# Chapter 9
# Nervous System

The nervous system is formed from the neural plate and the neural crest (see Chapter 4), and in the head region there are significant contributions from the ectodermal placodes.

## THE BRAIN

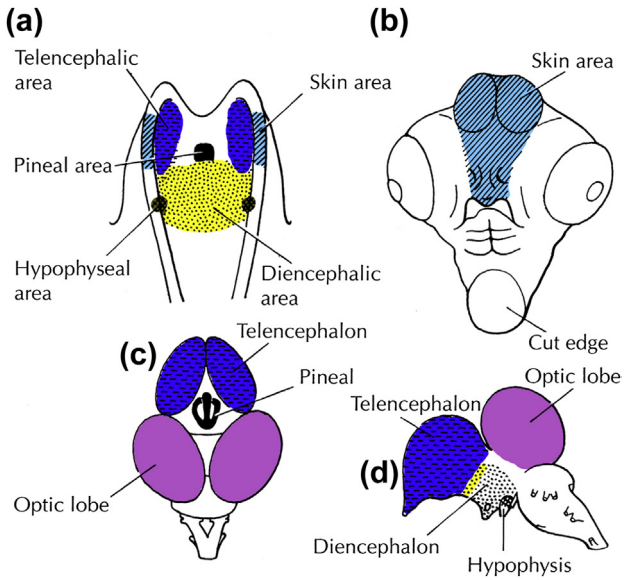
The anterior end of the neural tube enlarges to form the brain. *Text-Figure 62* shows a fate map of the most anterior region of the neural plate based on the results of chick–quail transplants. By stage 9, the primary optic vesicles (see below) have begun to form, and by stage 10 the brain has become divided into three primary regions, the **prosencephalon** (forebrain), the **mesencephalon** (midbrain) and the **rhombencephalon** (hindbrain) (*Plate 66*). By stage 11 the rhombencephalon has become subdivided into the **metencephalon** (cerebellum and pons) and the **myelencephalon** (medulla oblongata), which enclose the metacoele and the myelocoele, respectively (*Text-Figure 64*).

The division of the prosencephalon into telencephalon and diencephalon (thalamus) has begun to take place by stages 12–13. Paired swellings appear just anterior to the optic vesicles and form the telencephalic vesicles, each of which contains a lateral telocoele communicating with the median telocoele (the central lumen of the brain in that region) by the foramen of Monro. Fate maps of the developing forebrain have been published for stage 8 by Cobos *et al.* (2001) and Le Douarin *et al.* (2012) (*Text-Figure 63*), and for stage 10 by Pombero and Martinez (2008). At about the same time the cranial flexure has begun to appear as a bend at the anterior end of the mesencephalon; the cervical flexure begins at about stage 18 (day 3) (*Text-Figure 30*; Chapter 5). By stage 36 (10 days) the cranial flexure has almost gone, and the cervical flexure has become reduced so that the neck is less convex and the head no longer tucked against the thorax (see Normal Table in Appendix II). The walls of the telencephalic vesicles begin to thicken at about stage 13 (2 days) and will form the cerebral hemispheres, their lumina becoming the lateral ventricles. The cerebral hemispheres have expanded so much that by stage 37 (11 days) they overlap the diencephalon, and by stage 44 (18 days) the mesencephalon (*Text-Figure 65*).

The communication between the optic vesicles and the anterior end of the diencephalon has become reduced to a stalk by about stages 13–14 (*Plate 81*). The wall of the optic recess (*Plate 96*), a depression in the floor of the diencephalon between the optic vesicles, becomes very thin by stage 18 (3 days), but a thickened region immediately posterior to it marks the development of the optic chiasma (*Text-Figure 64; Plate 182*). The pineal organ begins to form at about stage 19 as an evagination from the median part of the roof of the diencephalon (*Text-Figure 64, Plate 108*). A description of its development is given by Calvo and Boya (1979). The infundibulum (see Chapter 12 and *Plates 108, 144*) develops posterior to the chiasma and combines with Rathke's pouch (*Plate 96*) to form the pituitary.

The mesencephalon has increased greatly in size by about stage 18 (3 days) and becomes clearly marked off posteriorly from the rhombencephalon by a narrower region, the isthmus (*Text-Figure 64*) located in the meso-metencephalic fold. By about stage 22 (3.5–4 days) the mesencephalon has grown so large that it projects over the metencephalon. The differentiation of the optic lobes from the mesencephalon begins to occur at about stage 27 (5–5.5 days), but even as early as 3 days, the surface of the mesencephalon is covered with fibres, foreshadowing the enormous size of the optic lobes in the adult, which itself is correlated with the importance of vision in birds.

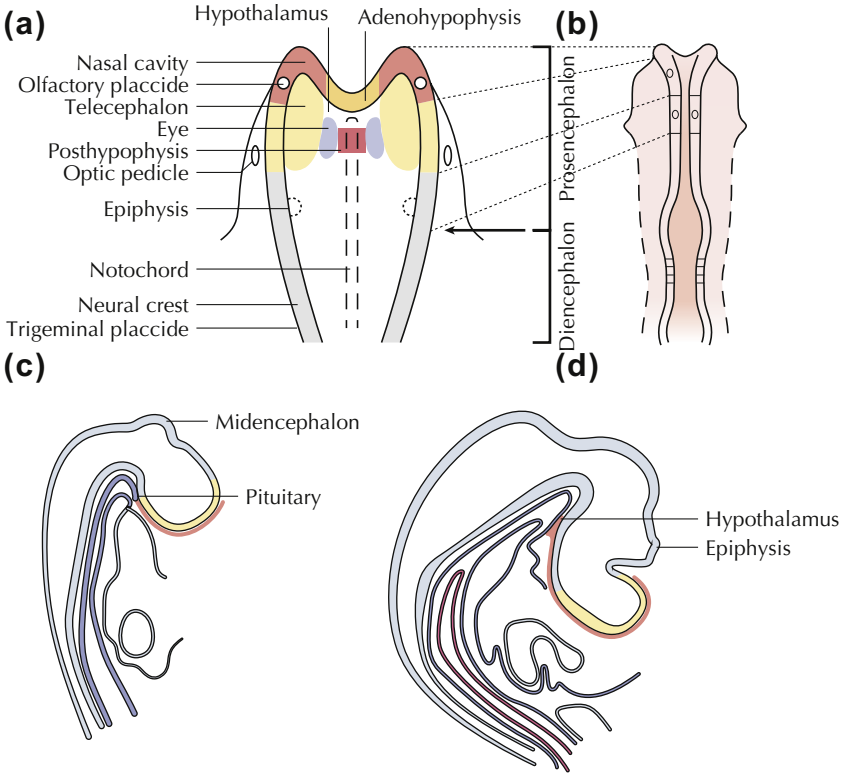
The Atlas of Chick Development. http://dx.doi.org/10.1016/B978-0-12-384951-9.00009-5



