present in very small numbers in the peripheral cytoplasm and dendrites. In contrast, coarser filaments abound in the cytoplasm of the satellite cells (Fig. 20).

In addition to the myelinated nerve fibres already seen with the light microscope, an abundance of unmyelinated axons occupies the neurolemmal sheaths in the core of the ganglia (Fig. 18). They measure 0·2–0·5 μm in diameter and the appearance of clusters of synaptic vesicles, 40–50 nm in width, and of neurotubules, in many of the axons indicates that these are terminal or preterminal (Fig. 21). The unmyelinated



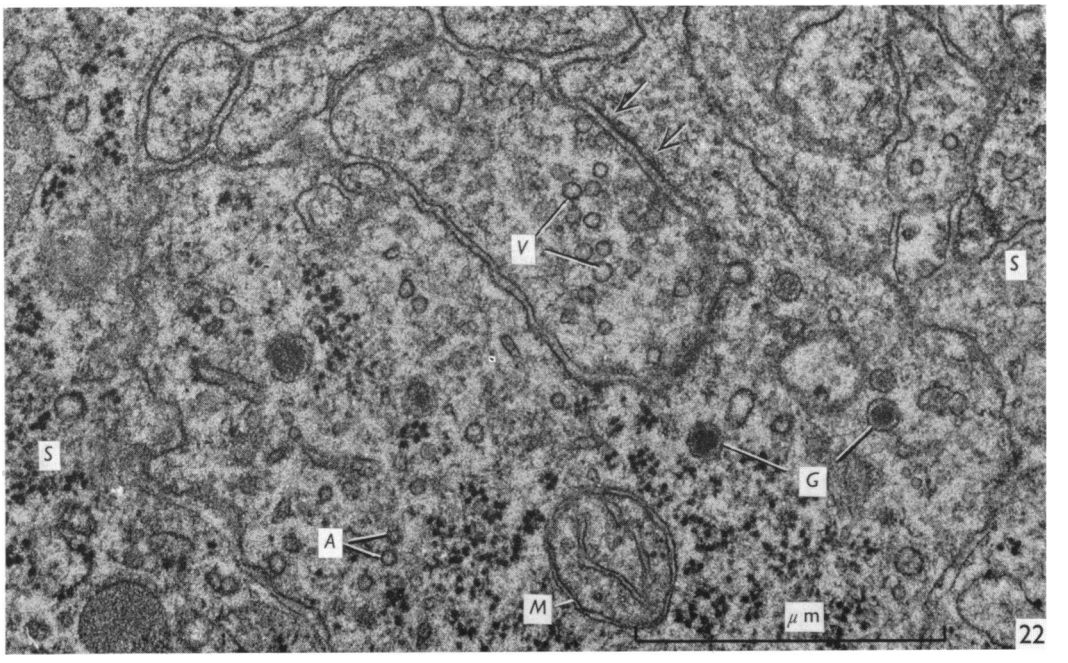


Fig. 21. Axodendritic contact. *B*, Basement lamina separating satellite cell (*S*) from connective tissue space. *G*, Granulated vesicles in dendrite. Microtubules (*Mt*) and synaptic vesicles (*V*) occupy the axon terminal.

Fig. 22. An axosomatic contact, enlarged from Fig. 19. The axon terminal displays synaptic vesicles (*V*). The increased electron density on the cytoplasmic aspect of the neuronal cell membrane is suggestive of a synaptic contact (arrows). *A*, Agranular vesicles in perikaryon; *G*, granulated vesicles; *M*, mitochondrion; *S*, satellite cytoplasm.

axons insinuate themselves between satellite cells and dendrites or nerve cell bodies. There the opposed plasma membranes frequently display asymmetrical thickening (Fig. 22). The space between the membranes here is about 30 nm wide. These features, together with aggregations of synaptic vesicles in the axon terminals, fulfil the structural criteria for a synaptic junction (Gray & Guillery, 1966).

Incubation of cryostat sections in thiocholine substrates reveals an intense reaction for cholinesterase enzymes. The response of intramuscular and glandular ganglia is the same: both follow promptly on the appearance of skeletal muscle end-plates, and, like the end-plates, their reaction is greater to acetyl than to butyryl substrate. With continued incubation the ramifications of the major intralingual nerve trunks give a relatively weak reaction, and ganglia are seen to be attached to them (Fig. 17).

