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[REVIEW]

Hagfish Development

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INTRODUCTION

Hagfish development is a largely unexploited research area. The reason for this is simply the unavailability of hagfish embryos. Myxinoids inhabit the sea bottom at depths that protect them from observation. Even breeding behavior and method of fertilization can only be guessed at. Developing eggs in their natural situation have never been seen, even from submersible vessels at depths down to 300 m in Monterey Bay, California and Barkley Sound, British Columbia (unpublished). An unusual species, *Eptatretus burgeri*, that can be found at depths as shallow as 10 m during the colder seasons of the year, migrates to breed at greater depths in the warmer seasons (Kobayashi *et al.*, 1972; Fernholm, 1974).

The only successful collections of hagfish embryos (*E. stouti*) took place at Monterey, California about 100 years ago (Dean, 1898, 1899; Doflein, 1899; Price, 1897; Worthington, 1905). These collections utilized the services of local fishermen who extended lines, each bearing about 100 baited hooks, from row boats in Monterey Bay. Fishing in this way for several summer months, they collected about 800 eggs, of which about 150 contained developing embryos (Dean, 1899). The eggs were in and on the masses of slime that were secreted by the stressed hooked hagfish (Fig. 1).

Three *Myxine glutinosa* embryos were obtained by N. Holmgren during a 24-year program of offering rewards to fishermen along the west Swedish coast. Two of these embryos were the basis for a description of their general structure (Holmgren, 1946) though Holmgren recognized that these specimens were poorly preserved. The third *Myxine* embryo, an advanced pre-hatching specimen 4.5 cm long, was the subject of a study on pituitary development by Fernholm (1974).

The more numerous Monterey embryos of *E. stouti* became the subjects of published studies by nine authors (see Table 1). It should be noted that for some of these few studies (e.g. Price, 1897; Neumayr, 1938; Holmgren, 1946; Fernholm, 1974) as few as three or less embryos were available, limiting their value for describing progressive changes. The major stock of embryos was Dean's, but his own published results (1898, 1899) were primarily superficial descriptions of stained whole-mount embryos; the results of his attempts to section the embryos apparently were not good. However, he gave specimens to his student Stockard for studies of general, pharyngeal and thyroid development, to Conel for studies of the brain and to Neumayr for a study of the head skeleton. The gift of some 30 embryos to Conel was made with the stipulation that he have them sectioned by a skilled microtechnician at Harvard University, and that the sections be retained at the Harvard Anatomical Museum to be available for study by others. As far as the author knows, he is among the very few to have eventually availed himself of this opportunity (Gorbman, 1983).

At the time in 1898, when the Dean collections were in progress, Doflein, in his twenties, and on a world tour, came to the Hopkins Marine Laboratory at Monterey as a scientist visitor. He brought about 30 Monterey Bay embryos to Munich for his own study of ovogenesis (Doflein, 1899) and he gave most of them apparently to C. von Kupffer. Von Kupffer became the author of the most definitive published study of hagfish development (von Kupffer, 1899), the principal cited source of information on the subject for the past century. It should be noted that von Kupffer's experiences with dissection and sectioning of the embryos were similar to those of Dean, as he describes them in the following citation from von Kupffer (1899).

".... the fixative only partially penetrated. Thus, a large part of the younger embryos were macerated; another part was flattened, and only a few are usable. These few my assistant, Dr. Neumayr, isolated after painstaking and prolonged effort. ....I do not consider Dr. Doflein responsible for the mishaps experienced by this valuable embryonic material" (my translation from the German).

The faultiness of the sectioned preparations available to von Kupffer is emphasized here not only to introduce a cautionary note before accepting unreservedly von Kupffer's descriptions, but also to show the need for additional study. Von Kupffer's findings were reported as a relatively informal communication, a "Sitzungsbericht" at a meeting of the Gesellschaft für Morphologie und Physiologie in Munich. In the Sitzungsbericht (von Kupffer, 1899) he promised a more definitive publication based on further study. Unfortunately he died after a lingering illness in 1902, three years later,

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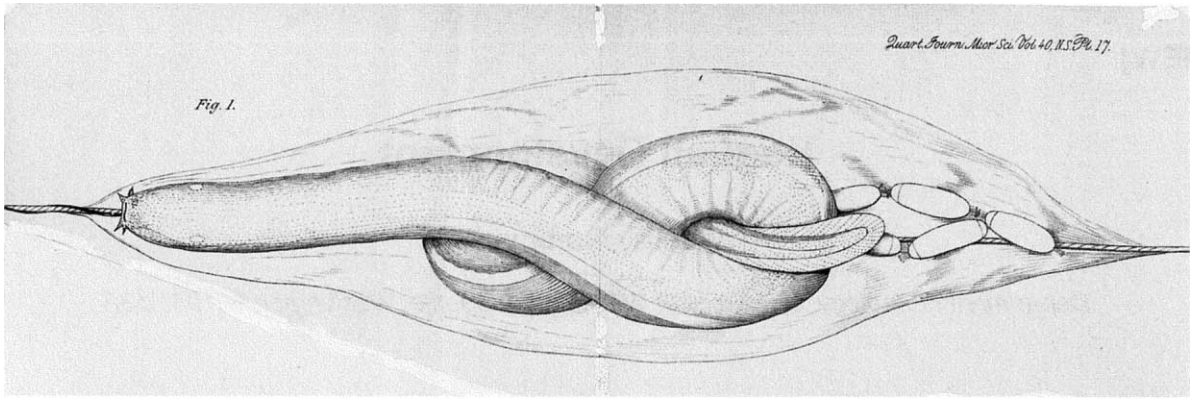


Fig. 1. This drawing by Bashford Dean (1899) depicts a hagfish freshly caught on a hook. The fishing line shows in front and behind the animal. The stressed hagfish has extruded a quantity of slime around itself as well as about 5 eggs that are linked to each other by their anchor hooks.

Table 1. Published studies of hagfish embryonic development

Author	Dates	Subjects	Species
G.C. Price	1896, 1897, 1904	General development Excretory organs	*Eptatretus stouti**
B. Dean	1898, 1899	General development Superficial features	*Eptatretus stouti*
C. von Kupffer	1899, 1906	General development Head development	*Eptatretus stouti*
F. Doflein	1899	General development	*Eptatretus stouti*
J. Worthington	1905	General development	*Eptatretus stouti*
C. Stockard	1906	Pharynx Thyroid gland	*Eptatretus stouti*
J.L. Conel	1929, 1931	Brain	*Eptatretus stouti*
L. Neumayr	1938	Head skeleton	*Eptatretus stouti*
N. Holmgren	1946	General development	*Myxine glutinosa***
B. Fernholm	1969	Pituitary gland	*Myxine glutinosa***
A. Gorbman	1983, 1985, 1990	Head development Pituitary gland Sex differentiation	*Eptatretus stouti*

* *Eptatretus stouti* in earlier studies was named *Bdellostoma stouti*.
** Holmgren studied two embryos; Fernholm studied one.

apparently without completing this intention. A partial review of von Kupffer's hagfish work was published posthumously in 1906.

FERTILIZATION

As mentioned above, the method of fertilization of the hagfish egg remains unexplained, but it has been the subject for speculation for a long time. Some of the older authors have assumed that eggs are fertilized in the coelomic space of females before oviposition. However, most authors agree that this is improbable because hagfish have no intromittent organ for sperm transfer, and the cloacal pore through which ovulated eggs are expelled under pressure would appear unlikely to admit spermatozoa from the exterior. Other factors which would affect fecundity and the success of external fertilization are as follows.

1. The number of eggs is small, usually less than 30. This would require that almost 100% successful fertilization must occur.

2. Testes are surprisingly small, and the consequent dilution of the small supply of spermatozoa in open sea water would make the likelihood of fertilization difficult or inefficient (Cunningham, 1886).

3. Access to the egg through its shell is limited to an opening, the micropyle, barely wide enough to admit one

spermatozoon at a time.

In considering these factors some authors have proposed that external fertilization must occur in an enclosed space where the confined spermatozoa would be in high concentration. Accordingly, one suggestion has been that oviposition and fertilization occur in a burrow in the mud. This, too, seems improbable for a number of reasons. It would require that males follow females into the same burrow, but the burrows are not permanent, and are only created by animals diving into the soft mud and reappearing from the mud (Strahan, 1963; Fernholm, 1969). Furthermore, eggs remaining in the collapsed mud burrow would become subject to anoxic conditions.

