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## THE ORIGIN AND DEVELOPMENT OF THE AGLOMERULAR KIDNEY OF THE MARINE PIPEFISH HALICAMPODES MACRORHYNCHUS (BAMBER)

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#### ABSTRACT

The aglomerular pronephric kidney of Halicampodes macrorhynchus (Bamber) begins to develop in the 2.2 mm long embryos as two symmetrical pronephric ducts in the form of solid cords of cell condensations originating from the intermediate cell-mass on either side. The cranial parts of the ducts end blindly giving rise to the aglomerular pronephros. The asymmetry of the kidneys appears in the 5.5 mm long embryos through the slight shifting of the left pronephric duct along its whole length towards the right side of the body as a result of the development and enlargement of the swim bladder. The aglomerular mesonephric kidney is formed in the 11-13:mm long embryos by the appearance of several irregularly arranged spherical condensations from the intermediate cellmass along the dorsal or lateral surface of the pronephric duct representing the anlage of the mesonephric tubules. In the larval stages the mesonephric tubules are elongated, curved and connected with the

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mesonephric ducts without formation of capsules or glomeruli. The successive mesonephric tubules develop from the same material and in the same manner. They open either in the early developed tubules or directly in the mesonephric duct.

#### INTRODUCTION

The ontogeny and phylogeny of the vertebrate kidney is either of the holonephric type, from which a small anterior part, the pronephros, is restricted from the remaining part, or opisthonephros, or of the tri-generation type in which pro-, meso- and metanephros are developed, Fraser [9]. In Anamniota, the pronephros is the embryonic functional kidney; this either persists, degenerate or disappears completely and becomes replaced in the adult stage by the functional mesonephros. In Amniota, however the persistent and functional kidney in the adult stage is the metanephric kidney, while the pro- and mesonephric kidneys generally disappear.

Some authors described, in detail, the origin and development of the aglomerular kidney in some teleosts in which the pronephric or/and the mesonephric kidneys possess no glomeruli. Audige [3] mentioned that the pronephric kidney was found to be degenerated or absent in the adult stage of <u>Lophius piscatorius</u>. Armstrong [1] described the appearance of the glomerular pronephric

kidney in the later stages of the aglomerular species,

Opsanus tau. Gérard [10], however, found glomerular pronephros in the early stages of Lophius piscatorius. Edward
[5 and 6], Marshall [20], Padovani [26], Grafflin [12],
Ogawa [24], and Khalil [16,17]gave more comprehensive
morphological and histological reports on the aglomerular
mesonephric kidneys of several adult marine teleosts. The
origin of the archinephric ducts, including the pronephric
and mesonephric ducts, has been described in a number of
lower vertebrates by Price [27], Tavolga [31], Holstvoogd
[14], Ford and Newstead [7], Harder [13], Bielek [4] and
Khalil and Agamy [18,19].

The development of the aglomerular kidney in Syngnathidae ( sea horses and pipefishes ) has been studied by Verne [32], Rauther [29] and Ogawa [25].

The present study has been undertaken to investigate the origin and development of the aglomerular pronephric and mesonephric kidneys of a holonephric kidney type in a marine pipe-fish, <u>Halicampodes</u> macrorhynchus (Bamber).

#### MATERIAL AND METHODS

Adult males of the Pipefish, <u>Halicampodes macro-rhynchus</u> (Bamber), 15-20 cm in length, carrying their embryos in their brood pouches were caught from the Red

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Sea, Hurghada. Each pouch contains 26-70 rounded eggs(in two rows) measuring 1.8-2 mm in diameter. Embryos were obtained from the brood pouches of the males.

The adult males were sacrificed and their brood pouches were opened to obtain the embryonic stages. All materials were fixed in 4% formol saline or aqueous Bouin's fluid. The embryos, containing large amounts of yolk, were embedded in terpineol paraffin (Kellner and Klenkhart[15]). Transverse, sagittal and frontal sections were cut serially at 5-7 mm thick and stained with Delafield's haemato-xylin and eosin, Heidenhain's iron haematoxylin and Mallory's triple stain.

