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The development of the human brain from a closed neural tube at stage 13* **

F. Müller and R. O'Rahilly
Carnegie Laboratories of Embryology, California Primate Research Center;
Departments of Human Anatomy and Neurology, University of California, Davis, California, USA

Summary. Twenty-five embryos of stage 13 (28 days) were studied in detail and graphic reconstructions of seven of them were prepared. Thirty or more somitic pairs are present, and the maximum is possibly 39. The notochord is almost entirely separated from the neural tube and the alimentary epithelium, and its rostral tip is closely related to the adenohypophysial pocket. Caudal to the cloacal membrane, the caudal eminence is the site of secondary neurulation. The eminence, which usually contains isolated somites, is the area where new notochord, hindgut, and neural tube are forming. The neural cord develops into neural tube without the intermediate phase of a neural plate (secondary neurulation). Canalization is regular and the lumen is continuous with the central canal. The neural tube is now a closed system, filled with what may be termed "ependymal fluid." The brain is widening in a dorsoventral direction. Neuromeres are still detectable. The following features are distinguishable: infundibular area of D2, chiasmatic plate of D1, "adult" lamina terminalis, and commissural plate (at levels of nasal plates). The beginning of the synencephalon of D2 can be discerned. The retinal and lens discs are being defined. The mesencephalic flexure continues to diminish. The midbrain possesses a sulcus limitans, and the tegmentum may show the medial longitudinal fasciculus. The isthmic segment is clearly separated from rhombomere 1. Lateral and ventral longitudinal fasciculi are usually present in the hindbrain, and the common afferent tract is beginning. Somatic and visceral efferent fibres are seen in certain nerves: 6, 12; 5, 7, 9–11. The first indication of the cerebellum may be visible in the alar lamina of rhombomere 1. The terminal-vomeronasal crest appears. Various cranial ganglia (e.g., vestibular, superior ganglia of 9, 10) are forming. The trigeminal ganglion may show its three major divisions. Epipharyngeal placodes of pharyngeal arches 2 to 5 contribute to cranial ganglia 7, 9, and 10. The spinal neural crest is becoming segregated, and the spinal ganglia are in series with the somites. Ventral spinal roots are beginning to develop.

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Offprint requests to: Prof. R. O'Rahilly, Carnegie Laboratories of Embryology, California Primate Research Center, Davis, CA 95616, USA

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Introduction

A review of stage 13 based on 26 embryos was published by Streeter (1945) and was revised by O'Rahilly and Müller (1987). The nervous system, however, has not been covered adequately and hence a thorough investigation will be presented here. Moreover, Streeter did not illustrate the whole of the neural tube. Furthermore, his example of a median section through the brain of No. 836 does not allow possible variations to be assessed. Some features of the brain have been prepared in computer-readable form (O'Rahilly et al. 1984, 1987). Although the focus here is on the brain, the phenomenon of secondary neurulation of the spinal cord is given the utmost attention because of the implications for possible malformations.

The earliest description of an embryo belonging to stage 13 is by Fol (1884a, b), who illustrated a specimen of 5–6 mm showing advanced features of the stage. Buxton (1899) published photomicrographs with little text of an embryo representing either an advanced stage 13 or a less differentiated sample of stage 14. Ingalls (1907) described a human embryo of 35 somites with painstaking accuracy and illustrated it with pictures of good tridimensional reconstructions. Beautiful pictures of Born reconstructions have been provided by Blechschmidt (1963, Plates 11 to 18) of a 4.2 mm embryo with probably 32 somites.

Stage 13 is characterized by the presence of 30 or more somitic pairs. Because the counting of somites is becoming increasingly difficult, however, their number is no longer used in staging. Four limb buds are present. Internally, lung buds have developed and the trachea is differentiating. The embryos are 4–5 mm in length, and the age is approximately 28 postovulatory days.

Material and methods

In Table 1 are listed the 25 embryos studied, as well as the details concerning embryonic length, stain, plane of section, and the enlargement of the graphic reconstructions. Most (23) of the embryos belong to the Carnegie Collection; the slides of No. 1954A and B are at the Anatomical Institute of the University of Basle. Three abnormal embryos were included in the study: No. 800 of the Carnegie

Table 1. Embryos of stage 13 studied

Embryo No.	Embryonic length (mm)	Magnification of reconstruction	Stain	Thinness of section (μm)	Plane
148	4.3	32	H.E.	10	Cor.
463	3.9		Al.coch.	10	Cor.
588	4.0	66.6	H.E.	15	Cor.
800	6.0		H.	10	Trans.
826	5.0		Al.coch.	20	Trans.
836	4.0	100, 66.6	Al.coch.	15	Trans.
1075	6.0		H.E. or G	20	Cor.
5541	6.0	63	Al.coch., E.	10	Trans.
5874	4.8	50	H.E.	10	Trans.
6473	5.0	50	Al.coch. & Ag	6	Cor.
7433	5.2	25	H.E.	8	Cor.
7618	4.8	63	H.E. & Ag	10	Cor.
7669	5.0		H.E. & Ag	6	Cor.
7889	4.2	39	H.E.	6	Cor.
8066	5.3	50	H.E. & Ag	8	Trans.
8119	5.3	63	H.E.	8	Trans.
8239	4.3		H.phlox.	8	Sag.
8372	5.6		Azan	10	Trans.
8581	4.8		Azan	8	Sag.
8967	5.7	63	H.E.	6	Trans.
9296	4.5	39	Azan	8	Cor.
9297	4.5	39	Azan	8	Sag.
D7805	5.0		Bodian	10	Cor.
1954A	5.0	100	Azan	6	Trans.
1954B	4.0	100	Azan	6	Trans.

Collection and embryo No. 1954B are examples of early anencephaly; No. 148 has fused nasal plates, which was not detected when it was first described by Gage (1905). For No. 5874 only bromide prints were at hand. Born reconstructions of three embryos were available. Graphic reconstructions of the whole neural tube were prepared from seven new embryos, using every or every second section. Furthermore, the caudal eminence of eight additional embryos was reconstructed graphically from photomicrographs of every section. In those same embryos, as well as in No. 8066, the mitotic figures of the different parts of the caudal eminence were counted in order to evaluate grades of activity in the area of secondary neurulation. The somitic count was not verified in all embryos. In the reconstructed specimens, 32 to 35 somitic pairs were present and 34 seems to be an average. High numbers are recorded for some embryos described in the literature: 35 pairs in the Ingalls (1907) and in the Hertwig embryo, Berlin; 39 for the R. Meyer embryo, Zürich. Because the occipital area can be particularly difficult in counting the four rostralmost somites, which are being transformed into the hypoglossal cord, differences in counting exist. For example, the number of somites for No. 836 was recorded as 30 by Streeter (1945) and as 32 by the present authors.

In order to delineate the different parts of the brain, the inner relief was reconstructed, which was especially important in identifying the isthmic segment. The external shape of the forebrain at stage 13 helps to localize the synencephalon (von Kupffer 1906). Brain and spinal cord were measured as a central axis from the rostral pole to the caudal eminence. The mesencephalic flexure was determined by using three points (Goodrum and Jacobson 1981): the floor of rhombomere 2, the midpoint of the floor of the mesencephalon, and the postoptic recess. In order to study fibre tracts and roots of spinal nerves, silver was added to five embryos (to one slide only in the case of No. 8066) by removing the cover-slips, bleaching, and applying the Bodian method.

Results

General. Photographs of the external form are available in Streeter (1945). In most embryos the head is bent towards the cardiac bulge and the whole embryo is U-shaped (Fig. 1). The caudal eminence is often bent to one side, so that reconstructions of the caudal region are more difficult. The surface ectoderm is still translucent, especially with formol fixation, so that features of the brain can be seen in the intact embryo (Streeter 1945, Plate 1, Figs. 10a and 16). Some 4 pharyngeal arches can readily be seen (Fig. 2), and the cervical sinus deepens at the lower level of pharyngeal arch 3, which forms the floor of a triangular area behind the second pharyngeal arch (Fig. 3C).

