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THE EMPEROR PENGUIN

Aptenodytes forsteri Gray

II. EMBRYOLOGY

By

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INTRODUCTION

BY far the most comprehensive account of the present state of our knowledge of the embryology of the penguin is to be found in Parson's publications of 1932 and 1934. These, however, by no means cover the subject completely. It is therefore proposed to give a concise review of this aspect of the subject before describing the present series of sixteen Emperor penguin embryos.

The material on which this paper is based has been deposited in the embryological collection of the Charing Cross Hospital Medical School, London.

EMBRYOLOGY OF THE EMPEROR PENGUIN

THE earliest reference to the embryology of the Emperor penguin is to be found in Pycraft's (1907) report on material collected by the National Antarctic Expedition 1901-4. This material collected in 1902 consisted of three fully incubated specimens and one younger embryo in which feather papillae were just appearing all over its surface. The wing of this younger embryo had attained all the principal features of the adult wing. Pycraft contended that his observations confirmed the opinion that the paddle of the modern penguin had been derived from a functional flying wing and that the tarso-metatarsus represented a primitive stage. From observations made on nestlings he concluded that the skull of *Pygoscelis* was more primitive than that of *Aptenodytes*. Wilson (1907) writing on broader ornithological aspects was of the

opinion that: "The possibility that we have in the Emperor Penguin the nearest approach to a primitive form not only of a penguin, but of a bird, makes the future working out of its embryology a matter of the greatest possible importance."

With this realisation in view, he set out to Cape Crozier with Bowers and Cherry-Garrard on the 27th June, 1911, to undertake "the weirdest birds'-nesting expedition that has ever been or ever will be". On the 20th July they collected five Emperor penguin eggs, three of which were brought back safely. They were delivered by Cherry-Garrard to the Natural History Museum in South Kensington in 1913.

In due course they passed into the hands of Assheton, who designated the embryos according to size as No. 1 (the smallest), No. 2 and No. 3. He prepared sections of the head and body of No. 1 and also cut and mounted a series of sections of the body of Embryo No. 2. The head of this embryo was embedded in paraffin wax, but it was not cut into sections. Assheton also made some notes and drawings. These were available to Cossar Ewart (1922), who gave a brief description of the three embryos in an appendix to *The Worst Journey in the World*. Cossar Ewart paid attention mainly to the external differences with special reference to the distribution and significance of the feather papillae. He compared these embryos with Chinese Goose embryos and concluded that scale papillae are fundamentally different from feather papillae.

The description of these embryos was delayed as a result of the death, first of Assheton and later of Cossar Ewart. It was not till 1934, twenty-three years after their collection, that their detailed description was published by Parsons.

It seems incredible that embryos of such rarity and biological importance, collected with such determination and under such appalling conditions, should have been treated with so little interest and sense of urgency.

However, to quote from Cherry-Garrard's vivid description of the expedition to Cape Crozier: "If you march your Winter Journeys you will have your reward, so long as all you want is a penguin's egg."

Parsons considers that Embryo No. 1 corresponds to a chick of ten days and Nos. 2 and 3 correspond to chick embryos of about thirteen days incubation. In his description of these embryos he remarks on the unsatisfactory state of their preservation. This is not altogether surprising in view of the fact that they were preserved under such difficult conditions.

The youngest embryo has reached a sufficiently advanced stage in development to possess a four-chambered heart and lungs with actively budding air-sacs.

Separate preparations were made of the limbs of this embryo. The shape of the humerus is already defined in cartilage, as that typical of the adult penguin. As pointed out by Lowe (1933) the lower end of this bone is triangular, in marked contrast with the rounded form of this part of the bone in the majority of birds. The radius, ulnar and cuneiform are also completely spheniscine.

The adult features, however, were lacking in the distal cartilages of the wing. The pollex has not yet fused with the metacarpal of the second digit, and between the metacarpals on one side and the radiale and cuneiform on the other, the semilunar cartilage of the distal carpal 2 is still perfectly distinct.

In the foot, the cylindrical metatarsals are characteristically free, a feature which is generally agreed to be a primitive condition.

The two older embryos do not differ from one another by more than a day of incubation.* There are, however, minor differences with regard to the distribution of feather papillae. Embryo No. 2 is without feather papillae on the breast, abdomen and on the pre-axial surface of the wing, whereas on Embryo No. 3 these parts are covered with them. There are no papillae as yet distal to the tarsal joints in either specimen. Parsons considered that the feather papillae were similar in type to those described by him in Gentoo penguin embryos (Parsons, 1932). He noted that the most striking feature of the Emperor penguin embryo was not its size but the high dome of the head, due to the mass of loose connective tissue around the pineal body. The arrangement of the thoracic and abdominal viscera in Emperor penguin did not differ in any important particular from embryos of other species of penguin at a corresponding stage. Parsons concluded that the examination of the three specimens had not greatly added to our knowledge of penguin embryology or ancestry.

Not until 1949 was Stonehouse able to collect a new series of embryos including some early stages. A preliminary communication on this series which forms the basis of the present communication has already been published elsewhere by the author (1953).

*No. 3 is only 0.5 mm. longer than No. 2. This, incidentally, is the only reference to any measurement of these embryos.

EMBRYOLOGY AND DEVELOPMENT OF OTHER SPECIES
OF PENGUIN

DATA on this aspect of the subject are also scarce. Menzbier (1887) was concerned with the osteology and integument of young birds, whilst Studer (1889) investigated the same aspects of the subject in relatively large embryos. Schauinsland (1890), who first examined early embryos, but made no mention of the species of penguin he was describing, drew attention to the marked development of a neurenteric canal in a 30 somite embryo and to the fact that this canal persists longer than is customary in reptiles. He pointed out that embryos with well-developed extremities in which only a cord of cells represents the tail gut still have an obvious neurenteric canal. Schauinsland states that the notochord develops from endoderm by a typical process of folding and that at the tailbud stage the caudal end of the neural tube develops a voluminous vesicle which he considered to be an embryonic sense organ. Pycraft (1907) refers to Adélie penguin embryos (*Pygoscelis adelia*) as well as Emperor penguin embryos, in the paper which has already been quoted in some detail in the previous section. In 1907 Anthony described the external appearance of one Gentoo penguin embryo (*Pygoscelis papua*) almost ready to hatch and collected by the Expédition Antartique Française 1903-5.

Waterston and Geddes (1909) described the embryos brought back by the Scottish National Antarctic Expedition. These embryos of the Gentoo penguin (*P. papua*) and the Adélie penguin (*P. adelia*) were fixed in formalin and were not in a satisfactory condition for microscopic examination; so only the external features of a series extending from the pre-primitive streak stage to hatching were described. About thirty stages were compared with a specially prepared series of duck embryos. Waterston and Geddes concluded that the early stages of development are exactly comparable to the corresponding stages of the chick or duck, though they take place more slowly. In the later stages the development of the down, the fore limb and foot differ from that in the duck. The foot of the penguin is clumsy and primitive throughout development and adult life, whereas in the duck it has a lighter appearance and is adapted as a paddle. They were of the opinion that the forelimb of the penguin is definitely progressive in its mesoblastic structures whereas the mesoblastic portion of a duck's wing is relatively regressive. Both have varied from the ancestral type, in the one case to form a paddle requiring rigidity, in the other case to form a wing requiring lightness—the reduced spread of bone being compensated for by an increased development of feathers. As a result of this embryological study Waterston and Geddes were of the opinion that the divergence of the *Aptenodytidae* from the main stem of the flying birds had occurred early in phylogeny, and the common ancestor of both had been a flying bird, though of a clumsy type.

Anthony & Gain (1915) described large series of embryos extending from the first to the last day of incubation of four different species of penguin, namely: *Pygoscelis adelia*, *P. papua*, *P. antarctica* and *Catarrhactes chrysolophus*. Their "Contribution à l'étude de l'embryologie des Spheniscidae" is devoted to three main points:

1. The development of the skeleton of the wing.
2. The development of the skeleton of the foot.
3. The pterylosis (feathering).

These aspects of the subject are described in detail but no conclusions are drawn.

Parsons (1932) reported on sixty-four Gentoo penguin embryos (*P. papua*) and fourteen Ringed penguin embryos (*P. antarctica*). His series of stages extended from a small 3.8 mm. Gentoo penguin embryo to a large Ring penguin embryo on the point of hatching and measuring 150 mm. from beak to tail. The earlier stages were defined in terms of the *Normentafel* of Keibel & Abraham for the chick.

Parsons (1934) also reported on thirteen Adélie penguin embryos (*P. adelia*), the youngest corresponding to a chick embryo of five days' incubation and the oldest to a chick embryo of about seventeen days' incubation. He paid particular attention to the pterylosis, the brain, the lungs and air sacs, the heart, the cartilaginous skeleton and the tail. He found that the skin is very scale-like, the feathers are of a primitive type and the thymus gland is disproportionately large at the time when feather filaments first make their appearance. The pineal body is developed to an exceptional degree in the early stages, and at one stage the heart has a transient reptilian flexure. Small papillae make a transitory appearance on the dorsal aspect of the lung and these are considered to be incipient air sacs. The primitive character of the tail persists.

Parsons stressed the fact that very early on in development the whole body of the embryo is that of a

creature specially adapted for an aquatic habit of life. He claimed that embryology supported the views of Kerr (1919) and Lowe (1933), that the avian stock arose from aquatic fish-eating reptiles and that penguins were probably an early offshoot of them that never succeeded in getting into the free air.

In 1952 Krabbe published his *Studies on the Morphogenesis of the Brain in Birds*. The brain of *Spheniscus* is among those described in this comparative work. Only three stages (15 mm., 26 mm., 35.5 mm.) of an unspecified type of *Spheniscus* are described but special note is made of the following facts:

The primordial brain is comparatively large and the rhinencephalon seems to develop later but, on the other hand, is comparatively large at a later stage, whilst stratification is less developed in *Spheniscus* than in the other birds examined. A paraphysis-like body is present during development and the epiphysis is well developed but not to such a degree as in *Melopsittacus* which shows a development which in certain particulars is comparable to the parietal eye of saurians. Krabbe considers that these special features in *Spheniscus* possibly confirm the conception that *Spheniscus* and *Dromicius* are the most primitive birds.

MATERIAL, METHOD OF COLLECTION AND INVESTIGATION OF THE PRESENT SERIES OF EMBRYOS

BETWEEN the end of the first week in June and the end of the first week in August, 1949, Stonehouse (1952, 1953) collected a series of Emperor penguin embryos from a rookery on the Dion Islets, fifty miles from one of the bases of the Falkland Islands Dependencies Survey. The blastoderms and embryos were dissected from the yolk and fixed in Bouin's fixative within a short time of collection. After fixation the specimens were transferred to 80% alcohol and placed in glass tubes which were corked and then sealed with paraffin wax. In this state they were delivered to this Department in early 1952.

Specimens E.526, E.527 and E.531 collected respectively on the 8th June, the 9th June and the 11th June consisted of yolky masses which were sectioned serially but revealed no embryonic tissue. All the remaining sixteen embryos (E.525, E.528, E.529, E.532, E.533, E.534, E.535, E.536, E.537, E.538, E.539, E.540, E.541, E.544, E.545) with one exception (E.530) are in an excellent state of preservation. This is due in no small measure to the skill and enthusiasm of Stonehouse, who collected and fixed these specimens in very difficult circumstances in the middle of the Antarctic winter. It should be noted further that the standard of preservation is so good that microscopical details are clearer in these preparations than is usually the case with freshly fixed chick or duck embryonic material. The one embryo that is in a poor state of preservation was frozen before fixation.

The nine smallest specimens which were younger than those brought back by Wilson have been sectioned serially at 10μ and stained with haematoxylin and eosin. The number of somites has been confirmed by examination of the serial sections and all measurements were taken after fixation.

From what has been written in the foregoing sections of this paper it will be seen that Waterston and Geddes compared their material with specially incubated duck embryos. Cossar Ewart compared his with Chinese Goose embryos, while Parsons compared his early embryos with the normal stages of development in the chick but this *Normentafel* only deals with the first half of the incubation period.

This lack of standardisation has led the author to compare his series with the latest data available on the normal stages of development in the chick for the whole incubation period. These data are to be found in *Lillie's Development of the Chick* revised in 1952 by Hamilton.

DESCRIPTION OF THE EMBRYOS OF THE PRESENT SERIES

SPECIMEN E.532 was collected on 11th June, 1949, and corresponds to a chick embryo of 12-13 hours of incubation (Stage 3). This specimen consisted of a blastoderm which appeared to consist of an amorphous, flattened mass of yolk in the centre of which an oval area of about 2-3 mm. at its greatest diameter was outlined vaguely. Although this "embryonic shield" was not distinct enough to be photographed, it was used as a guide when planning the plane of section of the blastoderm.

