THE EPIBRANCHIAL GANGLION OF THE GLOSSOPHARYNGEAL NERVE IN AMBLYSTOMA JEFFERSONIANUM.

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INTRODUCTION.

The epibranchial placode of the glossopharyngeal nerve in *Amblystoma jeffersonianum* has many characteristics in common with that of the facial nerve, but differs in the relation of the placode to the endoderm of the pharyngeal pouch. The second or glossopharyngeal pouch forms an open gill slit while that on the first or facial pouch does not become patent and withdraws from the ectoderm, leaving a detached mass of endoderm which later disappears. As a result of the presence of a permanent contact between the endoderm of the pharyngeal pouch with the ectoderm which forms the branchial cleft, the epibranchial placode of the glossopharyngeal nerve remains for a long time in contact with the endoderm and becomes detached relatively late while the placode of the facial nerve becomes detached from the endoderm relatively early.

The origin of the placodes of both nerves furnish very definite and positive evidence of the derivation of the placode from ectoderm from which the placode later, after a growth period, becomes detached from the ectoderm, moves medially and rests on the dorsal surface of the endoderm of the pharyngeal pouch. After this migration it joins the remainder of the glossopharyngeal ganglion derived from the neural crest and from the lateralis placodes. It is not possible in the case of either ganglion to follow the epibranchial placode after the fusion of the placode with the remainder of the ganglion occurs, until the differentiation of the definitive ganglion cells takes place. Kostir (24) has done this for the 11.5 mm. embryo and the description of the more difficult stages to analyze follows his description.

Stone (22) has shown that the removal of the neural crest does not prevent the formation of the placodal ganglia and that the removal of the ectodermal placodes does prevent the formation of the placodal ganglia. In contrast to the

experimental and embryological evidence derived from the urodeles, Adelman ('25) holds that in the white rat the placode arises as the result of the stimulus furnished by the neural crest and G. Elliot Smith ('28) quotes Hill to the effect that in echidna the placodes arise from the endoderm. Whatever value comparatively the evidence from fishes and urodeles may have it is positively against the theory that the epibranchial placodes arise as a result of the stimulus of the neural crest or that the placodes are endodermal in origin.

The author, Landacre ('10), has previously cited the evidence for the function of the epibranchial placode after joining the remainder of the cranial ganglia. It is briefly as follows: every nerve having gustatory fibers has an epibranchial ganglion; no nerve without an epibranchial ganglion has gustatory fibers; the size of the epibranchial ganglion is always proportional to the size of the epibranchial placode; in Ameiurus, which has an enormous epibranchial placode on the glossopharyngeal nerve and a small easily followed lateralis component and no recognizable neural crest component, the nerve is apparently aside from the lateralis component exclusively gustatory.

The differentiation of structures derived from neural crest or from ectoderm as compared with those derived from endoderm is relatively easy in the urodeles owing to differences in size of cells and in the number and size of yolk granules. Endoderm cells and structures derived from endoderm consist of large cells and contain numerous large yolk granules which stained with Delafield and orange G give a light yellow field with few nuclei. If stained with iron alum, such a field is dense black. Cells and structures derived from ectoderm and neural crest consist of smaller cells with small yolk granules presenting a darker denser mass when stained with Delafield and orange G. The distinction based on yolk granules exists even in mesenchyme, so that the two types of cells can be recognized even where they mingle in early embryos.

One of the striking features of the history of placodes in urodeles is the fact that the placodes grow as solid masses of ectoderm and become detached from the ectoderm as masses which are relatively easy to follow during the migration medially and during the time the placode rests on the endoderm of the pharynx. The distinction between ectodermal and endodermal derivatives persists until the absorption of yolk granules occurs, which is far beyond stages involved in the present problem.

MATERIAL.

The material consists of stages 26 to 45 of *Amblystoma jeffersonianum*. Sections were cut transversely 10 micra thick, stained in Delafield's hematoxylin and counterstained in orange G.

TABLE SHOWING AGE AND AGE INCREMENT OF STAGES INVOLVING EPIBRANCHIAL PLACODE IX.