Premandibular condensation. This mass of mesenchyme derived from the prechordal plate can still be distinguished from that forming in the first pharyngeal arch (Fig. 3). Whereas the condensation in stage 12 was restricted to the area caudal to the optic vesicle, it now extends more rostrally, filling the gap between brain and optic vesicle (Fig. 3A). It is difficult to identify a bridge between the condensations of the two sides; topographically it should be located between the rostral end of the notochord and the adenohypophysis.

Notochord. The notochord is separated from the neural tube and the intestinal epithelium in all but the caudalmost area (Fig. 1). The hindgut, notochord, and neural tube are in

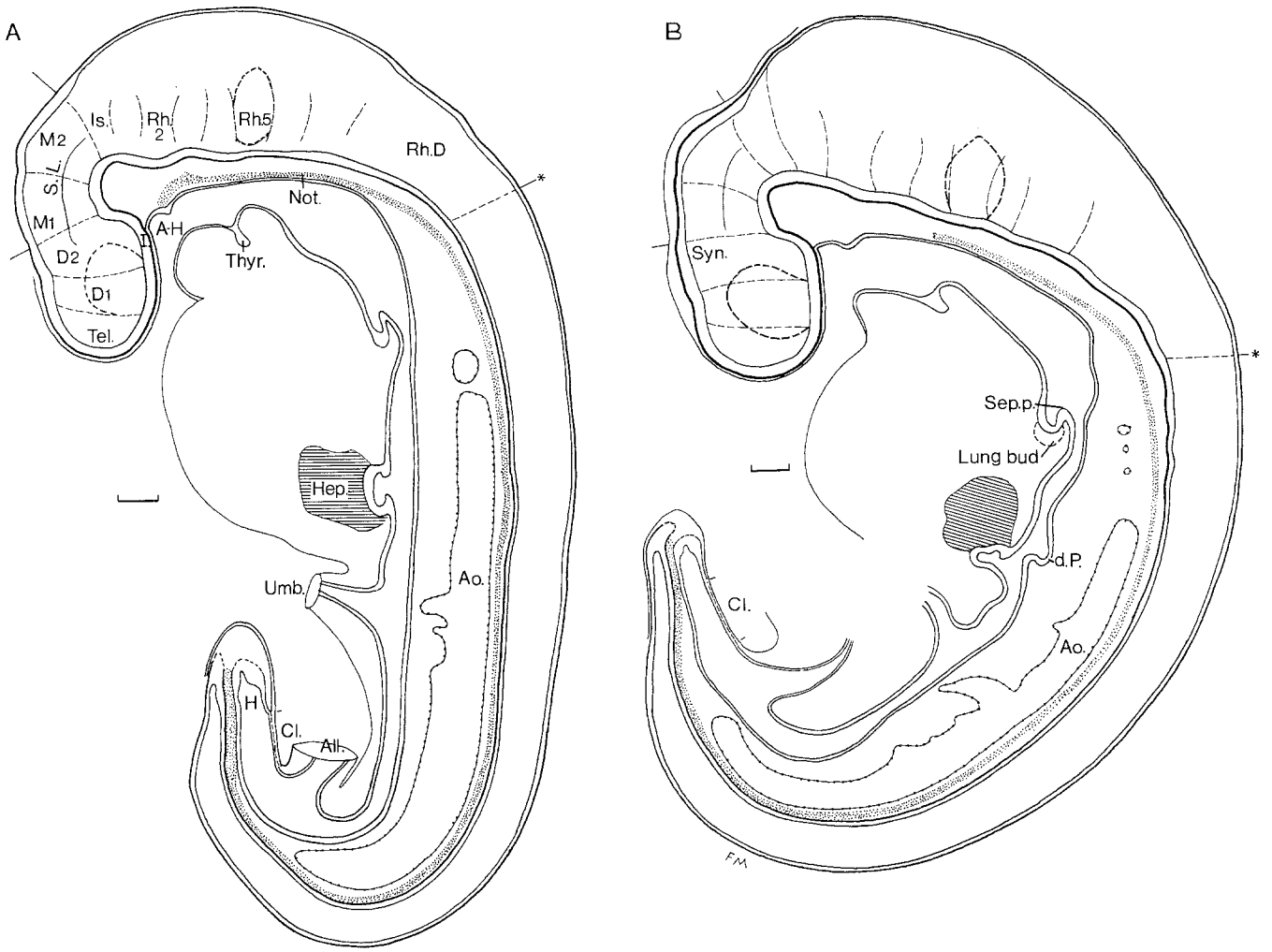

Fig. 1. Median sections of **A** embryo No. 8066 with 33, and **B** embryo No. 6473 with 34 somites. Characteristic features of the two embryos are: at the mesencephalic flexure, the head is bent towards the cardiac eminence; the three parts of the forebrain (*T, D1, D2*) are approximately equal in length; the mesencephalon has two segments; the rhombencephalon is increasing in height and possesses an isthmic segment. The adenohypophysial pocket is wide in both specimens. The digestive system is more advanced in **B**, where the dorsal pancreas is present. The separation point marks the site where respiratory and digestive tubes separate (O'Rahilly and Müller 1984b; Müller and O'Rahilly 1986b). The bars represent 0.2 mm

Abbreviations for Figs. 1–11: A-H, adenohypophysial pocket; *All*, Allantoic primordium; *Ao*, Aorta; *B.c.*, Boundary cap; *B.v.*, Blood vessel; *Cer.*, Cerebellum; *C 1*, cervical ganglion 1; *Ch.*, Chiasmatic plate; *Cl.*, Cloacal membrane; *Comm.*, Commissural plate; *C.sin.*, Cervical sinus; *D*, Diencephalon; *d.P.*, Dorsal pancreas; *End.*, Endolymphatic duct; *Ggl.*, Ganglion; *H*, Hindgut; *HC*, Hypoglossal cord; *Hep.*, Hepatic primordium; *I*, Infundibulum; *I.c.*, Internal carotid artery; *Int.-st.*, Interstitial nucleus; *Is*, Isthmic segment; *L.D.*, Lens disc; *L.L.*, Lower limb bud; *LLF*, Lateral longitudinal fasciculus; *L.T.*, "Adult" lamina terminalis; *M*, Mesencephalon; *Ma.*, Mamillary area; *M.D.*, Mesonephric duct; *MLF*, Medial longitudinal fasciculus; *N1*, Primary neurulation; *N2*, Secondary neurulation; *Nas.*, Nasal plate; *NC*, Neural cord; *N.Cr.*, Neural crest; *Not.*, Notochord; *Opt.*, Optic vesicle; *Ot.*, Otic vesicle; *Ph.*, Pharynx; *Ph.M.*, Pharyngeal membrane; *Pr.*, Mesenchyme of prechordal plate; premandibular condensation; *Rh*, Rhombomere; *R.D.*, Retinal disc; *Sep.p.*, Separation point; *s.*, Somite; *S.E.*, Somatic efferent; *S.L.*, Sulcus limitans; *S.P.*, Somitic plate; *Syn.*, Synencephalon; *U.L.*, Upper limb bud; *Tel.*, Telencephalon; *Thyr.*, Thyroid primordium; *Umb.*, Umbilical stalk; *VLF*, Ventral longitudinal fasciculus; *V.E.*, Visceral efferent.

Asterisk indicates borderline between brain and spinal cord inFigs. 1, 9, and 10. The plane and location of the sections in the photomicrographs of Figs. 8 and 11 are indicated in Fig. 10

close contact in the caudal eminence (Fig. 4), where most of the neural tube at this stage has formed through secondary neurulation and continues to form. Similarly notochord and hindgut seem to be derived from the undifferentiated most caudally-situated tissue. The rostral tip of the notochord extends towards the adenohypophysial primordium (Figs. 1A, 5C–F) but is probably always separated from the latter by some cellular strands that represent rests of the former prechordal plate. Sagittal sections of the rostral part of the notochord show a wave-like appearance (Fig. 5D), perhaps indicating a narrowing of space in the area of the mesencephalic flexure. Necrotic cells are present. A notochordal cellular sheath begins to form in the occipital area, as described by O'Rahilly and Müller (1984a).