The sectioned material revealed a presomite embryo with an already well-formed primitive streak

which extends a quarter of the way along the germinal disc. There is no indication of a primitive pit or a Hensen's node and as the blastoderm is folded along the long axis of the germinal disc it is difficult to ascertain whether the primitive groove is forming. There is, however, a well-developed subgerminal cavity and the relative arrangements of ectoderm, mesoderm and endoderm present the usual avian characteristics (Plate V, Figs 2 and 3).

SPECIMEN E.528 was collected on 9th June, 1949, and corresponds to a chick embryo of 51–56 hours of incubation (Stage 16). The greatest length across the curved embryo was 8 mm., while the total length was 11.7 mm. This specimen, which was sectioned transversely, is an embryo of twenty-seven somites (Plate I, Fig. 1).

External Form

The anterior amniotic fold extends back to the twenty-fourth somite, and the posterior fold of the amnion has just made its appearance. There are well-marked cranial and cervical flexures and the head is rotated to the right in such a way that its left surface is resting on the anterior part of the blastoderm. The thoracic region of this embryo shows a distinct ventral curvature. At this stage it is already evident that the head region is relatively smaller and the flexures less pronounced than in chick embryos at a corresponding stage of development (Plate I, Fig. 1). The lens vesicle, optic cup and otic pit form readily recognisable landmarks on the surface of the embryo. The heart tube forms a simple loop. The primitive streak is reduced to an area a quarter of a millimeter long at the caudal end of the embryo, and on histological examination four kinds of tissue are seen to be differentiating from the cranial end of the streak (Plate VI).

1. The neural tube.
2. The neurenteric canal.
3. The notochord.
4. Paraxial mesoderm.

The Nervous System and Organs of Special Sense

The three primary brain vesicles are established. The infundibulum is a shallow depression in the floor of the forebrain and the roof of the hindbrain is thinning out. The neural tube is closed completely but is being added to at its caudal extremity from a solid cord of cells in the tail bud which is being hollowed out to produce two lumina joining each other cranially (Plate VI).

No nerves are as yet established. The optic cup is shallow and the stalk is in wide communication with the forebrain vesicle. The choroidal fissure is wide open but the lens vesicle is in the process of being closed off from the surface. The otic pit still forms a cup-shaped structure. Opposite the unsegmented paraxial mesoderm and the caudal somites, the neural crest cells are confined to the vicinity of the dorsal portion of the neural tube. At the level of the twenty-second somite the neural crest cells are beginning to migrate ventro-laterally and opposite the cranial somites which are well differentiated into dermatome, myotome and sclerotome, the cells are seen to be streaming out in the direction of the somites and the dorsal aortae.

In the head region the epibranchial placodes in relation to the first visceral furrow are particularly well developed (Plate VII, Fig 1). These placodes are contributing cells to the neural crest tissue of the trigeminal and acoustico-facial complexes. The placode in relation to the second visceral furrow is less well developed.

Alimentary Canal

There is a well-developed stomatodaeum from the roof of which Rathke's pouch is growing to meet the infundibulum. The oral plate is in the process of breaking down and the notochord ends in an indistinct layer of cells in the roof of the pharynx—the prochordal plate.

The cellular partition between the first ectodermal furrow and the first visceral pouch is breaking down to form the first visceral cleft. The second visceral cleft has not yet appeared, the ectoderm of the furrow being in contact with the endoderm of the pouch. The first indications of the third furrow and pouch are present.

The rudiment of the thyroid gland has begun to develop and forms a saucer-shaped depression in the

floor of the pharynx in front of the ventral ends of the second visceral pouches; the laryngo-tracheal groove is forming in the floor of the post-branchial portion of the pharynx.

The anterior intestinal portal is opposite the sixth somite and the two liver buds are developing as out-growths from this region. The cephalic or superior bud is situated immediately above the fork of the omphalo-mesenteric veins at the caudal end of the sinus venosus, while the caudal or inferior bud is below the fork. The allantois has not yet developed and the tail fold has not formed.

The Cardiovascular System

The heart consists of a single tube with well-marked sinu-atrial and bulbo-ventricular loops. The bulbus arteriosus shows a slight independent curve to the left of the embryo. This curve is more marked than in a chick embryo at a corresponding stage. The wall of the bulbo-ventricular loop contains much cardiac jelly but there is no indication of septation of the heart. There are well-developed arteries in the first two arches and the third arch artery has made its appearance. The dorsal aortae fuse at the level of the fourth somite and the fused vessel divides at the level of the twentieth somite, giving rise to left and right dorsal aortae and omphalo-mesenteric arteries. The anterior and posterior cardinal veins and the ducts of Cuvier are well developed as are the omphalo-mesenteric veins. The omphalo-mesenteric arteries and veins are widely separated as in other species of penguin.

Head Mesoderm

Well-developed premandibular head cavities are present in close proximity to the developing eyes (Plate VII, Fig. 2). They are joined across the midline by a cord of mesenchymal cells passing dorsal to Rathke's pouch. The medial portion of the cavity is divided into compartments by epithelial bridges. The mandibular cavity is already obliterated in this specimen. Well-formed condensations of mesenchyme are present in the first and second arches and are beginning to form in the third arch.

Arrangement of Paraxial Mesoderm and the Urogenital System

As stated in a previous section, mesoderm is differentiating from the tail bud to form the caudal part of the embryo. The most caudal portion of the paraxial mesoderm is unsegmented but the lateral portion of it is split to form the caudal portion of the coelom. Cranial to this unsegmented paraxial mesoderm two or three pairs of somites are present which consist of undifferentiated masses of mesodermal cells connected to an intermediate cell mass. This intermediate cell mass is in turn connected to the lateral plate mesoderm which is split by the coelom. As the somites are traced cranially from this level they are seen to become more clearly differentiated into dermatome, myotome and sclerotome.

Opposite the sixth somite, the intermediate cell mass contains poorly defined collections of cells—the remnants of pronephric tubules. Opposite the sixth to the tenth somite poorly developed nephrostomes are present and the pronephric (mesonephric) duct starts at the level of the ninth pair of somites. The more caudal of these nephrostomes communicate, by way of a pronephric tubule, with the pronephric (mesonephric) duct. External glomeruli were found opposite the seventh, eighth and ninth somites. Opposite the tenth to the fourteenth somite well-developed but non-ciliated nephrostomes are found, the lumen of which communicate by way of a pronephric tubule with the lumen of the pronephric (mesonephric) duct (Plate VIII, Fig. 3). Opposite the fifteenth to the twentieth somite the mesonephric tubules are hollow and communicate with the pronephric (mesonephric) duct, but caudal to the twentieth somite the tubules have not differentiated beyond forming solid cords of cells. Caudal to the twenty-fourth somite the intermediate cell mass is undifferentiated. The pronephric (mesonephric) duct ends in a solid cord of cells at the level of the unsegmented mesoderm.

It is unlikely that this pronephros is functional at this stage as the glomeruli are situated at levels where the tubules are degenerating.

Opposite the twentieth somite and somites caudal to it, several primary germ cells were identified in the omphalopleure near the widely open gut.

SPECIMEN E.525 was collected on 7th June, 1949, and corresponds to a chick embryo of 3 to 3½ days of incubation (Stage 19). The embryo has thirty-eight somites and measures 24 mm. from the maxillary process to the tail; the greatest length across the curved embryo is 11.5 mm. (Plate I, Fig. 2).

External Form

The cranial and caudal amniotic folds have met to form a sero-amniotic connection at the level of the thirty-first somite. The embryo is lying on its left side and there is no curvature in the thoracic region; the cranial and cervical flexures are, however, more distinct than in the last specimen. The limb buds form sausage-shaped ridges on the side of the embryo.

The relative smallness of the head region and the less marked curvatures as compared with a chick embryo again form striking features. To these differences may be added the relative length of the tail.

The olfactory pits are now visible in addition to the eye and otic vesicle. The maxillary process has just appeared and the hyoid arch has begun to project over the surface and overlap the third arch. The foregut has lengthened considerably since the last stage, the anterior intestinal portal being situated opposite the fourteenth somite. The midgut is wide open inferiorly and extends to the posterior intestinal portal opposite the thirtieth somite; the formation of a well-developed tail-fold has led to the establishment of the tail gut.

At the level of the thirty-second somite there is a well-formed allantoic diverticulum from the hindgut. It is sausage-shaped and is curled upon itself.

The embryo has been sectioned in the transverse plane and examination of the histological material has revealed the following features:

The Nervous System and Organs of Special Sense

There is a definite telencephalic outgrowth from the forebrain vesicle resulting in the establishment of the velum transversum. The olfactory placodes now lie in the roof of olfactory pits. The lenses form closed vesicles which have lost all connection with the surface epithelium. The choroidal fissures of the optic vesicle are wide open and the lumen of the optic stalk is still in communication with the cavity of the diencephalon. The rudiment of the posterior lobe of the pituitary forms a marked depression in the floor of the diencephalon and Rathke's pouch is a well-developed outgrowth in wide communication with the roof of the stomatodaeum. The epiphysis is a simple outpouching of the diencephalic roof at this stage. The midbrain and the hindbrain show evidence of neuromeric segmentation, there being three segments in the midbrain and five in the hindbrain. The isthmus separating these two primary brain vesicles is well defined. The oculomotor nerve and the trigeminal and acoustico-facial complexes are very evident in the sections of the head region. The epibranchial placodes are still contributing cells to the neural crest tissue of these two complexes; epibranchial placodes are now evident at the dorsal angle of the second and third visceral furrows and these placodes are budding off cells to the subjacent neural crest tissue.

The otic vesicle is connected to the surface ectoderm in the region of the incipient endolymphatic duct. This structure has begun to develop from the supero-medial portion of the otic vesicle, which now forms a relatively thin-walled cyst.

The ventral and dorsal roots of the spinal nerves are well developed as far caudal as the twentieth somite. Well-defined collections of neural crest cells in relation to the dorsal aorta are also found opposite these cranial somites. Between the twentieth and twenty-fourth somites these nervous and neural crest structures are less clearly defined. Caudal to the twenty-fourth somite no spinal nerves are yet established and the neural crest tissue is seen to stream out antero-laterally from the region of the roof plate of the neural tube.

Alimentary System

In the roof of the pharynx there is a poorly defined prochordal plate in which the notochord terminates. The rudiment of the thyroid gland forms a solid cellular outgrowth from the floor of the pharynx opposite the ventral end of the second visceral pouches. Extending out laterally from the walls of the pharynx there are five endodermal visceral pouches and there are ectodermal furrows in relation to the four cranial ones. Only the first two pouches communicate by way of a cleft with the furrows in relation to them. In the region of junction between the foregut and midgut, the superior (left) hepatic diverticulum is well developed and budding off hepatic cells into the septum transversum. The inferior (right) hepatic diverticulum lies caudal to the ductus venosus opposite the twelfth somite and is well developed, but not quite so well as the superior diverticulum. The dorsal pancreatic outgrowth is an outpouching of the gut at the level of the twelfth somite, and the left anterior pancreatic bud has just appeared opposite the thirteenth somite.

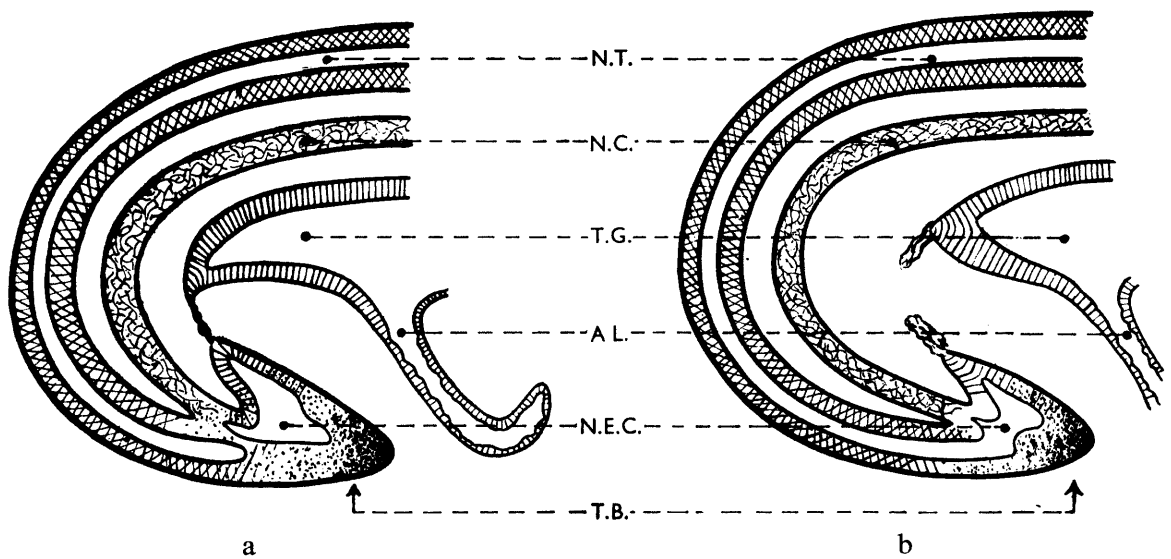


FIGURE 1

Diagram to show the arrangement of the structure in the tail region of (a) Specimen E.525 and (b) Specimen E.529

N.T. = Neural tube
A.L. = Allantois

N.C. = Notochord
N.E.C. = Neurenteric canal

T.G. = Tail gut
T.B. = Tail bud

The tail gut is separated from the surface of the embryo by a thick anal plate and ends in an ill-defined cord of cells which can be traced round in the tail to a dilated vesicle forming from the tailbud. This vesicle represents a persisting neurenteric canal, which, however, has lost its connection with the neural tube (Text Fig. 1a).