No. of series.....	26	27	28	29	30	31	32	33	34	35
Age in hours.....	238	243	249	255	261	267	273	280	287½	292
Increment.....	5 h	5 h	6 h	6 h	6 h	6 h	6 h	7 h	7½ h	4½ h

No. of series.....	36	37	38	39	40	41	42	43	44	45
Age in hours.....	297	302	308½	312	316	320	324	329	334	338
Increment.....	5 h	5 h	6½ h	3½ h	4 h	4 h	4 h	5 h	5 h	4 h

The history of the epibranchial placode on IX falls into two rather definite periods. The first period covers the time of growth of the placode in the nervous layer of the ectoderm and the second period the time during which the placode after completing its growth rests on the endoderm of the pharyngeal pouch during which the placode also becomes more or less completely fused with the remainder of the glossopharyngeal ganglion. This ganglion in Amblystoma has in addition to the contribution from the epibranchial placode definite contributions from the neural crest and from the dorsolateral placode.

The diagonal position of the ninth ganglion in Amblystoma makes it impossible in transverse sections to show except occasionally the fusion of the placode with the remainder of the ninth ganglion. This condition is in striking contrast to the condition of the facial ganglion. There is another rather striking difference due to the persistence of the pharyngeal pouch. The placodal portion of the IX ganglion remains in contact, resting on the dorsal surface of the pouch for a relative long period compared with the behavior of the placode of VII.

THE EARLY GROWTH PERIOD.

The early growth period is illustrated in Figures 1–4, inclusive. Figure 1, taken from series 26, is ten hours older than the first series in which the epibranchial thickening occurs, but is the earliest series in which there is marked evidence of growth as indicated by definite thickening of the primitive line. In stage 24, nine days and twelve hours after deposition,

3.75 millimeters in length and with twelve somites, the primitive thickening can be recognized,

After the epibranchial placode begins to grow there is a steady increase in its thickness and in its extension medially as a triangular solid mass. This epibranchial mass which arises from the nervous layer of the ectoderm rests with its base on the ectoderm and its apex directed medially. In the first section figured (Fig. 1, right side) it is not possible to separate the placodal thickening into epibranchial and dorsolateral because of the fusion of the two. The ventral portion of the placode on right side is epibranchial and the dorsal portion is dorsolateral and gives rise to the lateral line placode. In older stages this distinction can be made since the lateralis ganglion placode lies slightly caudal to the epibranchial placode, while the lateral line placode lies dorsal at least to the caudal portion of the epibranchial placode.

Part of the difficulty of separating the epibranchial and dorsolateral placodes is illustrated by a comparison of the right and left sides of Figure 2, where the section passes through the rostral portion of the placode on the right side and through the middle of the placode on the left side. The epibranchial placode of IX is situated well dorsal on the side of the body resulting in more or less fusion between epibranchial and dorsolateral placodes. The right side of Figure 2 illustrates also the presence of the placode rostral to the junction of the pharyngeal pouch with the ectoderm. The dorsal position of the epibranchial placode is illustrated in Figure 3. Here the section passes through the middle of the placode on both sides.

In Figure 4 the distinction between epibranchial placode and the lateral line placode is readily made. The lateral line placode lies dorsal to the epibranchial placode on the right side, while the lateralis ganglion is caudal to the level of this section. In the same figure the extension of the epibranchial placode medially is evident on right side. Both sides show the closeness of contact between the placode and the endoderm. The placode becomes detached from the nervous layer of the ectoderm except at its ventral border before any marked migration medially occurs.

During the growth period up to the time of complete detachment of the placode from the ectoderm it is evident that the placode is ectodermal in origin, that part of the placode lies rostral to the contact of the pharyngeal pouch with the

ectoderm and that the whole placode lies rostral to the neural crest portion of the IX ganglion.

THE PERIOD OF CONTACT WITH ENDODERM AND FUSION WITH THE REMAINDER OF VII.

During the second period the epibranchial placode becomes detached from the lateral ectoderm except at its caudal border where the truncus glossopharyngeus arises. At this level the epibranchial placode is continuous with the ectodermal mesenchyme lying ventral to it. This contact is similar to that in similar stages of the facial placode. As illustrated in Figure 5, the junction of the placode with the pharyngeal pouch is at the lateral border of the pouch. In later stages the placode comes to rest on the dorsal border of the endodermal pouch. The placode in Figure 5 is not completely detached from the ectoderm, at least a small group of cells lies between the two and in contact with both.

Figure 6 illustrates a constant condition in the relation of the placode to the lateral border of the pharyngeal pouch. The placode bifurcates over the lateral portion of the endodermal pouch and the medial portion of the placode seems to give rise to a ramus pharyngeus while the lateral portion later sends fibers into the truncus glossopharyngeus. This bifurcation is constant in all early series, but when the seventh ganglion becomes detached from the pharynx and lies more dorsal in the body the diagonal course of the pharyngeal ramus makes it difficult to follow.

