COMPARATIVE ONTOGENY OF THE PINEAL COMPLEX IN *ELEUTHERODACTYLUS COQUI* AND *XENOPUS LAEVIS*

Robert E. Corniea, Jr.

THESIS

COMPARATIVE ONTOGENY OF THE PINEAL COMPLEX IN *ELEUTHERODACTYLUS COQUI* AND *XENOPUS LAEVIS*

Submitted by

Robert E. Corniea Jr.

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY ROBERT E. CORNIEA JR. ENTITLED *COMPARATIVE ONTOGENY OF THE PINEAL COMPLEX IN ELEUTHERODACTYLUS COQUI AND XENOPUS LAEVIS* BE ACCEPTED AS FULFILLING PARTIAL REQUIREMENTS FOR THE SENIOR THESIS IN ZOOLOGY

Thesis Committee

Samuel I. Zeveloff

Earl A. Jennel

Gloria J. Wurst
Advisor

Samuel I. Zeveloff
Department Chair

Comparative Ontogeny of the Pineal Complex in Eleutherodactylus coqui and Xenopus laevis

Abstract

The pineal complex is present in different forms and performs varied functions in different vertebrates. All anuran amphibians possess an intra-cranial pineal body, but less than thirty percent possess an extra-cranial third eye or stirnorgan derived embryologically from the pineal anlagen. Studies have shown the stirnorgan to be left of the midsagittal plane, while the pineal body is found to the right of the midsagittal plane. This study concurs for both Eleutherodactylus coqui and Xenopus laevis. Additionally, lack of a stirnorgan in Eleutherodactylus coqui appears not to be owing to differences in rate of cranial ossification, or prevention of migration of the stirnorgan by the pre-chondral connective tissue.

Introduction

The pineal complex is found in some form in nearly all vertebrates. A pineal body or epiphysis is always present and there may also be a parietal eye and pineal nerve. Pineal complex function varies in different taxa. In lower vertebrates, the pineal complex is most often used as a photoreceptive dosimeter, while in higher vertebrates, such as mammals and birds, its function becomes more glandular. (Hamasaki and Eder 1968) My particular interest is in the photoreceptive third eye found in extant lampreys, amphibians, and reptiles. In frogs this extracranial third eye connects via the pineal nerve between the two parietal bones of the cranium to the pineal body. These structures make up the pineal complex. The pineal eye differs morphologically from the lateral eye in that the lateral eye has an eyelid, muscle attachments, and an inverted retina while the pineal eye has no eyelid, muscle attachments, and the retina is not

inverted. This pineal eye is much smaller than the lateral eyes, but studies by Edinger that measured the parietal foramen which correlates well with the size of the third eye (Edinger 1955 as cited by Eakin 1973), shows that the third eye was slightly larger in some fossil ancestors of fish and amphibians such as Ichthyostega, from the Carboniferous period, .

Early studies of anuran amphibians implied that all anurans possessed a third eye or stirnorgan, however these studies were performed on Hyla regilla (Eakin and Bush 1957 as cited by Eakin 1973), Xenopus laevis (Wakahara 1968) and others which have a stirnorgan. Wurst (1986) studied a large number of anuran genera to assess the frequency of occurrence of a stirnorgan based on external observation of a browspot. She found that less than 30 percent (20/74) of the anuran genera sampled possessed the stirnorgan. These previous reports led to the thesis question: is there a developmental reason why some genera of frogs have a stirnorgan and others do not? I began a comparative study to shed light on this question. Part of my goal was to confirm and extend the findings of Wakahara's (1968) study of X. laevis pineal complex development.