The Cardiovascular System

There are well-marked sinu-atrial and atrio-ventricular constrictions in the cardiac tube and cardiac muscle is differentiating in the bulbo-ventricular loop. The independent curvature of the bulbus arteriosus is now more pronounced and calls to mind the N-flexure described by Parsons in other species of penguin.

All the arch arteries are present and the dorsal aortae fuse opposite the fourth somite. The omphalo-mesenteric arteries are given off opposite the twenty-second somite and the aorta bifurcates at the level of the thirtieth.

The Respiratory System

The laryngo-tracheal groove, which is forming from the floor of the post-branchial portion of the pharynx, is deep and leads to a lung bud which is in the process of bifurcating, the caudal extremity of this bud reaching the tenth somite.

Well-developed pneumato-enteric recesses, and pleuro-pericardial and pleuro-peritoneal canals form a notable feature of this region of the embryo.

Mesoderm and Urogenital System

The premandibular cavities are voluminous and show duplication, the medial compartment being the smaller. A poorly defined cord of mesenchymal cells still connects the two cavities across the midline (Plate VII, Fig. 3).

All somites as far caudal as the thirty-third are well differentiated into their constituent parts. There is still a small tail bud giving rise to undifferentiated masses of paraxial mesoderm, neural tube, notochord and neurenteric canal (Text Fig. 1a).

External glomeruli, well-developed nephrostomes, degenerating portions of pronephric (mesonephric) duct and degenerating tubules are to be found opposite the ninth and tenth somites. Opposite the eleventh

somite the nephrostomes communicate with the tubules, but the pronephric (mesonephric) duct does not become a continuous channel till the twelfth somite is reached. Opposite the thirteenth somite there are well-developed external glomeruli and nephrostomes communicating with tubules which open into the pronephric (mesonephric) duct (Plate VIII, Fig. 4). The cells lining the nephrostomes have irregular borders so that it is difficult to be certain about the presence or absence of cilia. The first internal glomeruli are found at the level of the fourteenth somite, some fused external and internal glomeruli being seen at this level. Caudal to the level of the fourteenth somite all glomeruli are of the "internal" variety, the tubules are dilated, there are no nephrostomes and the outline in transverse section of the pronephric (mesonephric) duct becomes triangular caudal to the eighteenth somite. Caudal to the level of the twenty-fifth somite the tubules consist of solid cords of cells, and opposite the twenty-ninth somite the nephrostome is undifferentiated and forms a condensation in the region of the intermediate cell mass, which extends as far caudal as the thirty-second somite. The pronephric (mesonephric) ducts end as solid cords of cells which reach the wall of the cloaca opposite the thirty-third somite.

In this embryo primary germ cells are located at the root of the mesentery and extend further laterally round the coelomic bay. Primary germ cells are only found caudal to the twentieth somite.

SPECIMEN E.529 was collected on 10th June, 1949, and corresponds to a chick embryo of $3\frac{1}{2}$ to 4 days of incubation (Stage 22). The greatest length across the curved embryo was 10.6 mm., the total length being 35 mm. from the maxillary process to the tip of the tail. The embryo possesses forty-eight somites and was sectioned in the transverse plane (Plate I, Fig. 3).

External form

Although the embryo is now curved by a single convexity it still resembles a reptilian embryo far more than does a chick embryo at this stage. In the head, which is still relatively small, the most striking features are the nasal pits, the eyes, the maxillary process, the mandibular arch and the very large hyoid arch which overlaps the third arch. There is an ill-defined fourth arch and a fourth furrow is discernible. The otic vesicle is no longer identifiable on the surface. The limb-buds form obvious appendages which are developed to a degree comparable to those of a chick at this stage. The somites extend to the tip of the tail, which has curled round to the ventral aspect of the embryo.

Nervous System and Organs of Special Sense

The telencephalon now presents two developing hemispheres, the cavity of which is in wide communication with the cavity of the diencephalon. The infundibulum is still only a depression in the floor of the diencephalon and Rathke's pouch is in communication with the mouth region by a narrowing tube. This rudiment of the pituitary gland is budding off anterior and lateral outgrowths. The epiphysis is a flask-shaped structure, the cavity of which is in communication with the third ventricle. The optic lobes form prominent structures in the roof of the midbrain. The olfactory nerve fibres are established and connect the sunken olfactory placode to the under surface of the telencephalic hemispheres.

In the developing eye the lens vesicle has almost lost its lumen because of the great increase in length of the posterior lens cells. The retina is divided into a pars nervosa and a pars caeca, the choroid fissure is reduced to a slit and the optic stalk still has a lumen.

The endolymphatic diverticulum is a drawn-out tubular structure only just in contact with the surface at the junction of its distal and middle third. Nervous connections have been established between the acoustic complex and the brain stem on the one hand and the otic vesicle on the other. The trigeminal and facial nerves and post-otic complexes have also established their central and peripheral connections. The caudal end of the neural tube is dilated and in this specimen still communicates with the notochordal canal and the still persisting neurenteric canal (Text Fig. 1b).

Alimentary System

The prochordal plate is no longer identifiable in the roof of the pharynx and the notochord ends cranially in a mass of undifferentiated mesenchyme caudal to Rathke's pouch. The visceral pouches are well developed, including the fifth, but only the first three communicate by way of clefts which the corresponding

visceral furrow. The epithelium is thickened in the dorsal portion of the second pouch and there are definite cellular outgrowths from the dorsal and ventral portions of the third and fourth pouches. There appears to be a proliferation on the ventro-medial aspect of the fifth. The tuberculum impar forms a prominent elevation in the floor of the pharynx in front of the thyroid bud, which is still connected to the epithelium of the floor of the pharynx by an epithelial cord; the bud is now bilobed.

The anterior intestinal portal is situated opposite the sixteenth somite, whereas the posterior intestinal portal is opposite the thirty-third somite. The two hepatic lobes and ducts are now established and the gall bladder is a small evagination from the lower duct. The posterior pancreatic outgrowth is budding off cells into the mesentery, but the right and left anterior pancreatic buds are still simple outpouchings from the gut. The allantois is well developed and is spreading out into the extra-embryonic coelom. The anlage of the bursa fabricii has made its appearance and is beginning to show the characteristic vacuolation described in other birds. The tail gut as such has retrogressed but a cord of cells is attached to the caudal end of the gut. A similar cord extends forwards from the neurenteric canal but the two cords no longer form a continuous connection between the gut and the neurenteric canal (Text Fig. 1b).

Respiratory System

The laryngo-tracheal groove is closing up to form a tube. Apart from this the degree of development has not advanced much since the last stage.

Cardiovascular System

The bulbus arteriosus now shows a very marked independent curve which is very similar to the transient N-flexure of crocodilian embryos. The ventricular muscle is well developed now and the atrium is being divided by an as yet incomplete septum. The venous valves form a well-marked septum spurium and muscle is beginning to differentiate in the atrial portion of the heart wall. The first and second arch arteries appear to have retrogressed completely and the third arch artery is the first one to be conspicuous.

Head Cavities

The premandibular head cavities are still voluminous but not duplicated and are no longer connected across the midbrain by a bridge of mesenchymal cells (Plate VII, Fig. 4).

Urogenital System

External glomeruli are still present at the cranial end of the mesonephros opposite the fifteenth somite. Caudal to this level there is a sudden transition to internal glomeruli. The nephrostomes which are present in relation to the external glomeruli are not ciliated, and although they approach the tubules very closely they have not actually been observed to communicate with them, the walls of the tubule coming together at the point of contact with the coelomic funnel.

The mesonephric ridge is now being formed and at the caudal end of it tubules without glomeruli and cords of cells not yet differentiated into tubules are present. The caudal end of the mesonephric duct is very dilated and the ureteric bud has just appeared. The germinal epithelium is differentiating and forms a thickening on the medial aspect of the mesonephric ridge. Primary germ cells are found caudal to the twentieth somite in the mesothelium of the angle between the root of the mesentery and the mesonephric ridge. Some of these primary germ cells have reached the germinal epithelium but none are found at the caudal end of this tissue.

SPECIMEN E.530 was collected on the 10th June, 1949. The greatest length across the curved embryo was 10.6 mm., the total length being 35 mm. from the maxillary process to the tail.

This embryo is in a poor state of preservation as it was frozen before fixation. It appears to have reached a stage of development similar to specimen E.529, collected on the same day. The specimen still has its allantois attached to it and this, a large bladder-like structure, is in relation to the caudal end of the embryo (Plate II, Fig. 1). The specimen was sectioned serially in the sagittal plane but fixation was not good enough to permit the making of any useful observations.

SPECIMEN E.536 was collected on 18th June, 1949, and corresponds to a chick embryo of 5 days of incubation (Stage 26). The greatest length across the curved embryo was 13 mm., the total length being 47 mm. from the maxillary process to the tip of the tail (Plate II, Fig. 2).

External Form

This specimen is an embryo in which there is a very prominent hemisphere on the surface of the back of the head (Plate II, Fig. 2). This prominence is due to the high degree of development of the underlying optic lobes. The eyes are very large, prominent and surrounded by a sulcus, no lids having developed yet. The maxillary process is now prominent, the first and second arches are the only ones visible on the surface, and they present a series of protuberances, three being present on each arch. The limbs are subdivided into three segments arranged at a slight angle to one another. The external appearance is now much more like that of a chick embryo at a corresponding stage of development than was the case in the previous stages. This specimen was sectioned in the transverse plane.

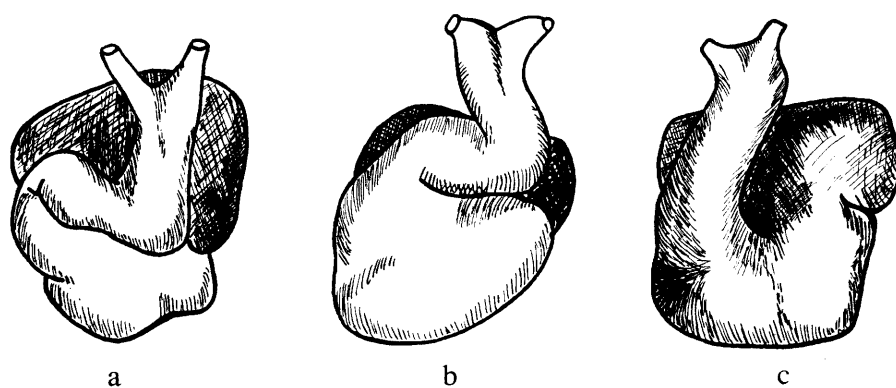


FIGURE 2

- (a) The heart of a penguin Embryo (Stage 24) (after Parsons)
 (b) The heart of a young crocodilian Embryo (after Hochstetter and Graham Kerr)
 (c) The heart of a chick Embryo (Stage 24) (after Patten)

The Nervous System

The neural tube and brain tissue are differentiated into ependymal, mantle and marginal zones. The cerebral hemispheres are considerably larger than in the last specimen and a basal thickened striatal portion is now evident. The pineal body forms a cellular bud connected to the roof of the diencephalon by a short stalk. The optic lobes are highly developed as mentioned above. There are well-marked optic recesses but the optic stalks have now become solid. The infundibulum is a funnel-shaped structure and Rathke's pouch has given rise to a much branched system of tubules. The pituitary duct (cranio-pharyngeal canal) is very drawn out though still patent. The choroid plexuses are beginning to form in the lateral, third and fourth ventricles. Neuromeres are no longer identifiable in the midbrain or the hindbrain and the cerebellum forms a couple of thickenings in the alar lamina of the metencephalon. The caudal end of the neural tube in the tail appears to be retrogressing and it is dilated at its caudal extremity. The neurenteric canal has been obliterated.

The olfactory pits are very deep and the eye is surrounded by a condensation of mesenchyme for the sclera and cornea. The retina is more clearly divided into its constituent parts than was the case in the previous stage. It is pigmented and the choroid fissure is now slit-like, with a developing hyaloid artery in it. The lens is well differentiated, having well-defined anterior lens epithelium and posterior lens fibres.

The endolymphatic duct has lost its connection to the surface epithelium and joins the otic vesicle at its upper and medial portion. The cochlear duct forms a ventro-medial outgrowth and outpouchings are present for the semi-circular canals.