In stage 39, Figure 7, the change in condition of the placode is slight, showing only a slight detachment from the pharyngeal pouch. Figures 8 and 9 from the same embryo are presented to show the differentiation into neural crest and lateralis portions of the ganglion. The cellular contact of the lateralis portion of IX with the ectoderm illustrated in Figure 9, right side, is interesting since it seems to persist in Amblystoma from the time the placode arises from the ectoderm.

In stage 42 (Fig. 10) the placode is detached from the endoderm of pharynx, but a few sections rostral rests definitely on the endoderm and further shows the bifurcated condition mentioned above. The placodal portion of the ganglion withdraws from the endoderm from caudal to rostral.

The bifurcated condition of the rostral portion of epibranchial IX is shown in Figure 11, right side, where the origin

of both ramus pharyngeus and truncus glossopharyngeus can be observed. In this stage the rostral portion of neural crest IX rests on dorsal surface of placodal IX. The age at which the epibranchial portion of IX ceases to rest on the pharyngeal pouch in contact with the endoderm varies. In a series four hours younger than stage 45 (Fig. 12) the rostral portion of IX, which is composed exclusively of cells derived from the epibranchial placode, lies well dorsal and has withdrawn completely from the pharynx. In two series four and one-half hours older than 45 (Fig. 12) the epibranchial placode touches the endoderm throughout the extent of a few cells.

Figure 12, right side, illustrates the last stage in which there is a well defined contact between the placodal ganglion and the endoderm. The placode is massive and the small group of cells resting on the dorsal surface of the placodal ganglion illustrate the plan of junction between the cells from the two sources. Either the neural crest cells shift rostral onto the dorsal surface of the placodal cells or the placode shifts caudal ventral to the neural crest cells.

SUMMARY.

The origin of the epibranchial ganglion of the glossopharyngeal nerve resembles closely that of the facial nerve. Each epibranchial placode is preceded by a primitive line which can be identified prior to the actual growth period in the formation of the placode. The growth period begins with a definite increase in the thickness of the placode which forms in the nervous layer of the ectoderm. This thickening lies in part rostral to the endodermal pharyngeal pouch and entirely rostral to the neural crest portion of IX. The epibranchial placode of IX begins its definite growth phase simultaneously with that of the dorsolateral placode which is continuous on its rostral border with the lateral line placode. The lateral line placode and the lateralis ganglion placode however have a more dorsal position than the epibranchial placode. In the earliest stages of the growth of all three structures there is some difficulty in separating them. This difficulty disappears when the three placodes assume definite histological structures.

The epibranchial placode during the growth period appears as a solid mass of cells, triangular in form with the base of the triangle resting on the ectoderm and with its apex directed medially. As the placode increases in size its medial portion

abuts against the lateral extension of the pharyngeal pouch and does not as in the case of VII come to rest at first on the dorsal border of the pharyngeal pouch. In later stages the placode loses its triangular form and consists of a rounded mass of cells which at its rostral end bifurcates over the lateral border of the pharyngeal pouch the medial arm of the ventral bifurcation seeming to give rise to a pharyngeal ramus and the lateral arm enters the truncus glossopharyngeus.

The caudal border of the epibranchial placode abuts at first against the neural crest portion of IX but in later stages there is a change in this relation and the neural crest portion of IX at its rostral border comes to rest on the dorsal border of the epibranchial portion. In still later stages the whole combination of IX withdraws dorsally and the ganglion is detached completely from the pharyngeal pouch. Since the epibranchial ganglion of IX arises entirely rostral to the neural crest the neural crest could not be considered as a stimulus to the formation of the epibranchial placode.

The differences in histological detail between ectodermal derivatives with their small cells and small yolk granules and the endodermal cells with their large size and large yolk granules leaves no doubt as to the ectodermal source of the epibranchial placode. This conclusion is further strengthened by the growth of the placode in the nervous layer of the ectoderm and by its compactness, since it is rare to find the borders of the placode irregular, and the gradual migration medially prior to its junction with the neural crest and dorsolateral portions of IX. The evidence points to the conclusion that the epibranchial placode is a ganglion forming structure, not dependent on the neural crest or even the pharyngeal pouch for stimulation and not a phylogenetically old sense organ and definitely ectodermal in origin like all other ganglia.