Materials and Methods

A developmental series of embryos and juveniles were obtained from Xenopus laevis and Eleutherodactylus coqui. Xenopus specimens ranged from stage one, the one cell stage, to stage sixty-six, with tail fully resorbed (Nieuwkoop and Faber 1956, as cited

by New 1966). A breeding pair of X. laevis maintained in the laboratory provided one hundred forty tadpoles stages one through forty-nine. Later stages, fifty through sixty-four, were purchased from Carolina Biological Supply Company (Burlington, N.C.). Five tadpoles had been fixed previously by Dr. Gloria Wurst. Thirty-seven E coqui embryos and juveniles were obtained from Dr. Daniel Townsend. These animals ranged in age from stage 5A through four day post hatchlings (Townsend and Stewart 1985). Tissues were fixed in formalin, dehydrated in an alcohol series, cleared in xylene, and embedded in medium hard paraffin. They were sectioned on a rotary microtome at seven microns and stained with Brookes' triple stain (Brookes 1967). All length measurements of tissues were averaged from at least three specimens using a stage micrometer or by multiplying the number of sections by section width (used for cross sections).

Results

In my study, the pineal complex of X. laevis originated as a thickening of the roof of the diencephalon in stage twenty-six. As the tadpole developed the pineal complex grew longer, thicker, and wider and tissues of stirnorgan and pineal differentiated and began to separate. Differentiation into stirnorgan and pineal body begins at about stage thirty-one. A line seems to separate stirnorgan and pineal into dorsal/ventral as well as left/right (See figs. 1 and 2). After differentiation the stirnorgan, while still proximate to the pineal body, appears to break through the pre-chondral connective tissue at about stage forty, separates physically into stirnorgan and pineal body (stage forty-nine), and

the stirnorgan migrates towards skin ectoderm (See fig. 3). From stages forty-nine through fifty-two the stirnorgan migrates anteriorly but remains connected to the pineal via the pineal nerve (See fig. 4). As it grows, the stirnorgan is oriented slightly to the left of the midsagittal plane , and the fully developed structure is located just left of the mid-sagittal plane between the lateral eyes slightly caudad of their anterior margin. It remains connected to the pineal body via the pineal nerve which runs through a foramen between the two parietal bones of the cranium.

In Eleutherodactylus coqui the pineal appears at stage seven. It begins as an outpocketing of the roof of the diencephalon and grows anteriorly juxtaposed with the pre-chondral connective tissue (See fig. 5). Growth occurs, lengthening the pineal body uniformly from approximately 140 microns at stage 9A to approximately 224 microns at stage 12A/B. At the next stage, stage 13, the pineal shortens to 112 microns and then resumes anterior growth. At the final stage of four days post-hatching, the pineal measures approximately 231 microns. As it grows, the pineal is oriented slightly to the right of the midsagittal plane, and it maintains this orientation at the final stage observed. Between the stage of one day post hatch and four days post hatch communication with the third ventricle is lost(See fig. 6). Four specimens at the four day post hatching stage were observed, two sectioned transversely and two sectioned sagittally. A communication with the third ventricle was not found in any of these four specimens.

Discussion

Generalized ontogeny of the pineal complex in anurans is a process in which the roof of the diencephalon thickens and evaginates; then tissues separate into a stirnorgan and pineal body, both of which grow and differentiate (Oksche 1965). Wakahara (1968) observed the emergence of the pineal system in X. laevis as a dorsal bulging of cells in the diencephalic roof at stage twenty-six. At stage twenty-seven this bud forms a pineal vesicle which communicates with the third ventricle through an orifice that Wakahara calls a "wide opening." Growth narrows the pineal lumen resulting in a massive cell-cluster at stage thirty-one. Segregation between stirnorgan and pineal body occurs at stage forty-two, and the orifice remains throughout development. From this point until the end of development the stirnorgan migrates anteriorly, the connection between stirnorgan and pineal body becomes longer and thinner, and the pineal body grows longer.

In X. laevis I found the bud-like appearance of the developing pineal complex at stage twenty-six, but I found no subsequent vesicle stage in the specimens I examined. I also found no obvious communication with the third ventricle. If communication does occur, the orifice in my specimens cannot be the "wide opening" reported by Wakahara (1968), but must be smaller than the section thickness of seven microns. The reason for the discrepancy between my study and Wakahara's about communication with the third ventricle at early stages of development may be explained in Wakahara's own study.