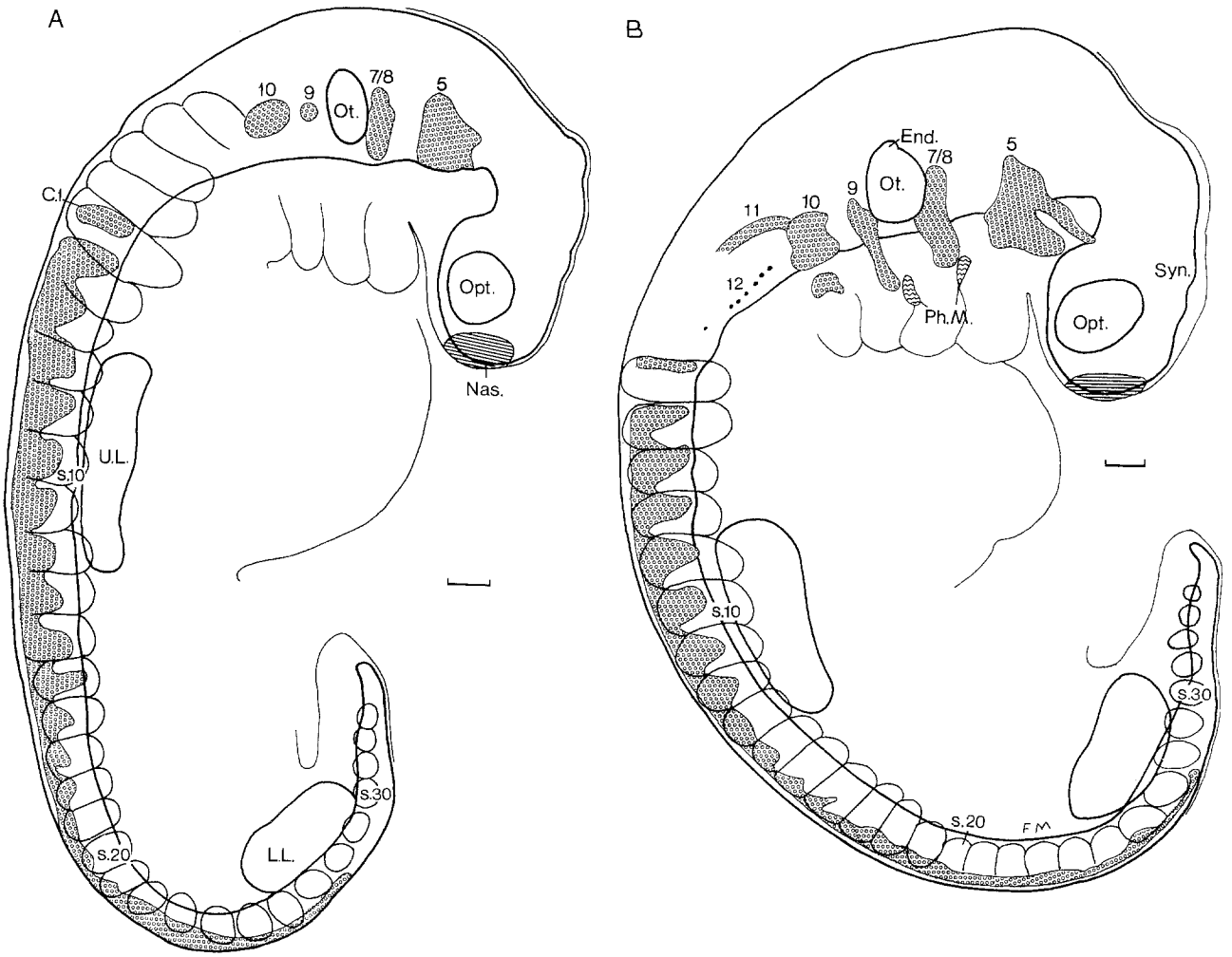

Fig. 2A, B. Lateral view of the embryos shown in Fig. 1, illustrating further typical features: upper and lower limb buds have formed; isolated somites are present in the caudal eminence; 4 pharyngeal arches can easiliy be seen; the neural crest of the trunk is beginning to segregate into ganglia, which are in series with the more laterally-lying somites. The slightly more-advanced embryo in **B** (No. 6473) possesses an endolymphatic duct, and the ophthalmic division is separating off from the trigeminal ganglion. The pharyngeal membranes are indicated. The neural crest material for the accessory nerve is shown. The bars represent 0.2 mm

Adenohypophysis. The adenohypophysial pocket is wide in many embryos (Fig. 1A, B), and the typical pocket representative for median sections in later stages is present in more advanced specimens of stage 13 (Nos. 588 and 1954A). Figure 5C shows how the two walls of the pocket wrap around the base of D2, which represents the infundibular area. The basement membranes of the rostral wall of the pocket and of D2 are in intimate contact. In sagittal sections, such as represented in Fig. 5F (an almost ideal median section), it is evident that the epithelium of the adenohypophysis, which consists of two cellular rows, is slightly thicker than that of the pharynx. Two branches of the internal carotid artery can be seen bilaterally in close relationship to the adenohypophysial pocket (Fig. 5C).

Caudal eminence. The caudal eminence lies caudal to the cloacal membrane (Figs. 1, 4). It contains somitic plate and in most cases one or more isolated somites. The somitic plates of both sides meet in the median plane in embryos of more than 34 somites (Figs. 4B, C, 6E). The caudal eminence in all embryos of stage 13 is the site of secondary neurulation. Furthermore, it is the area where new notochord is forming and the digestive tube is lenghtening. The high mitotitc activity to be expected there can be seen in Table 2, which shows the number of mitotic figures for the different areas of the caudal eminence. The highest activity is found in the somitic plate, the lowest in the notochord. No clear caudorostral gradient can be detected in individual embryos. A gradient is indicated, however, in the mean for caudal versus more rostral areas, and the activity in the caudal area seems to be lower. The number of developing somites in the caudal eminence varies from 0 to 5 pairs. In the cases represented in Fig. 4, regardless of the number of somites, the neural cord extends to the tip of the eminence and is in contact with the clearly delineated surface ectoderm. This relationship of somitic number to extent of the neural tube is represented schematically in Fig. 7.

The cloacal membrane lies opposite somites 30/31 (No. 6473), somites 31/32 (Nos. 588, 5874), somite 32 (No. 8066), or future somite 34 (No. 836).

Somites. The highest number of isolated somites in stage 13 is possibly 39 (R. Meyer embryo, Zürich, listed by Streeter

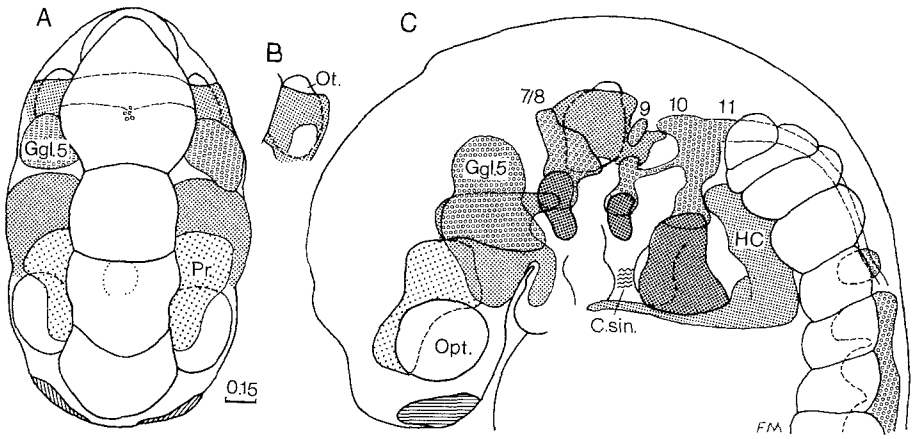

Fig. 3A–C Cephalic mesenchyme and pharyngeal placodes in No. 836 (32 s.). **A** Head-on view; **B** Isolated otic vesicle in a frontal view; **C** Lateral view. The premandibular condensation extends rostrally between forebrain and optic vesicle. The mesenchyme of pharyngeal arch 1, the otic sheet, and the hypoglossal cord are shown by light stippling. The mesenchyme of arches 2 to 4 is not indicated. The pharyngeal placodes of arches 2 to 4 are shown by heavy stippling. They form localized areas of highly active, dividing epithelium on the surface of the arches. Overlap with ganglia 7 to 10 is evident. The pharyngeal placode of arch 4 extends uninterruptedly to the area of arch 5. The cervical sinus caudal to pharyngeal arch 2 is indicated. Cranial ganglia 7, 9, and 10 are more complete than in stage 12, now possessing a superior part (indicated by circles) and consisting mainly of neural crest, and an inferior part formed by placodal cells. The otic vesicle is surrounded by the otic sheet in most parts. The white areas in **B** show (a) the future endolymphatic duct above and (b) the contact area with ganglion 7/8, where cells from the otic vesicle are being given off to the vestibular part of the ganglion