One or more strongly reactive nerve bundles emerge from each ganglion. An issuing bundle often returns to the related nerve trunk, to proceed distally within it. The largest ganglia, where the nerve trunks enter the tongue, characteristically behave in this way, and the lingual-hypoglossal plexus contains an abundance of reactive fibres. The smaller intramuscular ganglia are commonly linked to one another by reactive strands. Thick sections, and graphic reconstructions, show a striking formation of chains and networks which extend freely across the midline. From these, and from the remaining isolated ganglia, fine bundles ramify in the connective tissue spaces. The precise destination of the individual nerve fibres cannot be determined, but small bundles can frequently be traced into continuity with nerve plexuses on blood vessel walls (Fig. 12). The vessels concerned are small arteries with external diameters of 125–300 μm. The paravascular plexus is profuse in the middle two-thirds of the tongue musculature and is relatively scant at the root and tip (Fig. 16).

Table 3. *Reaction of intramuscular neurones to thiocholine substrates after 18 h incubation at pH 5·0*

Inhibitor	Substrate	
	Acetyl	Butyryl
None	++++	+++
DFP	+++	+
BW 284	++	+++
DFP+BW 284	±	±
Eserine	±	±

Reactions to the inhibitors are shown in Table 3. The response of the intramuscular ganglia to the various media paralleled that of the myenteric plexus. The neurones of vagal and sympathetic ganglia reacted in a typical manner to acetyl and butyryl substrates, the former displaying a reduced content of true cholinesterase and the latter being almost devoid of visible cholinesterase activity.

The lateropharyngeal ganglion is stationed at the point of entry of the lingual branch of the glossopharyngeal nerve into the tongue, and small ganglia are strewn along the farther course of this branch. Their appearance in silver preparations and their reactions to thiocholine substrates and inhibitors are indistinguishable from those of intramuscular ganglia in the body of the tongue. Cholinesterase-positive

processes pass from them to (*a*) the external surfaces of the acinar cells of the minor salivary glands embedded in the superficial musculature of the posterior two-fifths of the tongue, and (*b*) paravascular networks which invest the small arteries supplying these glands.

The distribution of ganglia in the frontal plane is given in Fig. 23. The distribution is uneven, two-thirds of their number being contained in the anterior half of the tongue. The minor salivary glands are restricted to the posterior two-fifths of the tongue. The records show no preferred distribution in the horizontal or sagittal plane.