While the text states that there is a wide opening at early stages of development, the figures supplied to illustrate this wide opening contradict rather than support his claim of such an opening. When looking at the photomicrograph, one may see an almost implied opening, but there is no glaringly obvious lumen or communication with the third ventricle. Other aspects of ontogeny closely followed that described by Wakahara.

In Eleutherodactylus coqui development of the pineal followed the generalized developmental plan for anurans except for the absence of separation of the tissue anlagen into pineal and stirnorgan, leaving only the pineal body. The lengthening/ shortening/ lengthening of the pineal body in E. coqui is not readily explained but it may be a growth pattern which developed trying to compensate for the loss of the stirnorgan. The pineal in E. coqui had a decidedly rightward orientation through all stages of development. This corresponds with the literature (Eakin 1973).

Early in my study I hypothesized the loss of the stirnorgan might have been due to differences in the pattern of ossification of the cranial bones in X. laevis an E. coqui (Trueb 1985). This proved not to be a factor because tissue migration occurs long before ossification in this area, and E. coqui has as much time and space to migrate to the epidermis as X. laevis. The only tissue between the pineal body of E. coqui and the epidermis is the pre-chondral connective tissue. This pre-chondral connective tissue which surrounds the brain seems to play a role in pineal ontogeny. In Xenopus laevis the stirnorgan end of the pineal complex apparently migrates through the connective tissue. In E. coqui though pineal body and pre-chondral connective tissue are in close

approximation at nearly all times the pineal tissue never breaks through. This may be due to differential growth rate of tissues in Xenopus. Such a differential growth rate has a developmental precedent in the chick heart (Waterson and Schoenwolf 1984) where heart tissue grows so much faster than the surrounding mesenchymal and epidermal tissues that it breaks through these two. It is possible the stirnorgan tissue may grow much faster than the pineal body tissue which may force it through the pre-chondral connective tissue.

Some fishes of the Devonian period (350 to 400 million years ago) had paired parietal eyes, one right and one left (Edinger 1956, as cited by Eakin 1973). As amphibians have evolved from fishes, this rightward orientation of the pineal in E. coqui may be explained by the loss of the left parietal eye, or stirnorgan as the animal evolved. Most probably the chemical evocator which triggers the stirnorgan anlage to grow, has been lost, or the enzyme needed to make that evocator, etc. This question might be addressed by future studies focusing on the chemical evocators present prior to differentiation of stirnorgan and pineal in an amphibian with a stirnorgan with comparisons made to the chemicals present in an amphibian that has only the pineal body.

Conclusion

This study has demonstrated the patterns of pineal development in X. laevis and E. coqui. I suggest the loss of the stirnorgan in E. coqui may be related to an evolutionary loss of an evocator for stirnorgan differentiation, but the search for reasons that a stirnorgan is present in X. laevis and absent in E. coqui continues. This initial comparative study will serve as a basis from which to study other genera of anurans with varying modes of development in order to pursue the reason for the loss of the stirnorgan.