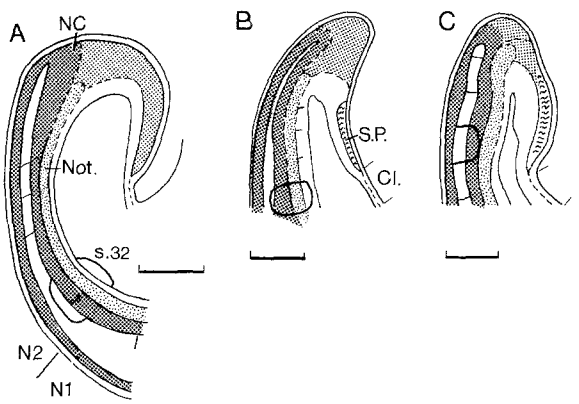

Fig. 4A–C. Reconstructions of the caudal eminence. **A** No. 836. **B** No. 7618. **C** No. 8119. The last isolated somite is marked by a rectangle. The caudal eminence comprising the material from the cloacal membrane caudally contains free somites in **B** and **C** only. The somitic plate extends further caudally and reaches the median plane. Three structures are derived from the undifferentiated mesenchyme of the caudal eminence and lack basement membranes where new material is being added: hindgut, notochord, and neural tube. The area of the definitive cloaca as part of the hindgut can now be delineated adjacent to the cloacal membrane

1945). Four of the reconstructed embryos have 34 pairs (Fig. 7). The occipital somites can still be reconstructed, although with difficulty, because they are being transformed into the hypoglossal cord (Fig. 3). However, even in this stage, sagittal sections show remains of somite 1 and some myotomic material. The epithelial character of the occipital dermatomes has become lost in three-fourths of the specimens. The cervical and thoracic somites are in series with the developing spinal ganglia (Fig. 2). The development of the caudal somites beyond the cloacal membrane has been mentioned.

Brain

Generalities. The brain for the first time is part of a closed system, the neural tube: the rostral neuropore closed in stage 11, the caudal in stage 12. The tube still shows some longitudinally arranged segments, the neuromeres. The limits between the segments are real, but only visible in a plane of section at a right angle to the slight furrows, i.e., coronal for the forebrain, horizontal for the rhombencephalon (Fig. 8A). It is clear, therefore, that the segmental organization cannot be clearly studied without reconstructions. The width of the neural tube is almost uniform in the brain area. Head-on views show that, with the exception of D1 and its evaginated optic vesicles/cups, the widest parts are forebrain and the rostral part of the rhombencephalon (Fig. 9A, C). The brain is bent at the mesencephalic flexure, which is a character in common with earlier stages but is now more pronounced. A striking feature in comparison with embryos of stage 12 is the dorsoventral enlargement of the rhombencephalon (Fig. 9B, D).

The relatively simple shape of the brain at stage 13 is matched by a still elementary histological organization of its walls. They are formed, in most parts, by the ventricular layer of pseudostratified neural epithelium. In more advanced areas, such as the rhombencephalon, a marginal layer had developed already in stages 11 and 12; formation of loose cellular areas (intermediate layer) in some distinct regions presages the formation of nuclei.

Forebrain. The telencephalon, mainly adjacent to the nasal plates (Figs. 2, 5E, 9), began its caudorostral extension in stage 12. It is of approximately the same length as D1 and D2 (Fig. 1) or slightly longer (Fig. 9D, Table 3). However, it is not wider (Fig. 9C). The differentiation of the olfactory area of the brain is indicated by increase in mitotic activity (Bossy 1980). The "adult" lamina terminalis and commissural plate can be distinguished by their topographical relationship: the commissural plate is the median area at