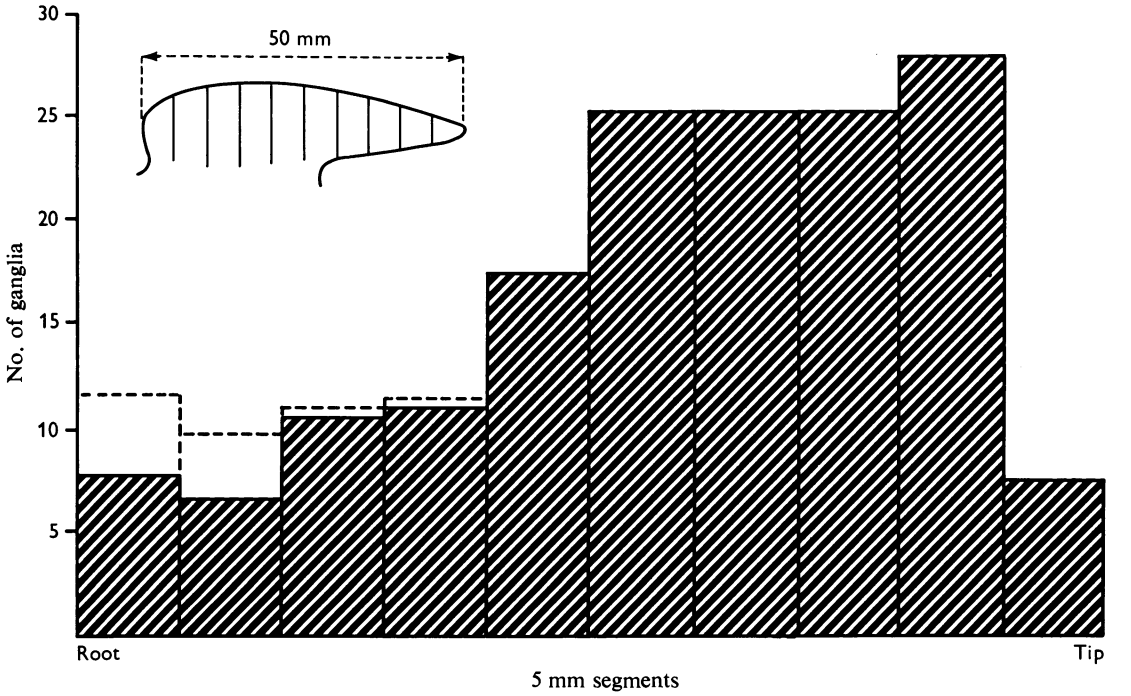
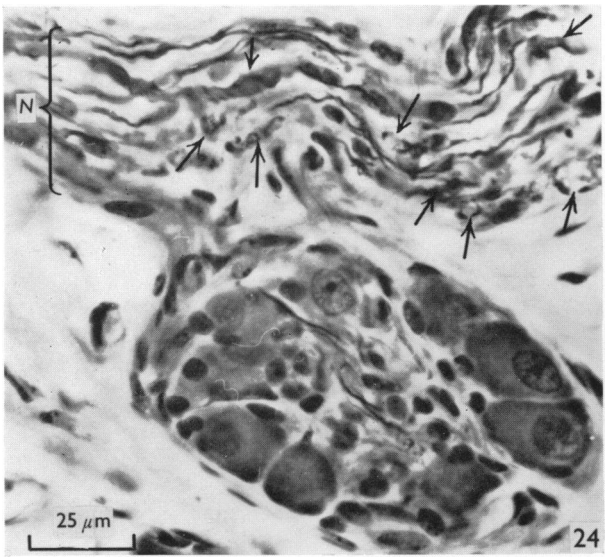
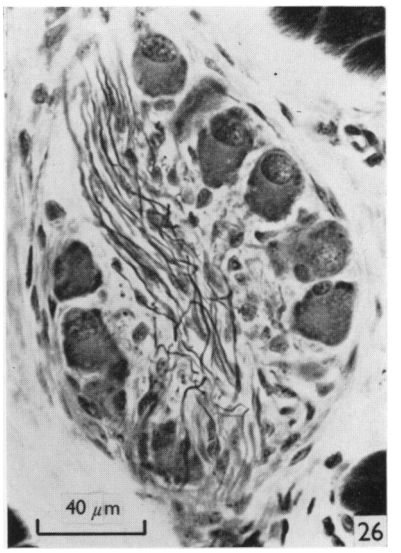


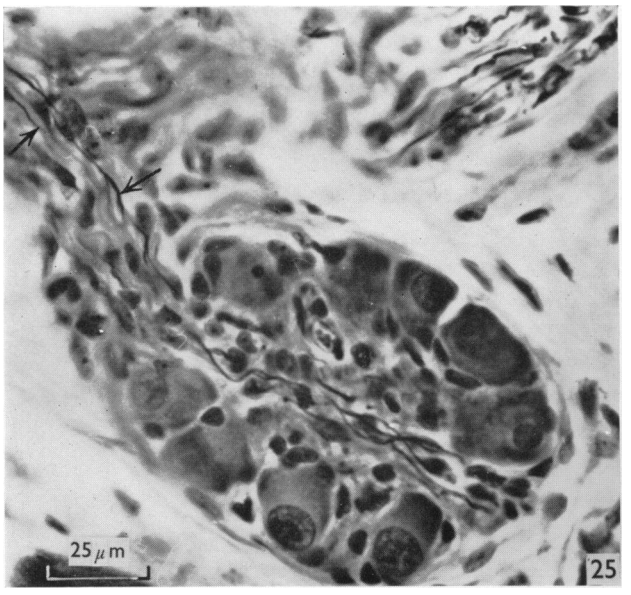
Fig. 23. Numbers and distribution of cholinesterase-reactive ganglia pooled from three cat tongues. The histogram was derived by allotting the ganglia to successive 5 mm segments. The areas enclosed by interrupted lines in the posterior segments represent ganglia which could be clearly identified as supplying the lingual salivary glands or related blood vessels.

