Morphological observations on the fate of the Lateral Appendix in the embryonic Olfactory Organ of Rana nigromaculata.

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With 12 Text-figures.

CONTENTS.

INTRODUCTION.

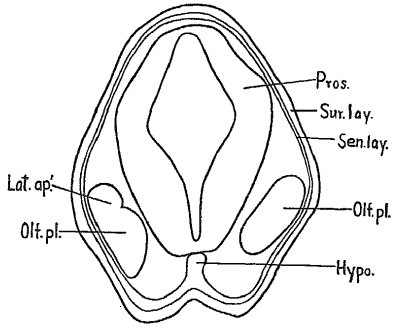
IN the early development of the olfactory organ of the Amphibia there appears an ephemeral structure, the lateral appendix, which persists for a time and degenerates in the later stages of the nasal development. The function of this organ is obscure. The present investigation is to give an account of the structure and development of the lateral appendix as a preparation for later experimental studies. For the development of the whole nasal organ the reader is referred to a previous paper by the writer (Tsui, 1946).

TECHNIQUES.

The techniques employed in the present investigation have been described in a previous paper (Tsui, 1942). For the purpose of studying the finer structures serial paraffin sections were cut 5μ in thickness. For every stage three specimens were used, and stained respectively with Delafield's haematoxylin, Mallory's triple stain and Heidenhain's haematoxylin in order to bring out the structure of every part of the cell.

Developmental Anatomy.

The formation of the lateral appendix in Rana nigromaculata Hallowell begins at the second stage of development of the olfactory organ. When the larva is 5·5 mm. in length, the olfactory pit is beginning to extend to form the middle lumen and its dorso-lateral part lengthens posteriorly



Text-fig. 1.

Cross-section of the head through the olfactory placode and the anlage of the lateral appendix. ×75. *Hypo*, hypophysis; *Latap'*, anlage of lateral appendix; *Olfpl*, olfactory placode; *Pros*, prosencephalon; *Senlay*, sensory layer of ectoderm; *Surlay*, surface layer of ectoderm.

Abbreviations for Text-figs. 1–12.

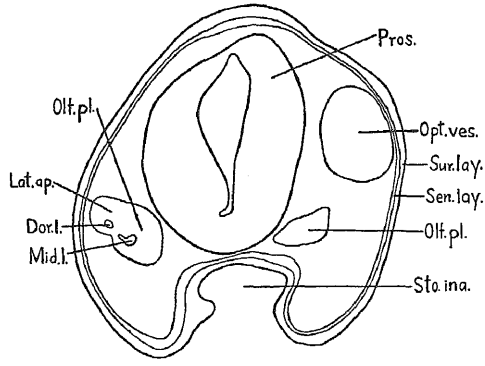
(Text-figs. 7–12 drawn with the aid of a camera lucida.)

Cil, cilia; *Cyto*, disintegrated cytoplasm; *Latap*, vestige of the lateral appendix; *Mito*, mitosis; *Nuc*, normal nucleus; *Nuc''*, degenerated nucleus; *Nuc'*, beginning of the degeneration of nucleus; *Olfep*, olfactory epithelium; *Piggr*, pigment granules; *Piggr'*, degenerated pigment granules; *Yogr*, yolk granules.

to form a small tube. The cells directly behind this tube are immediately differentiated into the anlage of the lateral appendix. In the beginning the anlage is not sharply marked off from the olfactory placode as the constriction between it and the placode is now barely perceptible (Text-figs. 1 and 7). The placode is at this time in the form of a thick disc and is still

appressed to the sensory layer of the ectoderm. With the placode in this position, the anlage of the lateral appendix is not yet lateral to it, but is situated on its dorsal part and is posterior to the olfactory pit (Text-figs. 1 and 7).

After the formation of the middle lumen (the length of the larva is now about 7 mm.), the blind end of the small tube enlarges and extends into the anterior part of the anlage of the

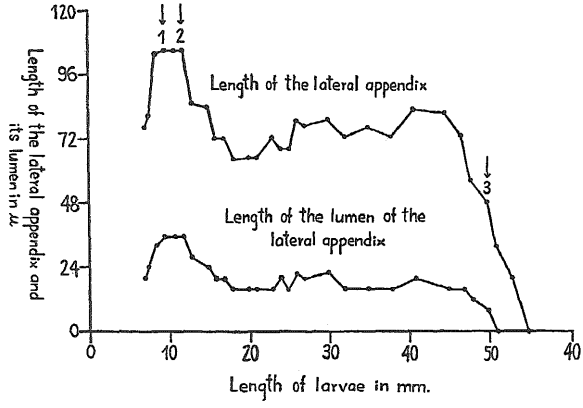


TEXT-FIG. 2.