Alimentary System and Derivatives

The tubo-tympanic canal forms a long, narrow recess extending from the dorsal portion of the lateral wall of the pharynx. The lateral extremity of this recess is in relation to the condensations for Meckel's cartilage and the palato-quadrate cartilage. The epithelium of the roof of this canal is thickened at its lateral end. The second pouch is still present, the dorsal portion giving rise to a proliferation of closely packed cells with dark staining nuclei. This proliferation represents the thymus II of *Selachia* and *Anura* (Plate VIII, Fig. 1). The remains of the third visceral pouch are still recognisable. The thyroid, parathyroid and thymus rudiments, however, are no longer connected to the pharynx and are to be found in the neck.

In the floor of the pharynx there is a well-defined tuberculum impar for the anterior portion of the tongue, and two swellings situated on either side of the midline posterior to the tuberculum for the pars copularis linguae. The laryngo-tracheal groove is closed and a solid plate of cells separates the trachea from the pharynx. Caudal to the laryngo-tracheal groove the lumen of the foregut is obliterated till the caudal portion of the neck is reached.

The midgut forms a looped tubular structure, loops of which have herniated into the extra-embryonic coelom. At the caudal end of the gut there is a well-developed anal plate and the anlage for the bursa fabricii presents appearances typical for a bird at this stage of development.

The pancreas forms a much branched system of ducts in the mesentery, and the anlage for the spleen is now present.

The Respiratory System

The solid laryngo-tracheal plate leads to a patent trachea. In the lungs the mesobronchi and entobronchi are now established.

Cardiovascular System

The atria and ventricles are well defined and contain well-differentiated muscle in their walls. Both horns of the sinus venosus are still prominent and well-developed venous valves project into the cavity of the right atrium. The free border of the interatrial septum has fused with the atrio-ventricular endocardial cushions and secondary perforations have been established in the septum. The interventricular septum is well developed but still has a concave free border, the dorsal extremity of which has reached the right side of the fused atrio-ventricular endocardial cushions. The truncus arteriosus and the distal portion of the bulbus arteriosus are completely separated by the septum aortico-pulmonale; in the proximal portion of the bulbus arteriosus, however, the bulbar ridges have not yet fused. The independent flexure of the bulbus arteriosus noted in the previous stages is still present.

Head Cavities

Head cavities are no longer present but condensations for the external ocular musculature are now identifiable in the region previously occupied by these cavities.

The Urogenital System

At the cranial end of the mesonephros, which is highly developed at this stage, some fused external glomeruli are still to be seen. Nephrostomes are, however, no longer found in relation to these glomeruli.

The gonad is now recognisable as a thickened layer of epithelium on the medial aspect of the mesonephric ridge. This epithelium consists of columnar cells from which epithelial downgrowths into the subjacent stroma are forming. Primary germ cells are rather scanty in both gonads of this specimen, especially so on the right side. The oviducts are present as infoldings of mesothelium at the cranial end of the mesonephric ridge. The ureteric bud forms a long tube, the cranial end of which communicates with a set of primary branches in a metanephric condensation of mesoderm.

Other Features

There are well-formed condensations for the long bones and the centra and neural arches of vertebrae; also for Meckel's cartilage, the palato-quadrate cartilages, and, to some extent, the hyoid cartilage. These condensations have reached the stage of differentiating to form precartilage. There are also less marked condensations for the cartilaginous bones of the skull.

SPECIMEN E.534 was collected on 14th June, 1949, and corresponds to a chick embryo of 5 to 5½ days of incubation (Stage 27). The greatest length across the curved embryo was 15 mm., the total length being 53 mm. from the maxillary process to the tip of the tail (Plate II, Fig. 3).

The specimen consists of an embryo which has reached a stage of incubation not much more advanced than the one reached by the previous specimen, E.536, and the external appearance presents few new features. The embryo possesses a recognisable beak, the mandibular process has grown forward and broadened ventrally and the external auditory meatus is now recognisable as such. The limbs are slightly longer than in the previous specimen.

The embryo was sectioned in the transverse plane and the main differences in internal structures when compared with the previous stage are as follows:

The pineal has a longer stalk. The lining of the cochlear duct is thrown into a fold, running along the inferior aspect of the canal, and the tympanic cavity is further expanded. The cellular proliferation on the dorsal aspect of the second pouch is larger, and the parathyroid glands are closer to the thyroid, which is situated just cranial to the bifurcation of the trachea. On the left side a post-branchial body was identified medial to the lower parathyroid. The pancreas contains small islets of cells which are more eosinophilic than the cells of the surrounding branching tubules. The bronchial tree has differentiated further to give rise to the first two ectobronchi and the mesobronchus terminates as an expansion—the incipient abdominal air-sac. Atrio-ventricular valves are beginning to form in the heart. The gonads of this embryo contain many more primary germ cells and these are more numerous on the left side. The groove for the oviduct extends further along the external surface of the mesonephros. No external glomeruli were seen in this specimen. All the condensations for cartilage described in the previous specimen now have the appearance of precartilage.

Specimens E.537 and E.535 share so many features that it is convenient to describe them together.

SPECIMEN E.537 was collected on 22nd June, 1949 and corresponds to a chick embryo of 6 to 6½ days of incubation (Stage 29). The greatest length across the curved embryo was 18.5 mm., the total length being 59 mm. from the tip of the beak to the tip of the tail (Plate II, Fig. 4).

SPECIMEN E.535 was collected on 17th June, 1949 and corresponds to a chick embryo of 6½ to 7 days of incubation (Stage 30). The greatest length across the curved embryo was 18.5 mm., the total length being 63 mm. from the tip of the beak to the tip of the tail (Plate III, Fig. 1).

External Form

The optic lobes still cause a bulge on the surface at the back of the head, the mandibular and hyoid arches have fused and there is a distinct external auditory meatus in relation to their caudal extremity. The beak forms a distinct outgrowth, but no egg-tooth has yet appeared. The eye-lids and nictitating membrane are beginning to develop and in E.535 the first scleral papilla is present. The wing is bent to form an elbow but the leg is almost straight. There are no feather papillae.

The head of Specimen E.537 was sectioned in the coronal plane but the body and limbs were sectioned in the sagittal plane. Specimen E.535 was sectioned in the sagittal plane.

The Nervous System and Organs of Special Sense

Mesenchyme forms a condensation which can be recognised as the meningeal coverings of the brain. An olfactory lobe is present on the under surface of the cerebral hemispheres in which the striatum forms a well-developed thickening. The hippocampus also forms a distinct swelling on the medial aspect of the upper part of the hemisphere. The epiphysis is a pedunculated swelling with a very long stalk, and in front of it a definite paraphysial outgrowth is identifiable in relation to the parencephalon. The infundibulum is funnel shaped and the cavity of the third ventricle extends into the root of the optic stalk. The optic lobes are relatively very large and the cerebellar swellings bulge into the fourth ventricle.

The spinal cord terminates caudally as a thin-walled expansion to which a cord of cells is attached ventrally, extending to the caudal end of the notochord and into the mesenchyme ventral to this structure (Text Fig. 3). The thickened olfactory epithelium is situated in the roof of a nasal cavity which is now

completely separated from the buccal cavity, external and internal nares and a palate having been established.

In the eye, condensations of mesenchyme for the cornea and sclera are well differentiated. Anterior and posterior chambers are identifiable, the choroidal fissure is still present and the hyaloid artery is well developed. The pars nervosa retinae exhibits stratification of the nerve cells. The eye-lids have made their appearance, the development of the lower lid being further advanced than that of the upper one. The nictitating membrane is beginning to develop in the specimen E.535.

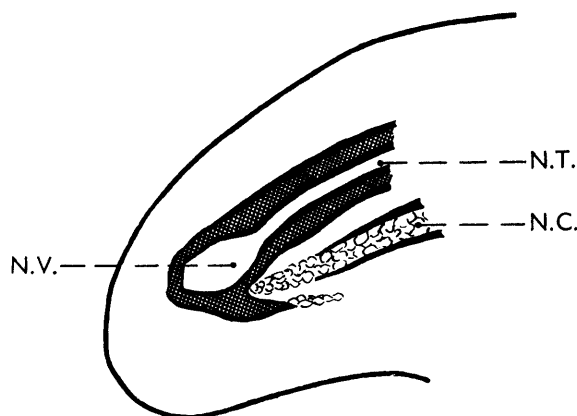


FIGURE 3

Diagram to show the relations of the dilated vesicle at the caudal end of the neural tube of Specimen E.535

N.T. = Neural tube N.C. = Notochord N.V. = Dilated neural vesicle

The otocyst forms a prominent feature of the base of the skull, and it is enclosed in cartilage. A perilymphatic space has been established in relation to all the parts of the inner ear. The semi-circular canals are well developed and the cristae and maculae are identifiable as localised thickenings of the epithelium lining the membranous labyrinth. The folding and thickening of the ventral part of the cochlea and lagena, identified in the previous stages, are more marked in these two specimens. The endolymphatic sac is still near the surface ectoderm but no longer connected to it. The tubo-tympanic recess is clearly subdivided into a pharyngo-tympanic tube and a tympanic cavity in relation to the cartilaginous precursors of the auditory ossicles.

Alimentary System

The beak has begun to develop and an epithelial ingrowth has made its appearance in the lower jaw. This is the "dental lamina" and is no more developed than in a chick embryo of a corresponding age. The pituitary has a definite cleft in the pars anterior and still communicates with the pharynx by a cranio-pharyngeal canal. The state of the first pharyngeal pouch has been described with the ear and the thickening in the dorsal portion of the second pouch has retrogressed so as to be hardly discernible. The parathyroids lie closer to the thyroid than they did in the previous stages, and no post-branchial bodies were seen. The upper part of the oesophagus and trachea still has no lumen. The constituent parts of the foregut are now identifiable. The gall bladder is a readily identifiable thick-walled structure and the ventral portion of the pancreas has rotated so as to lie in apposition to the posterior portion, but the separate ducts persist. At the tail end of the gut the bursa of Fabricius is beginning to have a glandular appearance.

Respiratory System

As already stated the cranial portion of the laryngo-tracheal tube is obliterated. Six ectobronchi are now present and their dilated terminations form papillae on the dorsal surface of the lung (Plate VIII, Fig. 2). These are comparable to the incipient dorsal air-sacs described by Parsons (1932) in other species of penguin. The abdominal air-sacs are well developed and an outpouching for the interclavicular and anterior thoracic air-sacs has appeared.

Cardiovascular System

The interventricular septum is complete and the pulmonary and aortic valves have developed.

Urogenital System

The mesonephros is highly developed at this stage and some external glomeruli are found at the cranial pole of the mesonephros. These glomeruli are associated with degenerate tubules, but no nephrostomes are found. A couple of the external glomeruli were present on each side in the Specimen E.537; five were found on each side in Specimen E.535. The paramesonephric ducts are still elongating caudally by a process of infolding of mesothelium at the lower end of the mesonephric ridge.

Well-marked rete cords and incipient sex cords were present in the gonads. The author was not able to determine the sex of these embryos from the histological picture presented at this stage.

In Specimen E.537 the ureter still joins the mesonephric duct but in the Specimen E.535 the ureter opens into the cloaca by a separate opening. Cranially the ureter divides into primary, secondary and tertiary branches. The metanephric condensation is well defined and now forms an elongated structure.

The remaining specimens were not sectioned as they consisted of embryos which had reached stages of development comparable to those described by previous authors.

SPECIMEN E.533 was collected on 12th June, 1949 and corresponds to a chick embryo of 10 days of incubation (Stage 36). The greatest length across the curved embryo was 32 mm., the total length being 85 mm. from the tip of the beak to the tip of the tail (Plate III, Fig. 2).

This specimen consists of an embryo, the external form of which is recognisable as that of a penguin. The embryo possesses a long beak on which a poorly developed egg-tooth can be made out. The mouth is partly opened and the tongue can be seen projecting freely in the floor of the mouth in relation to the proximal third of the beak. The ring of scleral papillae is complete and the lids are beginning to overlap the posterior half of the ring. The nictitating membrane extends half-way between the fused anterior margins of the lids and the scleral papillae. The external auditory meatus forms a small well-defined pit behind the caudal extremity of the mandible. The neck has greatly elongated since the previous stage and the upper limb has now assumed the appearance of a paddle, bent at the elbow and showing no surface indication of digits. The hind limb, however, is distinctly bent at the ankle and presents three large digits and with an additional small digit on the medial aspect of the foot. The claws can just be recognised. There are five rows of feather rudiments on the tail but otherwise no feather papillae are to be found on this specimen. There is a large pit on the dorsal aspect of the tail—the uropygial gland. A small genital tubercle protrudes from the anterior portion of the cloacal region.

SPECIMEN E.538 was collected on 26th June, 1949 and corresponds to a chick embryo of 11 days of incubation (Stage 37). The greatest length across the curved embryo was 35 mm., the total length being 92 mm. from the tip of the beak to the tip of the tail (Plate IV, Fig. 1).

The main differences between this embryo and the previous one, apart from size, are: The beak is relatively longer, the lids cover the scleral papillae, the nictitating membrane has reached the edge of the cornea, the neck is longer, the claws of the foot are more developed, the genital tubercle is more prominent and feather papillae are present on the back of the head and trunk, flanks and legs but not on the tarsus, and there are two rows of papillae on the post-axial border of the paddle. Fine filaments are protruding from the caudal two rows of papillae on the tail.