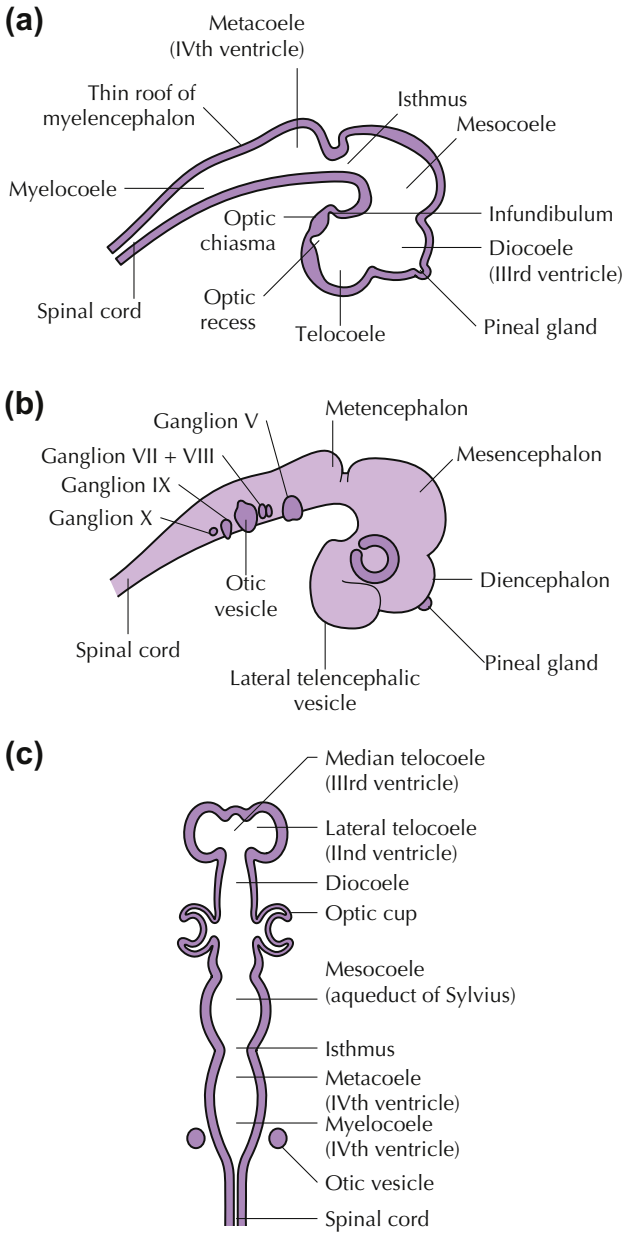

***Text-Figure 62.*** *Origin of the different tissues of the head. (a) Fate map of the anterior end of the neural tube at stage 7 (24 h). (b) Skin area of the face at stage 34 (8 days), which has originated from the lateral neural folds. (c,d) The telencephalic and diencephalic regions at stage 34 (8 days), including the hypophysis. (After Couly and Le Douarin 1988 with permission of Company of Biologists Ltd.)*

The rhombencephalon is characterized by a thin-walled roof in the early stages, though by about 4 days this becomes thicker in the anterior region as the metencephalon develops (*Plate 108*). A striking feature of the newly formed rhombencephalon is the development of a series of seven pairs of segmental bulges, each of which is known as a **rhombomere**. They extend from the anterior border of the rhombencephalon to its posterior end where they abut the first pair of somites. There is good evidence to suggest that each rhombomere is a 'compartment', that is to say that although the cells are able to mingle freely within their own rhombomere and form localized clones, they are unable to pass into adjacent rhombomeres. Young neurons migrate from the brain between the rhombomeres. This segmental pattern of the rhombomeres is subsequently lost, but is thought to be of significance in generating the neuronal patterns of the hindbrain (Lumsden, 2004).

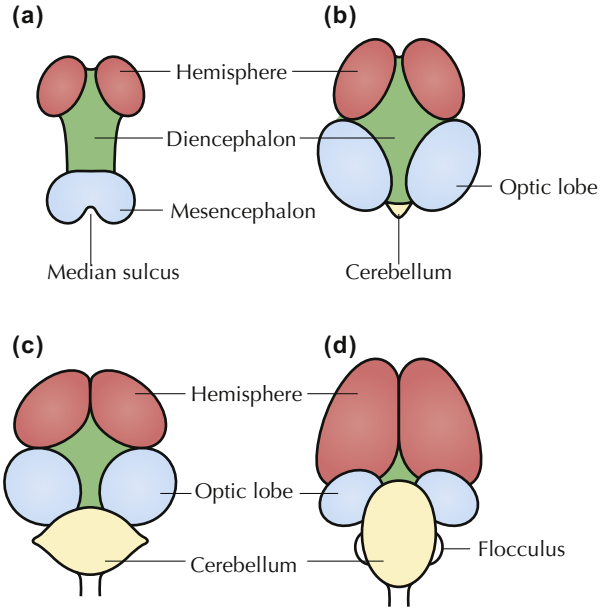
The walls of the metencephalon also become thicker as the two halves of the cerebellum begin to form. During the following days they enlarge, and by about stage 36 (9–10 days) they have fused together in the midline. During the remainder of the incubation period, the cerebellum increases in size and complexity, and by day 16 almost abuts the cerebral




***Text-Figure 63.*** *Fate map of the pre-otic region of the brain. The telencephalon is formed by the lateral-most regions of the anterior neural plate which develops between the two optic vesicles and in front of the notochord. (After Le Douarin and Kalcheim (1999) with permission of Cambridge University Press.)*




***Text-Figure 64.*** *Diagrams of the brain to show the topography of a 4-day (about stage 22) embryo. (a) Sagittal section. (b) View of brain from right side showing some of the cranial nerve ganglia. (c) Schematic frontal section plan with flexures straightened. (Modified after Patten, 1950.)*



