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On Histology and Sensory Nerve Supply of Larynx in Flying-squirrel.

ムササビ喉頭の組織と知覚神経分布に就て.

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At this laboratory, detailed studies have been undertaken on the innervation, especially, sensory innervation of the human laryngeal mucous membrane by SASAKI, MATSUMOTO, MOMONO et al. KOIZUMI, IRINODA et al. and LEE succeeded them with the study on the same subject concerning the larynx of dog, rabbit and goat, respectively, from the angle of comparative anatomy, and achieved many interesting results. The present author also was given the opportunity of making a similar study on the nerve supply, especially, the sensory nerve supply of the laryngeal mucous membrane of flying-squirrel.

The materials were fixed in 10% neutral formol solution for a long time, cut into serial frozen cross sections and stained by SETO's impregnation method in common use at this laboratory. The large series of preparations thus obtained were thoroughly examined, the findings were studied in comparison with those obtained by the abovementioned authors with the larynges of man and the other animals, with the results reported in the following.

I. Individual Findings.

Prefacing my description of the innervation of the larynx of flying-squirrel, I will touch on the histology of its larynx, for the larynx of this animal shows some morphological and histological features specific to the animal, as in the case of any other species of animals.

The mucous membrane on the lingual side of the epiglottis is much smaller than the same of man and dog, as shown in Fig. 1. This is due to the enormous development of the laryngeal sac as mentioned below. The epithelium of the lingual side here is a thick stratified flat epithelium and contains rather tall papillae growing out of the propria. A small number of mixed glands are found in the submucosa, lymph nodules being observed only very rarely (Fig. 1).

On the laryngeal side of the epiglottis, as shown in Fig. 1, the epithelium is a far thinner stratified flat epithelium, but in the vicinity of the median line of the side, it is a 5—6-rowed ciliated epithelium. Beneath the stratified flat epithelium of the laryngeal side the papillary formation is very poor, no papillae at all being found in formation in some places. Taste-buds were not found in the stratified flat epithelium of the laryngeal side of the epiglottis, unlike in the epiglottis of man, dog etc., but on the lingual side a few of them could be seen (Fig. 2). Mixed

Fig 1. A cross section through the cranial part of larynx of a flying-squirrel. *e* epiglottic cartilage, *l* and *l'* laryngeal and lingual sides of epiglottis, *p* pharynx, *s* laryngeal sac, *t* thyroid cartilage, *h* hyoid bone. Details in the text. SETO's impregnation. Photo $\times 10$.

Fig. 2. Some taste-buds (*t*) found in the thick stratified flat epithelium on the lingual side of epiglottis of a flying-squirrel. *p* cavum pharyngis. Same staining. Photo $\times 200$.

glands are found in the submucosa of the laryngeal side in a fairly large number. The epiglottic cartilage is not of elastic nature, as is that in man and the other animals, but of hyaline nature, as all the other laryngeal cartilages, and instead of being partitioned into several pieces, as in man, forms one integral plate (Fig. 1).

The *Ventriculus laryngis* of this animal, very deeply caved in lateralwards, makes transition into a huge laryngeal sac. As clearly seen in Fig. 1, this sac is bounded by a few layers of connective tissue from the inner surface of the thyroid cartilage on the ventral side, and juts out towards the pharyngeal mucous membrane on the dorsal side. On the cranial side, it extends beyond the upper border of the thyroid cartilage near to the hyoid bone (Fig. 1), and on the caudal side, beyond the level of the *rima glottidis* down to the vicinity of the incipient part of trachea. This laryngeal sac is generally lined by a one-rowed cubic epithelium, but in some places, a 2-rowed cylindrical epithelium lines it. The mixed glands beneath the mucous membrane here are generally very small in number, but on the dorsal wall, they are formed in exceptionally good development (Fig. 1).

The vocal fold is covered by a 2—3-rowed cylindrical or ciliated epithelium in their ventralmost part, but in general, by a 2-rowed stratified flat epithelium.

Fig. 3. A cross section through the vocal fold of a snapping-squirrel. *f* mucous membrane of the vocal fold, *c* vocal cord, *m* vocal muscle, *t* m. thyroarytaenoideus externus, *v* laryngeal ventricle, *a* arytenoid cartilage. Same staining. Photo $\times 24$.