Acknowledgements

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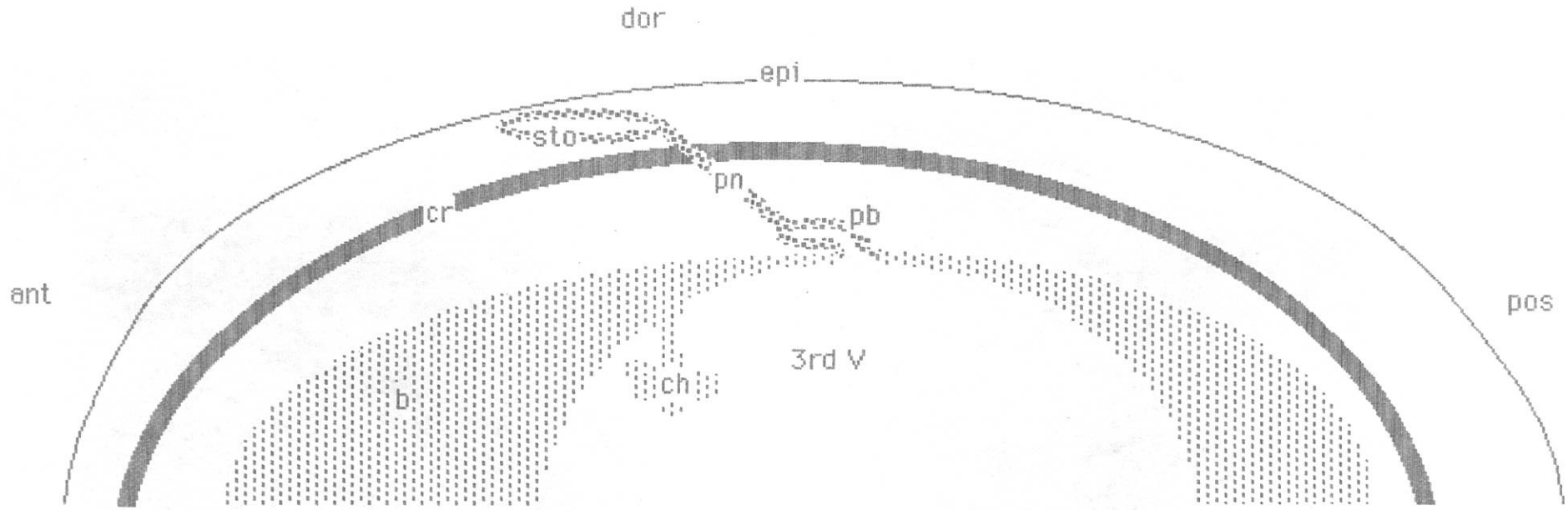
Generalized Anuran Pineal Complex



ant = anterior
dor = dorsal
pn = pineal nerve
b = brain tissue
epi = epidermis
pos = posterior
ch = choroid plexus
pb = pineal body
sto = stirnorgan
cr = cranium
3rd V = third ventricle