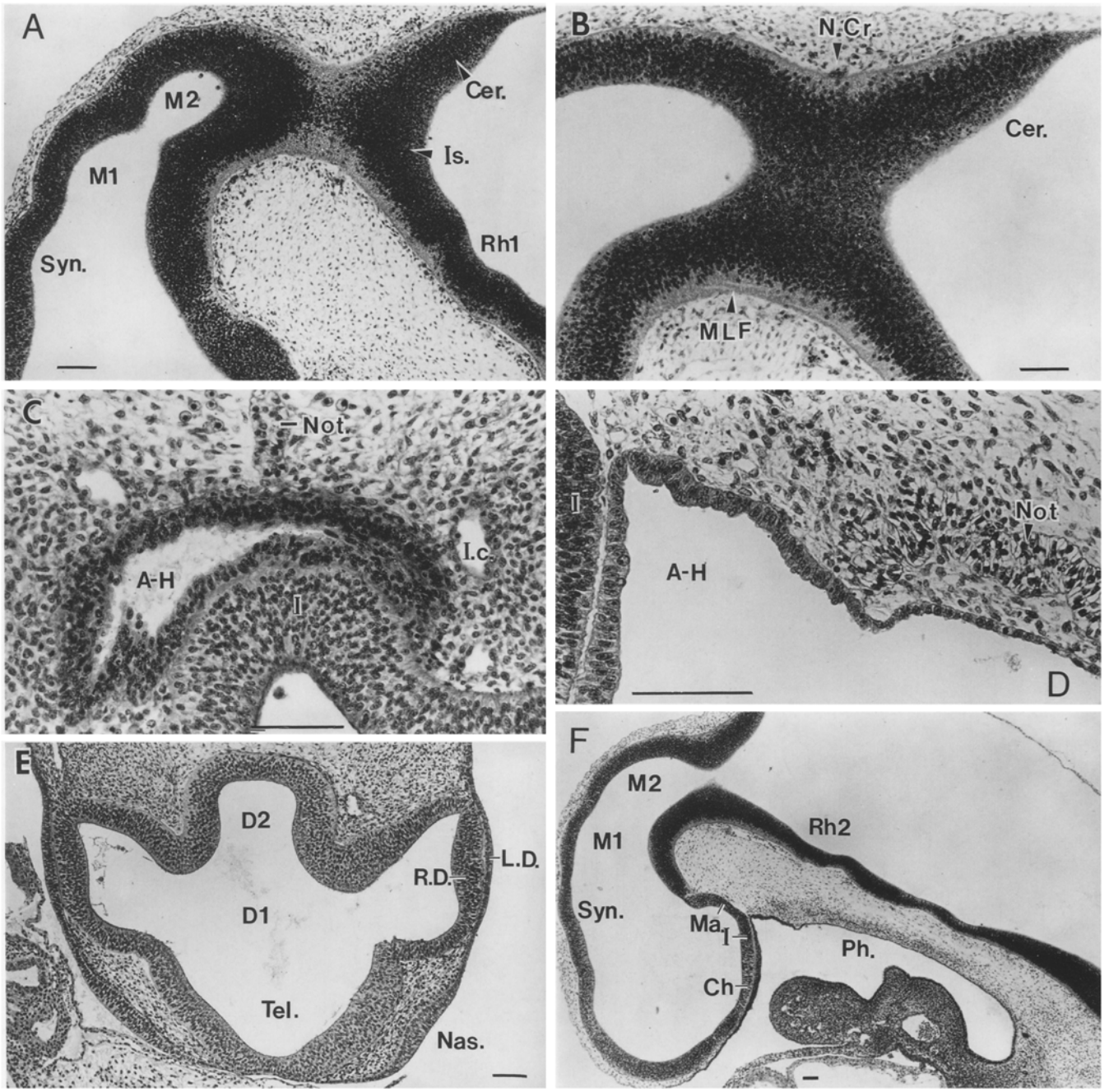

Fig. 5. A, B, D, and **F** are sagittal sections through embryo No. 9297 (33 s.). **A** (section 4-3-4) shows the synencephalon, M1, M2, and the isthmic segment. **B** (section 4-4-4) demonstrates neural crest at the level of the isthmic sulcus, and, in the basal marginal layer, some white neural fibres representing material of the medial longitudinal fasciculus. **C** (section 5-1-3 of No. 7889) is a horizontal section through the adenohypophysial pocket, from which the notochord (above in the picture) is separated by a few cells. The basement membranes of the rostral sheet of the pocket and of the infundibulum are close together; the epithelium of the pocket is denser than the wall of the infundibulum; 2–3 cell rows are present in the former, as seen also in **D** (section 4-4-8 of No. 9297). The notochord in this specimen is undulating in its rostral part. **E** (section 4-3-1 of No. 7889) shows the nasal plate adjacent to the telencephalon, the lens disc adjacent to the retinal disc in D1, and branches of the internal carotid arteries on both sides of D2. **F** (section 4-4-7) is an almost ideal median section showing the chiasmatic plate, infundibular and mamillary area, and synencephalon. The epithelium of the adenohypophysial pocket is clearly thicker than the pharyngeal epithelium. Scant and very delicate mesenchyme is visible between the dorsal surface of the brain and the surface ectoderm. The bars represent 25 μm

the level of the nasal plates (Müller and O'Rahilly 1984), whereas the "adult" lamina terminalis lies between the commissural plate and the chiasmatic plate. The latter is characterized by more widely-spaced cells (Fig. 5F) and belongs to D1, which is still closely related to the optic vesicle.

The infundibular area is a median part of D2. It lies adjacent to the adenohypophysial primordium, which becomes more distinct in stage 13 by formation of the adenohypophysial pocket (Figs. 1, 5C, D, F). The basement membranes of the rostral pocket-wall and the wall of D2 are

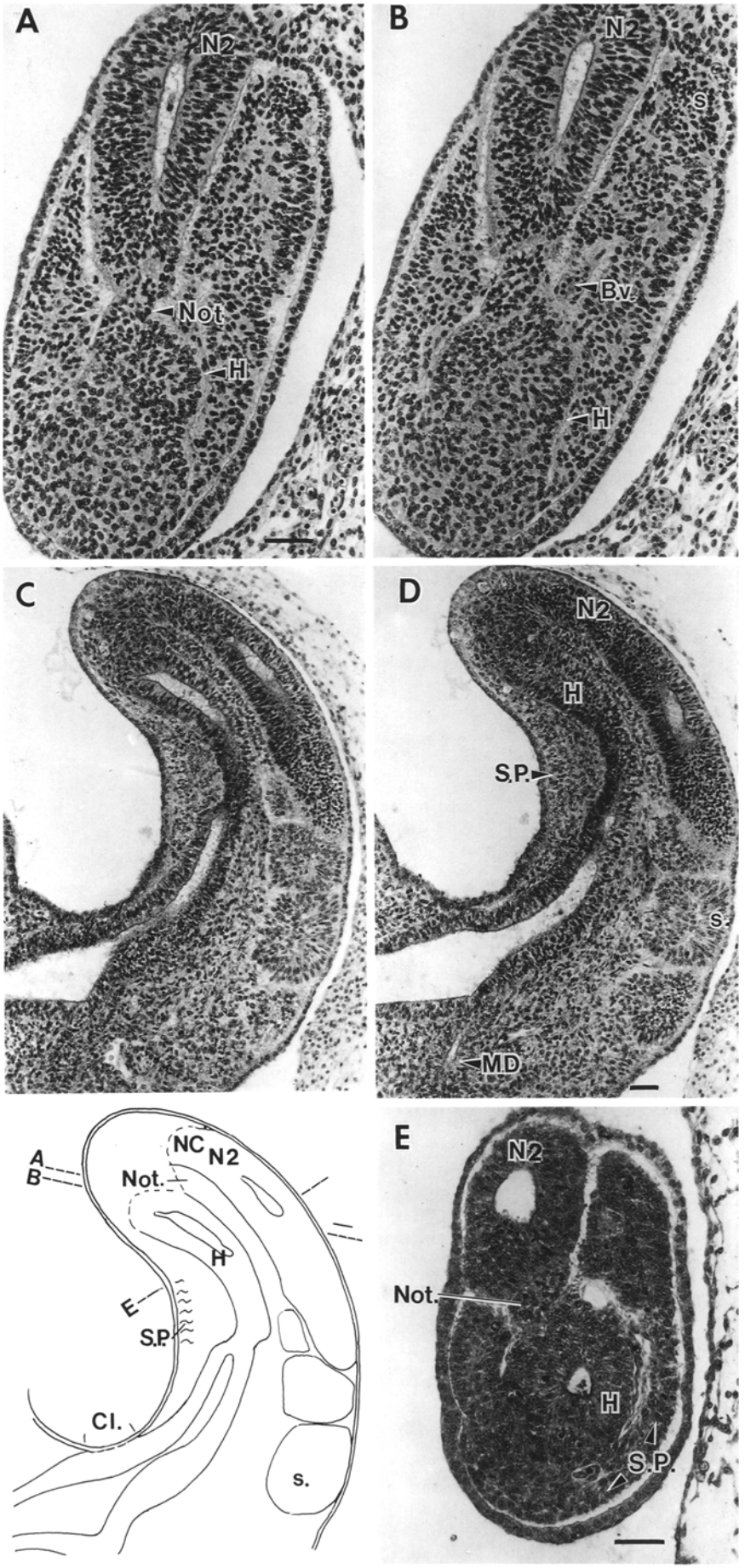

Fig. 6A–E. Secondary neurulation and caudal eminence in stage 13. The clearly delineated surface ectoderm indicates the area of secondary neurulation. **A** and **B** Oblique sections through the caudalmost part of the caudal eminence of No. 7889. Approximate levels of the sections are indicated in the key drawing. In **A** (section 4-4-11) the notochord and neural tube are not separated from each other by a basement membrane. In **B** (section 4-4-9) the notochord, neural tube, and hindgut can be distinguished. Blood vessels begin to appear along the notochord. **C, D** Sagittal sections of No. 9297 (33 s.), **C** being labelled in the key drawing. The caudalmost area of the caudal eminence shows undifferentiated mesenchyme and, differentiating from it, neural tube, notochord, and hindgut. In **C** (section 1-3-5) the cloacal membrane and the cloaca are visible. The mesonephric duct is indicated in **D** (section 1-3-2); it enters at a more lateral level. The approximate level of **E** (section 8-3-8 of No. 8967, 33 s.) is marked in the key drawing. The somitic plates of both sides join ventral to the hindgut. The bar represents 50 μm