Rather broad vocal cord oriented dorsoventral and vocal muscle are situated lateral to the narrow propria of the folds. No mixed glands are seen here (Fig. 3). The ventral one-third of the pars intercartilaginea of the rima glottidis is covered by a 2-rowed flat epithelium (Fig. 7) and its dorsal two-thirds by a 2—3-rowed ciliated epithelium (Fig. 5). The propria here is a very thin layer, so that the arytenoid cartilage here is covered by a very thin mucous membrane (Fig. 7), but as we go more dorsalwards, the subepithelial connective tissue becomes thicker and comes to contain some mixed glands, attaining fairly good development in the dorsal wall. In the part nearer to the trachea than the rima glottidis, the epithelium turns into a 2—3-rowed ciliated epithelium everywhere, the subepithelial connective tissue layer also becomes thicker and mixed glands may be found in it, though never in a large number.

The laryngeal mucous membrane of flying-squirrel, quite as in man and the other animals, is considerably well provided with nerve fibres, especially, sensory fibres, but in a number far smaller than those in the higher mammals. These nerve fibres are originated in the n. laryngeus superior of the n. vagus and the cervical sympathetic cords. The sensory fibres among them being thick and myelinated and the vegetative fibres thin and unmyelinated, these two sorts are very easily discriminated histologically.

These nerve fibres form very well-developed plexus submucosus and plexus proprius in man and other animals, but in flying-squirrel, the formation of the proprial plexus, not to mention that of the submucosal plexus, is very inferior, especially, in the parts where the connective tissue layer is thin. The sensory terminations found in this part are incomparably simpler than those in man and even far simpler than those in goat and dog, corpuscular terminations as found in the larynges of man and the other animals being entirely absent in the laryngeal mucosa of flying-squirrel and such branched terminations formed by very thick fibres as found specifically in rabbit being here out of the question.

The part richest in sensory fibres in the larynx of flying-squirrel is the laryngeal side of the epiglottis, in particular, its median portion rich in mixed glands and with thick mucosa and next in its peripheral portions, while in the areas between these, the sensory fibres and their terminations are far less abundant. In Fig. 4 are shown the submucosal and the proprial plexus formed in the vicinity of the median line of the laryngeal side of the epiglottis, but even those are incomparably worse developed than in man and the other animals.

In Fig. 5 is illustrated the nerve plexus formed in the subepithelial connective tissue in the dorsal half of the pars intercartilaginea of the rima glottidis. This is far poorer in development than the nerve plexus in the laryngeal side of the epiglottis. In Fig. 6 we see the plexus formation by small nerve bundles seen in the submucosa and the propria of the dorsal wall of the laryngeal sac. The plexus here is also ill developed. Some nerve fibres found in the very thin subepithelial connective tissue layer in the pars intercartilaginea of the rima glottidis are shown in Fig. 7. Here, no plexus is formed. Descending more distalwards nearer to the entrance of trachea below the rima glottidis, we find the sensory fibres gradually decreasing in number. Sensory fibres are found in a small number in the plica

Fig. 4. Submucosal (*s*) and proprial (*p*) nerve plexuses formed in the median part on the laryngeal side of epiglottis of a flying-squirrel. *c* multiple-rowed ciliated epithelium, *g* mixed glands. Same staining. Photo $\times 200$.

Fig. 5. Subepithelial nerve plexus found in the dorsal part of pars intecartilaginea of rima glottidis. *a* arytenoid cartilage. Same staining. Photo $\times 200$.

vocalis too and not rarely in the mucous membrane lining the laryngeal sac, but only very seldom in the part adjacent to the thyroid cartilage.

The sensory terminations found in the laryngeal mucous membrane of flying-squirrel consist in unbranched and simple branched terminations formed subepithelially or intraepithelially. Subepithelial terminations of the most primitive

Fig. 6. Submucosal (*s*) and proprial (*p*) nerve plexuses in the mucous membrane of the dorsal wall of the laryngeal sac of a flying-squirrel. *m* mixed glands, *d* gland duct. Same staining. Photo $\times 200$.

Fig. 7. Nerve fibres running in the narrow propria of the ventral part of pars intercartilaginea of rima glottidis of a flying-squirrel. *a* arytenoid cartilage, *e*, 2-rowed stratified flat epithelium. Same staining. Photo $\times 200$.

unbranched type are found in every part of the laryngeal mucous membrane, the terminations seen in the laryngeal sac being of this type in the largest majority of the cases. E. g., an unbranched sensory termination found subepithelially in the medial wall of the right laryngeal sac is shown in Fig. 8. The single fibre forming such a termination is usually comparatively thin, shows not much change in size, runs a course parallel to the epithelium just beneath it and ends sharply. Intraepithelial terminations are also very rare in the laryngeal sac, and in short, the sensory supply to the laryngeal sac is very poor on the whole.