#### **OBSERVATIONS**

A) The Development of the Pronephric Kidney: Stage 1 (1.2 - 1.5 mm long embryos):

At the begining of this stage, the somites are differentiated on each side of the body, but the intermediate cell-mass is indistinguishable from the lateral plate mesoderm. The gut is developed as a central tube extending beneath the notochord and possesses a small lumen in its middle region; its anterior and posterior ends are blocked with cells (PL. I, fig. 1 A-D). At the end of this stage, the dorsal aorta begins to appear ventral to the notochord. The intermediate cell-mass is increased in size

and scattered between the myotomes and the lateral plate mesoderm (PL. I, fig. 2A & B).

#### Stage 2 (1.6 - 2.2 mm long embryos):

The intermediate cell-mass is separated from the lateral plate mesoderm and scattered between the dorsal aorta and the gut. The myotomes are differentiated on each side of the neural tube and hotochord. In the 2.2 mm long embryos, the intermediate cell-mass, on each side of the body, is arranged as solid cord of cell condensation and gives rise to the pronephric duct anlage. The lateral plate mesoderm is differentiated into somatic and splanchnic layers and a narrow coelom appears in between. The dorsal aorta is more differentiated and contains blood cells. The gut is lined with columnar epithelium and possesses a clear lumen along its whole length (PL. I, fig. 3 A & B).

#### Stage 3 ( 2.4 - 2.9 mm long embryos ) :

The anlage of the pronephric duct is elongated and extends from the level of the second somite till the end of the gut. A narrow lumen appears in the centre of each pronephric duct. The two posterior cardinal veins develop in close proximity to the pronephric ducts; the left vein is much smaller than the right one. In the 2.7 mm long embryos, the two pronephric ducts are equal in length and symmetrical in position ventral to the dorsal aorta. Each

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pronephric duct runs along the corresponding posterior cardinal vein. The cranial parts of the pronephric ducts are blocked, while their caudal parts possess relatively wide lumina (PL. II, fig.4 A&B).

#### Stage 4 ( 3.2 - 3.8 mm long embryos ):

The pronephric ducts extend on either side of the body and each is differentiated into a cranial part lined with low cuboidal epithelium, and a caudal part lined with cubical cells. The nuclei are central in position, large in size, spherical in shape and poor in chromatin. No pronephric chamber or pronephric glomerulus appear in the cranial part of the pronephric ducts (PL.II, fig.5 A&B).

#### <u>Stage 5 ( 4.2 - 6.5 mm long embryos ) :</u>

The left pronephric duct is slightly shifted medially, leaving its corresponding posterior cardinal vein at the left side. The large right posterior cardinal vein extends caudally, forming the caudal vein (caudo-cardinal type). The left pronephric duct extends slightly anteriorly, so that the two ducts become asymmetrical (PL. II, fig. 6A). In the posterior region of the body, the two pronephric ducts occupy a dorso-median position (PL. II, fig. 6B). In the 6 mm long embryos, the swim bladder begins to develop in the middle portion of the body cavity.

#### Stage 6 ( 7.2 - 9.5 mm long embryos ):

The two pronephric ducts are located on the right side of the body, being more closely approximated and separted only by the large right posterior cardinal vein. In the posterior region, they extend straightly without any folding or coiling ( PL. II, figs. 7 and 8 ).

The swim bladder has expanded anteriorly for a short distance and its anterior portion is narrow (PL. III, fig. 9 A-F ).

### B) The Development of the Mesonephric Kidney: Stage 7 (11 - 13 mm long embryos):

Several spherical cell condensations from the intermediate cell-mass are developed along the dorsal or lateral surfaces of the pronephric ducts. These cells have small size, strong basophilic cytoplasm and relatively large nuclei. They give rise to the anlage of the mesonephric tubules (PL. IV, fig. 10 A-D).