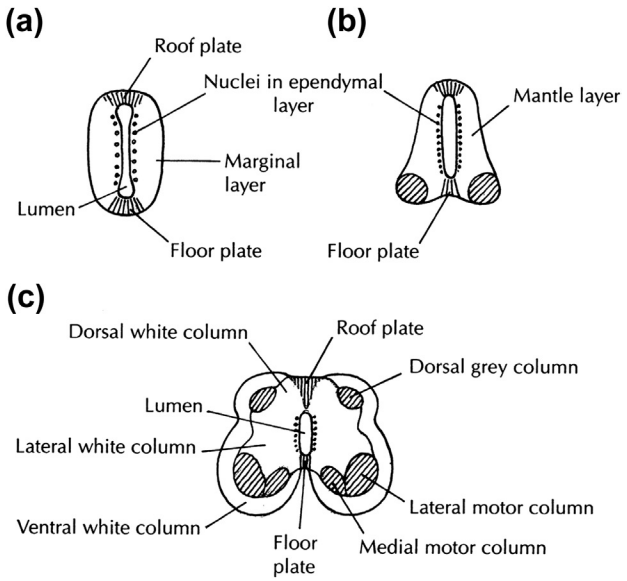

***Text-Figure 65.*** *Dorsal view of the brain to show how the cerebral hemispheres formed from the telencephalic vesicles expand and overlap the diencephalon. (a) Stage 18 (3 days); (b) stage 33 (7–8 days); (c) stage 38 (12 days); (d) stage 44 (18 days). (After Miller, 1903.)*

We have seen (Chapter 4) that a range of genes play a role in patterning of the brain and spinal cord. Expression of *Wnt8b* and *Wnt7b* are of importance in the patterning of the forebrain (Garda *et al.*, 2002), while *Wnt1* and *Wnt3a* may be implicated in the specification of the dorsal spinal cord (Muroyama *et al.*, 2002). The dorso-ventral patterning of the spinal cord is also dependent on *sonic hedgehog*; cells in chick neural plate explants differentiate into ventral cell types under the influence of *sonic hedgehog* (Robertson *et al.*, 2001).

hemispheres (day 18 shown in *Text-Figure 65a*). The myelencephalon has acquired the characteristic shape of the medulla oblongata by about stage 36 (9–10 days; *Plates 160, 191*). Many fibre tracts have developed in the brain by 5 days (stage 26). These are often visible in sections stained with haematoxylin and eosin, but better in silver preparations or after labelling with specific antibodies. The patterning of the developing axons in the chick brain appears to be organized in a way comparable to that in the mouse (Ware and Schubert, 2011).

## HISTOLOGY OF THE SPINAL CORD AND DEVELOPMENT OF THE FIBRE TRACTS

A fate map of the spinal cord in the thoracic, lumbar and caudal regions was published by Catala *et al.* (1996). At the time of its formation from the ectoderm, the neural tube consists of a thickened columnar epithelium, but changes soon take place in its shape and histology. All the cells of the neural tube are elongated with their long axes radiating out from the ependyma to the periphery. By stage 16 (2.5 days) the neural tube possesses two layers, the **ependyma,** which lines the neural canal and contains a large number of mitotic cells, and the **marginal**




***Text-Figure 66.*** *Development of the spinal cord at stage 16 (2.5 days); (b) stage 24 (4.5 days); (c) stage 34 (8 days). (After Romanoff, 1960.)*

**layer** (*Text-Figure 66*), and by stage 18 (3 days) the **mantle layer** is also recognizable. Neuroblasts are visible from about stage 15 (2 days) in the ventro-lateral part of the tube; this region is about six cells deep, whereas in the floor plate (the keel) there is only a single layer of cells.

By stage 16 spinal nerves have developed, and by stage 22 (3.5–4 days) regions of 'grey' and 'white' matter are recognizable. Nissl substance is visible in embryos of this stage stained with basic dyes. Dorsal and ventral horns can be seen in the grey matter from about stage 31 (day 7) and glial cells in the white matter. During the following days the spinal cord becomes larger in transverse section and there is a change in shape of the lumen from a longitudinal slit to an almost square or round shape. This is accompanied by the development of dorsal and ventral fissures. With the outgrowth of the spinal nerves the size and shape of the spinal cord varies along its length.

## SPINAL NERVES

Each spinal nerve has both a somatic and a splanchnic component, the former serving the somatopleure and the body axis and the latter the viscera, and each part has both motor and sensory components. By about stage 34 (8 days) there are 38 pairs of spinal nerves. The spinal ganglia are of neural crest origin (see Chapter 4).

The segmentation of the spinal nerves is associated with that of the adjacent somites so that eventually each spinal nerve emerges between two vertebrae. After leaving the neural tube, each axon passes through the anterior half of the sclerotome and is inhibited from going through the posterior half. The neural crest cells are similarly constrained to migrate through the anterior and not the posterior half (Keynes and Stern, 1984).

The ventral roots of the spinal nerves have emerged in the cervical region by about stage 13 and appear progressively in the more posterior regions during the following day. The spinal ganglia begin to appear early in day 3 and the dorsal roots that arise from them develop during days 3–4. The neurons in the dorsal root ganglia vary in structure. The size of the dorsal root ganglia themselves also varies, being especially large for the nerves innervating the limbs. By stage 20 (3–3.5 days), the ganglia of the brachial segments 14–15 are more than 80% larger than those in the cervical segments 5 and 6, and this has been correlated with the colonization of the brachial region by a larger number of neural crest cells (Goldstein *et al.,* 1995).

The motor axons of the spinal nerves innervate the myotomes to which they attach and become transported by the developing muscle. The sensory fibres subsequently migrate along the motor nerves. Lance-Jones (1988), who studied the somitic level of origin of the hindlimb muscles, found that the motor neurons supplying them arose from the same somitic level and, subsequently (Lance-Jones, 1990), that the axons were guided by the limb somatopleure. The dorsal root ganglia are derived from the neural crest cells, which differentiate into many subpopulations of sensory neurons as well as glial cells. The development of these sensory neurons has been analysed by George *et al.* (2010).

Spinal nerve plexuses develop in the brachial and lumbo-sacral regions from the 13th to 16th and the 23rd to 29th spinal nerve, respectively. Chick spinal nerves have been found to undergo a transient period of spontaneous activity during their development, with regularly occurring episodes interspersed with quiescent periods (Wenner and O'Donovan, 2001).

The meningeal layers of the spinal cord, and the endoneurium of the peripheral nerves are all formed from mesenchyme cells of non-neural crest origin, whereas the Schwann cells are of neural crest origin (Halata *et al.*, 1990).

Oligodendrocytes first appear in the ventral region of both the spinal cord and the metencephalon (Davies and Miller, 2001) under the influence of *sonic hedgehog*. Conversely, the specification of oligodendrocyte precursors is inhibited by bone morphogenetic proteins (BMPs) (Mekki-Dauriac *et al.*, 2002).