A better speculative scenario would be the one illustrated in Fig. 2. Here, first the mating pair couples, and the excitement would cause release of slime which envelopes the pair. The gametes are released into the slime mass in the interface between the mating pair and the slime. This would provide the requisite high concentration of spermatozoa. The mating pair then exits the slime mass by vigorous swimming motions. This method of escape from the slime mass is frequently observable in captive hagfish. The eggs remain centered in the slime, attached to each other by the anchor filaments at the ends of the eggs. Over the next several hours the mucus component of the slime dissolves in the sea water (also observable in aquaria). This leaves the eggs in a mass of keratinoid threads which gradually shorten and coarsen. The eggs are thus left in a basket of threads, allowing respiratory exchange, and protecting them from predation.

Although this scenario has not been actually observed it fits all of the requirements for successful fertilization and survival of the developing embryos. It also provides a new and rational functional value for the threads of hagfish slime. Abundant secretion of slime is known in other animals, usually as part of a defensive strategy, but none of these slimes has a thread cell component. If the threads in hagfish slime have a reproductive value, then they must be considered secondary sex features, and the thread cells in the slime glands are possibly the only known secondary sex structures in hagfish. It would be of interest to know if there is endocrine regulation of the specialized thread cells. The biochemical nature of hagfish slime threads has been studied, and they have been found to be keratin-like (Ferry, 1941; Koch *et al.*, 1991).

CLEAVAGE AND EARLY DEVELOPMENT

The only description of cleavage of the hagfish egg is that by Dean (1899). Cleavage furrows in the cytoplasmic cap are visible at first near the micropyle. They extend into the underlying yolk for a short distance as in other highly telolecithal eggs. Continued cleavage produces a cellular layer over the yolk, the blastoderm. The blastoderm gradually extends toward the lower pole of the egg, almost covering it. Eventually, a primitive streak is formed, marking the posterior end of the developing embryo. In Dean's descriptions and figures it appears that the primitive streak forms some time after neurulation already has begun at the anterior end. To cite Dean (1899) directly, "....no blastopore could be determined; and what appears to be the hinder portion of the neural axis, two thirds of the entire length, is as will later be shown, to be interpreted as 'primitive streak', the blastopore having closed precociously".

It must be remembered that most of Dean's conclusions and interpretations are based on surface studies of whole embryos on the egg, or sheets of cells dissected off of the egg. However he did make some histological sections of early embryos.

Dean's use of the term "blastopore" appears to be applied to situations in which two lateral wings of the posterior margin of the blastoderm are approaching each other, forming a

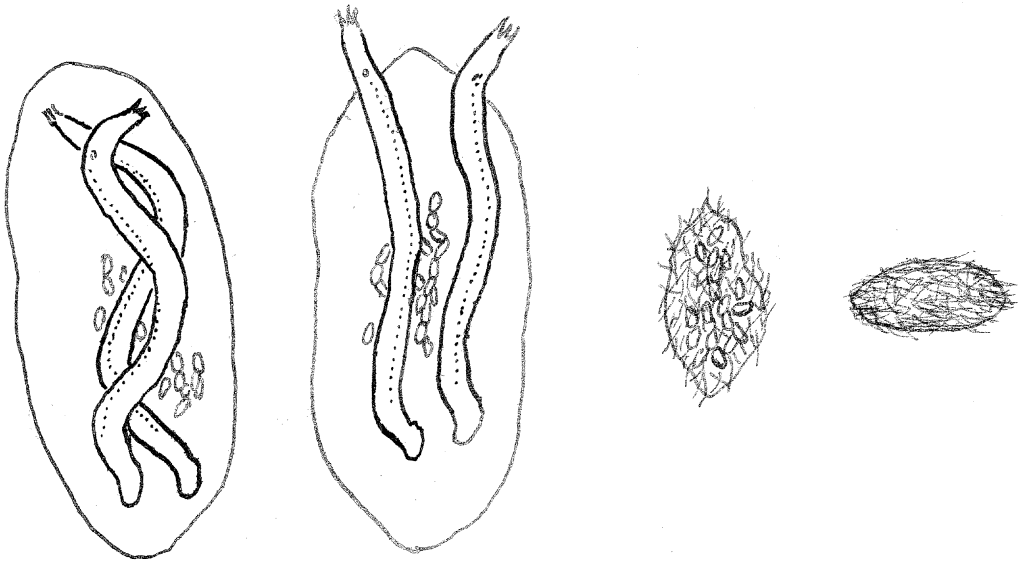


Fig. 2. Presumptive method of fertilization and protection of fertilized eggs. Further discussion in the text.

reversed V, with the acute angle in the midline. At the angle of the V, as illustrated in Fig. 24 of Dean (1899), the margin of the blastoderm is thickened, about eight cells in depth, giving the appearance of inturning of blastoderm cells, as in gastrulation of the avian embryo. As the posterior wings of the blastoderm continue to come together in the midline they form a primitive streak which is visible in many of Dean's figures.

At this stage neurogenesis at the anterior end of the embryo is already far advanced and the differentiated head is already beginning to rise above the blastoderm. This sequence of developmental events would indicate that there are some differences in the process of gastrulation and mesoderm formation between the hagfish and some higher vertebrates, including lampreys. It is clear that these questions require further study.

ORGANOGENESIS

An important problem in describing hagfish development is that successive stages cannot be assigned an age since the actual and relative times for these stages are not known and cannot be estimated. Another problem facing workers with the Monterey Bay hagfish material is due to the osmotic entry of fluid from the hypotonic fixatives used. This has squeezed the embryos against the shell and it has radically changed the shapes of hollow organs. This artefact can readily be seen in Figs. 3 and 6.

The Brain. Neurulation appears to be more or less typical of the vertebrate pattern. Neural folds begin in the anterior part of the embryo (Dean, 1899; Conel, 1929) and progress posteriorly. The folds fuse in the same order in the midline to create an initially hollow brain and spinal cord (Fig. 3). This is of interest because most of the ventricular spaces of the adult brain are eventually lost except in the hypothalamus and hindbrain.

Although Price (1896), Dean (1899), von Kupffer (1899, 1906), Worthington (1905) and Holmgren (1946) all have contributed descriptions of hagfish brain development, that of Conel (1929, 1931) is the most complete. His descriptions are based on 20 fixed and alcohol-preserved embryos given to him by Dean. Conel's initial procedure was to make reconstructions of the brains from the shapes of successive sections. Conel stated in 1931 that he had gone to Munich to examine von Kupffer's slides only to be told that they had been lost. However, he was given access to 10 more uncut Monterey *E. stouti* embryos by L. Neumayr. The fate of this material also is unknown. Most of the specimens that Conel describes are late embryos, already with an eel-like external form. However, Conel's youngest embryo has neural folds in the brain area which still have a wide opening between them. The youngest embryo seen by Gorbman (1983) has neural folds that appear to have fused in the brain area, but the brain is still a tubular structure with a shallow expansion in the ventral wall, the future infundibulum (Figs. 4, 7a).

It is probably not appropriate to go into greater detail concerning brain differentiation in this review. It may be enough to say that hagfish brain development does not differ in an essential way from that of other vertebrates. That is, the anterior neural tube at first differentiates into prosencephalon, mesencephalon and metencephalon. Later development produces a prosencephalon of which the olfactory bulb is unusually large, reflecting the importance of olfaction in the life style of the adult. In older embryos the ventricular system is only partly reduced from its earlier form. Yet, in the adult the only ventricular spaces that remain are the preoptic and infundibular recesses, the aqueduct and the medial portion of the fourth ventricle. Solidification of the brain to the adult form, therefore, must be partly a post-hatching event.