SPECIMEN E.541 was collected on 6th July, 1949 and corresponds to a chick embryo of 11 days of incubation (Stage 37). The greatest length across the curved embryo was 35 mm., the total length being 93 mm. from the tip of the beak to the tip of the tail (Plate III, Fig. 3).

This embryo presents the same features as Specimen E.538 except that the pterylosis is not quite so far advanced, no filaments protruding from the tail region and there being no papillae on the post axial borders of the paddle.

SPECIMEN E.540 was collected on 26th July, 1949 and corresponds to a chick embryo of 12 days of incubation (Stage 38). The greatest length across the curved embryo was 40 mm., the total length being 99 mm. from the tip of the beak to the tip of the tail (Plate III, Fig. 4).

This specimen consists of an embryo and foetal membranes. The latter are shown on Plate V, fig. 1, and require no special description. The embryo differs from the previous specimens in that the beak is longer and the egg-tooth is further developed, though a very blunt structure. The upper eye-lid covers about a third of the cornea, the lower lid just overlaps the margin and the nictitating membrane has reached the anterior edge of it. Feather papillae are also found at this stage, on the upper lid and edge of the lower lid, around the auditory meatus, on the front and back and sides of the neck, front of the chest and abdomen and four rows on the post-axial border of the paddle. The head is showing pigmentation in the region immediately above the upper eye lid.

SPECIMEN E.539 was collected on 26th June, 1949 and corresponds to a chick embryo of 12 days of incubation (Stage 38). The greatest length across the curved embryo was 41 mm., the total length being 110 mm. from the tip of the beak to the tip of the tail (Plate IV, Fig. 2).

This embryo differs from the previous one in that it is larger, the lids cover a third of the cornea each, five rows of feather papillae are present on the post-axial border of the paddle and there are two rows of feather filaments on the tail.

SPECIMENS E.544 and E.545 were collected on 1st and 2nd August, 1949 respectively. The greatest length across the curved embryo was 105 mm. in E.544 and 122 mm. in E.545. The length from the tip of the beak to the tip of the tail was 299 mm. in both specimens (Plate IV, Figs. 3 and 4).

Both chicks are hatching or newly hatched and are similar in appearance except that Specimen E.544 is still lying in its membranes. Both chicks are covered in down, have pigmented heads and have the same features as those described by previous authors in hatching chicks or nestlings.

DISCUSSION AND CONCLUSIONS

IT is evident from the examination of the history of this subject that a need exists for the description of the normal stages of development of the penguin, in some readily available species of this bird. This will enable workers in this field to work on a common basis and enable them to produce reliable comparative data.

The main features of the embryos described in this paper are given in the following table:

Serial No.	Date of collection	Corresponding stage in chick	Incubation period in chick	Greatest length across curved embryo	Total length from beak or maxillary process to tail
E.532	11.6.1949	3	12 -13 hr.	—	—
E.528	9.6.1949	16	51 -56 "	8 mm.	11.7 mm.
E.525	7.6.1949	19	3 - 3½ days	11.5 "	24 "
E.529	10.6.1949	22	3½- 4 "	10.6 "	35 "
E.530	10.6.1949	Poor state of preservation		10.4 "	34 "
E.536	18.6.1949	26	5 "	13 "	47 "
E.534	14.6.1949	27	5 - 5½ "	15 "	53 "
E.537	22.6.1949	29	6 - 6½ "	18.5 "	59 "
E.535	17.6.1949	30	6½- 7 "	18.5 "	63 "
E.533	12.6.1949	36	10 "	32 "	85 "
E.538	26.6.1949	37	11 "	35 "	92 "
E.541	6.7.1949	37	11 "	35 "	93 "
E.540	26.6.1949	38	12 "	40 "	99 "
E.539	26.6.1949	38	12 "	41 "	110 "
E.544	1.8.1949	Hatching	20 -21 "	105 "	299 "
E.545	2.8.1949	Hatching	20 -21 "	122 "	299 "

All measurements taken after fixation.

The embryos collected on 26th June and 6th July, 1949 have reached a stage of development similar to that reached by Wilson's largest specimens. This observation seems to confirm the ones made by Stonehouse (1953) on measurements of hatched chicks, by which he concludes that the chicks of the rookery at Cape Crozier were hatched about three weeks after the ones from the rookery from which the present series was obtained. Comparison of the development of the external form of the Emperor penguin with that of the chick and with that of penguins already described reveals that in the earlier embryos, the head region is relatively smaller, the neck and tail regions relatively longer, and the curvatures less well marked in penguin than in chick embryos. These features are more marked in Emperor penguin embryos and result in early penguin embryos resembling early reptilian embryos more closely than do early chick embryos.

Examination of the serially sectioned material has revealed some hitherto unknown and emphasised known primitive characters of penguin embryos.

The head cavities are well developed and persist for a comparatively long time (cf. Wedin, 1953, on the Development of Head Cavities in *Ardea cinerea*).

Sections through the tail region of the embryo E.528 present a picture remarkably reminiscent of the one on p. 52 of Vol. 2 of J. Graham Kerr's *Textbook of Embryology*. This figure (Fig. 32) is a diagrammatic representation of sections through the region of the neurenteric canal of a *Chelonia* embryo with about sixteen segments. These drawings are comparable with photographs of sections through the tail bud region of this penguin embryo (Plate VI), if it is borne in mind that in *Chelonia* (tortoise, turtle) the caudal end of the neural tube is formed by a process of infolding, whereas in the penguin the caudal end of the neural tube is formed by the hollowing out of a solid cord of cells.

It should be noted that the neurenteric canal which is a normal feature in selachian, amphibian and reptilian embryos is only present in some birds such as *Anser*, *Anas*, *Motacilla*, *Melopsittacus* and *Sterna*, and never develops as such in *Gallus* embryos. It was also observed in this embryo and in E.525 that the epibranchial placodes are highly developed, and as these ectodermal thickenings are rudimentary structures representing parts of the system of branchial sensory organs of the head of aquatic vertebrates (Hamilton 1952), the marked development of these placodes is a further indication that penguins are primitive birds.

The cellular proliferations in relation to the dorsal portion of the second pharyngeal pouch found in the embryos E.529, E.536 and E.534 probably represents the thymus II of *Selachia* and *Anura*. Although a similar proliferation occurs in chick embryos on the fourth day, in Emperor penguins it is particularly well developed and persists for a considerable time, thickening of the epithelium still being identifiable in the embryos E.537 and E.535.

The observation made by Parsons on a Gentoo penguin embryo at stage 24, that there was an independent flexure in the bulbus arteriosus of the same nature as the temporary N-shaped flexure of reptilian embryos, has been confirmed in this series of embryos. It does, however, appear earlier in the Emperor penguin (E.528) and persists longer (still present in E.534).

The incipient dorsal air-sacs in the embryos E.537 and E.535 are at least as well developed as in the stage 34 Gentoo penguin embryo described by Parsons. They therefore appear earlier in the Emperor penguin and the author sees no reason for interpreting these incipient air-sacs differently from the way Parsons did, namely, a bird developing them is a primitive form approaching, more closely than other birds, an aquatic reptile.

The highest degree of development of the pronephros throughout its extent was found in the embryo E.528 (twenty-seven somites) where it extends from the fifth to the fifteenth somite, the cranial end undergoing degeneration. A relatively highly developed pronephros with a highly developed caudal portion still persists in embryo E.529, a remarkable fact when it is realised that a chick embryo of twenty-one somites only retains isolated pronephric rudiments cranial to the eleventh somite (Hamilton 1952).

In 1950 Davies compared the pronephros of duck embryos with reptilian embryos (turtle, alligator and crocodile) and with chick embryos. He subdivided the excretory organ of the birds and reptiles described into three regions:

1. A region anterior to the first true internal glomerulus of the mesonephros, which contains glomeruli, nephrostomes and tubules. It is rudimentary in the chick and duck. In the crocodile and turtle it contains well differentiated tubules and ciliated nephrostomes as well as external glomeruli.

2. An intermediate zone in which fused external and internal glomeruli are found. This area is well developed in the reptiles described, extensive and probably functional in the duck but mainly rudimentary in the chick.

3. The mesonephros proper.

In the Emperor penguin zone No. 1 is more highly developed than in the duck and differs from the reptiles only in that the nephrostomes are not ciliated. Zone No. 2, however, although not rudimentary and possibly functional, has not been found to extend over more than a localised area corresponding to one somite in these penguin embryos.

No clear indication of a developing Jacobson's organ has been found in the embryos examined. Jacobson's organ is a characteristic of reptiles but not all reptiles possess one, a notable exception being the crocodile, the modern reptile said to be most closely related to modern birds. A rudiment for Jacobson's organ may, of course, make a transient appearance as in the chick, and indeed, in specimen E.536 there is a slight medial outpouching of the nasal pit, and the stages when Jacobson's organ is manifest may be missing in this series. Its absence from these embryos therefore detracts little from the hypothesis that penguins are the most primitive group of birds living today.

All the other findings discussed so far confirm this hypothesis, but although there are suggestive indications to support the view, it is not as yet possible to state, from the embryological findings, that the Emperor penguin is the most primitive type of penguin. It may, however, be concluded that when a primitive characteristic has been found in other penguins, it has been found as well marked, or more so, in the Emperor penguin; some primitive characteristics observed in the Emperor penguin have not yet been observed in other penguins. From the information available Wilson's conjectures appear to have been justified.

Two further observations should be noted: As development proceeds beyond the stage when the parathyroid and thyroid glands of the penguin have reached the neck, the parathyroid glands come to lie closer to the thyroid gland. It seems likely, therefore, from the few stages examined that in the penguin the parathyroids develop from the pharyngeal pouches, this theory fitting in with the traditional view of their origin and not confirming the view of Venzke (1947), who considers that they bud off from the posterior portion of the two thyroid lobes.

From the examination of the present series of embryos it would seem that the penguin may be added to the list of vertebrates in which primordial germ-cells migrate from the endoderm of the germ wall to the genital ridges.

SUMMARY

1. Sixteen Emperor penguin embryos collected on the Dion Islets in 1949 are described and compared with embryos of other species of penguin, and chick embryos.
2. Several features have been found which suggest that penguins are the most primitive type of bird living today, and that possibly the Emperor penguin is the most primitive penguin.
3. These features are:
 - (a) The external form.
 - (b) The presence of well-developed head cavities which persist for a comparatively long period.
 - (c) The presence of a highly developed neurenteric canal which persists for a long time.
 - (d) The presence of highly developed epibranchial placodes.
 - (e) The transient appearance of a thymus II.
 - (f) The transient appearance of an N-flexure in the bulbus arteriosus.
 - (g) The development of a pronephros not unlike that of reptilian embryos.
 - (h) The development of incipient dorsal air sacs from the ectobronchi.

ACKNOWLEDGMENTS

I wish to record my thanks to Professor W. J. Hamilton of Charing Cross Hospital Medical School for his valuable criticism.

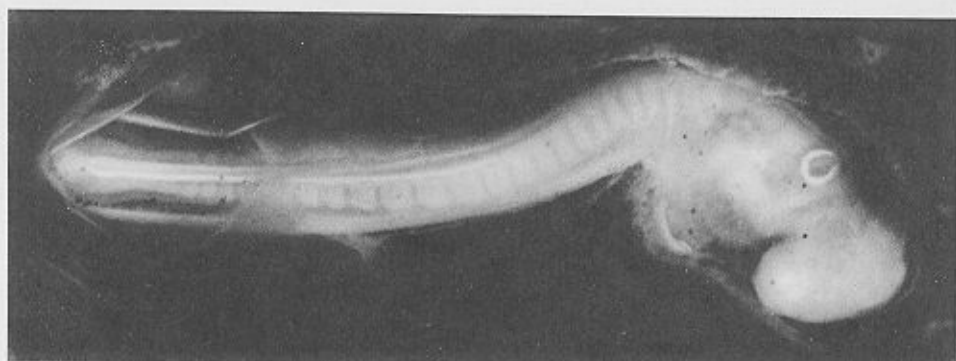
The technical work has been carried out by Mr. R. H. Watts; the photographs have been taken by Mr. E. V. F. Pittock, F.R.P.S.