The left side of the body contains lymphoid tissue around the dorsal aorta and the small left posterior cardinal vein, whereas the right side contains only the two pronephric ducts which surround the large right posterior cardinal vein ( PL. IV, fig. 10 A ). The anterior and posterior ends of each pronephric duct possess small lumina, while the middle portions have relatively wide

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lumina.

At the end of this stage the embryos have attained 13 mm long, they begin to leave the brood pouches of some males.

#### Stage 8 (14 - 20 mm long larvae):

At the beginning of this stage, the mesonephric tubules anlage are oval in shape and some of them are even elongated. The cells of the elongated tubules are arranged to form central lumina ( PL. IV, fig. 10 A ). The early developed tubules are curved towards the wall of the underlying pronephric duct and their distal portions connect with the duct, while their proximal ends are still blid. At the end of this stage, the early developed mesonephric tubules increase in number and length and finally open into the pronephric ducts which are now called mesonephric duct ( PL. IV, fig. 11 A & B ). The mesonephric tubules which are distributed at the whole dorsal or lateral sides of the mesonephric ducts are aglomerular and have narrow closed anterior parts, the tubules are lined with cuboidal epithelium. Lymphoid tissue appears also on the right side of the body around the mesonephric ducts and the right posterior cardinal vein. ( PL. IV, fig. 11 C & D ). The swim bladder is situated in the centre of the body cavity and deleated slightly towards the left side of the

body ( PL. IV, fig. 11 B & C ).

#### <u>Stage 9 ( 22 - 28 mm long larvae ):</u>

The two mesonephric ducts are present on the right side of the body; the left mesonephric duct is slightly longer than the right one ( PL.. V, fig. 12 A & B). More lymphoid tissue is formed on both sides of the body. Another generation of the mesonephric tubules appears at the beginning of this stage as intensively stained spherical condensations from the intermediate cell-mass. They develop in the same way as those of the early developed tubules ( PL. V, fig. 12 C & D ). In 26 mm long larvae, the second mesonephric tubule generation open into the mesonephric ducts either directly or in the collecting segments of the early developed mesonephric tubules ( PL. V, fig. 13 A-D ). Neither Bowmann's capsules nor glomeruli are developed at the proximal ends of both first and second generation of mesonephric tubules ( PL. V, fig. 13 B & C ).

#### Stage 10 ( 32 - 60 mm long youngs ):

The third generation of mesonephric tubules originates and develops from the same sourse and in the same way as the early developed generations. They either open directly into the mesonephric ducts or into the early developed mesonephric tubules ( PL. VI, fig. 14 D). The first formed tubules are differentiated, each into two

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portions, a blind cranial part with cubical cells and small lumen and a caudal one with low cuboidal cells and wide lumen representing the collecting duct segment which opens into the mesonephric duct. The epithelium of the cranial part of each mesonephric tubule is provided with a weakly developed brush border. The whole kidney is restricted to the right side of the body, while the swim bladder extends in the left side ( PL. VI, fig. 14 A-C ). The kidney in the young stages is surrounded by a thin coat of connective tissue to give its definitive form as the adult kidney. The two mesonephric ducts are situated underneath the large right posterior cardinal vein and around which the aglomerular mesonephric tubules are distributed. The left side of the body is occupied by the dorsal aorta, which extends caudally as the caudal artery, and large amounts of lymphoid tissue. The mesonephric tubules are arranged along the whole length of the mesonephric ducts to which they are attached by the collecting duct segments ( PL. VI, fig. 15 A & B ).