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ABBREVIATIONS USED.

L. P.—Lateral line placode.
E. P.—Epibranchial placode.
A. V.—Auditory vesicle.
N. C. G.—Neural crest portion of ganglion.
L. G.—Lateralis portion of IXth ganglion.
R. IX—Root of IX.
E. G.—Epibranchial portion of IXth ganglion.
G. IX—Glossopharyngeal ganglion.

DESCRIPTION OF PLATES.

All figures are untouched photographs of transverse sections 10 micra thick of *Amblystoma jeffersonianum*. Sections were stained in Delafield's hematoxylin and orange G and photographed at a magnification of ×75 and reduced to ×35 for printing.

PLATE I.

Figures 1–4, inclusive, cover the growth period of the placode in the nervous layer of the ectoderm.

Fig. 1. Series 26, Section 123, right side rostral to left. Section or right side in middle of placode which is ten sections long. The caudal tip of the auditory vesicle and the root of the IX nerve on right side. Neural crest portion of IX on left side of section. Lateral line placode on dorsal surface of epibranchial placode on right side.

Fig. 2. Series 28, Section 120, right side slightly rostral to left. Left side through middle of placode. Right side through rostral portion of placode which here extends three sections rostral to the contact of pharyngeal pouch with the ectoderm and is fused on its dorsal surface with the primordium of the lateral line placode.

Fig. 3. Series 29, Section 132, right and left sides symmetrical and at the middle of the epibranchial placodes. Epibranchial placodes in Figures 1, 2, and 3 lie at same dorsoventral level as the lateralis ganglion placodes which are located more caudal. These two placodes do not appear in the same transverse section of the IXth ganglion as they do in the case of the VIIth ganglion.

Fig. 4. Series 32, Section 165, right side slightly caudal to left. Epibranchial placode on right side taken through middle of placode which is twelve sections long. Compared with Figure 3 the placode on the right side has grown medially but is still attached to ectoderm. On left side placode extends ventrally continuous with nervous layer of ectoderm. Lateral line placode appears on right side dorsal to and in contact with the epibranchial placode.

Figs. 5–12, inclusive, cover the period of contact with the endoderm and fusion with the remainder of IX.

Fig. 5. Series 35, Section 152, right side caudal to left. Epibranchial placode eight sections long. Right side through middle of placode which is detached from ectoderm except for a small group of cells still lying between placode and ectoderm. Placode closely attached to lateral border of pharyngeal pouch. Lateral line placode on right side.

Fig. 6. Series 38, Section 150, right side slightly caudal to left. Placode which is seven sections long free on right side from IX. Two sections caudal to right side placode is fused with IX. Placode on right side rests definitely on pharyngeal pouch. On left side, which is slightly rostral to right, and two sections from rostral end of placode, the placode shows the typical bifurcation of placode over the lateral border of pharyngeal pouch.