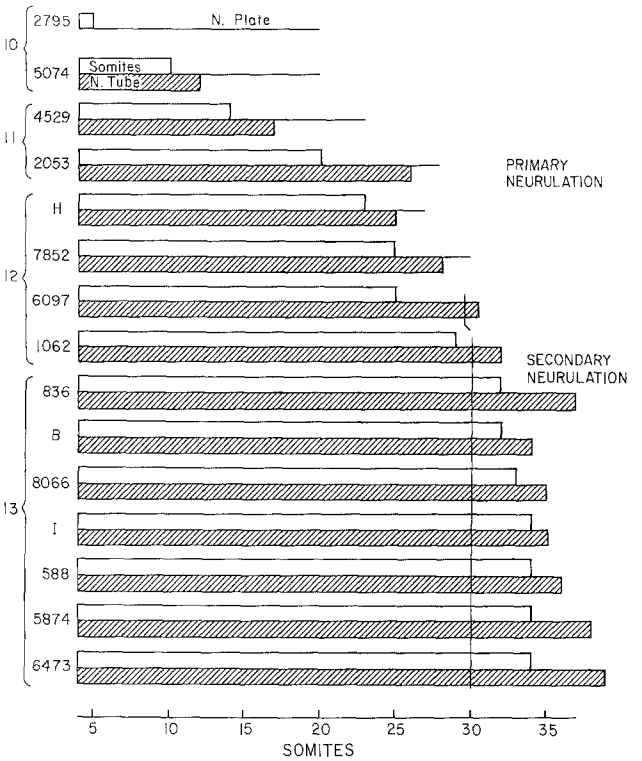

Fig. 7. Relationship of neural tube and somitic column in stages 10 to 13. The neural plate in early stages (9 and 10), the neural tube with the neural plate (stages 10 to 12), and finally the neural tube (13 and following stages) are always longer then the somitic column. The relationship, however, is not rigid. Embryos possessing the same number of somites (e.g., 34) have neural tubes of various lengths. The situs neuroporicus is marked at the level of somites 30/31, corresponding to level of future vertebrae S1/S2. Embryos possessing 34 somites at stage 13 need to form 4 or 5 more to build up the whole complement for a vertebral column characterized by 4 or 5 coccygeal vertebrae. Hence the caudal eminence is not a "tail" but corresponds to the sacral area in stage 13. Primary neurulation, which lasts up to and includes stage 12, is now completely succeeded by secondary neurulation in the area caudal to somite 30, which corresponds to future vertebra S1. *H*, Holmdahl (1934) embryo; *B*, Blechschmidt (1963) embryo; *I*, Ingalls (1907) embryo

in close contact. Branches of the internal carotid arteries for the adenohypophysial primordium, together with branches of the same artery to the optic primordium, are the earliest arteries to the forebrain. The mamillary area caudal to the infundibular region (Figs. 5F, 9D) may possess a mamillary recess. Other recesses of the forebrain present in some embryos are the infundibular, postoptic, and preoptic. The roof of D2 shows a slight bulge, which indicates the synencephalon of later stages (Figs. 5A, F, and 9). The synencephalon of von Kupffer is the caudal part of D2 in which prerubrum and pretectum will develop in future stages. It can be seen best in head-on reconstructions (Fig. 9C) and in sagittal sections (Fig. 5A, F). Signs of differentiation in the prerubral area can be seen in the development of fibres forming the medial longitudinal fasciculus (Fig. 10), and these are derived from the interstitial nucleus. Those fibres, present in seven embryos, may already reach the isthmus rhombencephali.

Mesencephalon. Two segments are present in the midbrain from stage 12 onwards, and they last until approximately stages 15/16. The sulcus limitans, which reaches almost as far as the optic sulcus in stage 12, is now more restricted (Figs. 1, 9). A marginal layer forms in the tegmental area at stage 13 (Fig. 5A). The nuclei of cranial nerves 3 and 4 can be distinguished from each other (O'Rahilly et al. 1984, Fig. 1) but no nerve fibres are yet present in either nerve. The limit between mesencephalon and rhombencephalon, the isthmic sulcus, is not well marked in stages 12 and 13, except in lateral sagittal sections, such as shown in Figure 5A, B. The medial longitudinal fasciculus is forming in the tegmentum in half of the embryos of stage 13. The fibres start from cells of the interstitial nucleus (Fig. 10A, B), which lies partly in D2 and partly in M1. Some of the fibres are visible in Figure 5B, where they appear as white lines basally in the marginal layer.

Rhombencephalon. Most embryos of stage 12 possess 8 rhombomeres, whereas in stage 13 a new segment is added. It lies between the midbrain and Rh1, and was named the isthmic segment by His (1893). This segment is clearly separated from Rh1, especially at the ventricular surface. It can be seen in Figures 1, 3, 5, 9 and 10. The characteristic expansions of the floor of Rh2, 4, and 6 (Fig. 10A, B) still help in identifying those neuromeres. In addition, ganglion 7/8 characterizes Rh4, cranial ganglion 9 Rh6, the vagal ganglion Rh7, and the otic vesicle Rh5. The marginal layer expands to the alar plate (Fig. 11D–F).

The first fibre tract of the brain began to form in the rhombencephalon of stage 12 from unipolar cells representing the neurons of the nucleus of the lateral longitudinal fasciculus (Müller and O'Rahilly 1987, Fig. 5B). This nucleus is more evident in stage 13 (Fig. 10C). The loosly arranged cells extend slightly into the alar plate; their fibres run along the level of the sulcus limitans, slightly ventral to the exit of the motor fibres that will be described below. The lateral longitudinal fasciculus extends from M1 to Rh3 in No. 6473 (Fig. 10A), from the isthmic area to RhD in No. 8066 (Fig. 10B). Fibres of the ventral longitudinal fasciculus are present in most embryos of stage 13. The bundle forms from unipolar cells that are situated ventral to the nucleus of the lateral longitudinal fasciculus (Fig. 10C) in the area that will become the reticular formation in later stages. Most of the fibres run ventrally in the marginal layer, and, in more occipital areas, some cross to the other side, forming the ventral commissure (Fig. 8C). Some of the fibres running ventrally to the ventral longitudinal fasciculus are shown in a projection to the lateral wall in Figure 10A. In general they accompany the intramedullary fibres of the visceral efferent nerves. A fasciculus-like formation is formed by the spinal part of the accessory nerve. However, the fibres, still accompanied by neural crest cells, are clearly extramural while ascending from the cervical area (Fig. 10A). A few afferent fibres of the ganglia of the cranial nerves begin to form the common afferent tract, which contains descending fibres of nerves 5, 7, 9, and 10. It will change name at the time that the tractus solitarius will be separating off in later stages. The vestibular fibres then will form the vestibulospinal tract, and the trigeminal fibres will form the trigeminospinal tract. In the transverse sections of No. 8066 the fibres are quite long for the trigeminal ganglionic cells, rather short for nerves 9 and 10, and intermediate for nerve 7.

The rhombencephalon of stage 13 is further characterized by the development of the motor component of the

Table 2. Mitotic figures in the caudal eminence in percentages

Specimen No. and area	Surface ectoderm	Somitic plate and somites	Neural tube	Notochord	Digestive tube
588					
caudal	20.8	24.5	10.2	1.4	6.9
rostral	8.3	17.6	7.4	0.5	2.3
total	29	42	17.6	1.9	9.3
836					
caudal	7.5	39	11.7	1.0	8.8
rostral	3.6	14.8	3.9	0.8	8.8
total	11.2	53.8	15.6	1.8	17.7
7618					
caudal	6.8	11.8	6.5	0.9	6.2
rostral	7.4	19.8	6.8	1.8	12.1
total	14.2	31.7	13.3	2.7	18.3
7889					
caudal	15.3	4.3	12.3	0	0
rostral	12.3	25.8	20.3	2.5	7.4
total	27.6	30	32.5	2.5	7.4
8066					
caudal	3	23.3	3.4	0.7	7.7
rostral	6	38	9.2	0.5	8
total	9.2	61	12.6	1.2	15.8
8119					
caudal	5.2	16.2	11.4	0	1.9
rostral	5.7	32.9	16.7	1.4	8.6
total	10.9	49	28	1.4	10.5
8372					
total	17.8	30.9	37.4	1.7	12.2
8976					
caudal	8.9	13.5	11.3	2	3.2
rostral	9.2	28	15.6	2	6
total	18	41.5	27	4.3	9.2
mean of 7					
caudal	9.6	13.5	11.3	1	3.2
rostral	9.2	28	15.6	1.4	6
mean of 8 total	17.3	42.5	23	2.2	12.8

cranial nerves. The intramural fibres of nerve 12 begin to show already in the most advanced embryos of stage 12 (Müller and O'Rahilly 1987). By now the nerve fibres reach the occipital somites (Fig. 8E). As can be seen in Figure 10A and B, those hypoglossal roots are not strictly segmental: some somites receive two roots. The fibres are accompanied by cellular strands already present in stage 12 and probably representing neural crest cells. The nucleus of nerve 12 can be seen in Fig. 8D (Cf Fig. 10D). As is the case also for nerve 6, the cells of the somatic efferent nuclei have a more lateral position than those of the visceral efferent nuclei of nerves 5, 7, and 9 to 11 (Fig. 10D). In the case of the hypoglossal nucleus (Fig. 8D), nerve fibres of several unipolar neurons seem to unite to form one single fibre, which then leaves the wall; or the fibres may unite extramurally. The intramedullary (intramural), visceral efferent fibres of the cranial nerves are projected onto the lateral wall of the rhombencephalon in Fig. 10A; they can be seen in coronal sections of the same embryo in Figs. 8B (glossopharyngeal), 8C (vagal and accessory), and 11C (facial nerve). The fibres arise from motor cells that are situated closer to the median plane than the hypoglossal nucleus (Fig. 10D). They ascend in the marginal layer and leave the rhombencephalic wall at the level of the sulcus limitans. The nerve fibres projected onto Rh5 in Figure 10A are believed to belong to nerve 6 (which has no extramural fibres at this stage). All those motor fibres are accompanied by fibres of unipolar cells that occupy the area of the future reticular formation (Fig. 8B) and which form the ventral longitudinal fasciculus. The extramural fibres of nerve 11 (spinal part) are the longest. They are visible as a longitudinal bundle extending from the cervical area to Rh7, and joining in this area the extramural fibres of the cranial part of nerve 11 (Fig. 10A). Neural crest of the spinal part of nerve 11 is shown in Fig. 2B.