Even in this laryngeal sac, however, in the vicinity of its entrance subepithelial simple branched terminations are often observed and some intraepithelial fibres too are demonstrable. In Fig. 9 is shown a simple branched termination formed by terminal fibres showing some change in size. Some of its terminal fibres run further up into the epithelium and end in intraepithelial fibres. Another simple branched termination found in the same place is shown in Fig. 10. Here, the comparatively thick terminal fibres showing some change in size run very peculiar winding courses.

As mentioned above, the laryngeal side of the epiglottis is the part most abundantly supplied with sen-

sory fibres and their terminations in the larynx of flying-squirrel as well as of man and the other animals. The terminations here comprise some of even somewhat complex type. Now, in the laryngeal side of the epiglottis, the portion richest in sensory fibres is the area adjoining its median line, for here the propria as well as the submucosa containing many mixed glands are thick.

The sensory terminations found in the median part of the laryngeal side of the epiglottis are in most cases of rather complex branched type composed of several terminal branches. Not rare, however, are very simple branched terminations having only 2 or 3 terminal fibres, either. Very frequently, some of the terminal fibres of such branched terminations run into the epithelium to end therein. The stem fibres are mostly medium-sized fibres but sometimes thin fibres form the stem

Fig. 8. An unbranched sensory terminal fibre running directly beneath the epithelium of the medial wall of the laryngeal sac of a flying-squirrel. Same staining. $\times 400$.

Fig. 9. Simple branched sensory termination found in the mucous membrane of the orifice of the laryngeal ventricle of a flying-squirrel. Fine terminal fibres pass over into the ciliated epithelium. Details in the text. Same staining. Photo $\times 400$.

Fig. 10. Ditto. Nerve fibres rich in change in size show specific looping courses. Details in the text. Same staining. Photo $\times 800$.

fibres. Their terminal fibres usually do not show much change in size and comparatively rarely run winding courses.

In Fig. 11 is shown a simple branched sensory termination originated in the plexus proprius, found near the median line of the laryngeal side of the basis of the epiglottis. Its terminal fibres, showing not much change in size end always

Fig. 11. Some simple branched sensory terminations originated in a nerve bundle of the proprial plexus found in the median part of the laryngeal side of epiglottis of a flying-squirrel. *i* intraepithelial fibre. Same staining. $\times 400$.

Fig. 12. An intraepithelial branched termination composed of several fine terminal fibres originated in the weakly formed proprial plexus in the median part on the laryngeal side of the basis of epiglottis of a flying-squirrel. Details in the text. Same staining. $\times 400$.

sharply, partly subepithelially and partly intraepithelially. Fig. 12 shows a somewhat more complex branched termination with about half a dozen terminal fibres, formed by a medium-sized sensory fibre from the plexus proprius. In this terminal fibres, hardly showing any change in size or peculiar winding in their courses, gradually taper off into sharp points, either subepithelially or intraepithelially.

In the peripheral parts of the laryngeal side of the epiglottis, the mixed glands are worse developed than in the median area and the subepithelial connective tissue layer is thinner, so that here the plexus formation is less prominent and the number of sensory fibres smaller, while their terminal mode is also simpler. E. g., you see in Figs. 13 and 14 rather thin stem fibres branching out into 2 or 3 thin

Fig. 13. A simple branched intraepithelial sensory termination found in the peripheral part on the laryngeal side of epiglottis of a flying-squirrel. Stem fibre runs parallel with the basis of the ciliated epithelium. Details in the text. Same staining. $\times 400$.

terminal fibres and ending in intraepithelial simple branched terminations. As shown in these figures, the terminal fibres of the sensory terminations found in the laryngeal mucous membrane of flying-squirrel run comparatively long courses beneath the epithelium on very frequent occasions.

A bifurcated sensory termination found in the peripheral part of the upper laryngeal side of the epiglottis is shown in Fig. 15. Here, a myelinated medium-sized fibre, after losing its myelin sheath, divides into 2 terminal fibres, which, after looping once each, run toward and into the epithelium to end there in sharp points. Terminations with terminal fibres showing rather perceptible windings as

Fig. 14. A simple branched intraepithelial sensory termination composed of fine terminal fibres found in the peripheral part on the laryngeal side of epiglottis of a flying-squirrel. Details in the text. Same staining. $\times 400$.

Fig. 15. A bifurcated intraepithelial sensory termination found in the peripheral part on the laryngeal side of epiglottis of a flying-squirrel. 2 terminal fibres describe a large looping course respectively. Same staining. $\times 400$

seen here are very rare in the laryngeal mucous membrane of flying-squirrel. In Fig. 16 is shown a bifurcated termination with 2 rather thick terminal fibres

Fig. 16. A bifurcated sensory termination composed of thick fibres found in the same place as in Fig. 15. Details in the text. Same staining. $\times 400$.

originating in a thick fibre found in the same place as the preceding. Such terminations formed by comparatively thick fibres are not rare in the larynx of flying-squirrel, but terminations formed by such enormously thick fibres as found in the human and more frequently in the rabbit's larynx were never found in the larynx of my flying-squirrel.