#### DISCUSSION

Fraser [8], Tavolga [31], and Holstvoogd [14] emphasized that the glomerular pronephric kidney is not developed in fishes having no larval stages (Elasmobranchs) or in those whose embryos develop inside the bodies of their mothers (Amniotes) or in viviparous teleosts (Platy-

poecilus). It has been suggested that, this type of kidney is absent also in Syngnathifrom fishes whose embryos remain in the brood pouches of males till hatching.

In <u>Halicampodes macrorhynchus</u> the pronephric ducts originate as condensations of cells in the form of two solid cords from the intermediate cell-mass. This agrees with Gihr [11] in <u>Esox lucius</u>, Ogawa [25] in <u>Syngnathus</u> and Khalil and Agamy [18] in <u>Tilapia zillii</u>. However, it differs from the findings of Price [27] in <u>Coregonus clupeaformis</u> and Bielek [4] in <u>Thymallus thymallus</u>. No pronephric glomeruli as those described by Tavolga [31] in the platyfish, <u>Platypoecilus</u>, Ford and Newstead [7] in the Pacific pink Salmon, <u>Oncorhynchus gorbuscha</u>, and Bielek [4] in <u>Thymallus thymallus</u> or large glomerulus as that observed by Holstvood [14] in <u>Chanos chanos</u> have been recorded in the species under investigation.

The present study revealed that the left pronephreic duct is longer than the right duct and the anterior ends of the ducts are blind throughout the whole period of their development. The asymmetrycal disposition of the kidneys observed in <u>Halicampodes macrorhynchus</u> seems to be a characteristic feature of Syngnathiform kidneys ( Verne [32], Edwards [5], Ogawa [25] and Arru [2]). The kidneys of Halicampodes macrorhynchus, in the early stages of develop-

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ment are symmetrically located on both side but in 5.5 mm long embryos the left kidney becomes slightly shifted towards the right side of the body to permit a suitable place for the development and extension of the swim bladder. The two kidneys, therefore are present in the right side of the body around the large right posterior cardinal vein of both young and adult stages.

The mesonephric tubules of the mesonephric kidney are developed either from mesonephric blastema (Marschkowzeff, [21]) or mesonephric bridges ( Moghe, [22] and Newstead and Ford, [23]) or from the intermediate cell-mass ( Bielek, [4] and Khalil and Agamy, [19]. In the present investigation the mesonephric tubules develop as cell condensations from the intermediate cell-mass. These cell condensations are irregularly distributed; they become elongated, attach to the lateral or dorsal walls of the mesonephric ducts and their proximal portions end blindly without formation of any glomeruli or definition into segments. Therefore, the mesonephric kidney in the species under investigation is of the aglomerular type and its mesonephric tubules are unisegmen= tal. The epithelium of the mesonephric tubules has a poorly developed brush border and is similar to that of the second portion of the proximal convoluted tubule of the fresh water teleosts. The above mentioned observations agree with those of Marshall [20], Padovani [26], Edwards [6], Grafflin

[12], Ogawa [24], Somayajulu and Kamat [30] and Khalil [19].

The data presented in this investigation supports the concept that the vertebrate kidney is a holonephros with a homogeneity of pronephros and mesonephros as both develop from a continuous intermediate cell-mass lying between the myotomes and the lateral plate mesoderm on the sides of the body (Fraser [9], Rangarajan [28] and Bielek [4]).

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#### LIST OF ABBREVIATIONS

CE = coelom, CL = cloaca, CLA = cloacal anlage, CLO = cloacal aperture, CS = collecting tubule segment, CT = connective tissue, DA = dorsal aorta, G = gut, HG = hind gut,

IM = intermediate cell-mass, L = liver, LMD = left mesonephric duct, LPD = left pronephric duct, LV = left posterior cardinal vein, LY = lymphoid tissue, M = myotomes.

MD = mesonephric duct, ML = muscular layer, MS = mesoderm,

MT = mesonephric tubules, MT1 = lst generation of mesonephric tubules,

MT3 = 3rd gneration of mesonephric tubules, N = notochord,

NT = neural tube, P = pancrease, PD = pronephric duct,

RMD = right mesonephric duct, RPD = right pronephric duct,

RV = right posterior cardinal vein, S = somites, SW = swim bladder, SWA = swim bladder anlage, Y = yolk.