## Cranial Ectodermal Placodes

Cranial ectodermal placodes are thickened patches of ectoderm which invaginate. They each break away from the ectoderm and contribute to a specific special sense organ (see below) or to one of the cranial nerves (V, VI, IX or X). The precursors of these placodes are arranged in 'a horse-shoe shaped region around the anterior neural plate, at the border of the neural plate and non-neural ectoderm' (McCabe and Bronner-Fraser, 2009). Initially there is some overlap between the precursors of the different placodes, though eventually they become established in a clear linear arrangement (*Text-Figure 67*). Specific inductions from adjacent tissues appear to play an important role in their differentiation (reviewed by McCabe and Bronner-Fraser, 2009). For example, Wnt signals derived from the isthmus act together with FGF in the development of the trigeminal placode (Canning *et al.*, 2008).

## THE CRANIAL NERVES AND GANGLIA

The development of each of the cranial nerves is closely associated with that of fibre tracts and nuclei in the brain. The following cranial sensory nerve ganglia are partially derived from the neural crest and partially from epidermal placodes: the trigeminal (V), geniculate (VII), vestibular acoustic (VIII), and the proximal ganglionic components of the petrosal (IX) and the nodose (X). The head neural crest cells migrate between stages 9 and 11, and some condense lateral to the brain and join with cells from the epidermal placodes which will become neuroblasts. McCabe and Fraser (2009) have mapped the position of the neural crest and placodal anlagen (*Text-Figure 67*) from stages 7–15, and the position of the cranial ganglia at 12 days.

## Cranial Nerve I (Olfactory Nerve) (*Plates 163, 193*)

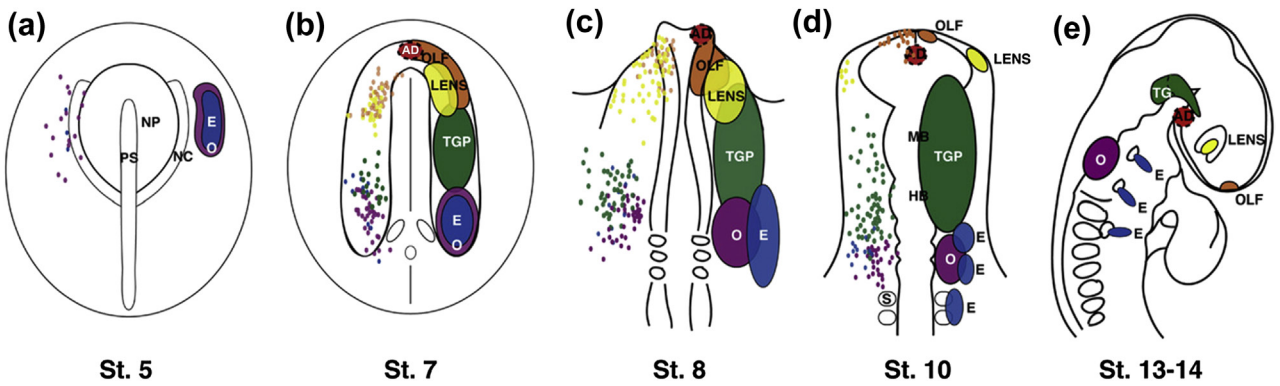
The sense of smell is poor in birds, and this is reflected in the small size of the olfactory nerve. It forms by the growth of cells from the nasal placode (*Plate 27c,d*) to the olfactory lobes, which begins about stages 17–18 (3 days). Fibres become visible at about 3–4 days.

## Cranial Nerve II (Optic Nerve)

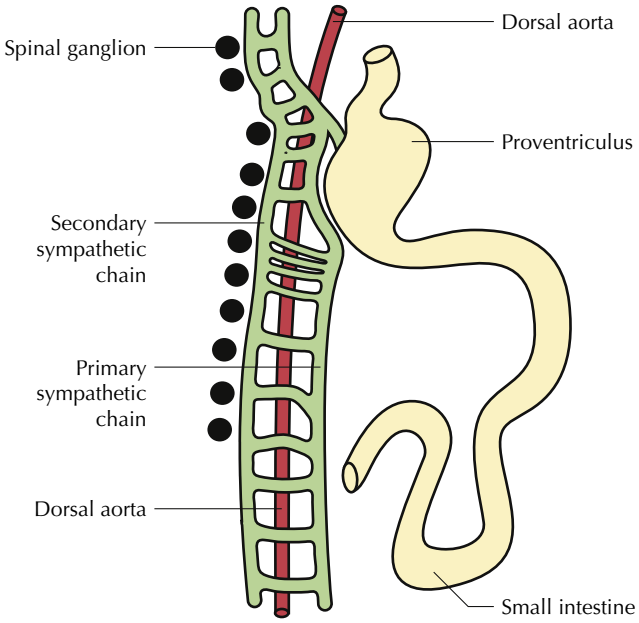
Vision is highly developed in birds, and this is indicated by the large optic nerves and optic lobes. The optic nerves grow from the retina toward the ventral region of the diencephalon, where they cross to form the optic chiasma (*Plates 182, 192*) and terminate on the contralateral side of the brain. The retinal fibres enter the optic stalk at about stage 18 (3 days) and cross through the optic chiasma at about the beginning of day 4, reaching the surface of the optic lobes on day 5. By day 7 many fibres have spread over the surface of the optic lobes.

## Cranial Nerve III (Oculomotor Nerve) (*Plate 113*)

This is the main nerve to the extra-ocular muscles and is especially large in birds because of the great size of




*Text-Figure 67. Placode development. The right side of each diagram shows the placodes which form by stage 13–14, whilst the left indicates the overlapping areas that give rise to them. AD: adenohypophysis (red); E: epibranchial (blue); Lens (yellow); OLF: olfactory (orange); O:otic (purple); TGP: trigeminal placode (green); HB: hindbrain; NP: neural plate; PS: primitive streak; NC: neural crest. (From McCabe and Bronner-Fraser (2009), with permission of Elsevier.)*




***Text-Figure 68.*** *Autonomic nervous system showing primary and secondary sympathetic chains at 10 days. (Modified after Romanoff, 1960.)*

the eyes. The left and right oculomotor nerves grow from the ventral side of the midbrain immediately posterior to the hypophysis and innervate the superior and inferior rectus muscles of the eye. The ciliary ganglia appear at about stage 10, and each is situated two-thirds of the distance from the proximal to the distal end of the oculomotor nerve. The ciliary nerve, which runs from the ganglion to the iris, is visible according to some authors at 4 days (stage 23).