The observed mean diameters of the ganglia from two tongues was 56 and 51 μm respectively. Schwartz's correction increased these values by 4 μm. The estimates of total numbers yielded values of 1490 and 1340 respectively for the intramuscular ganglia of the entire tongue (excluding those related to glands).

Degeneration experiments. Positive data have been provided by fragmented axons, which reveal the extralingual source of ganglionic connexions. Axonal fragmentation in the larger nerve bundles (in excess of 100 μm in diameter) is unexpectedly late. The ultimate ramifications of the severed nerve trunks have undergone almost complete degeneration by the end of the second postoperative day; however, at this time the larger bundles show only occasional axonal breakdown, the remaining fibres being swollen and somewhat irregular, but not grossly abnormal. Degenerative







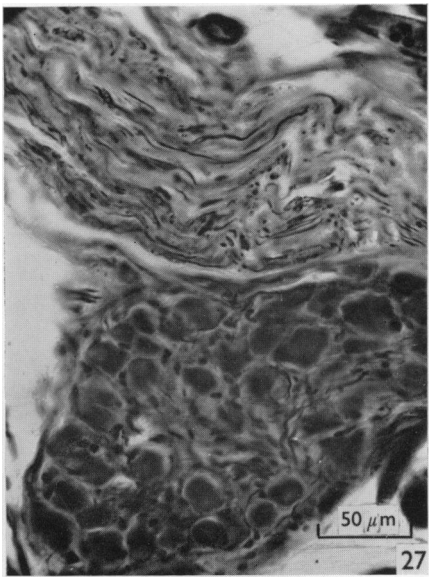


Fig. 24. Four days after hypoglossal nerve section, many axons in the intramuscular nerve (*N*) are undergoing degeneration (arrows). The ganglion appears to be attached to the nerve; however, no axons connect the two, and only normal axons occupy the core of the ganglion. Double silver impregnation.

Fig. 25. The next section of the series. The nerve is seen at the upper corners of the field. From the left part of the trunk, normal axons (arrows) descend to the ganglion. Double silver impregnation.

Fig. 26. Ganglion from another animal, 4 d after hypoglossal section. The core is occupied by many coarse axons. Double silver impregnation.

Fig. 27. Ganglion immediately adjacent to a salivary gland in the posterior third of the tongue, 4 d after glossopharyngeal nerve section. Degenerating axons impart a granular appearance to the nerve trunk. They could be traced into the interior of the ganglion in adjacent sections. Double silver impregnation.

changes in the larger bundles are at their height on the fourth postoperative day; by the seventh day the entire fibre complement has disintegrated and the argyrophil remnants have been removed. Negative data are provided on and after the seventh day: intact axons seen after this period of survival are judged not to have been contained in the peripheral stump of the severed main trunk.

The effects of chorda-lingual and hypoglossal nerve section are reciprocal. The severed nerve undergoes Wallerian degeneration as far distally as the points of union of chorda-lingual and hypoglossal nerve branches. About one-third of the more peripheral nerve bundles show varying admixtures of normal and degenerate fibres, because of plexus formation between the chorda-lingual and both divisions of the hypoglossal (cf. FitzGerald & Law, 1958).

In the anterior two-thirds of the tongue the intramuscular ganglia can be assigned with confidence to the chorda-lingual nerve. In the rare instances in which ganglia are located within epineurial sheaths near the inferior surface of the tongue, their relation to the chorda-lingual nerve can be established at once because they are surrounded by neuronal debris, or by empty neurolemmal sheaths, after appropriate periods of postoperative survival. In the usual arrangement the medium-sized nerve bundles from which they are suspended prove to be mainly composed of fibres of the chorda-lingual, and the pedicle of fibres passing to the ganglion is purely of chorda-lingual origin. A small minority of ganglia lie alongside nerve bundles which prove to have been derived almost exclusively from the hypoglossal nerve. However, in every such instance careful study of serial sections again establishes the chorda-lingual as the parent trunk (Figs. 25, 26).