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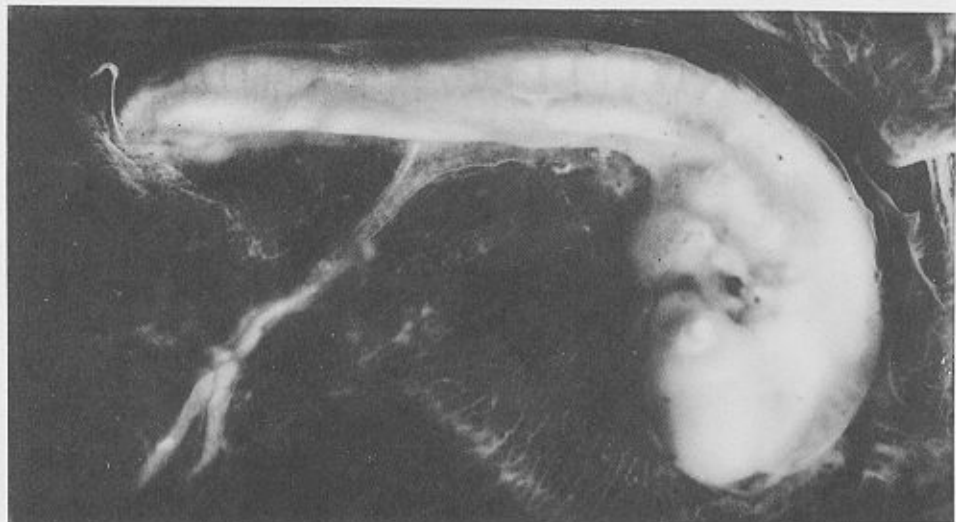
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PLATE I

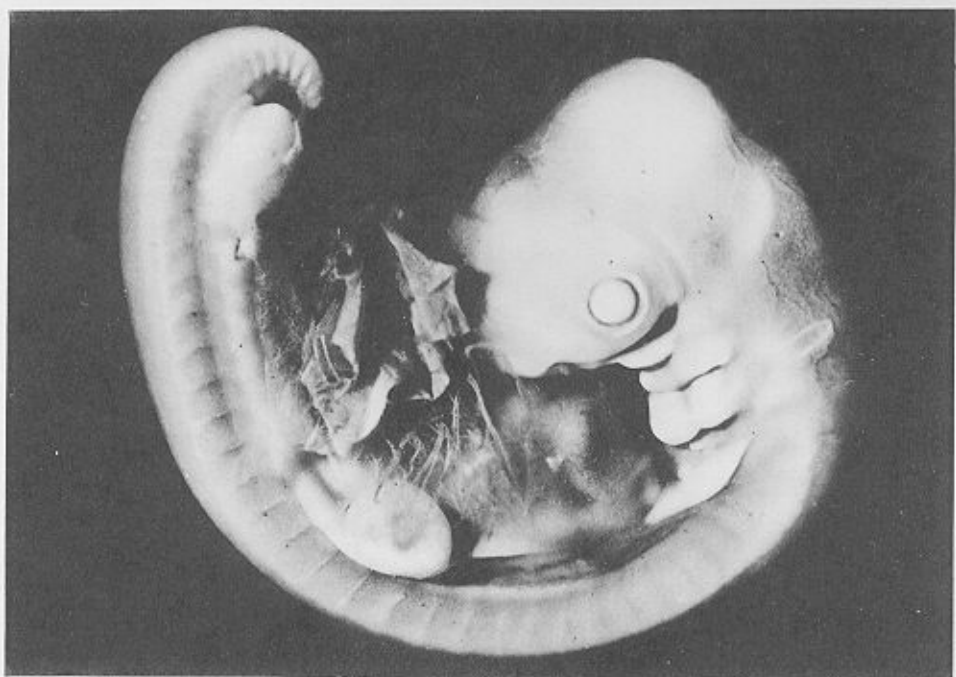
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2. Specimen E.525. \times 9.3 (p. 6)
3. Specimen E.529. \times 9.7 (p. 9)



1



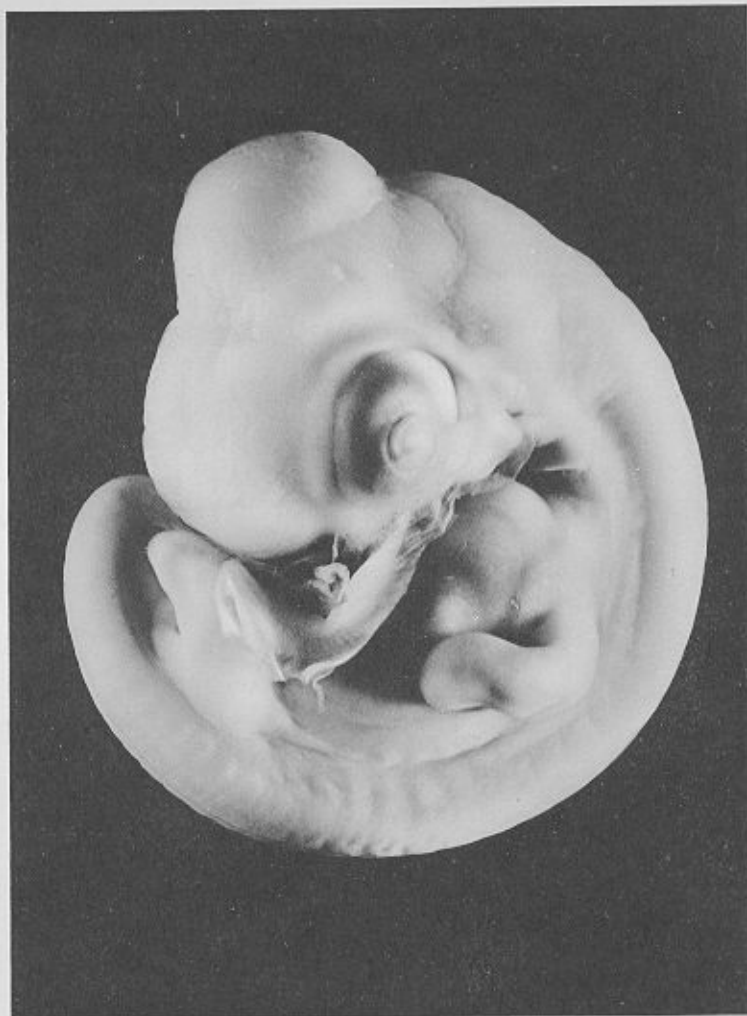
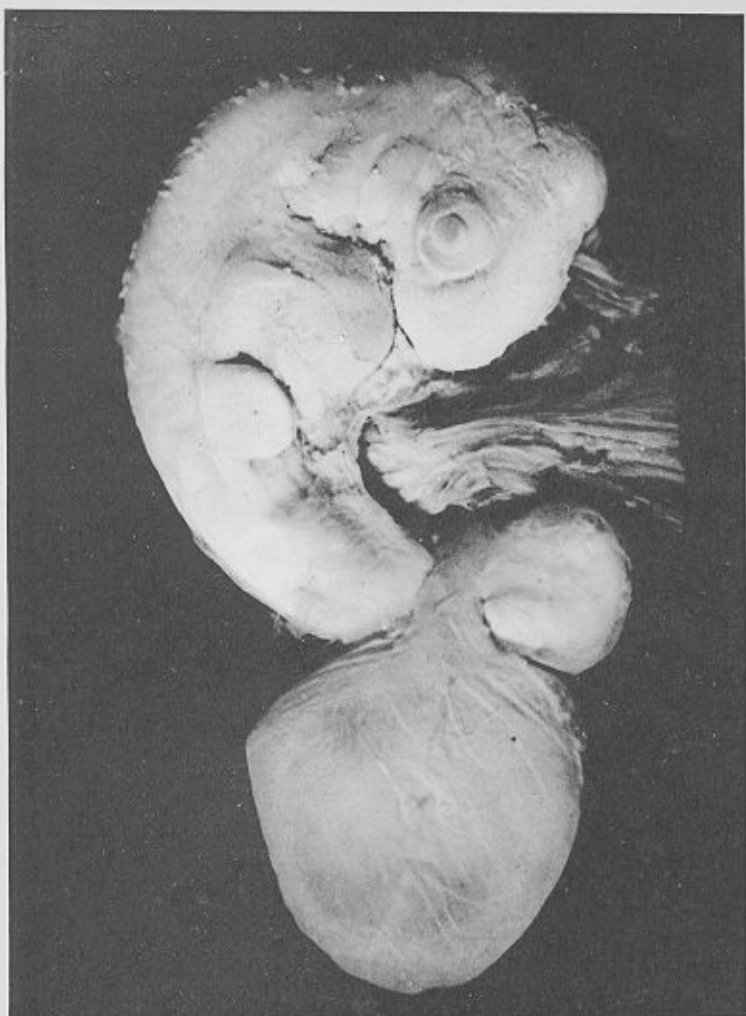
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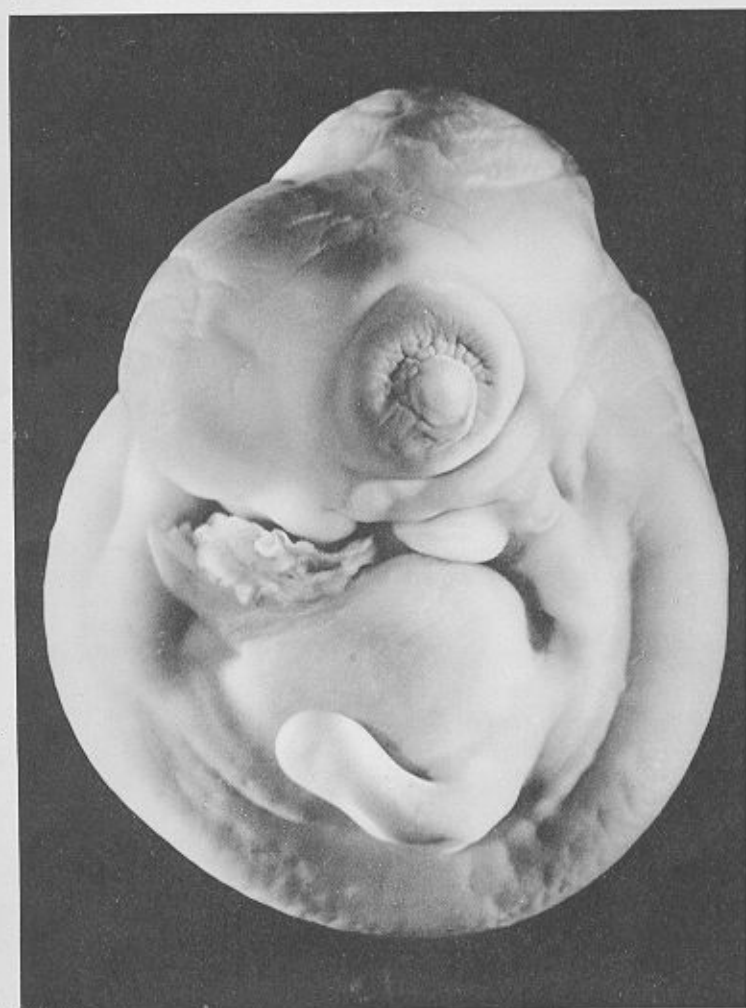
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PLATE II

1. Specimen E.530. $\times 8.3$ (p. 10)
2. Specimen E.536. $\times 7.5$ (p. 11)
3. Specimen E.534. $\times 7.5$ (p. 13)
4. Specimen E.537. $\times 6.5$ (p. 13)