Cross-section of the head through the olfactory placode and the lateral appendix. ×75. *Dorl*, dorsal lumen; *Latap*, lateral appendix; *Midl*, middle lumen; *Optves*, optic vesicle; *Stoina*, stomodeal invagination. For other abbreviations see Text-fig. 1.

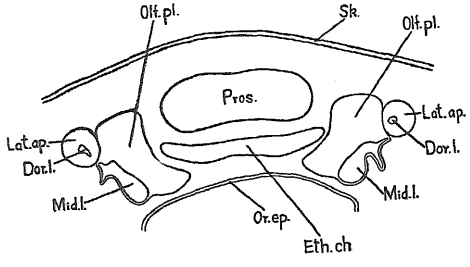
lateral appendix, thus converting it into a blind sac. In this way the lateral appendix proper comes into being (Text-figs. 2 and 8). Its length is now about 76 μ, while its lumen, which is termed the dorsal lumen, is about 20 μ (Text-fig. 3). The relative size of the lumen to the lateral appendix is small and remains at the anterior part of the latter throughout its developmental history.

In the course of development, the olfactory placode expands more rapidly at the medial region and thrusts the lateral appendix from its original position to a new one lateral to the



TEXT-FIG. 3.

Graphic representation of the length of the appendix and that of the lumen during growth and degeneration. Arrow 1 indicates the appearance of the anterior lower sac; Arrow 2, appearance of the medial nasal gland; Arrow 3, the beginning of the metamorphosis.

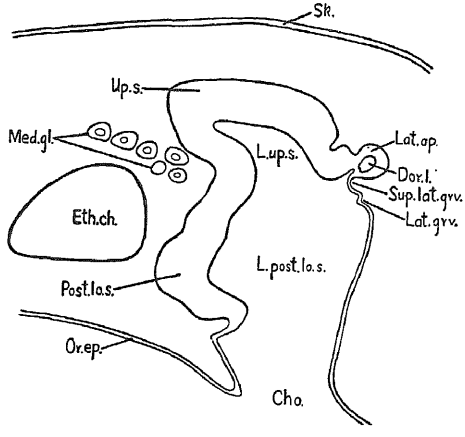


TEXT-FIG. 4.

Cross-section of the upper half of the head through the olfactory placode and the lateral appendix. ×75. *Ethch*, ethmoid part of the chondrocranium; *Orep*, oral epithelium; *Sk*, skin. For other abbreviations see Text-figs. 1 and 2.

placode. A prominent constriction now appears between these two structures (Text-figs. 4 and 9).

Immediately after the formation of the lateral appendix, its posterior end lengthens out hanging free of the placode. The body of the lateral appendix together with its posterior extension increases in dimensions along with growth of the placode



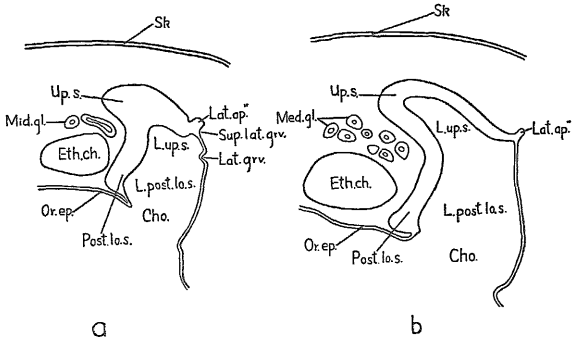
TEXT-FIG. 5.

Cross-section of the right olfactory organ through the lateral appendix and the choana. ×75. *Cho*, choana; *Dorl*, dorsal lumen; *Ethch*, ethmoid part of chondrocranium; *Lpostlos*, lumen of posterior lower sac; *Lups*, lumen of upper sac; *Latap*, lateral appendix; *Latgrv*, lateral groove; *Medgl*, medial nasal gland; *Orep*, oral epithelium; *Postlos*, posterior lower sac; *Sk*, skin; *Suplatgrv*, super-lateral groove; *Ups*, upper sac.

up to the beginning of the third developmental stage, when the larva has reached a length of 9·5 mm. The growth of the appendix is then suddenly arrested and remains stationary for a very brief period. After this it begins to degenerate. The easily ascertainable evidence of degeneration is the diminution of its dimensions (Text-fig. 5). The curves in Text-fig. 3 represent the whole life-history of the lateral appendix in the course

of nasal development in terms of its length and the length of its lumen. Their widths both in growth and degeneration correspond with their lengths.