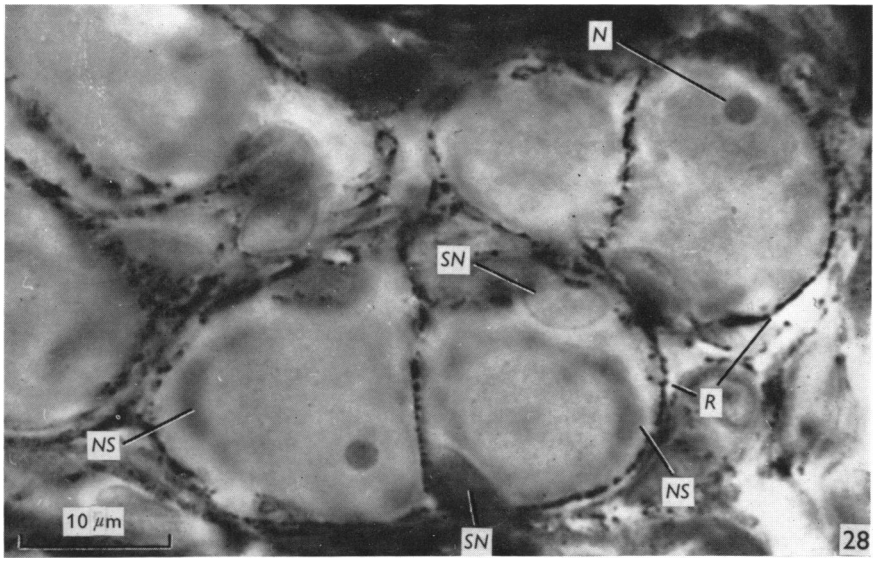
The degenerative changes following chorda-lingual section extend into the interior of the affected ganglia. Terminal axonal clubs develop on the surface of some nerve cell bodies and persist for 1–2 d after the other axonal remnants have been removed. The neurones themselves appear to be unaffected (in light microscopy), as are the fine fibre bundles that pass from the ganglia to blood vessels or to connective tissue spaces where they become lost to view. The small proportion (about 15 %) of intramuscular ganglia that occupy the posterior tongue appear to be unattached to either nerve because Wallerian degeneration cannot be traced to them after chorda-lingual or hypoglossal section, either alone or in combination.

The course of the lingual branch of the glossopharyngeal nerve is clearly shown in the obliquely cut material taken on the fourth day after glossopharyngeal section. Prescinding from its contributions to the mucous membrane, this nerve appears to expend itself entirely in the ganglia related to the minor salivary glands (Fig. 27). No descending fibres can be traced into the underlying musculature.

Superior cervical ganglionectomy is without any apparent effect upon intralingual neurones, or upon their central or peripheral connexions.

DISCUSSION

The evidence of axosomatic and axodendritic contacts on electron microscopy, together with the preganglionic degeneration elicited by appropriate nerve section, shows that the intramuscular ganglia of the cat's tongue belong to the autonomic nervous system. Failure of silver techniques to reveal the fine dendritic processes finds



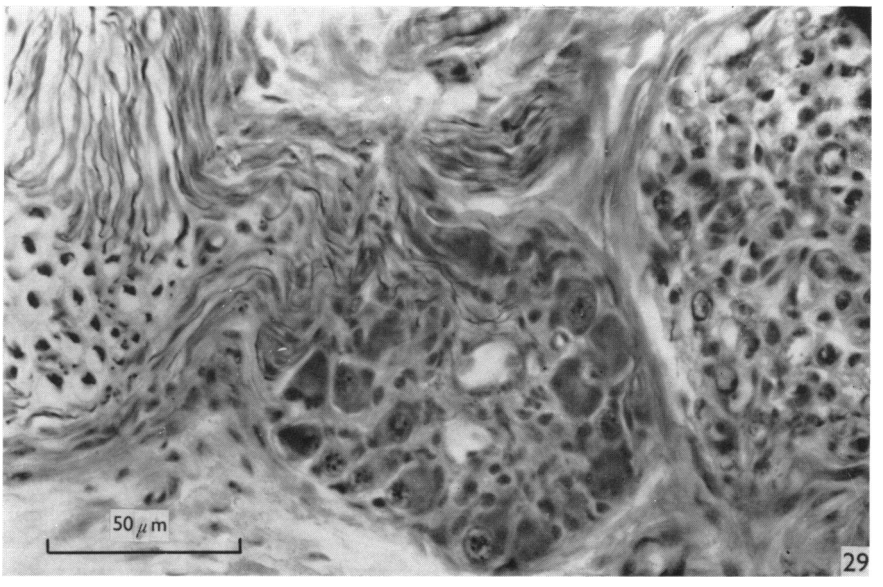


Fig. 28. Detail from a normal intramuscular ganglion. The nucleoli (*N*) of two nerve cells are prominent, but the nuclei are faint. Nissl substance (*NS*) occupies the outer part of the perikaryon. The nuclei of satellite cells (*SN*) lie within the investing web of reticular connective tissue fibres (*R*). Buffered osmium; Epon; 0·2% protargol-S 18 h; hydroquinone-sulphite; gold toned; counterstained with toluidine blue and basic fuchsin.