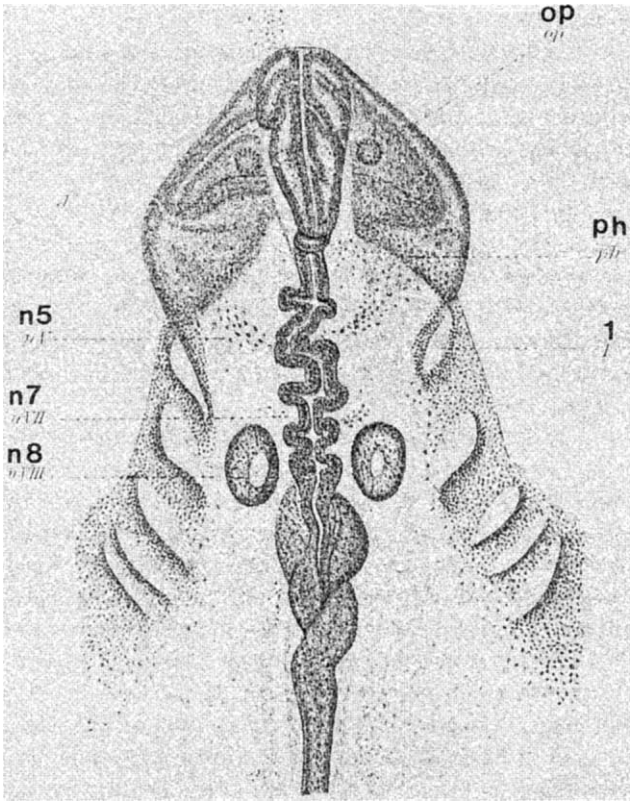
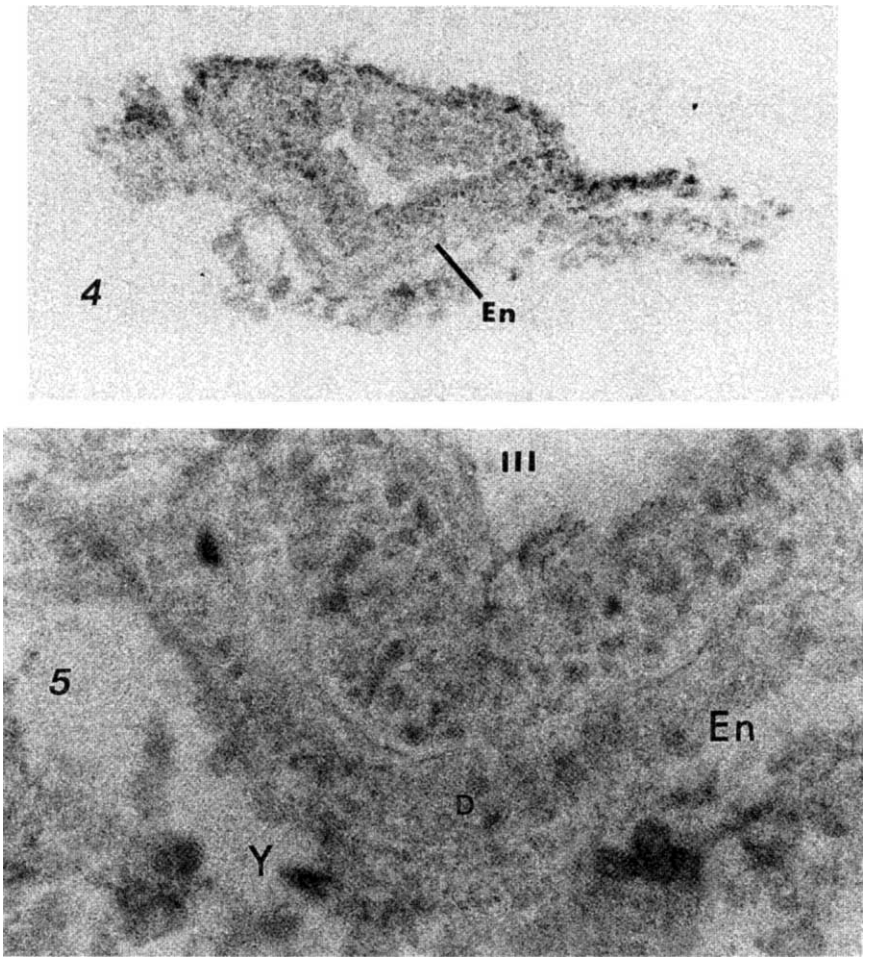


Fig. 3. Dorsal view of a head-fold stage *Eptatretus* embryo. The drawing was made by Dean of a stained whole-mount preparation. The brain is well differentiated and has a lumen. The hindbrain and spinal cord are distorted due to pressure caused by entry of the hypotonic fixative and consequent swelling of the embryo against the rigid shell. The spreading of the gill area of the pharynx may be due to the same artefact. Labels added due to the faintness of the originals: 1, first pharyngeal pouch; n5, n7, n8, cranial nerves (not actually visible in the drawing or the photograph); op, optic cup; ph, edge of the pharynx. Original Figure 100 in Dean (1899).

ENDODERMAL CONTRIBUTIONS TO HEAD STRUCTURES

One of the most unusual and characteristic features of hagfish development is the topographic relationship of the superficial endoderm to differentiating head structures. The



Figs. 4, 5. Cross section through the infundibular region of the brain of a young hagfish embryo that is represented in full length as Fig. 7a. In Fig. 7a a vertical line designates the position of sections 4 and 5. The hazy cellular outlines indicate poor fixation. However, the respective germ layers are clear. Figure 5 is at a higher magnification of part of Fig. 4. The infundibulum, the shallow ventral part of the brain in this section, is in direct contact with the cellular layer of endoderm (En), which lies above the yolk (Y). Below the infundibulum the endoderm is thickened where it will split to form the nasopharyngeal canal (D) shown in Fig. 7b, 7c and 7d. Additional label III, third ventricle of the infundibulum. From Gorbman (1983), with permission.

closure of the neural folds (Fig. 4) apparently occurs before there is a head fold. At this time surface ectoderm covers the entire dorsal surface of the embryo, but does not advance beyond the dorsal surface (see Fig. 7a). Consequently, the newly formed neural tube rests directly upon the cellular layer of endoderm which bounds the yolk. In Fig. 7b a head fold has just begun by continued growth and rising of the embryo above the surface ectoderm. At this time most of the brain is still underlain and in contact with yolk endoderm (Fig. 6). A horizontal splitting of the endoderm layer under the forebrain creates the first visible archenteron, or future nasopharyngeal cavity (Fig. 7c).

Further horizontal splitting of the single endoderm layer (Fig. 7c, d) extends this endodermal cavity from the brain area back to the future pharyngeal space and becomes continuous with it. However, at this time the endoderm beneath the infundibulum and forebrain becomes separated from the archenteric space by a new horizontal septum that begins to grow forward from the dorsal wall of the endodermal cavity below the infundibulum (F in Fig. 7c). Continued growth of this septum then divides the most anterior endodermal lined space into a nasopharyngeal canal above and an oral cavity below (Fig. 7d). This septum appears to have no counterpart in other vertebrates. An analogous structure in the lamprey embryo, which comes to separate a nasohypophyseal canal above from a stomodeal cavity below it is the dorsal lip of the mouth (Gorbman and Tamarin, 1985b). Meanwhile, the head fold, by continuing forward growth, brings the surface ectoderm below the head, and movement of mesenchymal mesoderm from more lateral areas begins to fill spaces in the head and to envelope the gut endoderm (Fig. 7d).

Figure 7d may be compared with an equivalent figure by von Kupffer (1899), here reproduced in Fig. 8, and often adapted by later reviewers because no more recent information was available. It is clear from both diagrams that the now separated nasopharyngeal and stomodeal spaces do not yet

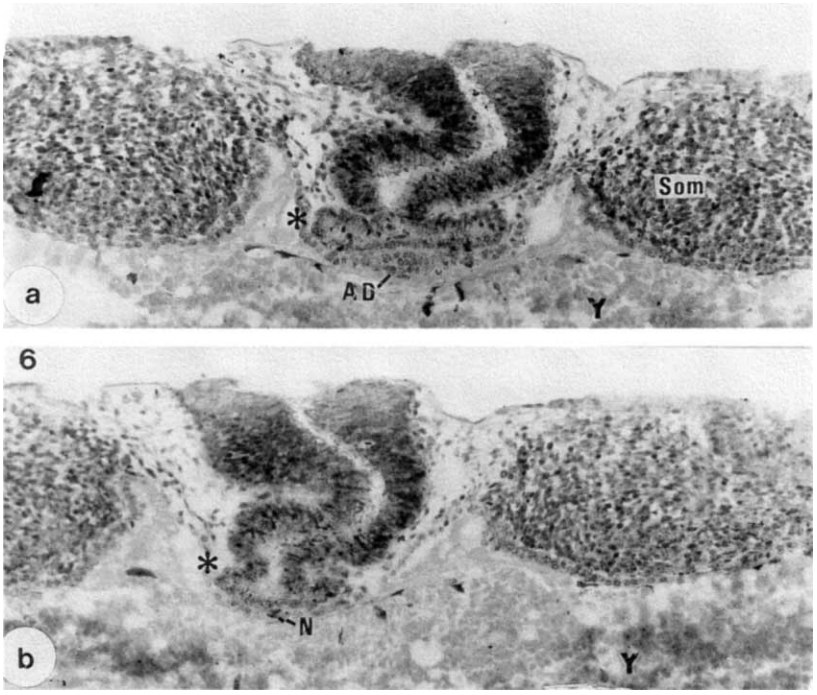


Fig. 6. Cross sections through the diencephalon and infundibulum of a hagfish embryo approximately of the age represented by Fig. 7b. Compression of the embryo after fixation has altered the shape of the brain so that it bends first to the right and then to the left. The neural folds have not yet fused, or possibly the fixation distortion has separated them. The infundibulum has been squeezed so that it has lost its lumen and has a flattened shape. The most superficial layer of endoderm is marked with an asterisk (*), in each photograph. The brain is in direct contact with endoderm. Section **a** is through the adenohypophyseal anlage, a thickened layer of endoderm just beneath the infundibulum (labeled AD). Somites (Som) are on both sides of the brain at this level. Section **b** is only slightly posterior to **a**. At this level the infundibulum is reduced in size, near its end. The endoderm, since it is beyond the adenohypophyseal anlage, is thin again. The anterior tip of the notochord (N) has appeared. The yolk is labeled (Y). From Gorbman (1983), with permission.

open to the exterior, and that the ectoderm is excluded from the interior. What this means is that the oral cavity, olfactory epithelium and adenohypophysis are formed from endoderm in the hagfish, differing in this respect from all other vertebrates.