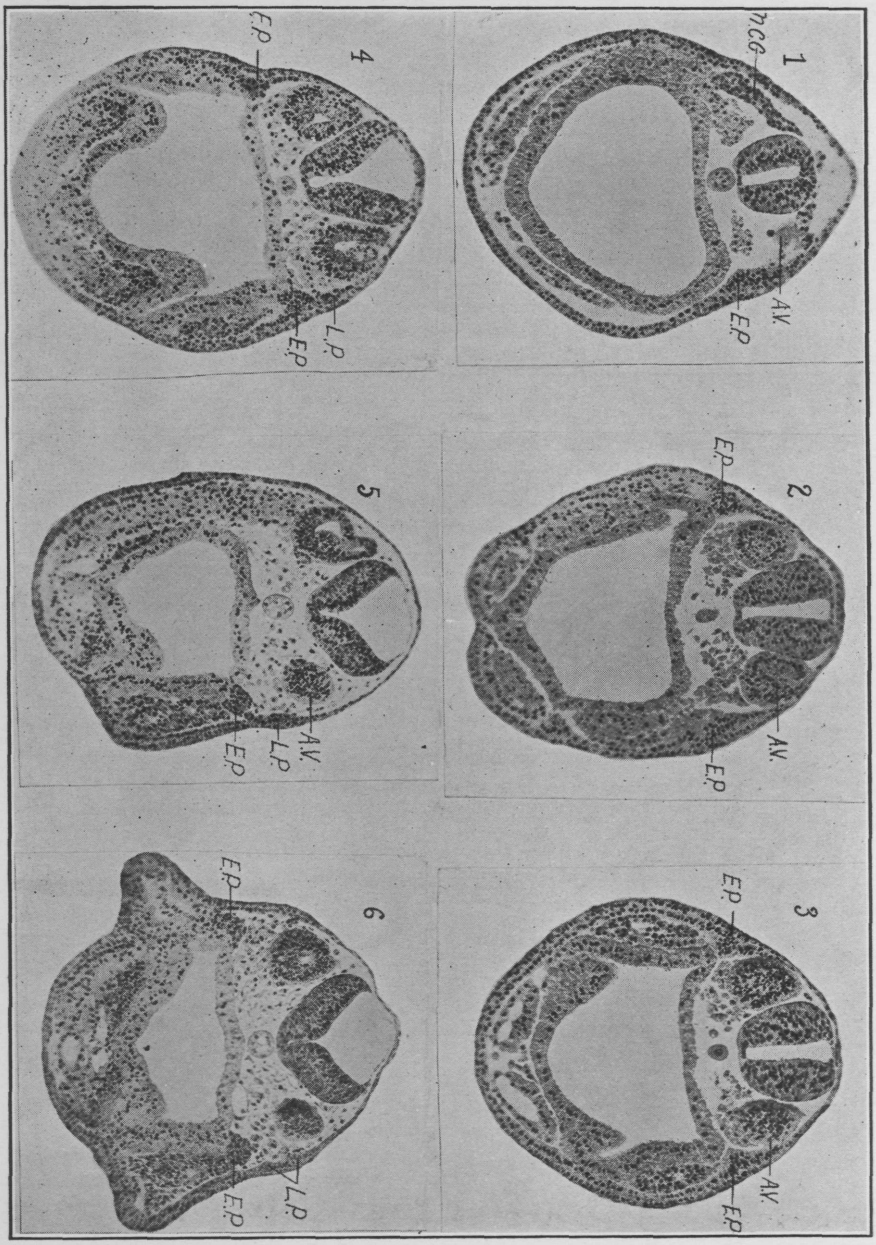

1
mcG.
A.V.
E.P.
2
E.P.
A.V.
E.P.
3
E.P.
A.V.
E.P.
4
E.P.
L.P.
E.P.
5
A.V.
L.P.
E.P.
6
E.P.
L.P.
E.P.

PLATE II.

Fig. 7. Series 39, Section 169, right side rostral to left. Free portion of placode on right side six sections long. Figure 7 is taken through the fifth section of placode on right side, on left side section passes through the junction of the placodal and neural crest portions of IX. The placodal portion of the ganglion lies medial to neural crest portion. The placode on right side still rests on pharyngeal pouch, but the line of contact between placode and endoderm is reduced and at the lateral border of the placode the placode is becoming detached.