In the peripheralmost margin of the laryngeal side of the epiglottis where the laryngeal and the lingual sides of the epiglottis meet, sensory terminations are still observable and some complex branched terminations are found here and there, but upon passing over into the lingual side, the number of sensory terminations decreases rather steeply and their terminal mode becomes much simpler. In Figs. 17 and 18 are shown sensory terminations found in the peripheralmost parts of the laryngeal side of the epiglottis. In Fig. 17 we see unbranched and simple branched intraepithelial terminations originating in medium-sized sensory fibres. In Fig. 18, a comparatively thin stem fibre, after a rather strangely winding course, divides into 3 fine fibres, which run long courses directly beneath the epithelium before ending sharply. The sensory supply to the taste-buds found in the epithelium of the

Fig. 17. Unbranched and simple branched intraepithelial terminations found in the peripheralmost part of the laryngeal side of epiglottis of a flying-squirrel. Details in the text. Same staining. $\times 400$.

Fig. 18. Simple branched sensory termination formed beneath the stratified flat epithelium of the peripheral part of the laryngeal side of epiglottis of a flying-squirrel. Stem fibre runs specific looping courses. Details in the text. Same staining. $\times 400$.

lingual side of the epiglottis is very poor, most of them being devoid of sensory terminations in them, suggesting that these taste-buds are merely rudimentary formations.

The submucosa being rich in mixed glands and the propria being considerably

Fig. 19. 2 unbranched sensory terminal fibres and a simple branched intraepithelial termination found in the plica aryepiglottica of a flying-squirrel. Details in the text. Same staining. Photo $\times 400$.

Fig. 20. A sketch of Fig. 19. Terminal fibres are represented very clearly.

thick in the plica aryepiglottica in the upper pars intercartilaginea of the rima epiglottidis, the sensory fibres are here rather abundant and their terminations are often rather complex. An intraepithelial branched termination originating in a medium-sized stem fibre and 2 subepithelial unbranched sensory terminations are shown in Fig. 19. Fig. 20 is a hand-drawn sketch of the same, to bring out the ramification and the running courses of the terminal fibres of the branched termination with particular clarity.

In the plica vocalis of flying-squirrel, the propria is very ill developed, so that the incoming sensory fibres are very small in number, their terminations consisting in mere unbranched or very simple branched terminations found very sporadically. These terminations are formed subepithelially in most cases, but more rarely intraepithelially.

II. Summary.

The epithelium of the laryngeal side of the epiglottis of flying-squirrel is a stratified flat epithelium much thinner than that on its lingual side, but near its median line, it is a 5—6-rowed ciliated epithelium. No papillae are formed beneath this stratified flat epithelium, as are found on the lingual side of the epiglottis. Taste-buds are found on the lingual side alone in small number. The mixed glands are well developed in the median and the peripheral parts of the laryngeal side. The epiglottic cartilage is of hyaline nature and forms one integral plate.

The ventriculus laryngis forms a huge laryngeal sac, which reaches the inner surface of the thyroid cartilage on the ventral side, juts out toward the pharyngeal mucous membrane on the dorsal side, reaches the vicinity of the hyoid bone beyond the upper border of the thyroid cartilage on the cranial side and extends down to the upper end of the trachea on the caudal side. The sac is generally lined by a one-rowed cubic epithelium and partly by a 2-rowed cylindrical epithelium. The propria is very ill developed and mixed glands are found only in a limited area.

The vocal cord consists in the main of 2-rowed stratified flat epithelium and a thin propria, but their ventralmost part alone is covered by a 2—3-rowed cylindrical or ciliated epithelium. The ventral one-third of the pars intercartilaginea of the rima glottidis is composed of a 2-rowed flat epithelium and a thin propria, but its dorsal two-thirds are of a 2—3-rowed ciliated epithelium and subepithelial connective tissue gradually gaining in thickness dorsalwards and containing some mixed glands. The lower part of the larynx between the rima glottidis and the entrance to the trachea is composed of a 2—3-rowed ciliated epithelium and a subepithelial connective tissue layer containing mixed glands.