Von Kupffer (1899) recognized this possibly remarkable difference in germ layer origin of these head endodermal structures and was apparently troubled by it. His search for a way out of this seemingly exceptional situation is expressed in the following paragraph.

(Translation from the German) "From these facts several questions stand out. First of all is the question whether the epithelial layers which close off both canals from the exterior are primary structures, or whether they are secondary closures of earlier openings. In the first case the stomodeal closure plate would be formed from pharyngeal epithelium and that of the nasohypophyseal canal would be of the same type. Then the epithelium of both canals would be endodermal and this would lead to the paradoxical conclusion that the epithelium of the olfactory organ is exclusively endodermal. It would be much simpler to assume that both canals have closed secondarily" (von Kupffer, 1899).

In the series of embryos illustrated in Fig. 7 there is no evidence that the stomodeal and nasopharyngeal spaces were ever open prior to the stage shown in von Kupffer's figure. Dean (1899) and Stockard (1906a) also state that in their studies they never saw the openings of the mouth and nasopharyngeal duct that von Kupffer speculated should occur. Thus, it seems clear that the stomodeal and nasopharyngeal spaces and their organ derivatives (olfactory organ, adenohypophysis) in fact have an endodermal origin in the hagfish (Gorbman, 1983). The significance of the difference in germ layer origin of these head structures from their origins in all other vertebrates is difficult to assess, but it opens up a number of interesting and important questions. Unfortunately the unavailability of hagfish embryos for further study or experimental analysis at this time makes these questions impossible to approach.

Pharynx and Thyroid Gland

Stockard, as a student of Bashford Dean, had access to Dean's extensive collection of hagfish embryo material. His study of the development of the mouth and pharynx (Stockard, 1906a) is summarized in Fig. 10. He described extensive changes of the shape of the pharynx, and elongation of one area of the head that resulted in distancing of the gill region from the more anterior part of the embryo. The earlier gill structures, according to Stockard, are in broadly laterally expanded parts of the pharynx termed "lappets". The lappets are drawn inward as the pharynx and gills continue development. It is quite possible that the "lappets" were

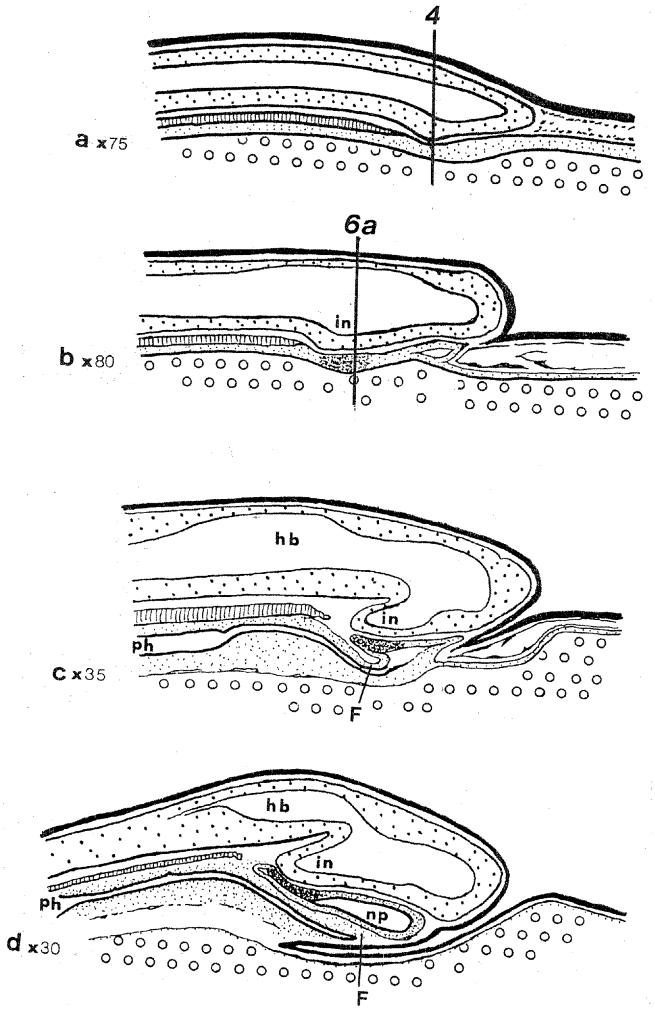


Fig. 7. These four sagittal drawings are reconstructions based on studies of serial sections of four hagfish of different ages. (**a**) is the youngest. It has no head fold, but the brain has differentiated to the extent that a shallow infundibulum has formed as a ventral pouch-like swelling. The position of the cross section in Fig. 4 is indicated by a vertical line. (**b**) is slightly older and shows several significant changes. Growth of the head over the surface ectoderm has produced a head fold. A horizontal split of the endoderm under the brain has produced the first archenteric space. The endoderm beneath the infundibulum (in) has thickened and is indicated here by heavy stippling. The position of a photograph of this thickening (Fig. 6a), the anlage of the adenohypophysis, is indicated by a vertical line labeled 6a. (**c**) represents an older embryo in which the split in the endoderm has progressed posteriorly creating a pharyngeal cavity continuous with the anterior mouth-nasopharyngeal area. The horizontal septum that eventually separates the nasopharyngeal canal from the mouth is just forming (labeled F). The adenohypophyseal anlage has thickened and remains in contact with the enlarging infundibulum (in). Other labels: hb, hindbrain; ph, pharynx. (**d**) An older embryo comparable to von Kupffer's figure of the head, shown here in Fig. 8. The horizontal septum (F) at this stage has grown further and has fused at its distal end with the subcephalic ectoderm, fully separating the mouth from the nasopharyngeal canal. The nasopharyngeal canal has grown posteriorly, beyond the infundibulum (in), and will soon open into the pharynx (ph). At this time it is clear that the adenohypophysis is formed from the dorsal side only of the epithelium of the nasopharyngeal canal (see Fig. 9). From Gorbman (1983), with permission.

artefacts due to compression of the embryos and distribution of skeletal supporting structures between the gills.

The thyroid gland primordium described by Stockard (1906b) is a long groove in the floor of the pharynx, between the first and the fifth pharyngeal pouches. It forms relatively late in development when the head and pharyngeal regions are included in a long head fold that is separate from and extends over the yolk. From the bottom of this groove grape-like clusters of cells grow ventrally and become detached while at the same time they are breaking up into smaller groups of cells. These smaller groups of cells become thyroid follicles, and by the time of hatching they already contain colloid. The broad pharyngeal origin of hagfish thyroid follicles contrasts with the single pharyngeal diverticulum, generally between the first and second pharyngeal pouches, that forms thyroid follicles in other vertebrate embryos, including teleosts. However, it is reminiscent of the broad origin of thyroid follicles from the endostylar epithelium of larval lampreys.

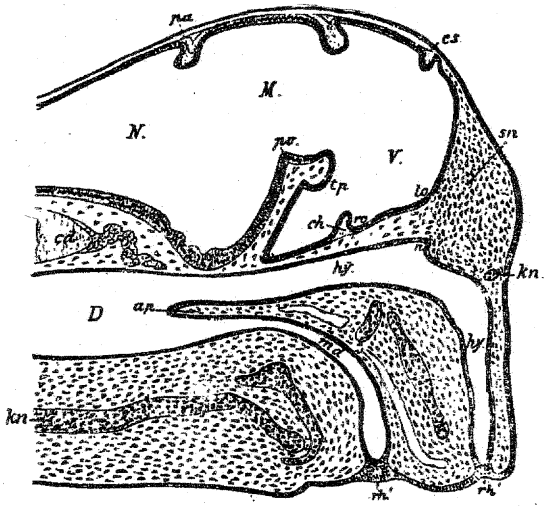
Stockard (1906b) states that between the time of their origin from the pharyngeal floor and the adult location, hagfish thyroid follicles do not move very far. However, in adult hagfish thyroid follicles have spread in the pharyngeal region as far as the bases of the gills. The migratory nature of thyroid follicles is even more strongly expressed in some teleosts in which they can move into areas like the pericardium, the kidney and even the eye (Baker-Cohen, 1959).