## Cranial Nerve IV (Trochlear Nerve)

This special somatic nerve arises at about 3–3.5 days (stage 20) at the dorsal surface of the midbrain posterior to the oculomotor nerve, and innervates the superior oblique muscle of the contralateral eye. The left and right nerves cross at about 3.5 days (stage 21) at the dorsal surface of the midbrain, and then start to grow ventrally over the lateral face of the mesencephalon.

## Cranial Nerve V (Trigeminal Nerve)

The various branches of the trigeminal nerve appear at about stages 13–14 together with the neural crest-derived trigeminal (Gasserian/semilunar) ganglion, and is easily recognizable (shown at later stages *Plate 108*) by its large size and its situation at the widest part of the mesencephalon. The sensory parts of the nerve are formed from the ganglion, and the motor components are derived from the rhombencephalon. The major branches of the trigeminal nerve are the ophthalmic (*Plate 107*) and the maxillary-mandibular, the latter subdividing into its maxillary and mandibular branches (*Plates 109*) during day 4.

## Cranial Nerve VI (Abducens Nerve)

This cranial nerve (*Plate 192*) arises during day 3 in the midline from the ventral side of the myelencephalon, and has migrated to the primordium of the lateral rectus muscle by stage 24 (4.5 days). There is no ganglion associated with it. The main nucleus, which is situated ventral to the floor of the fourth ventricle, appears at 5 days. The accessory nucleus, which lies between the descending root of the trigeminal and superior olive nerve, is derived from cells which have left the main nucleus.

## Cranial Nerve VII (Facial) and Cranial Nerve VIII (Acoustic or Vestibulocochlearis Nerve)

The development of these two nerves is closely associated. The facial nerve, which becomes recognizable at about stage 11, is formed partially from neural crest and partially from the epibranchial placode of the first pharyngeal groove. A branch from the facial ganglion has reached the upper end of the hyoid arch by stage 13, and has extended into the third rhombomere by stage 15. Cells leave the auditory epithelium at about stage 18 to form the acoustic ganglion (shown at stage 37 in *Plate 192*) and at about the same time the facial and acoustic ganglion columns fuse with one another (shown at stage 23 in *Plate 109*). Meanwhile cells bud from the placode over the hyoid arch and fuse with the facial and acoustic columns to form the geniculate ganglion (shown at stage 23 in *Plate 108* and stage 26 in *Plate 118*). By about 3.5–4 days (stage 22) the geniculate ganglion is attached to the rhombencephalon.

Early on day 4 the acoustic ganglion is present as two parts and has become independent of the facial nerve, but by day 5 it is a large bilobed structure, closely applied to the auditory epithelium. The vestibular ganglion is derived almost entirely from the epibranchial placode. At 5 days the motor nucleus of the facial nerve is a single group of cells, but by day 7 this has become divided into a dorso-medial and a ventro-lateral group.

## Cranial Nerves IX and X (Glossopharyngeal and Vagus Nerves)

The glossopharyngeal nerve (*Plate 109*) innervates the pharyngeal muscles and the salivary glands. The vagus nerve (*Plate 118*) supplies the cervical and thoracic regions and much of the abdominal viscera. The peripheral differentiation of the vagus nerve was described by Kuratani and Tanaka (1990). Sato *et al.* (2002) found that electrical stimulation of these nerves between days 4 and 8 of incubation elicited a pattern of optical recordings which changed with advancing development.

A large ganglionic complex begins to form at about stage 13 at the root of the two nerves, the most rostral part being the superior ganglion of the glossopharyngeal nerve (shown at stage 23 in *Plate 108*), and the more caudal part the jugular ganglion of the vagus (shown at stage 23 in *Plate 110*). In addition, a ganglion develops on the trunk of each nerve a short distance from the brain, the **petrosal ganglion** on the glossopharyngeal and the **nodose** on the vagus. The nodose ganglion provides sensory innervation to the heart and other viscera (Harrison *et al.,* 1995).

Both the petrosal and the nodose ganglia receive a contribution of cells from the ectoderm (the nodose from the ectoderm dorsal to the fourth pharyngeal cleft and the petrosal from a more anterior region), in addition to that from the neural crest. The nodose ganglion begins to appear at stage 18 (3 days) as a group of cells lateral to the dorsal aorta, where it is joined by the anterior cardinal vein (D'Amico-Martel and Noden, 1983), but it subsequently shifts posteriorly.

By stage 13 the cells of the glossopharyngeal–vagus part of the neural crest have already migrated under the ectoderm and over the first and second somites. By stage 15 the distal part of the migrating column of cells has started to bifurcate, the anterior part going to the third visceral arch and the posterior part to the fourth visceral arch.

The ganglia of the IXth and Xth cranial nerves form just posterior to the auditory vesicle at the dorsal side of the second and third visceral furrows.

## Cranial Nerve XI (Spinal Accessory Nerve)

This nerve supplies the pharynx and shoulder muscles. In the 4–5 day embryo the strand runs from the ganglion and dorsal root of the third cervical nerve to the root of the vagus.

## Cranial Nerve XII (Hypoglossal Nerve)

This nerve supplies the muscles at the base of the tongue. It leaves the ventral side of the rhombencephalon by several roots opposite the third and fourth somites, which unite to form a single trunk. The first axons appear at about stages 17–18 (3 days). During days 4–5 the nerves extend posteriorly and enter the pharynx.

# THE AUTONOMIC NERVOUS SYSTEM

This is of neural crest origin. The levels of the neural crest that give rise to the various autonomic ganglia as well as to the adreno-medullary cells were determined experimentally by exchanging strips of neural tube, together with the associated neural crest, between chick and quail embryos (Le Douarin *et al.,* 1984a, b). All the sympathetic ganglia arise from the neural crest posterior to the level of the fifth somite. The adreno-medullary cells form from the neural crest between levels of the 18th and 24th somites. All the parasympathetic enteric ganglia receive neural crest cells from somite levels 1–7, but those parasympathetic ganglia that lie posterior to the yolk sac stalk receive additional neural crest cells from the sacro–lumbar region. The ganglion of Remak is derived from the lumbro–sacral crest posterior to the level of the 28th somite.