fig. 1 * indicates division between dorsal and ventral

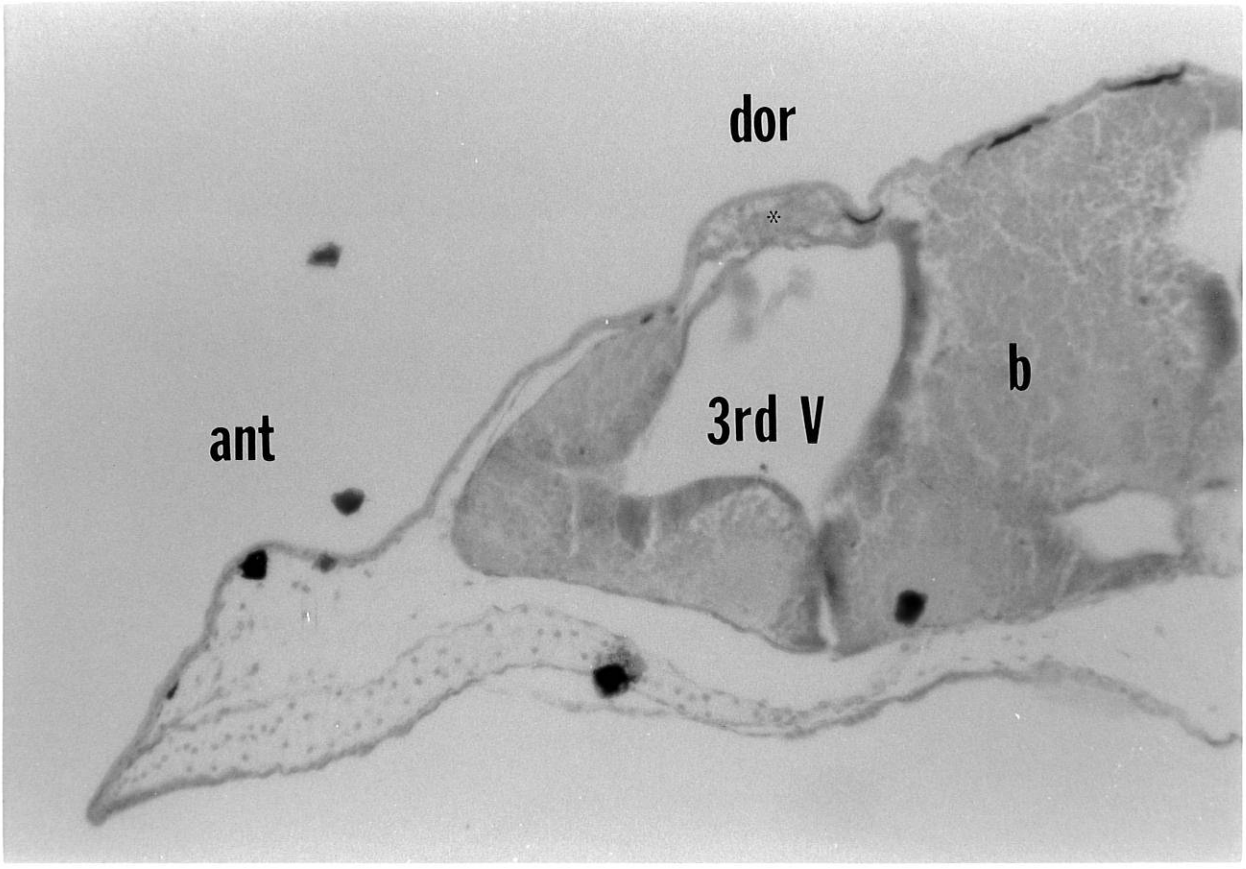