#### EXPLANATION OF FIGURES

#### Plate I:

- Fig. 1: Photomicrograph of T.S. of the anterior (A), middle (B), posterior (C) and posteriormost (D) trunk region of 1.2 mm long embryo, showing the early development of the neural tube, notochord, gut and mesoderm.
- Fig. 2: Photomicrograph of T.S. of the anterior (A) and middle (B) trunk region of 1.4 mm long embryo, showing the differentiation of mesoderm on each side, the first appearance of the dorsal aorta, the blocked anterior end of the gut and the intermediate cell-mass.
- Fig. 3: Photomicrgraph of T.S. of the anterior (A), and middle (B) trunk region of 2.2 mm long embryo, showing the development of the myotomes and coelom. The intermediate cell-mass forms the anlage of the pronephric duct as solid condensation of cells on each side of the body ventral to the well-developed dorsal aorta.

#### Plate II:

- Fig. 4: Photomicrograph of T.S. of the anterior (A) and middle (B) trunk regions of 2.7 mm long embryo, showing the appearance of the two posterior cardinal veins and the more differentiation of the two pronephric ducts.
- Fig. 5: Photomicrograph of T.S. of the anterior (A), and

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- middle (B) trunk regions of 3.8 mm long embryo, showing the pronephric ducts, the left and right posterior cardinal veins and the dorsal aorta. Notice the absence of pronephric chamber or glomerulus.
- Fig. 6: Photomicrograph of T.S. of the anteriormost region

  (A) and near the posteriormost region (B) of the trunk of 5.5 mm long embryo, showing the elongated left pronephric duct and its shifting towards the right side and the enlarged right posterior cardinal vein (A). In the posterior-most region, the two pronephric ducts extend in the median line, the veins and the aorta are found near each other in the median line (B).
- Fig. 7: Photomicrograph of a frontal section of the trunk region of 7.8 mm long embryo, showing the wavy pronephric ducts extending in the right side of the body and surrounding the large right posterior cardinal vein, while the small left posterior cardinal vein is found close to the left pronephric duct.
- Fig. 8: Photomicrograph of a sagittal section of the trunk region of 9 mm long embryo, showing the straightly extended left pronephric duct which is found in the right side anteriorly and converged medially posteriorly. The epithelial cells of the ducts are cuboidal.

#### Plate III:

Fig. 9: Photomicrograph of T.S. of six different regions
(A-F) of the trunk of 9.5 mm long embryo, showing
the anlage of the swim bladder, the anlage of
the cloaca which opens to the outside by the
cloacal aperture and the intermediate cell-mass
is distributed around the two pronephric ducts.

#### Plate IV:

- Fig. 10: Photomicrograph of four different regions (A-D) of the trunk of 11 mm long embryo. Cell condensations developing from the intermediate cell-mass give rise to the first generation of mesonephric tubules. In (B) appears two generations of these tubules, one of them possesses lumen, while the other is solid and intensely stained.
- Fig. 11: Photomicrograph of T.S. of four different regions
  (A-D) of the trunk of 14.5 mm long larva. The
  mesonephric tubules are attached to the pronephric (mesonephric) ducts by the collecting
  duct segment. The lymphoid tissue appears on
  both sides of the body.