The first signs of the primary sympathetic chain are visible at the end of day 3 as two rows of cells lying one on either side of the neural tube, ventral to the somites and immediately lateral to the aorta. Ganglia form on the chains about days 4–5. The secondary sympathetic chains, which start to appear early on day 5, grow out as sprouts from the primary chain and then become connected with one another to form the secondary chain, which runs close to the spinal ganglia (*Text-Figure 68*). The primary chain then becomes reduced in size and it eventually disappears. There are about 38–39 secondary sympathetic ganglia (*Plate 166*), consisting of 13 cervical, 7 thoracic, 14 lumbro-sacral and 4–5 coccygeal. The rami communicantes are visible in silver preparations by 5–7 days, and pass from the secondary sympathetic ganglia to the roots of the spinal nerves.

The secondary chain (*Text-Figure 68*) becomes a continuous nerve cord (the paravertebral) between days 4 and 8, running from the superior cervical ganglion to the coccyx. It bifurcates around each rib, but remains as a single structure between the ribs. It connects with the plexuses in the following regions: the aortic plexus

(which becomes modified further into the coeliac plexus around the coeliac artery, as well as the pelvic and hypogastric plexuses), the medullary plexus of the adrenal gland, the splanchnic plexus and Remak's ganglion in the rectal region.

The times at which enteric neurons first appear in different regions of the gut have been plotted by Fairman *et al.* (1995). Most of the enteric nervous system is formed from neural crest cells of the vagal region, though there is also a contribution from the sacral neural crest (Burns and Le Douarin, 2001).

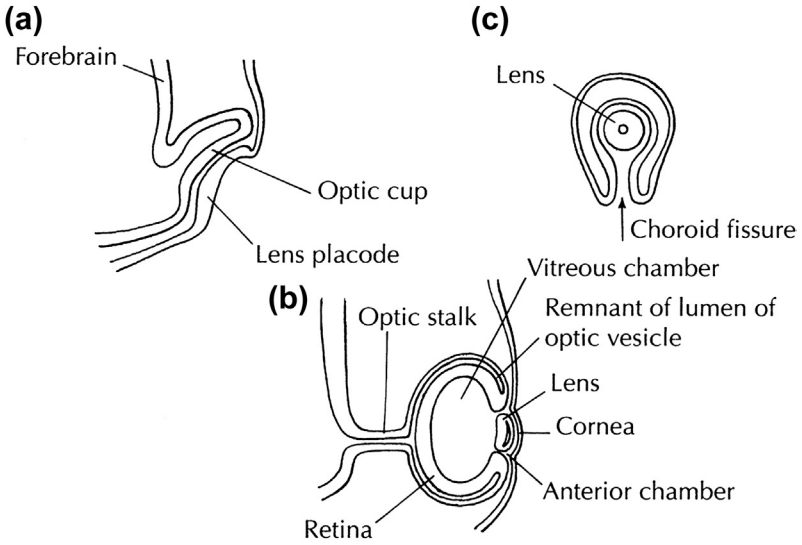
## ORGANS OF SPECIAL SENSE

### The Eye

The first sign of the development of the eyes is a bulging at the lateral sides of the prosencephalon at about stage 9 (*Plate 64*). These are the rudiments of the optic vesicles which lie beneath the head ectoderm. By stage 10 each vesicle has begun to constrict at its base (*Plate 68*) until by stages 12–14 its connection to the brain has been reduced to a narrow stalk (*Plates 74, 81*); but the lumen of the optic vesicle is still in communication with the prosencephalon via the stalk.

Meanwhile, the distal part of each optic vesicle (the future sensory layer) invaginates and presses against the proximal part (the future pigment layer of the retina, iris and ciliary body). This results in the formation of the **optic cup** *(Text-Figure 69, Plate 81*), the elimination of the original lumen of the optic vesicle and the formation of a new lumen, the future **vitreous chamber.** The invagination of the optic cup does not start at the extreme lateral border of the optic vesicle, but from a more ventral position. The wall of the cup is therefore lacking on the ventral side and the invagination is thus able to continue along the inferior (ventral) side of the optic stalk, so that a groove forms, along which the optic nerves and blood vessels subsequently pass. The gap in the ventral wall of the optic cup is the **choroid fissure** (*Text-Figure 69, Plate 107*). By the time the optic cup has formed, regional differences are already marked within it.

The lens is formed from the lens placode (*Plate 28*), a thickening of the ectoderm formed in response to an inductive signal from the optic cup. It is first visible at about stage 12 (Bancroft and Bellairs, 1977) and invaginates at about stage 14 to form the lens vesicle (*Plates 81, 100*), which sinks beneath the surface of the ectoderm, the latter becoming the cornea. By about stage 18 (3 days) the wall of the proximal side of the lens vesicle has greatly thickened, whereas the wall at the distal side has become thinner. The thickening is brought about by an increase in the length of the individual cells that stretch across the entire thickness of the walls, a band of nuclei being visible within the thickening (seen as a dark band in *Plate 145b*). A high mitotic rate at the periphery of the lens results in its continuing growth. By day 4 the lens cavity has been obliterated and at the same time the lens has begun to acquire its characteristic, lentoid, shape. Both the lens and the optic cup require the function of the gene Pax6 to develop (Canto-Solev and Adler, 2006).




***Text-Figure 69.*** *Development of the optic cup and lens. (a) Stage 14, longitudinal section through the developing lens and retina. (b) Stage 18, transverse section through the eye to show retina and developing lens. (c) Stage 18, frontal section through the eye to show the choroid fissure.*

As the lens continues to grow, the cells in the thickened region lose their ability to divide and become converted into fibres that will become the core of the adult lens. Fibroblast growth factor (FGF) signals appear to play a role in the conversion to fibres (Le and Musil, 2001). New fibres are formed from the cells at the periphery of the lens which divide rapidly and become arranged in concentric circles around the original core. By the time of hatching there are three concentric layers of fibres, the core (0.8 mm in diameter), the intermediate layer of irregularly arranged fibres, and the radial layers which continue to grow after hatching. The major lens protein is Δ-crystallin (not δ-crystallins as in mammals), though α- and β-crystallins can be detected when the lens placode has only just begun to form (Zwaan and Ikeda, 1966). The lens capsule, which is an extracellular material with a high collagenous component, starts to form about day 7. The ciliary body develops close to the lens, its role being to secrete the fluid of the vitreous chamber. It appears to be induced by lens FGF and BMP, each playing a critical role (Dias da Silva *et al.*, 2007).

As the lens loses contact with the ectoderm a space is formed, the **anterior chamber of the eye** (*Text-Figure 69*). Neural crest cells, which have reached the margin of the optic cup by stage 18, have migrated into the anterior chamber by stage 23 (Beebe and Coates, 2000). The corneal epithelium develops from the ectoderm covering the anterior chamber, whilst the corneal stroma forms from the mesenchyme and becomes visible on day 4 as a thin layer beneath the epithelium. It becomes thicker as mesenchyme cells migrate into it during day 7, and by the formation of Bowman's membrane from 11 days, followed by Decemet's membrane from about 13 days.