fig. 2 / indicates division between right and left

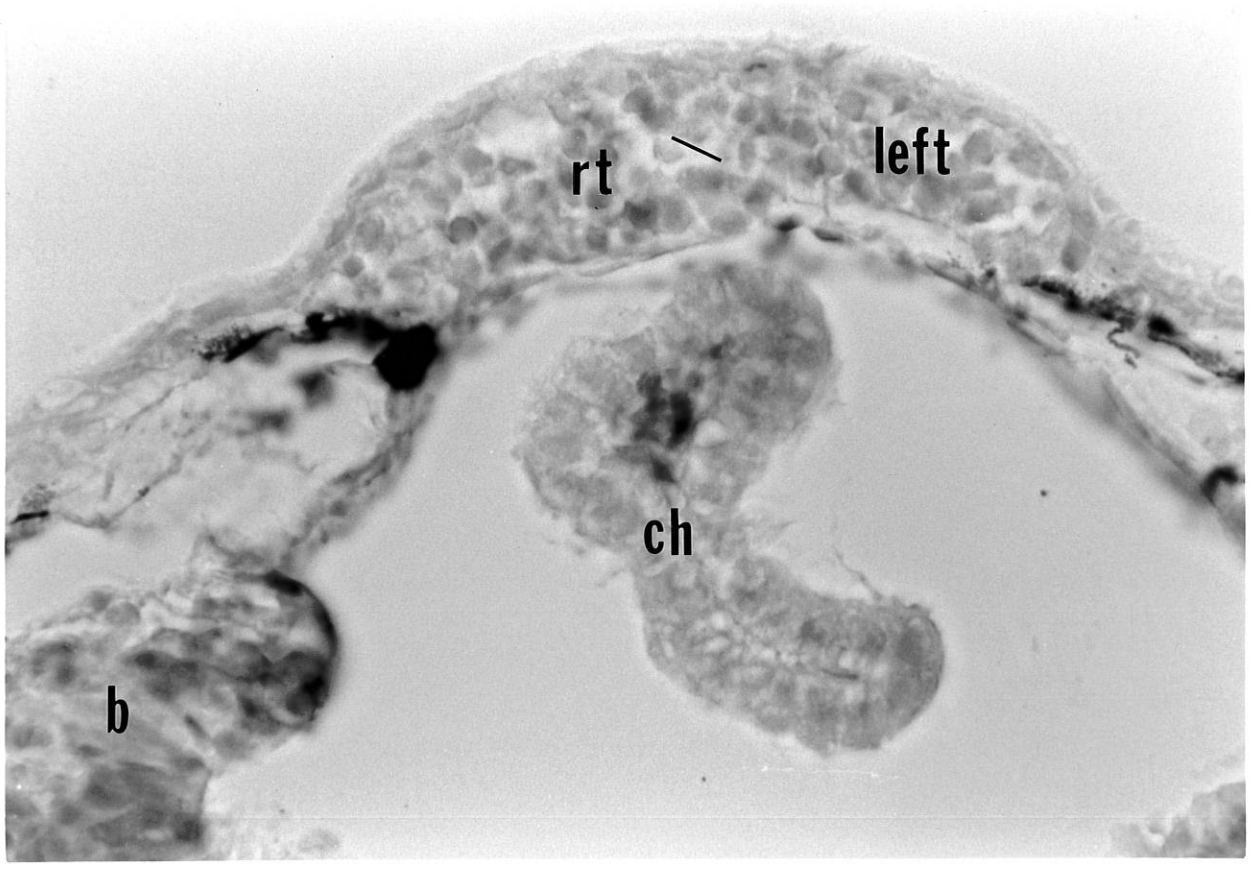