Pituitary Gland

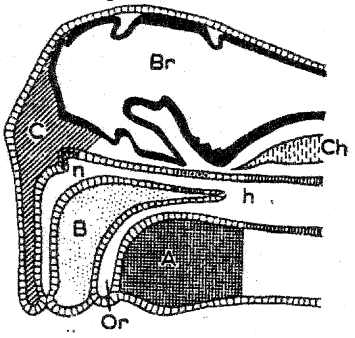
Earlier authors and reviewers (e.g., Fig. 8) considered the entire nasopharyngeal canal to be the adenohypophyseal precursor, and usually labeled it *hy* or hypophysis in their figures. However, Gorbman (1983) and Gorbman and Tamarin (1985b) clearly showed that the adenohypophysis forms from the dorsal epithelium of the canal only, and from the limited area where it is in contact with the infundibulum. The actual separation of the adenohypophysis from the nasohypophyseal epithelium is by multiple budding of groups of cells and delamination of a flat thin organ rudiment (Fig. 9). This mode of formation of the adenohypophysis is very similar to that in the lamprey (Gorbman and Tamarin, 1985a, b), and it differs from the majority of vertebrates in which the adenohypophyseal anlage is a Rathke's pouch or a solid ingrowth of cells (e.g., Amphibia).

Somites and Mesodermal Differentiation

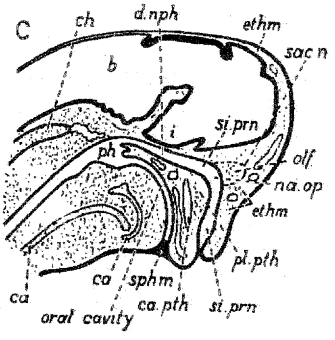
Development of mesodermal structure is poorly described in the limited older literature. Dean describes the lateral and anterior progression of the "mesoblast", the sheet of early mesoderm, as seen in whole-mounted stained hagfish embryos (Dean, 1899). The origin of the mesoblast is assumed to be by inrolling of cells at the "blastopore". In Dean's terminology the blastopore is the V-shaped gap between the advancing posterior edges of the blastoderm. Somites appear early, just lateral to the still open neural tube, presumably from the medial part of the mesoblast, and their number is about 75 pairs in an embryo with five gill slits.



Kupffer 1899



Heintz 1963



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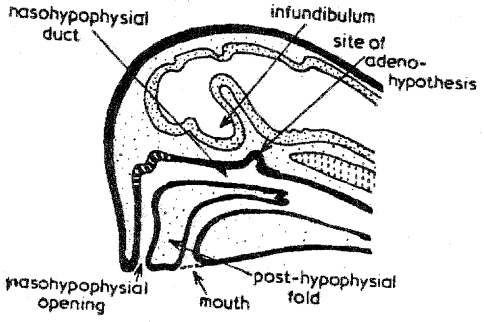


Hardisty 1979

Fig. 8. This figure illustrates some of the difficulties created by the lack of new information about hagfish development since 1899. More recent reviewers of this field, who appear to have depended largely on von Kupffer's (1899) crude drawing of a sagittal view of a mid-section of the head of a later hagfish embryo, have variously, and sometimes incorrectly, modified it. Von Kupffer's diagram (top) is comparable to Fig. 7d. In von Kupffer's figure the horizontal septum that divides the original archenteric space into mouth (md) below and nasohypophysial space (hy) above is well developed and contains skeletal elements. The later reviewers label this septum B, or "posthypophyseal fold". The greatest creativeness is shown in Hardisty's (1979) drawing in which the future external openings of the two spaces are shown open. Von Kupffer shows them closed by two membranes labeled rh'. Most original is the addition of a Rathke's pouch-like structure by Hardisty labeled appropriately "site of the adeno-hypothesis". From Gorbman and Tamarin (1985a).

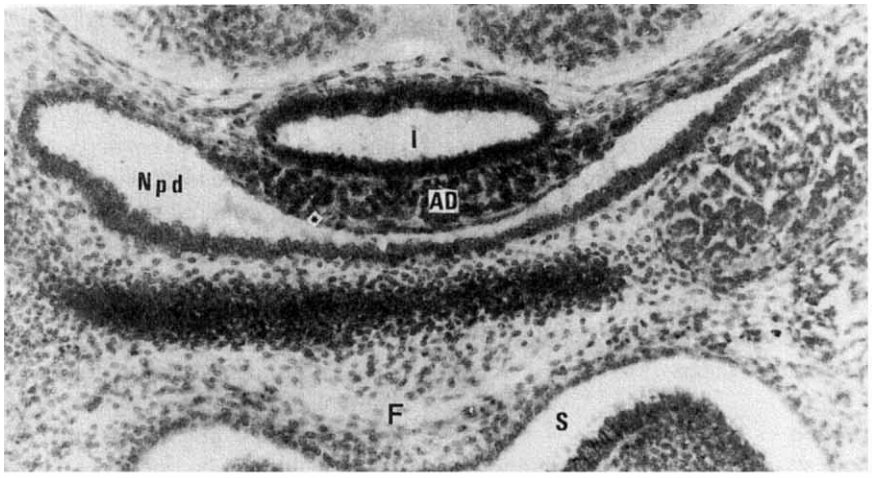


Fig. 9. Cross section through the pituitary region of an embryo slightly older than the one in Fig. 7d. The adenohypophysis (AD) has separated from the dorsal side of the nasopharyngeal canal (Npd). A layer of follicular groups of cells lies between the nasopharyngeal canal and the floor of the infundibulum (I), which will become the flat neurohypophysis of the adult. The thin regenerating dorsal epithelium of the nasopharyngeal canal is marked by an asterisk (*). Below the nasopharyngeal canal is the thickened horizontal septum (F), now well developed and containing a dark concentration of mesenchyme that will form head skeleton. Below that is the mouth (S) and the forming "tongue" in its floor.

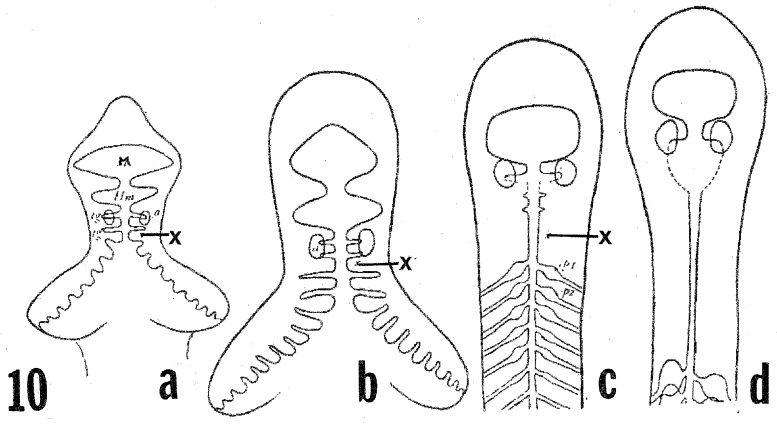


Fig. 10. Diagrams indicating the changes in position of pharyngeal structures during development of the gills. (**a**) The condition in young embryos when all of the branchial gills are out on the gill lappets. Note a pair of otocysts just behind the hyomandibular arch (Hm). X marks an apparent center of growth and elongation. (**b**) The first few gill pairs are drawn in from the lateral lappets. Otocysts are larger. (**c**) The beginning of pouch formation in the branchial gills. (**d**) Apparent growth and elongation of the neck region shifts the gill area further posterior behind the otocysts. From C. R. Stockard (1906).

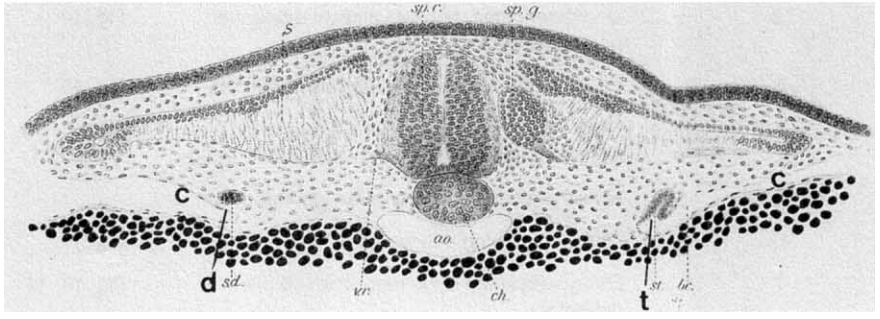


Fig. 11. A drawing by G. Price (1897) of a cross section in the trunk region of a late hagfish embryo. There is a well organized somite on either side of the neural tube, with recognizable differentiated dermatome and myotome regions. Below the neural tube in the midline are the notochord and dorsal aorta. The section must be slightly oblique because a spinal ganglion is on one side of the neural tube, and a ventral root emerges on the other side. On one side below the somite and above the coelom (c) is the undifferentiated anlage of the pronephric (or mesonephric) duct (d). At an equivalent position on the other side is a segmental kidney tubule (t) with an opening, the nephrostome. Slightly modified (labeling only) from Price (1897).