The **iris** arises from cells at the margin of the anterior chamber at about day 7. Removal of the lens results in disorganization of the components of the anterior chamber (Beebe and Coates, 2000).

The **retina** is formed from the optic cup. Its inner layer becomes the neural retina and its outer layer the pigmented retina. FGF8 is associated with the differentiation of the neural retina, and BMP7 with that of the pigmented retina (Vogel-Hopker *et al.*, 2000).

The **choroid** and **sclera** differentiate from the mesenchyme around the optic cup, forming the inner pigmented vascular layer, and the outer, fibrous layer, respectively. The melanophores of the choroid are derived from cells of the neural crest that reach the eye during day 2 and develop pigment on day 7. Cartilage starts to form in the sclera on day 8. On day 9 (stage 35–46), 14 papillae become visible on the conjunctival sclera; each is composed of a solid cord of epithelium and becomes associated with a condensation in the underlying mesenchyme, which is derived from the neural crest. By 13 days the papillae have disappeared and the condensations have become 14 scleral ossicles (*Plate 232*). Jourdeuil and Franz-Odendaal (2012), who produced a detailed map of the blood vessels of the eye, concluded that there was no correlation between blood vessel development and the patterning of the conjunctival papillae. The development of the sclera ossicles is described by Zhang *et al.* (2012).

The **eyelids** start to form at about 7 days (stage 31) from a circular fold of skin surrounding the eye which becomes modified to form the upper and lower eyelids. A semicircular fold within this circular fold becomes the nictitating membrane. The Harderian gland begins to develop on day 11 (stage 37) from epithelial cones on the conjunctiva (Niedorf and Wolters, 1978).

The **choroid fissure** usually begins to close in the region near the lens about day 4 (stage 23), though accounts vary. At this time a ridge of mesoderm, carrying with it a blood vessel, migrates along the choroid fissure into the posterior chamber of the eye and enlarges during day 5 (about stage 26) to form the **pecten** (seen at stage 29 in *Plates 160, 165*). Subsequently it becomes wrapped around by the ridges of the choroid fissure in the optic stalk and is totally covered by day 8. The pigment cells of the pecten are derived from the pigmented retina (Yew, 1978). The pecten is a structure characteristic of birds, and it is thought that it acts not only by bringing oxygen and nutritive materials to the eye but that it may also play a role in vision. Its development has been described by Uehara *et al.* (1990) (not seen).

The development of the pecten, the neural retina and the retinal pigment epithelium are all influenced by retinoic acid and *sonic hedgehog*. BMPs also play a role in the development of these structures. Adler and Belecky-Adams (2002) showed that overexpression of *noggin,* which binds several BMPs at the optic vesicle stages, led to microphthalmia, together with abnormalities of the retinal pigment epithelium and lens. Similar treatment at the optic cup stages resulted in extensive anomalies, including coloboma and pecten agenesis. BMP4 is expressed in the developing neural retina, where it appears to be responsible for apoptosis (Trousse *et al.*, 2001). The **optic nerve** is derived principally from the retina, its axons growing along the choroid fissure on their way to the brain (see p. 98).

The vitreous humour is secreted by the cells of the optic cup. For a general review of the molecular

events in the development of the vertebrate eye, including that of the chick, see Graw (2010).

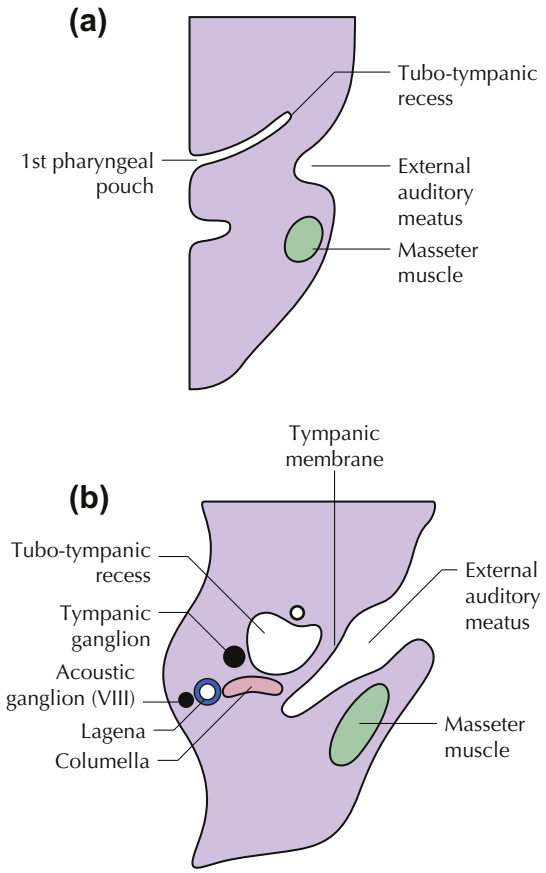
## The Ear

The **inner ear** is derived from the otic placode (*Plates 26, 27, 85*). The **middle ear** is formed from the first pharyngeal pouch, and the **outer ear** from the adjacent ectodermal groove. The ectodermal groove becomes the external auditory meatus (Eustachian tube) (*Text-Figures 53, 70*). The tissue between these two regions develops into the tympanic membrane.

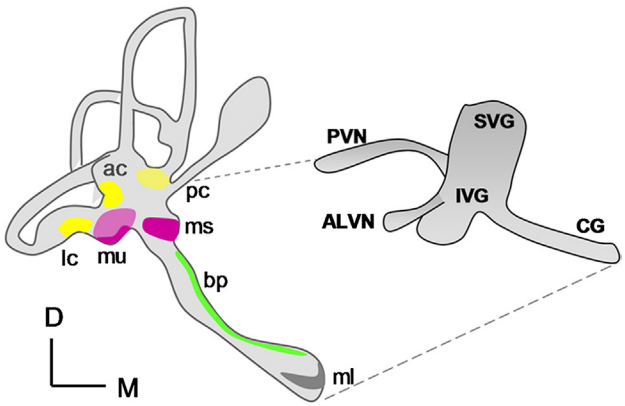
The left and right **otic placodes** are visible in embryos at about stage 10 (Bancroft and Bellairs, 1977) as thickened regions on either side of the head just anterior to the somites. Precursors for vestibular and auditory cells are already regionally segregated (Bell *et al.*, 2008). By stage 12 each has invaginated to form an otic pit, probably as a result of induction by FGF3 (Vendrell *et al.,* 2000), and by stage 14 the opening of the pit has become greatly restricted and the whole structure now becomes known as the **otic vesicle** or otocyst (*Plates 79, 89*). If the basal lamina associated with the otic placode is enzymatically disrupted, the placode fails to invaginate properly (Moro-Balbás *et al.,* 2000). The otic vesicle loses its communication with the outside of the embryo at about stage 18. The endolymphatic duct is a blind-ended outgrowth from the dorsal wall of the otic vesicle. The precursors of both the vestibular and auditory cells occupy different regions of the otic placode. The otic neurons are formed from the anterior medial region of the placode (Alsina *et al.*, 2004) and give rise to the cochlear vestibular ganglion (d'Amico-Martel and Noden, 1983). Bell *et al.* (2008) showed by the use of labelling experiments that the sensory organs and their nerves each formed from the same region of the otic placode and that the development of the neurons preceded that of the sense organs. Similarly, the differentiation of the vestibular system took place before that of the cochlea.