After a sharp decrease, the length of the appendix and its lumen remains again more or less stationary (the fluctuations are greater for the body of the appendix than for the lumen).



TEXT-FIGS. 6 A AND 6 B.

6 A. Cross-section of the right olfactory organ through the lateral appendix and the choana. × 50.

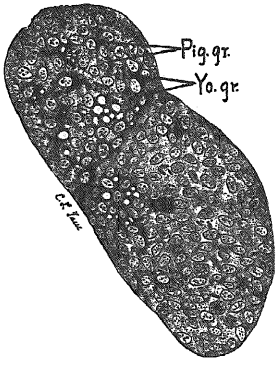
6 B. The same, at a later developmental stage. × 50. *Latap"*, vestige of the lateral appendix. For other abbreviations see Text-fig. 5.

Towards the end there is again a rapid decrease. At the beginning of metamorphosis the lateral appendix is reduced to a very small remnant attached to the lateral posterior part of the upper sac above the super-lateral groove (Text-figs. 6 A and B). Later it disappears completely in the nasal cavity.

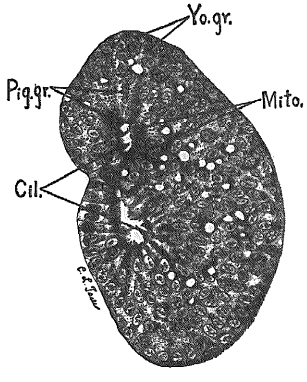
The cause of the degeneration of the lateral appendix is not known. It is remarkable, however, that the sudden arrest of its development is just at the beginning of differentiation of the anterior lower sac. Furthermore its degeneration starts at the point when the medial nasal gland makes its appearance.

Developmental Histology.

The anlage of the lateral appendix consists of undifferentiated epithelial cells. Their nuclei are ovoid in shape and are crowded together so that the outline of individual cells cannot be made out. Chromatin granules are comparatively small. Large yolk-granules and numerous fine pigment granules are present in the



Text-fig. 7.



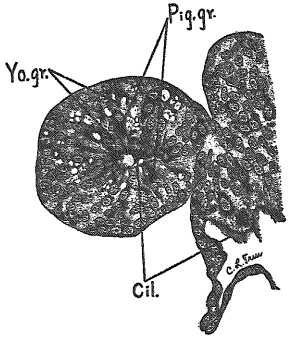
Text-fig. 8.

Fig. 7.—Enlarged and detailed view of the left olfactory placode with the anlage of the lateral appendix shown in Text-fig. 1. ×350.
Fig. 8.—Enlarged and detailed view of the left olfactory placode with the lateral appendix shown in Text-fig. 2. ×350.

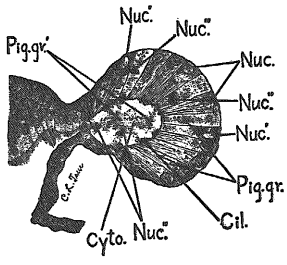
cytoplasm. The granules are more or less evenly distributed at this stage (Text-fig. 7).

As soon as a blind sac is formed out of this anlage, the cells are differentiated into stratified epithelium containing about four layers of cells at the height of the development of the lateral appendix and about three layers in the earlier stages (Text-figs. 8 and 9). Around the dorsal lumen there is a layer of columnar cells provided with cilia on their free surface. Their nuclei are ellipsoid. The layers of cells in the basal part are not well defined and are represented only by their ovoid nuclei.

Yolk and pigment granules are still present in the cytoplasm; the former decrease in quantity in the course of development, and are completely absorbed when the lateral appendix reaches its full growth. The distribution of pigment granules is no longer so even as in the anlage. A large number of these granules become concentrated in the neighbourhood of some of the nuclei, their aggregate assuming more or less the form of



TEXT-FIG. 9.



TEXT-FIG. 10.

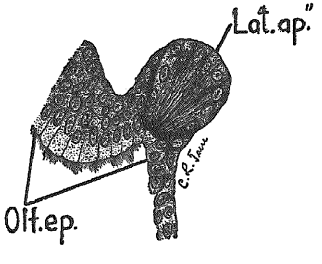
Fig. 9.—Enlarged and detailed view of the left lateral appendix with a part of the olfactory placode shown in Text-fig. 4. ×350.
Fig. 10.—Enlarged and detailed view of the lateral appendix with a part of the upper sac shown in Text-fig. 5. ×350.

a spindle with the nucleus in the middle. Such aggregates are especially prominent in the advanced developmental stages (Text-fig. 9). Mitotic figures are often seen in the early stages but not later (Text-fig. 8).