Somite organisation in *E. stouti* embryos studied by Price (1897) appears typical in showing a dermatome and myotome, but it leaves in question whether there is at some time a nephrotome. In Price's illustration of a somite in the youngest embryo available to him (Fig. 11) there already is a pronephric tubule with a nephrostome, and the anlage of the pronephric duct. According to Price, the pronephric and mesonephric ducts form as continuous structures rather than, in other vertebrates, as segmental units which must join to form a continuous duct. He indicates that he found it difficult to distinguish between pronephros and mesonephros other than by their relative anteroposterior positions.

Gonadal Sex Differentiation

The adult hagfish gonad is a single long membranous structure that extends the full length of the body cavity. The male and female elements of the gonad are differentially distributed, the testis occupying the extreme posterior end, and the ovarian structures occupy the much longer anterior part of the membrane. In the mature male the anterior gonad is degenerate, and in the female the posterior (male) portion is degenerate. The anteroposterior distribution of the hagfish sexual elements therefore differs from the cortico-medullary localization of the separate sexual elements seen in most other vertebrate gonads.

Sex differentiation in the hagfish occurs in young animals long after hatching from the egg, so sample supplies of study material are available. The study described here (Gorbman, 1990) is the only one in which body length of the animal is correlated with the anteroposterior position of the tissue sample taken and the stage of sex differentiation found. From *E. stouti* between 12 and 45 cm in length, three gonad-containing tissue samples were taken; one was from the presumptive male region, in the five most posterior abdominal body segments; one was between segments 6 to 10 anterior to this (transitional region); and one was from segments 24 to 28 further anterior to the presumptive testicular area (presumptive ovarian area). Each of the gonadal areas was classified as one of the progressive stages of gonad differentiation outlined in Table 2. The results of these tissue classifications are summarized in Fig. 12 and illustrated in Figs. 13 to 24.

It is clear from Fig. 12 that all hagfish less than 20 cm in length progress through a series of ovogenic stages. Sex differentiation in *E. stouti* is therefore progynous. In animals larger than 20 cm further sex differentiation involves development of one portion of the gonad, either anterior or posterior, and involution of the other. In male differentiation, as Fig. 12 shows, the posterior region develops spermatogenic lobules while oocytes in the long anterior section degenerate and disappear. In female differentiation the initial oocytes continue to grow and become vitellogenic, while the posterior presumptive male segments involute. In a few animals of intermediate length both testicular and ovarian elements can be found at the same time. In these young animals it is uncertain whether, or for how long, the bisexual nature of the gonad will remain. Since adult hermaphroditism is rare (Fig. 25) these bisexual gonads most likely are developing males in which involution of the oocytes is delayed.

Sex ratios in populations of hagfish appear to vary geographically (Gorbman, 1990). Accordingly, there may be epigenetic influences over hagfish gonadal differentiation. This question deserves further study.

Table 2. Numerical designation of stages of gonadal differentiation

Stage of gonadal differentiation	Description	Illustration
1	Completely undifferentiated. Small thin gonad	Fig. 13
2	Undifferentiated, but with a slight amount of parenchyma	Fig. 14
3	Small gonad with a few cell cysts or follicles	Fig. 15
4	More numerous gametocysts and a few oocytes (cells surrounded by thin follicle cells)	Fig. 16
5	Mostly oocytes, and only a few gametocysts	
6	Ovary containing only oocytes	Fig. 17
7	Ovary with some eggs more than 60 μm in diameter	Fig. 18, 19
8	Ovotestis	Fig. 20, 21
9	Solid rounded cellular testis with early spermatocysts	Fig. 22, 23
10	Testis, usually with meiotic spermatocytes	Fig. 24

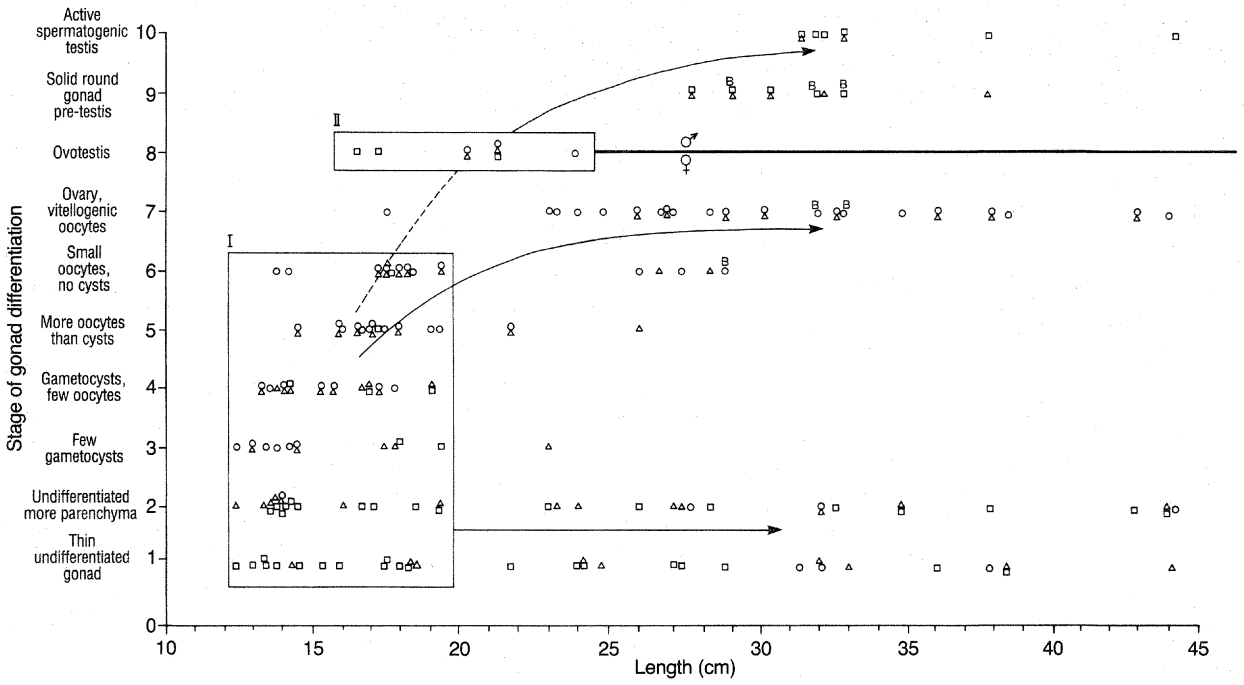


Fig. 12. This figure summarizes all of the determinations of stage of sex differentiation of all levels of the gonads of all hagfish studied. Circles represent the anterior, A level, body segments 20-24 anterior to the posterior end of the body cavity. Squares represent the most posterior level of the developing gonad, in body segments 1-5. Triangles represent the usually transitional level, body segments 6-10. A rectangle, I, encloses the determinations for all animals under 20 cm in length, with gonads developed up to stage 6. For definitions of the stages of sex differentiation see Table 2. It is obvious that in animals in the under-20 cm group sex differentiation stages gradually move from 1 to 6, to the right, with increase in body length. A second rectangle, II, encloses the determinations for six animals in which intersexual (both male and female) gonadal elements were found in the same tissue block. Three long arrows indicate the three general tracks of gonadal differentiation in hagfish over 20 cm in length. In the upper part of the figure are six Bs placed next to six gonadal blocks. These indicate that in three animals fully differentiated male and female elements were both found, but they were at different levels of the gonad. From Gorbman (1990), with permission.

Figs. 13-24. All photographs are cross sections of designated regions of gonads of hagfish of different lengths and they illustrate the successive stages of gonadal differentiation described in Table 2. All are at the same magnification unless otherwise stated.

Fig. 13, stage 1. The gonad, G, is thin with almost no parenchyma. The oval structure above the gonad is the "ureter", U. The more solid structure below it is the wall of the intestine, INT.

Fig. 14, stage 2. A long indifferent gonad characteristic of larger animals. The parenchyma is mostly fibrous and vascular tissue. No apparent germinal cells.

Fig. 15, stage 3. Transitional gonadal level of a small specimen. Two undifferentiated gametocysts are visible and germinal tissue is on one side of the gonad only.

Fig. 16, stage 4. Anterior (20-24) level of a 14 cm long animal. Approximately nine cystic structures are visible. The most distal (ventral) cysts are not sex-differentiated. However, proceeding dorsad (toward the mesentery) a second, smaller, cell type is seen within some cell nests. At the hilum of the mesentery is a group of about eight oocytes breaking out of the cyst pattern. Accordingly, this section shows the sequence: gametocysts, oocysts, oocytes.