Fig. 29. Ganglion situated at the entry of the chorda-lingual nerve (to the left) and hypoglossal nerve, 4 d after hypoglossal nerve section. The nerve trunks appear to be 'pure', degeneration being confined, and complete, within the hypoglossal nerve. The ganglion belongs exclusively to the chorda-lingual nerve.

an explanation in the relative lack of neurofilaments contained in these neurones: several studies have suggested that neuronal argyrophilia may be proportional to the numbers of neurofilaments (Gray & Hamlyn, 1962; Guillery, 1965). That the ganglia belong to the parasympathetic system is indicated collectively by (*a*) the histochemical reactions to thiocholine substrates and inhibitors, which are typical of parasympathetic ganglia in general; (*b*) by their demonstrable attachment to the chorda-lingual nerve, whose efferent component is mainly or entirely parasympathetic; and (*c*) by their resemblance to known parasympathetic ganglia in foetuses (submandibular, otic, sphenopalatine) and in postnatal animals (gland-related ganglia of the posterior part of the tongue). In view of their distal connexions to blood vessel walls, however, their acceptance as parasympathetic is tentative at this time because histological evidence for the existence of vasomotor neurones in the cranial parasympathetic system has hitherto been lacking.

The significance of the granulated vesicles in perikarya and dendrites is uncertain. Several lines of evidence indicate that vesicles of this morphological type, situated in sympathetic nerve terminals, are storage organelles of neuronal norepinephrine (Bloom & Aghajanian, 1968). However, they have been observed also in presynaptic terminals of the sphenopalatine ganglion (Grillo & Palay, 1963) and in the nerve cells of the otic ganglion (Dixon, 1966).

The chorda-lingual nerve appears to be the exclusive source of preganglionic fibres in the anterior two-thirds of the tongue. In normal foetal and postnatal material a significant number of ganglia seem to be attached to the hypoglossal nerve, but the degeneration experiments implicate the chorda-lingual, which makes varying contributions to the hypoglossal via the lingual-hypoglossal plexus. In retrospect, the argyrophil cell infiltrate accompanying the intralingual course of the chorda-lingual nerve in the 11·0–13·0 mm embryos is considered, in view of the other evidence, to represent neuroblasts which have migrated to the tongue in this nerve. If this interpretation is correct, the neuroblasts that enter the distal branches of the hypoglossal nerve do so between the twenty-fourth and twenty-seventh day, since the adult pattern of distribution is already evident on the twenty-eighth day. The lingual branch of the glossopharyngeal nerve is also accompanied by argyrophil cells on the twenty-fourth day. If these, too, are neuroblasts they do not seem to form the intramuscular ganglia in this part of the tongue. The experimental findings indicate that the preganglionic component of this nerve is destined only for the nerve cells supplying the local minor salivary glands. The source of preganglionic fibres to the underlying intramuscular ganglia has not been identified. The vagus nerve, by exclusion, is considered the most likely alternative to the glossopharyngeal.

The observed tendency of the ganglia to link together to form chains and networks is typical of the autonomic nervous system. The occasional union of fibre strands across the midline in the anterior part of the tongue is not unexpected, for it is in this region that examples of transmedian somatic neuromuscular innervation have already been detected (Alexander & FitzGerald, 1967).