Cerebellum. The first indications of the cerebellar primordium can be seen in the alar lamina of Rh1 in most specimen of stage 13 (Fig. 5A, F). Loosly arranged cells begin to constitute its intermediate layer (Fig. 5A).

Optic vesicle. The wall of the optic vesicle related to the surface ectoderm becomes flattened (Fig. 5E) and forms the retinal disc. Concomitantly the lens disc becomes evident.

Growth of the brain. The percentages of longitudinal measurements of ten specimens belonging to stage 13 (Tables 3, 4) indicate the following: (a) the brain still occupies 40% of the whole neural tube, as was the case in stage 12; (b) the proportions of the individual parts are almost the same as in stage 12. However, at the level of the rhombencepha-

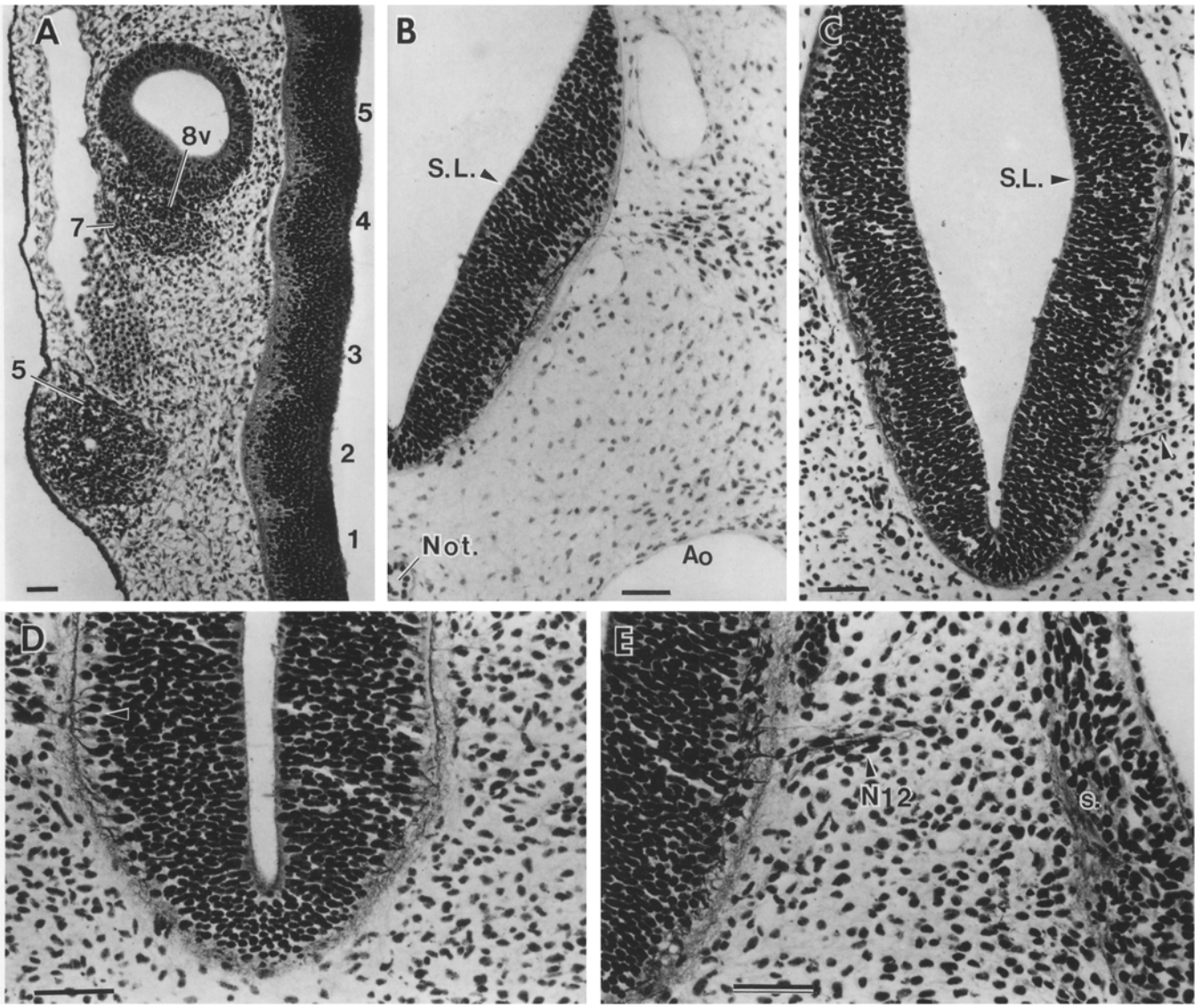

Fig. 8A–E. Cranial nerves in stage 13. **A** (section 2-2-8 of No. 8066) shows the trigeminal ganglion with its surface ectoderm giving off placodal cells, and the otic vesicle in the area where cells are being contributed to the vestibular ganglion. The large vessel is the head vein. Rh1 to 5 are clearly indicated. **B** (section 3-4-3 of No. 6473) is at the level of the glossopharyngeal nerve. Cells of the superior ganglion are visible. Some nerve roots leaving at this level are motor fibres of nerve 9, and these can be followed back along the marginal layer to a ventral area in the section. **C** (section 4-1-12 of No. 6473) is at the level of nerves 10, 11, and 12. Motor fibres of the hypoglossal nerve are seen leaving the basal plate ventrally (*arrowhead*). Some fibres opposite the sulcus limitans and leaving the brain are accessory and vagal efferent fibres. **D** (section 4-3-3 of No. 6473) shows the hypoglossal nucleus formed by unipolar neurons. **E** (section 4-3-5 of the same embryo) shows the hypoglossal nerve root to somite 3. The nerve fibres are accompanied by neural crest cells. The somite has lost the epithelial nature of the dermatome, but clearly shows some myotomic structure. The levels of all sections are indicated in Figure 10. The bar represents 50 μm

lon, differences from stage 12 include: (1) the newly added isthmic segment occupies 4% of the rhombencephalon and presents a relative lenghtening of the rostral part of the rhombencephalon (RhA of earlier stages); (2) Rh4 becomes further reduced, from 13% in stage 11 to 6% in stage 12 to 4% in stage 13. A gradual decrease in the total length of the rhombencephalon and a slight increase in the length of the spinal cord are apparent in Table 3. Lateral expansion of the brain occurs mostly in Rh2, which is slightly wider than the prosencephalon (without optic vesicles, Fig. 9). The most striking difference in comparing the rhombencephalon with that of stage 12 is its widening in dorsoventral direction (Figs. 1, 10B).

Mesencephalic flexure. The angle formed by the mesencephalic flexure (Table 4) continues to diminish: from a mean of 127° in stage 10 and 117° in stage 11, to 89° in stage 12 and 53° in stage 13. The decreasing angle is associated with a bending of the head towards the cardiac area and contributes to the U-shape of the whole embryo.