fig. 3 * indicates division between stirnorgan and pineal body

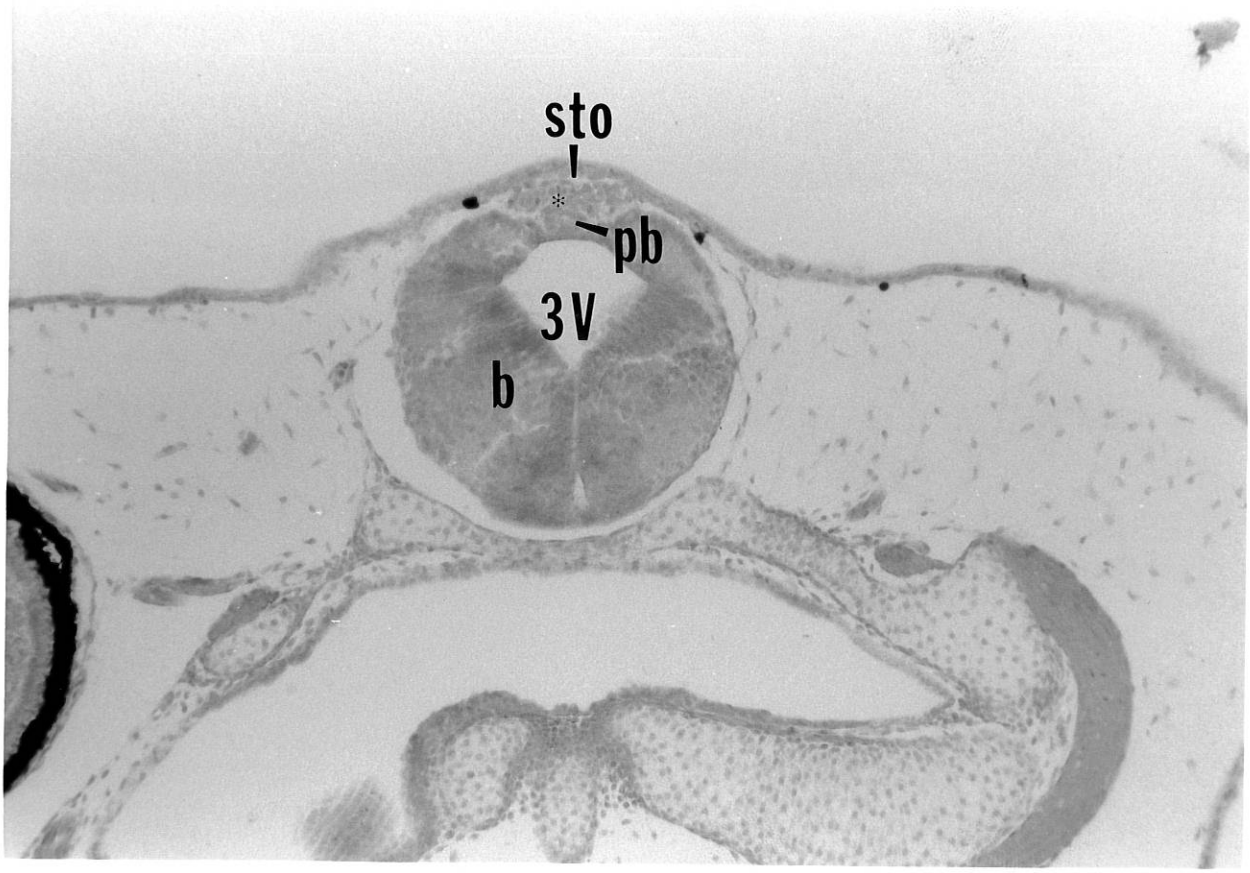


fig. 4