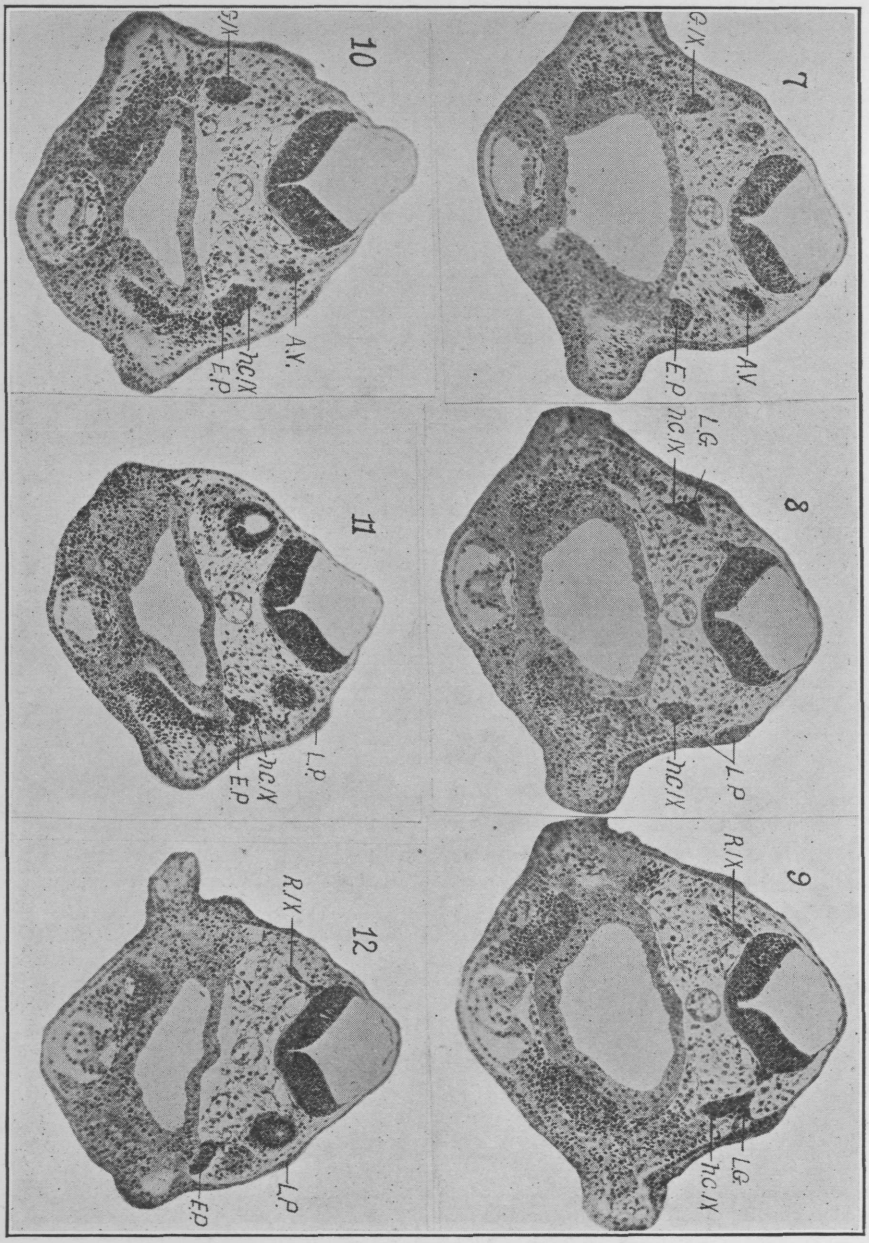
Fig. 8. Series 39, Section 175. On right side section passes through the neural crest portion of IX anterior to the junction of neural crest and epibranchial portions. On left side section passes through the root of IX and the combined neural crest situated medially and lateralis portions of IX situated laterally.

Fig. 9. Series 39, Section 178. On right side section passes through the root of IX, the lateralis ganglion of IX situated dorsally and laterally, and the neural crest portion of IX situated ventrally. The lateral portion of the ganglion consists of lateralis IX from which arise the cellular rami. The more dorsal ramus is ramus auricularis IX and the more ventral ramus is ramus supratemporalis IX. The right side of this figure illustrates a characteristic condition of the early cellular composition of lateral line nerves. On left side the section passes through the root of IX.

Fig. 10. Series 42, Section 166, right side rostral to left. Right side passes through the combined epibranchial and neural crest portions of IX, the epibranchial placode lying more ventral and lateral to the neural crest portion. The lateralis ganglion is caudal to this section. On left side of section epibranchial and neural crest portions are closely fused. On right side the close relation of the ganglion with the ectodermal mesenchyme lying ventral to the ganglion is evident. At this level the truncus glossopharyngeus arises. The detachment of the epibranchial placode from the pharyngeal pouch at this level is evident.

Fig. 11. Series 43, Section 160, right side caudal to left. IXth ganglion on right side, three sections from rostral end, consists of the epibranchial ganglion bifurcated over the pharyngeal pouch and the neural crest portion lying dorsal to epibranchial portion. The lateral portion of the epibranchial ganglion lies at the level of the truncus glossopharyngeus and the medial portion gives rise to a small nerve that is apparently the ramus pharyngeus IX. On right side only a few cells of neural crest IX rest on epibranchial IX.

Fig. 12. Series 45, Section 166, right side rostral to left. On right side the section passes one section rostral to truncus glossopharyngeus and three sections from rostral end of ganglion IX. The placodal ganglion is here very compact and readily distinguished from endoderm of pharyngeal pouch. This is the last stage where there is a constant contact between epibranchial IX and the endoderm of pharyngeal pouch.


7
G.IX.
A.V.
E.P.
8
L.G.
hC.IX
L.P.
9
R.IX
L.G.
hC.IX
10
G.IX.
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