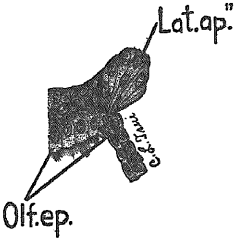
As long as the lateral appendix remains stationary, its microscopic anatomy is as described above, but structural changes immediately take place when it begins to degenerate. The most easily detectable change is the diminution in size. Histologically, this is effected at first by the reduction in the number of cell layers. As described above, the fully developed appendix consists of about four layers of cells. These layers

gradually diminish through the destruction of their cells. In the dorsal lumen or somewhere near it are often found small amounts of remnants of nuclei and cytoplasm, as well as pigment granules and cilia. The stages in the process of degeneration cannot be made out, however. They are probably obscured by the crowded condition of the basal nuclei.

At last the degenerated appendix consists of only one layer of well-defined cells. In this greatly simplified picture, the disintegration of various elements are more easily observable. This



TEXT-FIG. 11.



TEXT-FIG. 12.

Fig. 11.—Enlarged and detailed view of the vestige of lateral appendix shown in Text-fig. 6 A. ×190.
Fig. 12.—The same shown in Text-fig. 6 B. ×190.

last layer of cells is highly columnar (Text-fig. 10). Their nuclei are ovoid in shape and are always situated at the basal part of the cells. The cytoplasm is fibrillar and takes the stain very faintly. The free surface of most of the cells is devoid of cilia, or, when they are present, they are scanty and appear stuck together. In most cases, the lumen is filled with disintegrated cytoplasm containing fragments of nuclei, cilia, and pigment granules. Sometimes the lumen is so filled with débris that the surrounding columnar cells are flattened and become squamous in shape.

The columnar cells as described in the preceding paragraph appear still normal under the microscope. When their degeneration is evident, their nuclei become kidney- or horseshoe-

shaped. The chromatin granules are very scarce and take the stain faintly. They finally disintegrate. Their cytoplasm loses the fibrillar structure and appears disorganized. Cilia are totally absent. The pigment granules are few and scattered, and are finally discharged into the lumen (Text-fig. 10).

As the olfactory organ further develops, the degeneration of the lateral appendix continues. At its last stage, its lumen becomes obliterated, and the remainder of its cells become partially embedded in the olfactory epithelium as a small vestige (Text-fig. 11). The last trace of it (Text-fig. 12) disappears completely towards the end of the metamorphosis.

Discussion.

The role which the lateral appendix plays in the embryonic olfactory organ in Rana nigromaculata is not clear. The histological structure of the appendix is similar to the early undifferentiated olfactory placode. Each possesses a lumen lined with cells which possess cilia. They are probably alike in function. The presence of the lateral appendix may have the advantage that the inhalant water can make a considerably longer circuit and thus react with a greater surface of the olfactory epithelium.

The development of the lateral appendix is suddenly arrested at the appearance of the anterior lower sac and it starts to degenerate after the formation of the medial nasal gland. This synchronism is probably more than a coincidence. The portion of the anterior lower sac connected with the medial nasal gland together with the gland develops into the recessus medialis in the adult stage (Tsui, 1946). This structure is identified by Mihalkowicz (1898) as the vomero-nasal or Jacobson's organ in the nose of the Amphibia. This view has been accepted by most anatomists. There have been researches to ascertain the function of this vomero-nasal organ. Broman (1920) made various experiments with reptiles and mammals and came to the conclusion that it is an aquatic organ of smell (Wassergeruchsorgan) which persists in the nose of terrestrial vertebrates. Kerkhoff (1924) repeated Broman's experiments on the horse and came to the same conclusion. Observations of

the recessus medialis or vomero-nasal organ reveal that it contains more olfactory cells than any other part of the nasal cavity. The lumen of it is always filled with the secretion from the medial nasal gland. It is fairly certain that it is an aquatic organ of smell in the Amphibia. Why does the lateral appendix degenerate when the beginnings of the recessus medialis appear? A plausible view is that the lateral appendix is a forerunner of the vomero-nasal organ in early life of the Amphibia, and is soon replaced by the latter organ. It is proposed to conduct experimental studies to test the validity of this view.

Summary.

1. The anlage of the lateral appendix is formed from the dorso-lateral part of the olfactory placode in Rana nigromaculata Hallowell.

2. The lateral appendix, a blind sac, is formed when the dorsal lumen is differentiated in its anterior part.

3. It increases in dimension as the olfactory placode further develops, but growth stops as soon as the anterior lower sac appears; it begins to degenerate when the medial nasal gland makes its appearance; and completely disappears towards the end of metamorphosis.

4. Histological changes are described.

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