#### Plate V:

Fig. 12: Photomicrograph of the anteriormost region of the kidney (A), few sections caudally (B) and the region of the swim bladder (C,D) of 22 mm long larva. The left mesonephric duct is much

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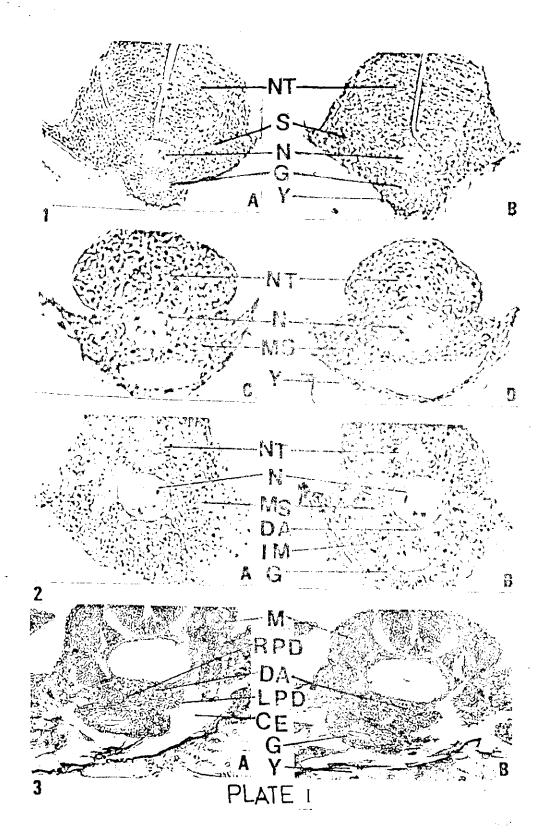
longer than the right. (A,B). The swim bladder has a wide extension in the right side of the body (C,D).

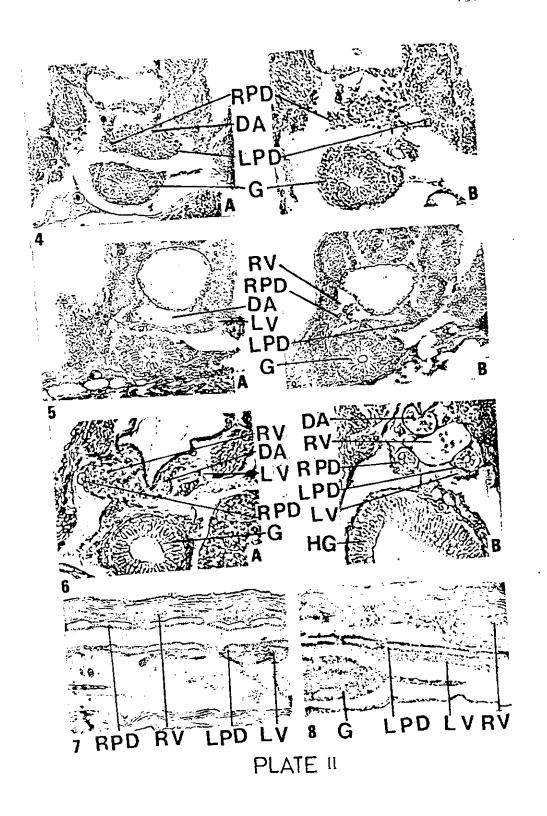
Fig. 13: Photomicrograph of T.S. of four different regions (A-D) of the trunk of 26 mm long larva. The second generation of the mesonephric tubules appears as round condensed cells (A,B), while the first generation of them are elongated and attached with the mesonephric duct by the collecting duct segment (C,D). The intermediate cell—mass is continued to form new tubules.

#### Plate VI:

- Fig. 14: Photomicrograph of T.S. of four different regions (A-D) of the trunk of 40 mm long larva, representing three generations of the mesonephric tubules originating from the intermediate cell-mass. The first generation is attached to the mesonephric duct (A,B), the second is either attached to the first generation (C) or to the collecting duct segment (D) and the third generation appears intensely stained near the mesonephric duct (D).
- Fig. 15: Photomicrograph of T.S. of two different regions
  (A,B) of the trunk of 55 mm long young. The two
  mesonephric ducts are present in the right side
  of the body ventral to the large right posterior
  cardinal vein. The mesonephric tubules are found

around this vein, while the left side of the 'body contains only the dorsal aorta and large amount of lymphoid tissue.