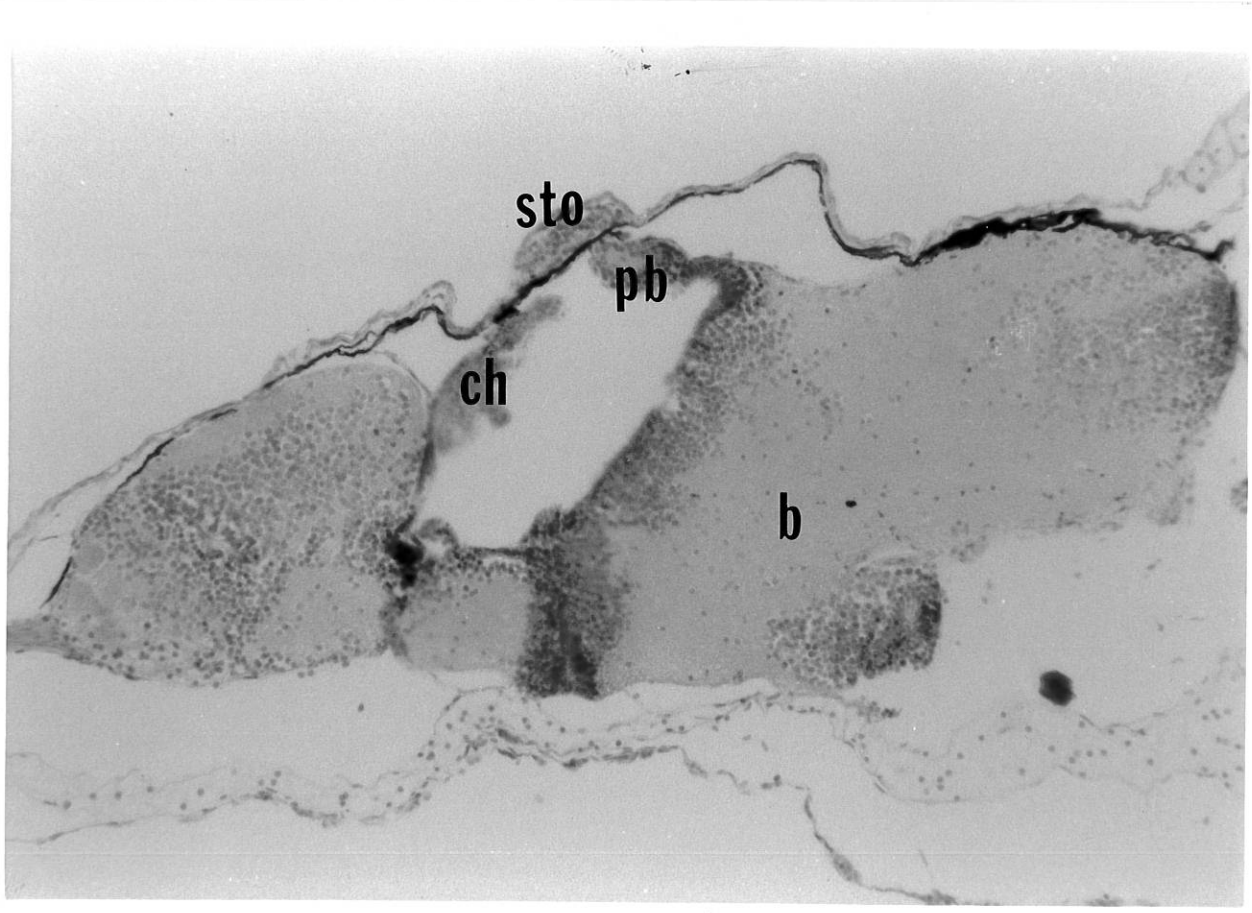


fig. 5

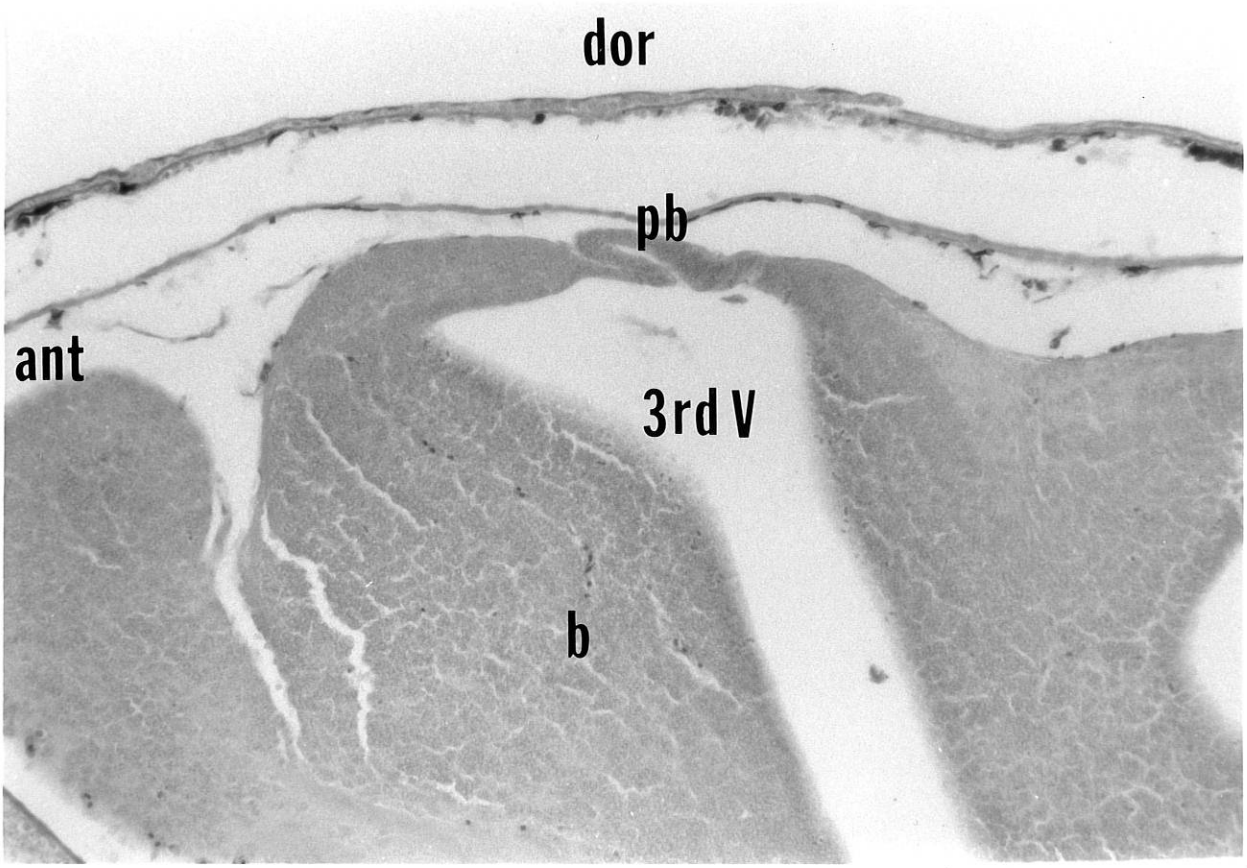


fig. 6