2



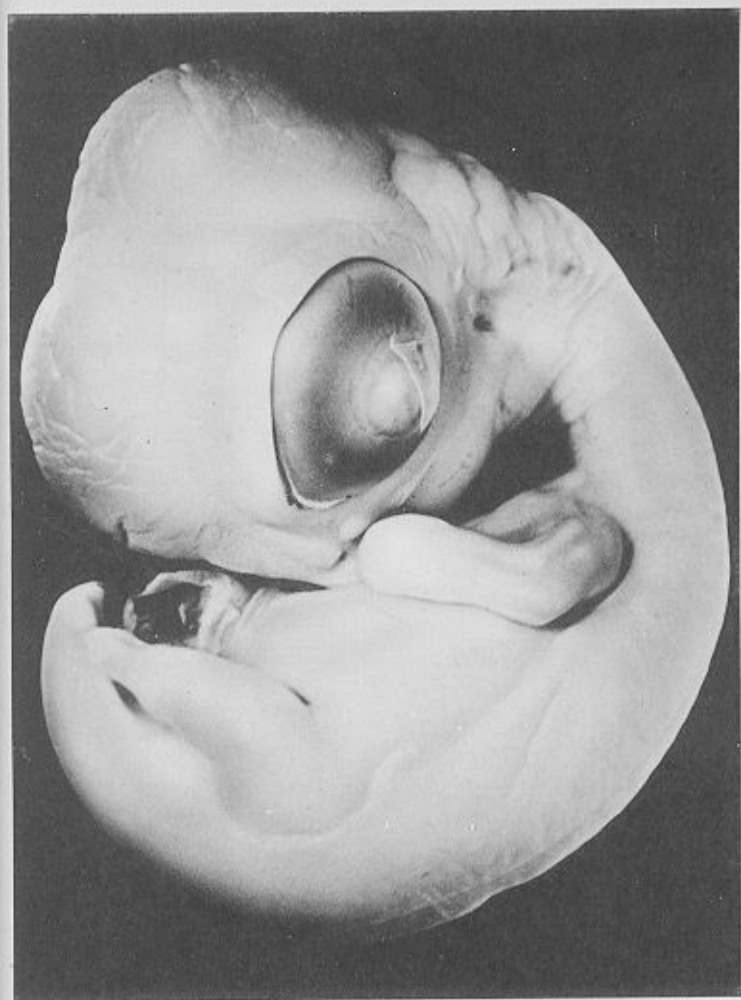
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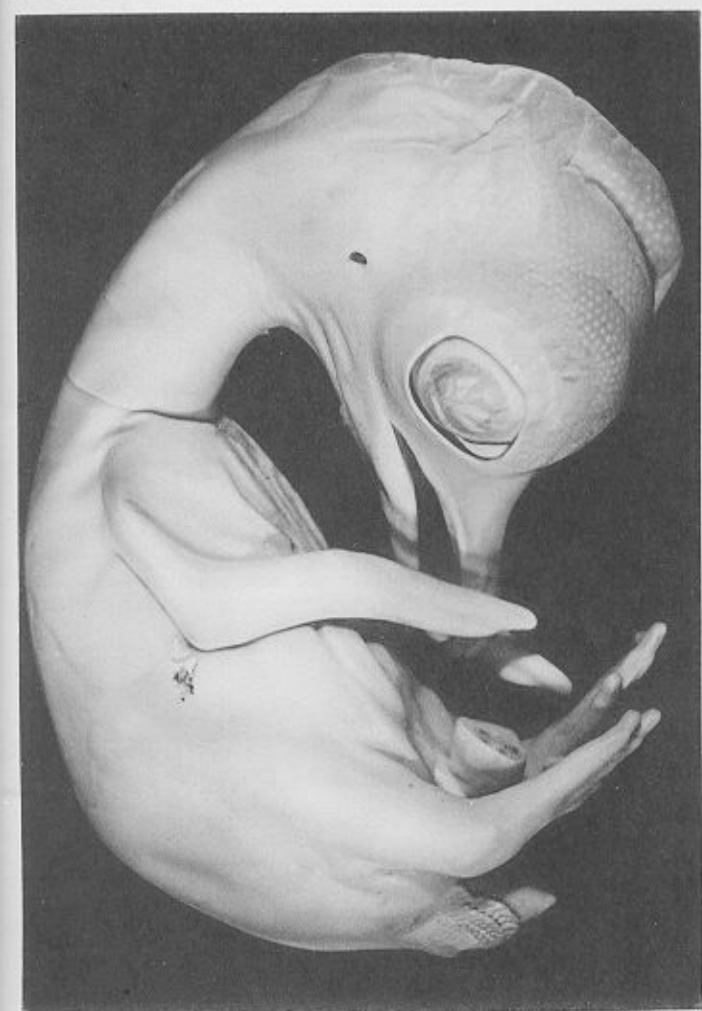
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PLATE III

1. Specimen E.535. × 5.9 (p. 13)
2. Specimen E.533. × 3.6 (p. 15)
3. Specimen E.541. × 3.4 (p. 15)
4. Specimen E.540. × 2.6 (p. 16)



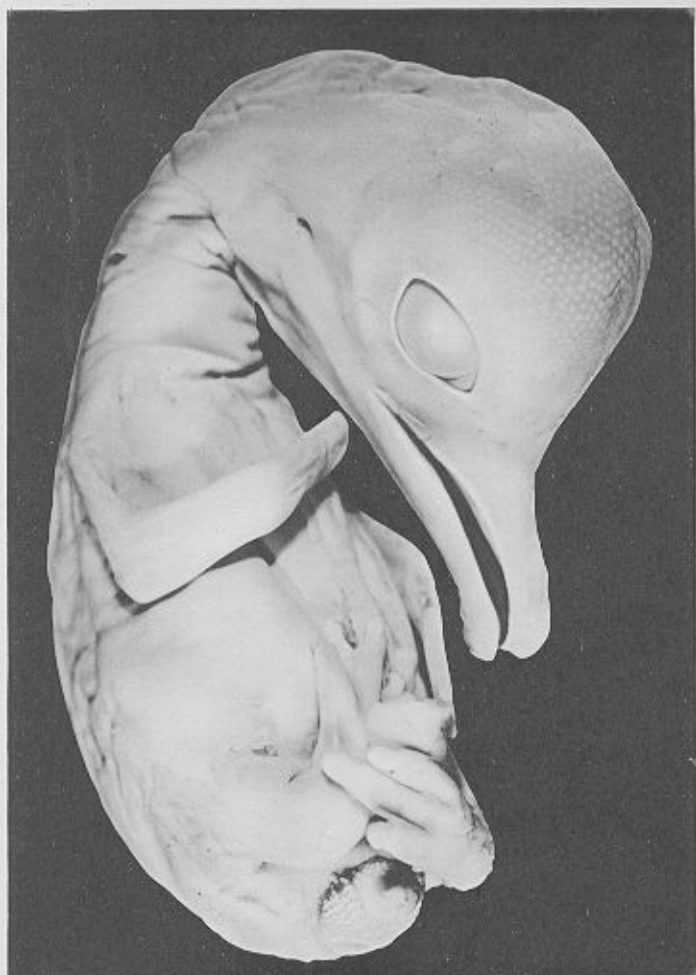
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PLATE IV

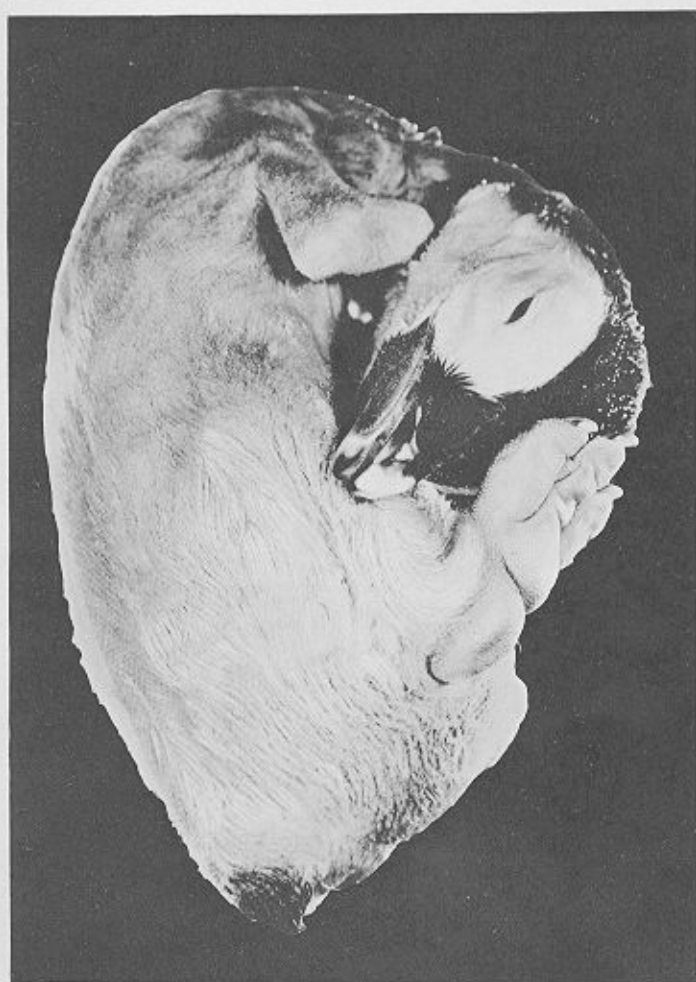
1. Specimen E.538. $\times 3.4$ (p. 15)
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3. } Specimen E.545. $\times .9$ (p. 16)
4. }



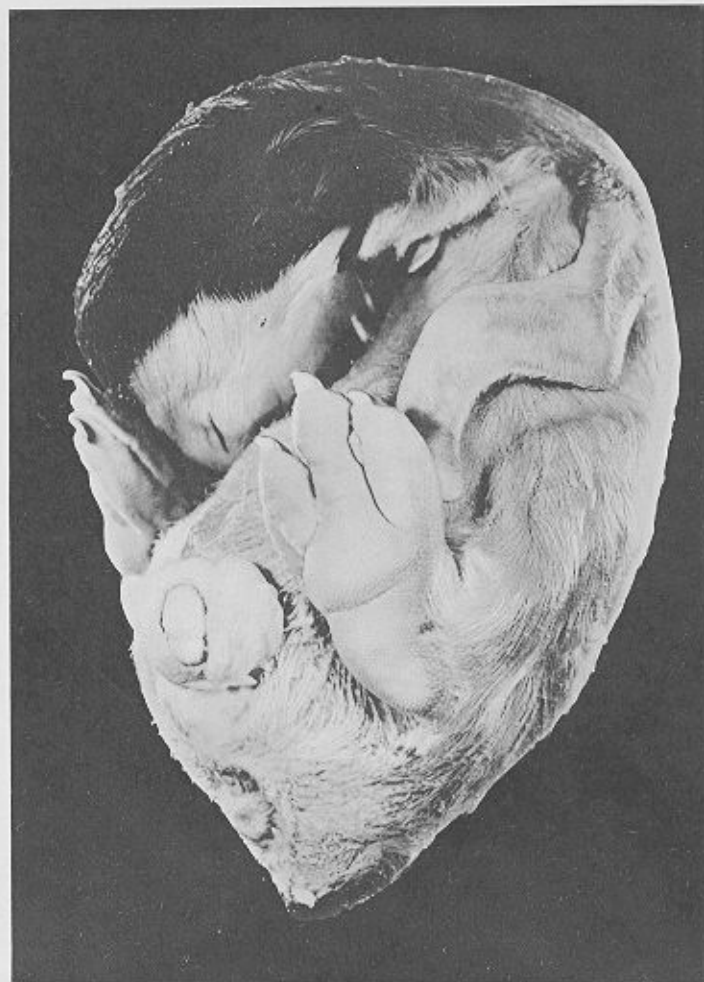
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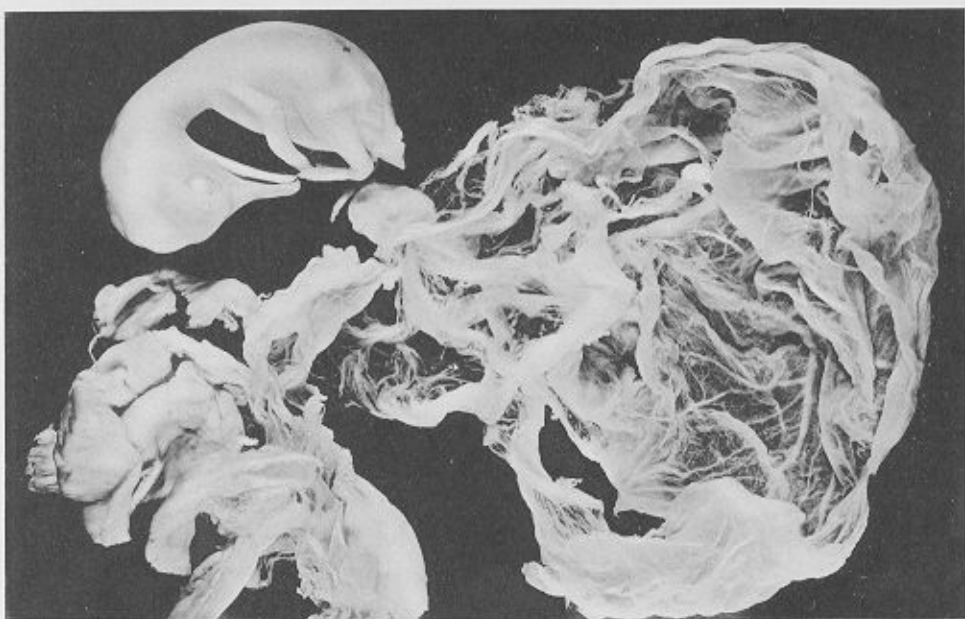
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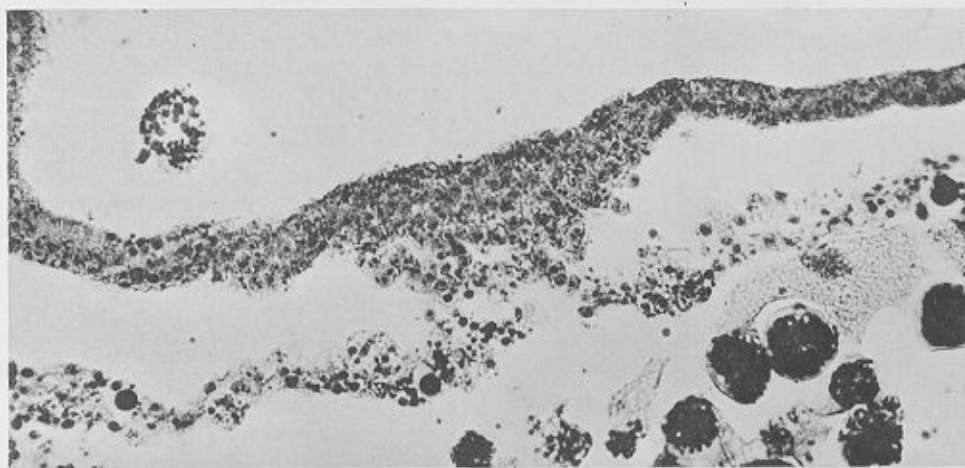
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PLATE V