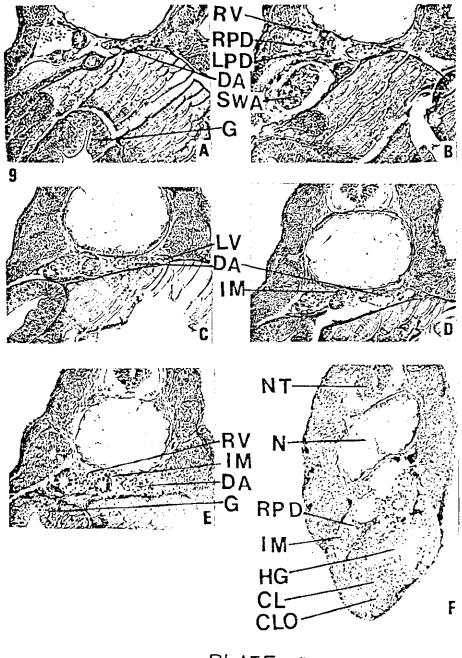
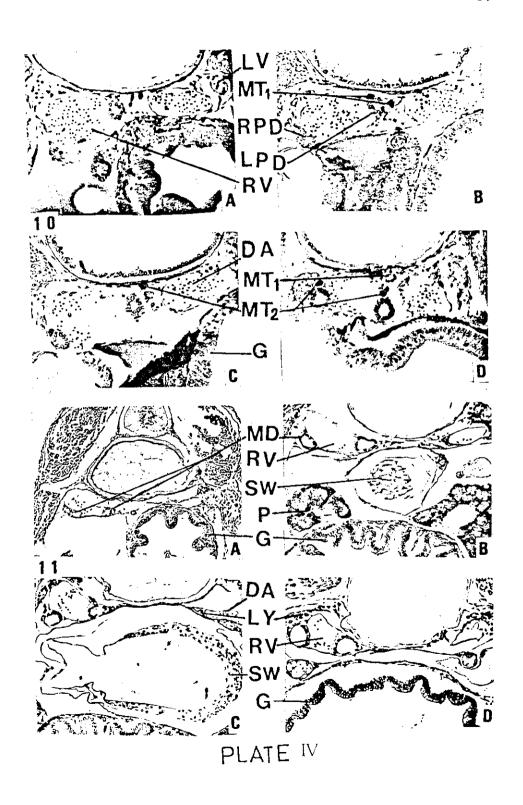
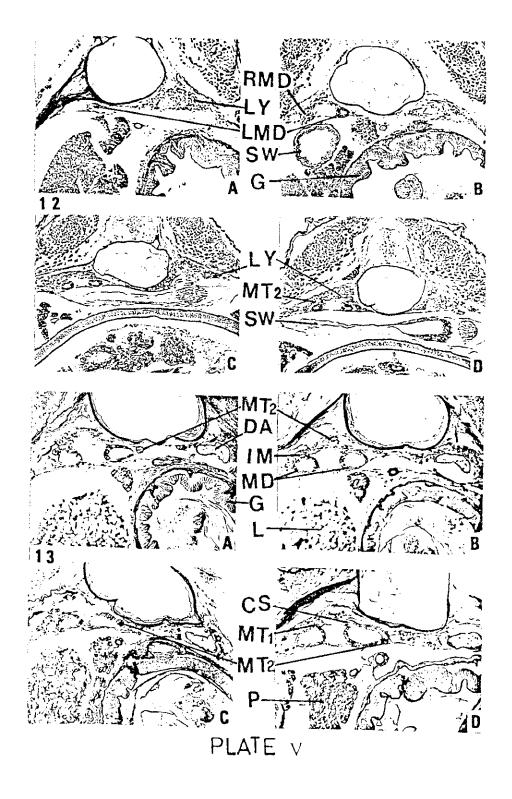
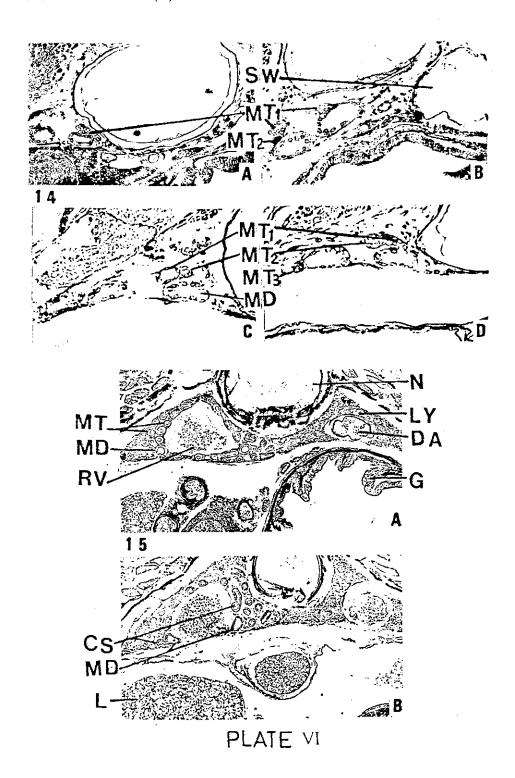