Fig. 17, stage 5-6. Anterior level of gonad of a 23 cm specimen. Oocytes near the free end of the gonad are separating out of an oocyst. All other large cells are oocytes. The germinal epithelium is limited to one side.

Fig. 18, stage 7. Anterior gonadal level of a 17.4 cm specimen. This was the smallest animal that attained stage 7 in any part of the gonad.

Fig. 19, stage 7. A more advanced stage 7 (large vitellogenic eggs) at the anterior gonadal level of a 24 cm animal.

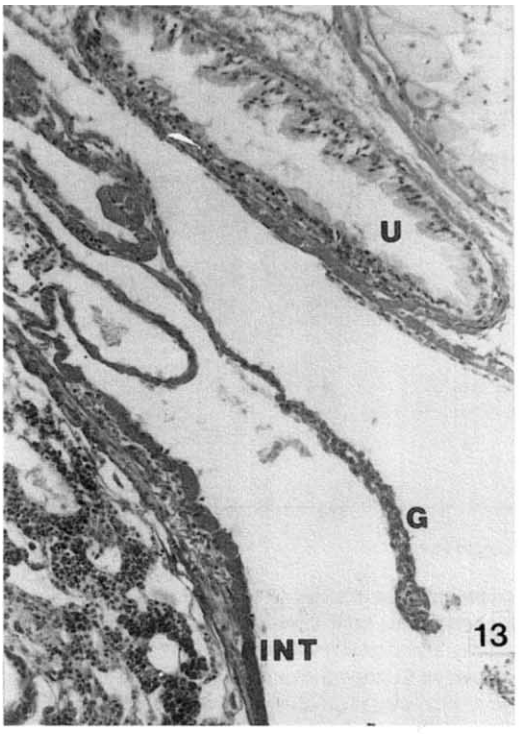
Fig. 20, stage 8. An intersexual gonad at the anterior level of a 24 cm specimen. A testicular follicle appears in the upper part of the photo. Most of the gonad is ovarian.

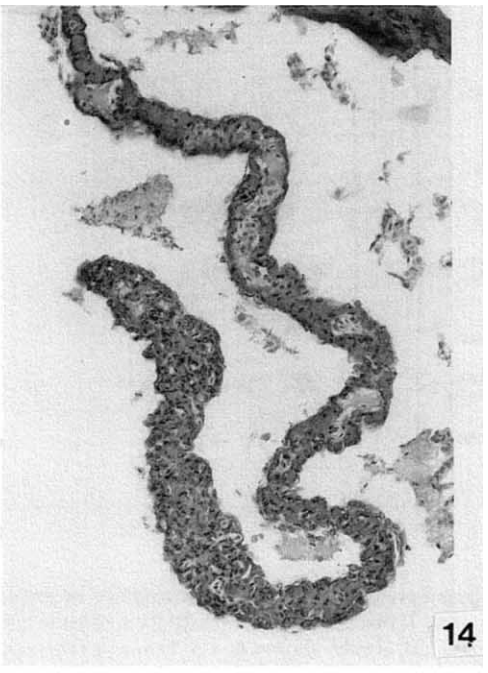
Fig. 21, stage 8. An intersexual gonad at the transitional level (6-10) of a 20.1 cm specimen. In contrast to Fig. 20 this intersexual gonad is mostly testicular.

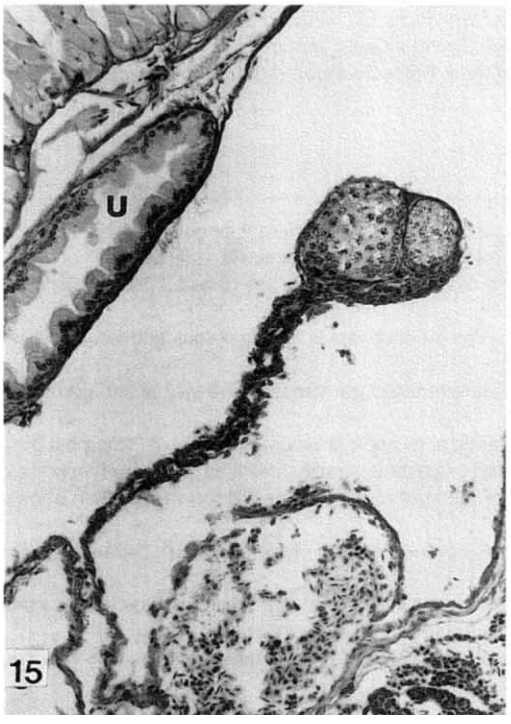
Fig. 22, stage 9. (early). Posterior end of the gonad of a 27.9 cm specimen. Numerous small spermatocysts are forming from the thickened germinal epithelium at one side of the testis.

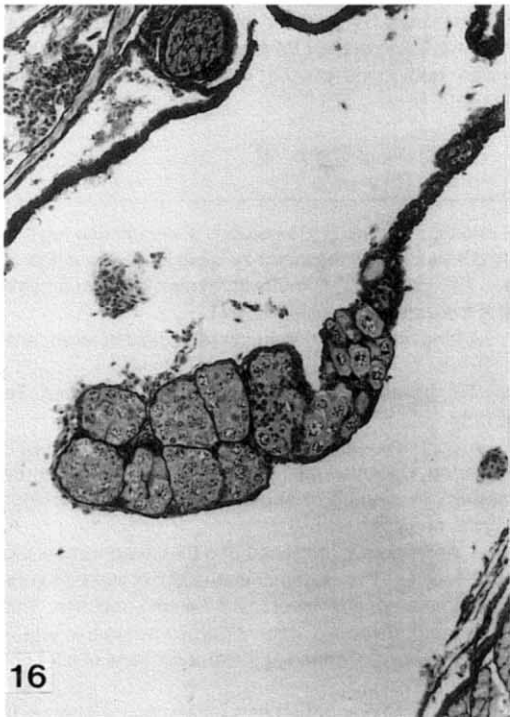
Fig. 23, stage 9. (more advanced). The germinal epithelium is characteristically deeply folded and thicker on one (right) side. This gonad is at the most posterior level of a 32 cm male.

Fig. 24, stage 10. Testis of a 45.5 cm male specimen. Only a small part of the testis is shown. Active spermatogenesis is in progress as indicated by the meiotic activity. Posterior level of the gonad. From Gorbman (1990), with permission.