The laryngeal mucous membrane of flying-squirrel is supplied with sensory fibres as in man, dog, goat and rabbit, but much less abundantly than in these, the submucosal and the proprial plexus found very well developed in these mammals being much inferior in flying-squirrel. The sensory nerve terminations are also much simpler in formation, no such corpuscular terminations or complex branched terminations as found in the above animals being ever to be found here.

The sensory terminations found in the laryngeal mucous membrane of flying-squirrel are of unbranched and simple branched types formed either subepithelially or intraepithelially. Most primitive subepithelial unbranched terminations are found everywhere in the mucous membrane and in the laryngeal sac in particular most of the terminations are of this type, found here and there in sporadic existence. Thus, the sensory supply to the laryngeal sac may be called very poor in general, but near the entrance of the ventriculus laryngis subepithelial and intraepithelial simple branched terminations are not rare either. The terminal fibres of these terminations are sometimes comparatively thick and sometimes run wavy courses,

The laryngeal side of the epiglottis is richest in sensory fibres, rather complex branched ones being found among their terminations, especially near its median line. Branched terminations with several terminal fibres are not rare here and some complex intraepithelial branched terminations are present, but the terminal fibres are usually medium-sized or thin, showing not much change in size. In the peripheral parts of the laryngeal side of the epiglottis, the sensory fibers are fewer and their terminations are simpler, but in these parts, simple branched terminations originating in thick fibres may be observed. Terminations formed by extraordinarily thick fibres, as found in man and rabbit, are never found in flying-squirrel.

Sensory terminations are found on the border part between the laryngeal and the lingual sides of the epiglottis but their number decreases rapidly as we pass over into the lingual side, while their terminal mode also becomes simpler. The sensory nerve supply to the taste-buds in this part is so very poor as to suggest that these taste-buds represent nothing but rudimentary formations.

In the plica aryepiglottica, the submucosa is rich in mixed glands and the propria rather thick; so the sensory supply is rather abundant and their terminations are often of rather complex branched type. In the plica vocalis, the incoming sensory fibres are very few and their terminations are only of unbranched and very simple branched type and found only far between in sporadic existence. A few intraepithelial fibres are also found here. A small number of sensory fibres and their terminations are found in the mucous membrane between the rima glottidis and the upper end of the trachea, but these lose gradually in development, the nearer the distal end of the larynx is approached.

内 容 自 抄.

ムササビの喉頭蓋の喉頭面の上皮はその舌面のものよりも遙かに背の低い重層扁平上皮から成るが、その正中部丈は絨毛上皮から成る。味蕾は舌面上皮のみに少量に発見される。この部の粘膜では混合腺は良好な発達を示すが、淋巴集合は極めて少い。

喉頭室は甚だ大きい喉頭嚢を作る。前方は甲状軟骨、上方は舌骨、下方は気管上部に達する。その粘膜は概ね1列性立方上皮と狭い固有膜とから成る。

声皺襞は概ね2列性扁平上皮と狭い固有膜とから成る。声門の軟骨間部の前 $\frac{1}{2}$ は同様2列性扁平上皮で蔽われるが、後 $\frac{1}{2}$ は2—3列性絨毛上皮で蔽われ、固有

膜も増大，中に混合腺を含む．声門より下方気管に至るまでの粘膜は前者同様な組織構成を示す．

ムササビの喉頭粘膜も人や2—3動物に於けると同様に知覚線維に恵まれているが，粘膜下膜及び固有膜神経叢の劣勢な形成に伴ってその量も又知覚終末の構成も人等に於けるよりは遙かに劣勢である．

この喉頭粘膜に見られる知覚終末は非分岐性及び単純性分岐終末として上皮下及び上皮内に形成される．喉頭嚢粘膜では最単純の非分岐性終末を而も散在性に見るに過ぎず，従ってここでは知覚神経分布は甚だ劣勢である．唯喉頭室の入口附近では単純性分岐性終末も稀ならず発見される．

喉頭蓋喉頭面の粘膜は喉頭粘膜の中では最も知覚線維に富む．そして少々複雑な分岐性終末も所々に発見される．特に正中部で然りである．然しこの部の終末は一般に中等大又は細い線維に由来する．反之その周辺部では前者に於けるよりも終末の量は少いが，太い線維に由来する分岐性終末も発見される．

喉頭蓋舌面の粘膜には知覚線維の進入は劣勢，その終末様式も甚だ単純，又この部にのみ見られる味蕾に対する知覚神経分布も甚だ劣勢である．

声皺襞にも極く少量の知覚神経の進入を見る．その終末は非分岐性及び単純性分岐性終末で表わされ，又稀ならず上皮内線維も発見される．声門から下方の粘膜にも知覚終末が見られるが，之は下方に向って愈々劣勢を示す．

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