PLATE III





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# منشا وتطور الكلية غير الجمعية للسمكه المزمارية البحرية هاليكامبونس ماكروريهينكس ( بامبر ) فواد عفيفي أبو زيد قسم علم الحيوان ... كلية العلوم ... جامعة طنطا- مصر

يبدا طهور الكلية الأمامية غير الجمعية في السمكة المزمارية البحرية هاليكامبودس ماكروريهينكس ( بامبر ) في الأجنة التي يبلغ طولها ٢٠٢ مم كتناتين جانبييتين متماثلتين على شكل حبلين مصمتين من تغلظات خلوية تنشأ من كتلة الخلايا المتوسطة وينتهيان نهاية مغلقة في الأمام ليكونا الكلية الأمامية غير الجمعية و

يبدأ ظهور حالة عدم التماثل في الكلية لهذا النوع من الأسماك في الأجنة التي طولها ٥,٥ مم على هيئة أزاحة طغيغة لقناة الكلية الأمامية اليسرى ناحية الجانب الأيمن من الجسم نتيجة لتكوين وتعدد المثانة الهوائية المجانب الأيمن من الجسم نتيجة لتكوين وتعدد المثانة الهوائية المجانب الأيمن من الجسم نتيجة لتكوين وتعدد المثانة الهوائية المجانبة المجانبة المجانبة المجانبة المجانبة المجانبة المجانبة المجانبة المجانبة المحانبة المح

تتكون الكلية الوسطى غير الجمعية أيضا فى الأجنة عندما يبلغ طولها من 11-11 مم وذلك بظهور عدة تغلظات كروية غير منتظمة من كتلة الخلايا المتوسطة على طول السطح الظهرى أو الجانبى لقناتى الكلية الأمامية مكونة الجيل الأول لانابيب الكلية الوسطى، تستطيل هذه الأنابيب فى الأطوار اليرقية وتنحنى وتتصل بالقناتين بدون تكوين محافظ أو جمع بولية، تتكون أجيال ثانوية وثالثة من هذه الأنابيب من نفس المادة وبنفس الطريقة التى تكونت منها وبها أنابيب الجيل الأول وتكون جميعها عديمة الجمع وهذه الأنابيب الأخيرة اما أن تفتح في أنابيب الجيل الأول أو تفتح مباشرة فى قناتى الكلية الوسطى ( قناتى الكلية الوسطى ( قناتى الكلية الوسطى ( قناتى الكلية الأمامية )،