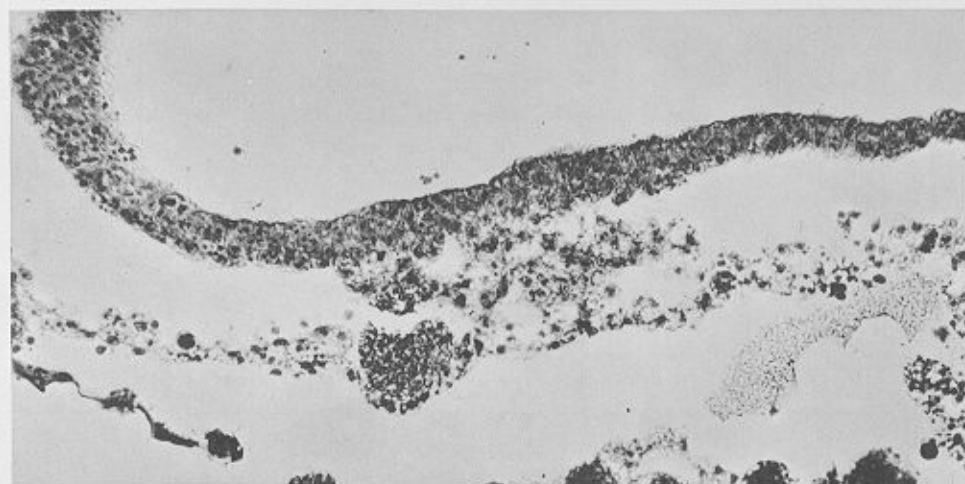
1. Specimen E.540. Embryo and membranes. $\times 1$ (p. 16)
2. Specimen E.532. Transverse section of the blastoderm at the cranial end of the primitive streak. $\times 250$ (p. 5)
3. Specimen E.532. Transverse section of the blastoderm at the caudal end of the primitive streak. $\times 250$ (p. 5)



1



2

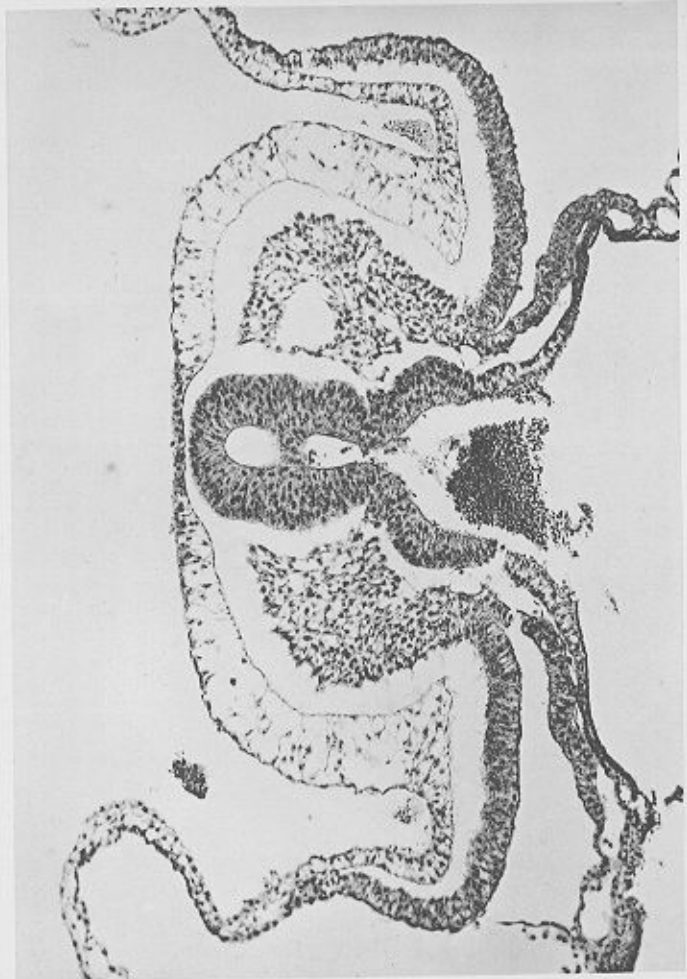


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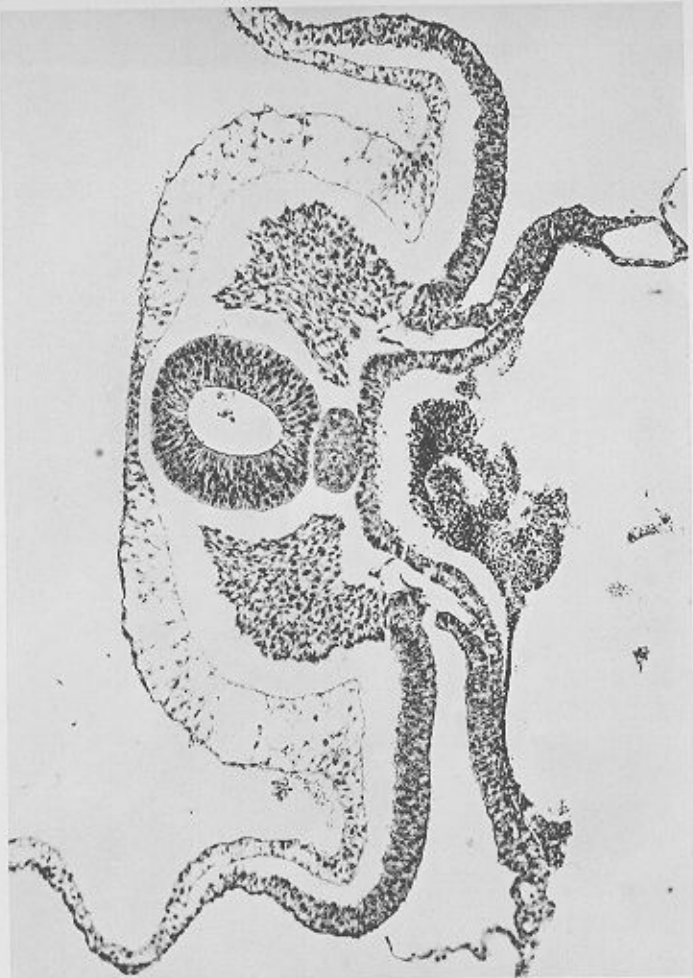
PLATE VI

1. Specimen E.528. Transverse section through the tail bud region showing the neural tube, gut and paraxial mesoderm differentiating from a solid cord of cells. $\times 130$ (p. 5)
2. Specimen E.528. A more cranial section showing the double lumen of the neural tube and the neurenteric canal. $\times 130$ (p. 5)
3. Specimen E.528. Transverse section through the most cranial portion of the neurenteric canal. $\times 130$ (p. 5)
4. Specimen E.528. Transverse section cranial to the neurenteric canal showing the relations of the neural tube, the notochord, the gut and the paraxial mesoderm to one another. $\times 130$ (p. 5)

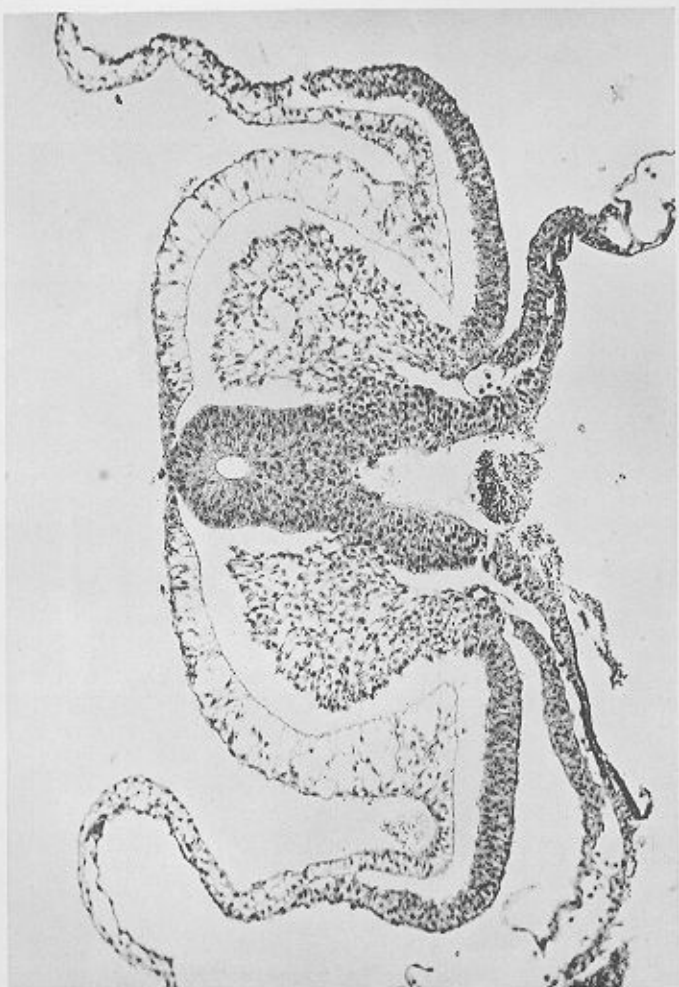
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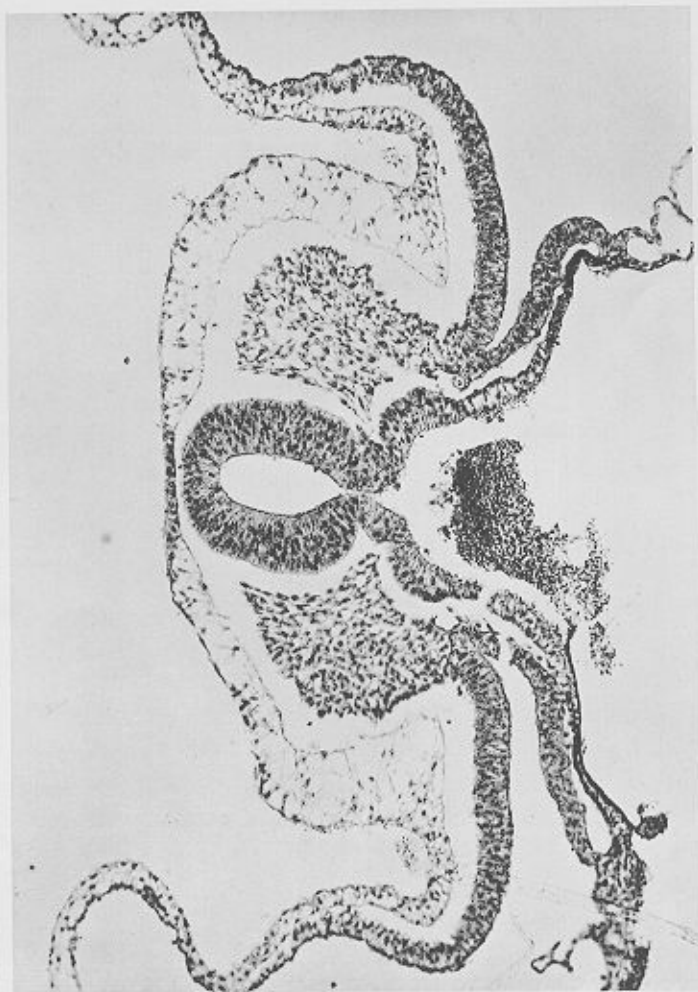


PLATE VII

1. High power photomicrograph of a section through the portion of the first epibranchial placode in relation to the trigeminal neural crest cells (Specimen E.528). The first visceral pouch is seen at the lower right corner of the picture. $\times 375$ (p. 5)
2. Transverse section through the head region of Specimen E.528 showing a premandibular head cavity and the bridge of mesenchymal cells dorsal to Rathke's pouch. $\times 210$ (p. 6)
3. Transverse section through the head region of Specimen E.525 showing the two premandibular head cavities and the bridge of mesenchymal cells connecting them across the midline. $\times 100$ (p. 8)
4. Transverse section through the head region of Specimen E.529. The two premandibular head cavities are in relation to the floor of the forebrain (bottom of the photograph). Part of the ocular globe is seen at the bottom left corner and the hindbrain is seen at the top of the photograph. $\times 55$ (p. 10)



PLATE VIII

1. Transverse section through the head region of Specimen E.536, showing the epithelial proliferation in the dorsal portion of the second visceral pouch. $\times 185$ (p. 12)
2. Sagittal section through the developing lung of Specimen E.535, showing the dilated terminations of the ectobronchi forming papillae on the dorsal surface of the lung. $\times 55$ (p. 14)
3. Transverse section through Specimen E.528 at the level of the twelfth somite, showing a nephrostome, a pronephric tubule and the pronephric duct. $\times 250$ (p. 6)
4. Transverse section through Specimen E.525 at the level of the thirteenth somite showing external glomeruli, nephrostomes, pronephric tubules and the pronephric ducts. $\times 85$ (p. 9)



1



2



3



4