During days 5–7 the distal end of the endolymphatic duct (*Plate 119*) enlarges to form the **endolymphatic sac** (*Plate 157*), which grows above the brain and comes to lie at the dorso-lateral side of the myelencephalon. The remainder of the otic vesicle meanwhile has become loosely divided into a **superior chamber**, which will form the semicircular canals and utricle, and an **inferior chamber**, which will give rise to the saccule and cochlea. The development of the cochlea duct is, at least partly, controlled by rhombomeres 5 and 6 (Liang *et al.*, 2010).

The **semicircular canals** (*Text-Figure 71*) start to develop on days 4–5 as three grooves in the wall of the superior chamber, which each invaginate and close over to form a tube. The lumen of each tube is continuous




***Text-Figure 70.*** *Transverse section through the right middle and outer ear at (a) 4 days, (b) 12 days. (After Romanoff, 1960.)*




***Text-Figure 71.*** *Schematic diagram of the inner ear and its innervations at stages 30–31 (day 7). ac: anterior crista (yellow); bp: basilar papilla; lc: lateral crista (orange); mu and ms: utricular and saccular maculae (purple); pc: posterior crista. The anterior vestibular nerve (ALVN) innervates ac and lc; the inferior vestibular ganglion (IVG) projects to the maculae; the posterior vestibular nerve (PVN) to the pc; and the cochlear ganglion (CG) to the bp. SVG = superior vestibular ganglion. (From Bell et al. (2008), with permission of Elsevier.)*

with that of the superior chamber of the otic vesicle, which itself will become the utriculus. The anterior semicircular canal (which lies in the sagittal plane) forms first, followed by the external semicircular canal (which lies in the horizontal plane) and finally by the posterior canal (which lies in the frontal plane). The canals then move out from the main body of the otic vesicle, each carrying with it a thin fold of otic wall. By days 7–8 this thin sheet has perforated so that the canals remain attached to the otocyst only at the ends. Swellings which form along the canals during days 5–6 form the ampullae. There is some evidence that BMP2, secreted by the epithelium of the otocyst, is involved in the early stages of the formation of the semicircular canals and may affect the chondrogenesis of the otic capsule around the canals (Chang *et al.,* 2002). BMP4 is essential for normal development of the semicircular canals (Gerlach *et al.,* 2000).

The **saccule** begins to appear on day 7 as a swelling on the dorso-medial wall of the inferior chamber. The cochlea and the cochlear duct start to form during day 6 by growing out from the ventral region of the inferior chamber.

The **middle and outer ear** are formed from the first pharyngeal pouch and the surrounding wall of the pharynx (see Jaskoll and Maderson, 1978). The dorsal part of the first pharyngeal pouch gives rise to the tubo-tympanic recess, which later becomes the auditory (Eustachian) tube (*Text-Figure 71*). The **external auditory meatus**, which forms from the first ectodermal groove, is apparent by day 6 and further elaborated by day 12 when it then lies in juxtaposition to the tympanic cavity. The thickened region of the wall which separates them is the primordium of the tympanic membrane. Birds possess a single auditory ossicle, not three as in mammals. The cartilaginous predecessor of this bone, the **columella** (*Plate 161*), is present by 6 days and lies between the tympanic membrane and the cochlea, and the tubo-tympanic recess is directed towards it. Wood *et al.* (2010) have provided a detailed account of the columella at different stages. For a full description of the development of the middle ear and tympanum see Jaskoll and Maderson (1978).

The maturation of the tympanum layers is discussed by Chin *et al.* (1997). For a report on the changing sizes of the individual components of the inner ear between 10 days of incubation and adulthood see Cohen *et al.* (1992), and on patterns of chondrification and ossification see Cohen and Hersing (1993).

For an atlas on the gross anatomy of the chick inner ear see Bissonette and Fekete (1996). The neural relationships in the vestibular region of the 11-day-old chick embryo are described by Diaz *et al.* (2003).

## The Nose

The nasal (olfactory) placode (*Plates 27, 110*) becomes visible at stage 16 (Bancroft and Bellairs, 1977) as two thickened regions anterior to the eyes. Like the optic and otic placodes, each nasal placode invaginates, and it forms the **nasal pit** on days 3–4. A slight depression on the medial wall of each pit at about 4–5 days is thought to be the **organ of Jacobson**. Each nasal pit remains open to the surface of the head during its earliest stages of development, this opening eventually becoming the **external naris** (see Chapter 5). It becomes closed from about day 6, however, by the proliferation of epithelial cells forming a plug, and does not reopen until hatching, by which time the epithelial plug has degenerated.

The pits deepen, partly due to the bulging up of the ectoderm around the opening, forming the lateral and median fronto-nasal processes. The cavities of the nasal pits initially open into the oral cavity, but when the palate forms between days 5 and 7, the upper (nasal) part of the oral cavity becomes partly separated off from the lower part, and as a consequence the internal nares (*Plate 151*) become displaced to the posterior end of the mouth. In birds, however, the palatine processes do not fuse completely along the length of the **median palatine fissure** (*Plate 193*), even though they meet in the midline on day 4. Birds are therefore said to have a split palate.

Three **conchae** (turbinals) (*Plate 193*), the superior, middle and inferior, develop from the lateral wall of the nasal cavity and project into the lumen, the middle appearing on day 5, the superior at days 5–6 and the inferior (or vestibular) during day 7. The inferior turbinal is found only in birds, although not in all birds.

The lateral nasal glands form between days 8 and 14, starting as a solid mass in the septal wall of the nasal cavity as designated.