It has been known since Heidenhain (1883) that stimulation of the chorda tympani nerve causes vasodilatation in the tongue as well as in the submandibular gland. Hilton & Lewis (1958) found the vasodilatation in the cat's tongue to be abolished by hexamethonium bromide, indicating that the effective fibres of the chorda tympani

are preganglionic. However, they did not acknowledge specific vasodilator neurones; the vascular response was attributed instead to the release of small amounts of a vasoactive substance (bradykinin-forming enzyme) by glands embedded in the tongue. The same authors (1956) had retrieved bradykinin precursor in the venous outflow from the submandibular gland on chorda stimulation, and had deemed it responsible for vasodilatation there. The cat's submandibular gland has been re-investigated by Bhoola, Morley, Schachter & Smaje (1965); on the basis of temporal and quantitative comparisons between chorda-lingual stimulation and infusion of bradykinin precursors, they consider that bradykinin or related peptides contribute little or nothing to the observed vasodilatation, and that the effect is mediated by parasympathetic vasodilator nerve fibres. Pharmacological evidence indicates that kinins are not concerned in the physiological regulation of vascular resistance in skeletal muscle (Webster, Skinner & Powell, 1967).

The cat's tongue appears to be unusual among mammals in the restriction of glandular elements to its posterior part. The ganglia attached to the chorda-lingual nerve greatly outnumber the rest, and appear to be quite unrelated to glandular innervation. The morphological and histochemical findings are complementary, and are entirely in favour of the view that the ganglia house the cell bodies of cholinergic neurones whose axons run to small intramuscular arteries.

The existence of a vasodilator innervation of skeletal muscle, in subprimate mammals at least, is well known. This innervation is considered to belong to the sympathetic nervous system, however, and not to the parasympathetic: it is activated in the 'alarm reaction' induced by appropriate stimulation of the sympathetic outflow from the hypothalamus—presumably providing optimal conditions for muscular effort. The vasodilatation can be blocked with atropine and is, therefore, cholinergic. Whether this system extends to the tongue is uncertain; Erici, Folkow & Uvnas (1952) have induced vasodilatation there (in the cat) by stimulating the superior cervical ganglion, following ergotamine paralysis of the vasoconstrictor nerve supply. However, the effect was not reversed by atropine.

Since ganglia of the chorda-lingual nerve are concerned with secretomotor activity in the submandibular gland, it is reasonable to assume that the ganglia under investigation are also concerned in digestion. The simplest explanation would be to regard the entire cholinergic component of the chorda-lingual nerve as activated by the intake of food, the lingual fibres ensuring an adequate blood supply to the vigorously active tongue musculature during mastication. At the morphological level, validation of this role for the intramuscular neurones will require the identification of appropriate nerve terminals in the arterial walls, and their persistence following cervical sympathectomy. Available evidence indicates that cholinergic nerves ending upon smooth muscle contain only agranular vesicles while adrenergic terminals contain both granulated and agranular (Simpson & Devine, 1966).

SUMMARY

1. The intramuscular ganglia of the cat's tongue have been studied in order to determine their morphology, their distribution, and their central and peripheral connexions. Techniques have included graphic reconstructions, histochemistry, electron microscopy, and degeneration experiments.

2. Discrete ganglia are first identifiable in 30 mm embryos. Prior to this stage the intralingual fibres of the chorda-lingual nerve are invested by argyrophil cells. At 60 and 105 mm most intramuscular ganglia can be assigned to the chorda-lingual nerve; their morphology closely resembles that of the submandibular ganglion.

3. In postnatal material the ganglion cells are rounded and dendritic processes cannot be defined in silver preparations.

4. The nerve cells contain true cholinesterase, and pseudocholinesterase in smaller amounts. Reactive processes can be traced from the ganglia to the walls of small arteries.

5. The ganglia are distributed throughout the tongue musculature, but two-thirds of their number are found in the anterior half. Estimates of the total numbers in tongues have yielded 1490 and 1340, respectively.

6. Electron microscopy shows the neurones to be multipolar. Both perikarya and dendrites contain granulated and agranular vesicles. Preganglionic nerve fibres in the ganglia form unmyelinated terminals containing synaptic vesicles. Both axo-somatic and axodendritic synapses can be identified. A dearth of neurofilaments may account for the lack of silver-affinity in light microscopy.

7. Degeneration studies following section of the chorda-lingual, hypoglossal, glossopharyngeal, and sympathetic nerves show the chorda-lingual to be the main source of preganglionic nerve fibres. The small number of intramuscular ganglia in the posterior third of the tongue appear to be unaffected by nerve section.

8. In the light of the known physiological effects of chorda-lingual stimulation, the ganglia are tentatively considered to house cholinergic vasodilator neurones of the parasympathetic system. They may serve to adjust local blood flow in response to tongue movements during mastication.

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