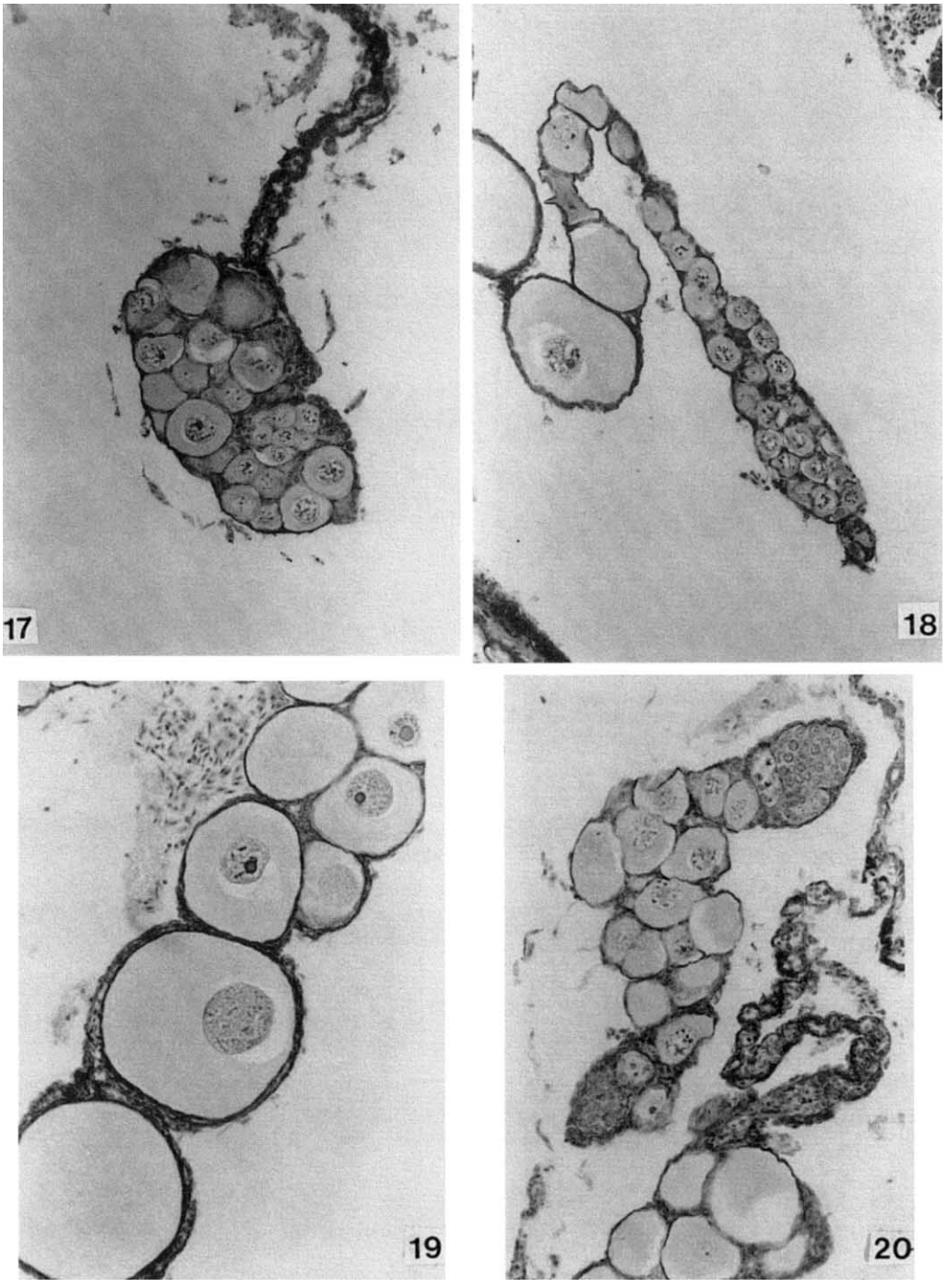




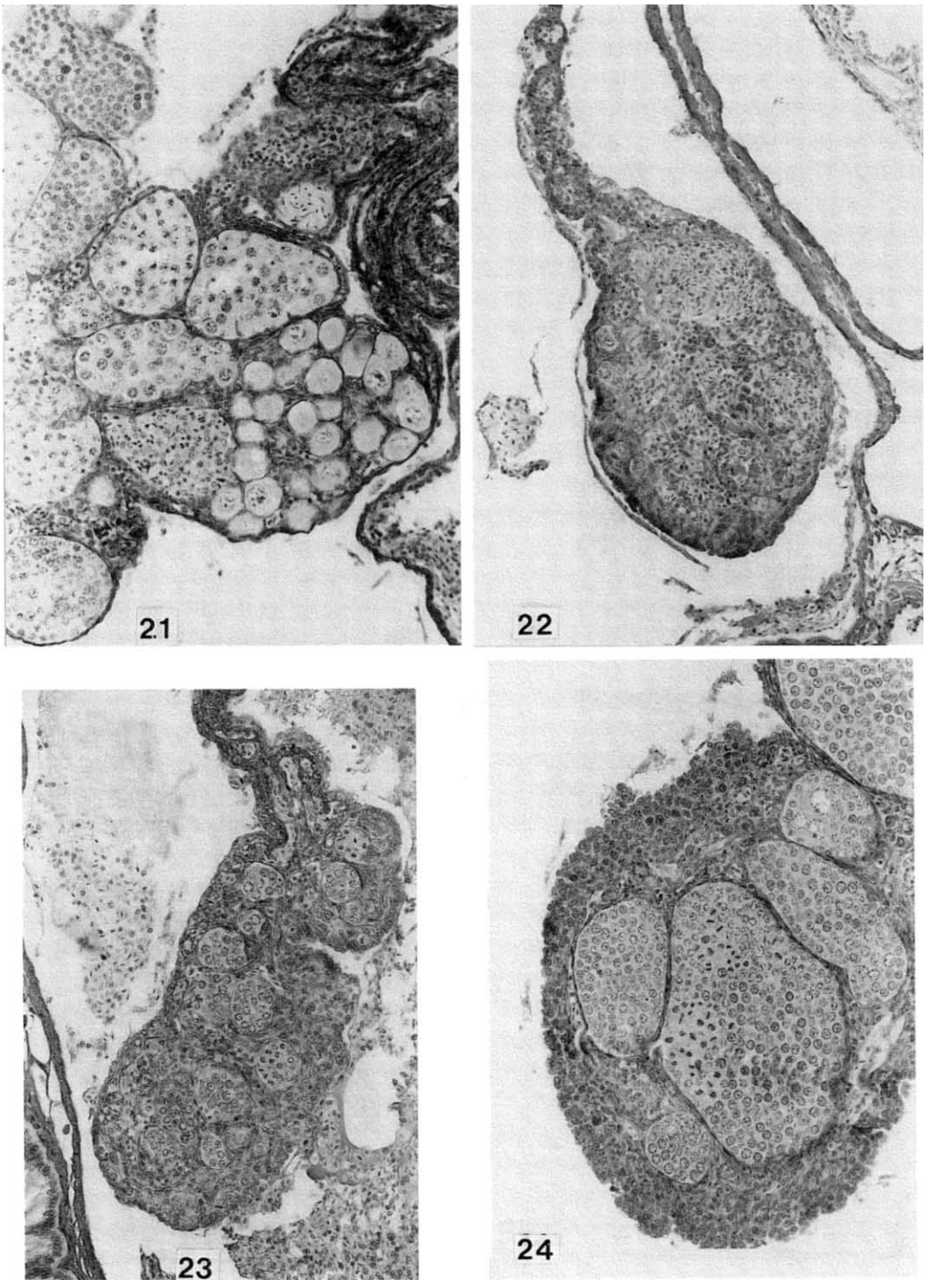




Figs. 13-16



Figs. 17-20



Figs. 21-24

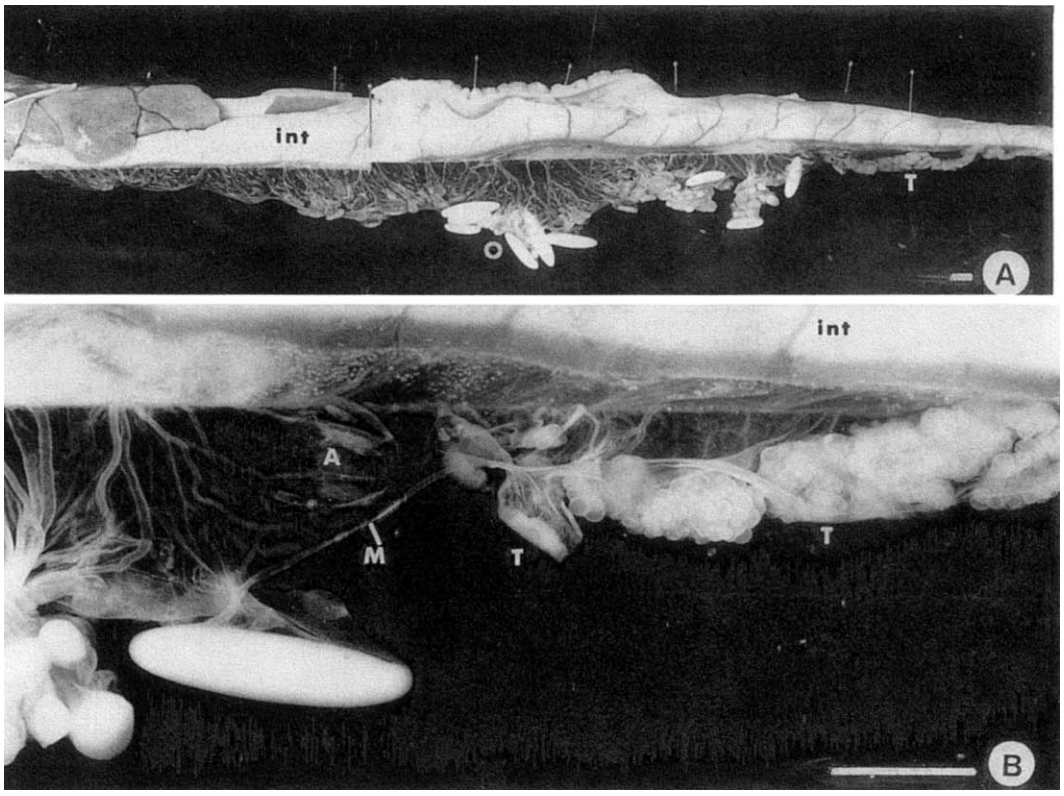


Fig. 25. Gonad of an adult hermaphroditic *E. stouti* 54 cm long. (**A**) General view of the entire gonad. (**B**) A more magnified view of the junction area between the ovary and testis of the same animal. The white scale bars at the lower right of each photo are equivalent to 5 mm of actual size. Labels: A, atretic follicle; int, intestine; M, mesentery (mesovarium); O, ovary with vitellogenic eggs; T, testis. There is no apparent overlap of ovary and testis in this animal. T occupies a normal posterior position. The number of large maturing eggs (14 to 18 mm in length) is unusually small. The number of atretic egg follicles is unusually high. At the higher magnification spermatic vesicles are discernible in the testis. From Gorbman (1983), with permission.

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