Neural crest and ganglia. The terminal-vomeronasal crest appears in stage 13 (O'Rahilly 1965). Cells leave the still convex or flat (Fig. 5E) nasal plate and form cellular buds (Bossy 1980). The adjacent olfactory area of the telencephalon is characterized by increased mitotitc activity (ibid.). Some crest material is present in the roof of the isthmic

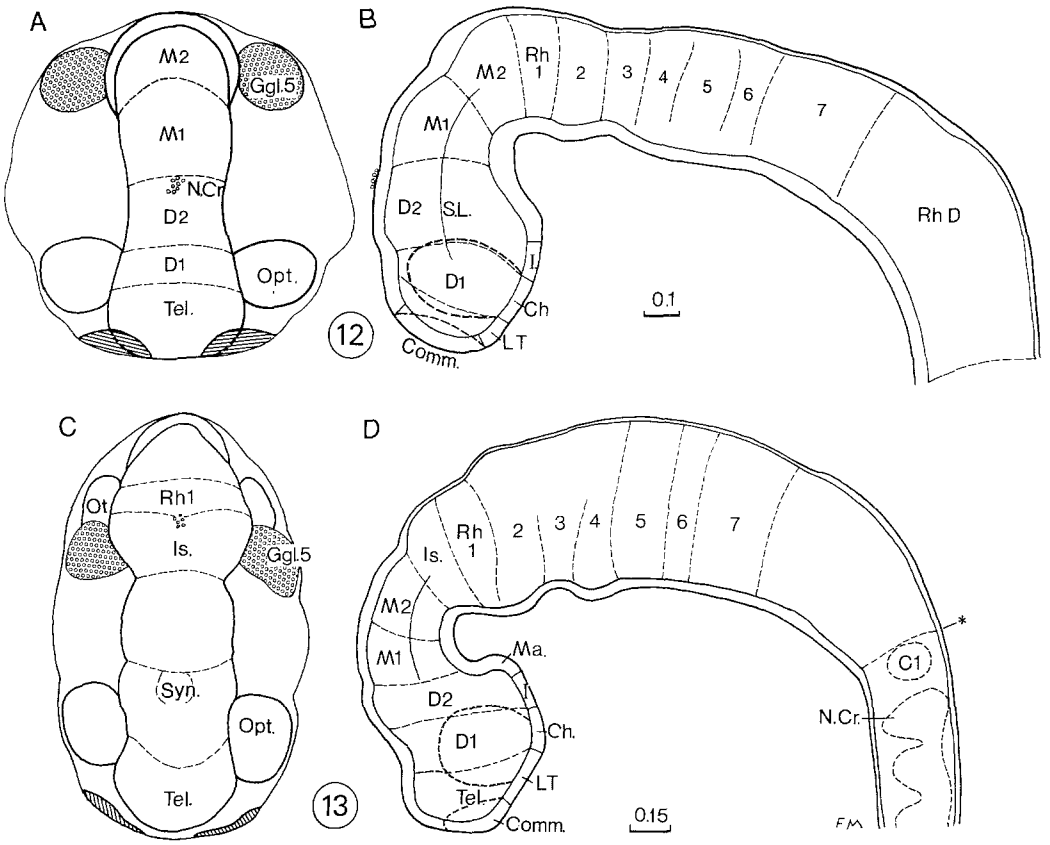

Fig. 9A–D. Head-on views combined with median sections of stages 12 and 13. **A** Head-on view of the brain of No. 6097 (25 s.), stage 12. **B** Median section of the same embryo. The brain is widest at the level of the telencephalon and Rh2. Neural crest material is present on the roof of D2. The optic vesicle is related mainly to D1. (Compare with **C** and **D**, where it extends more rostrally towards the telencephalic part of the brain.) **C** Head-on view of the brain of No. 836 (32 s.), stage 13. **D** Median section of the same embryo. The widest part of the brain is the isthmic segment. Some neural crest material can be seen between the isthmic segment and Rh1. The head-on view shows a slight bulge in D2: the synencephalic area. The median section shows the increasing height of the rhombencephalon. This and other examples indicate that the still-forming neural crest material develops irregularly in relation to the various neuromeres

segment, e.g., in No. 836 (Fig. 9B) and No. 9257 (Fig. 5B). Neural crest material is still forming in the caudal areas of the spinal cord. Cranial ganglia that were not completely formed in stage 12 were the superior ganglia of nerves 9 and 10. Both are present now, but the superior ganglion of the glossopharyngeal nerve is still not compact in many embryos (Fig. 8B).

Some of the ganglia of the cranial nerves still contain some cellular inclusions although, compared with stage 12, they are diminishing. In some ganglia, especially 5 and 7/8, a so-called "boundary cap" develops towards the brain wall (Fig. 11B). Cellular strands from brain wall to ganglion 7/8 as seen in stage 12 are no longer present; a clear marginal layer has developed instead. The trigeminal ganglion sometimes shows a beginning division into its future three divisions. The clearest is the ophthalmic, whereas distinction of the maxillary and mandibular divisions from the pharyngeal arch-mesenchyme is scarcely possible without special methods. Ganglion 7/8 contains a lighter, more differentiated part rostrally, and a darker part (possessing smaller cells) towards the otic vesicle (Fig. 8A). The latter part is still being added to from the otic vesicle, as indicated in Figure 3B. Nerves 7/8 and 9 show a difference between superior and inferior parts, the latter being more condensed (Fig. 11D, E). The accessory nerve, consisting of neural crest and nerve fibres, can be followed to the first cervical ganglion and further.

The spinal neural crest material, present as an almost continuous thick sheet and extending from somite 1 to approximately somite 18 in stage 12 begins to be segregated (Fig. 2). The developing spinal ganglia are clearly in series with the somites. Ganglion C1 is isolated and smaller than the following cervical ganglia. Most other ganglia are connected to each other dorsally (Fig. 2). Dorsal roots of the spinal nerves have not developed by stage 13. Ventral roots are clearly visible in more advanced embryos (No. 1954A: Müller and O'Rahilly 1984, Fig. 2) down to thoracic nerve 8. Less advanced specimens such as No. 836 have no ventral roots yet.

Otic vesicle. The otic vesicle now possesses such a well-developed otic sheath that it can be reconstructed (Fig. 3B). With the exception of the apex, which develops into the endolymphatic duct, the medial surface touching the wall of Rh5 and the contact area with the faciovestibular ganglion, the sheath surrounds the entire vesicle. The contact area with ganglion 7/8 is rostroventral. No basement membrane is present here, and cells are given off from the otic vesicle to the ganglion. Those cells contribute mainly to the formation of the vestibular ganglion, which forms a

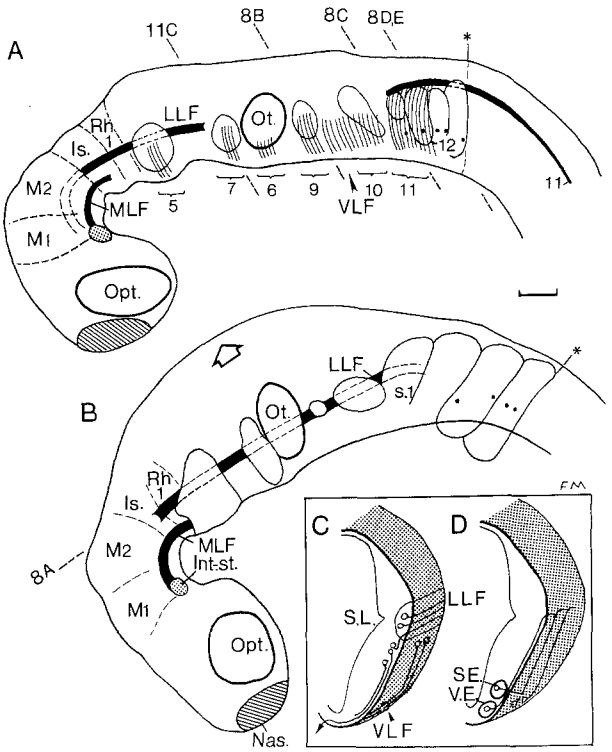

Fig. 10A–D. Development of the long fibre tracts and the motor fibres during stage 13. **A** Brain reconstructed from coronal sections (No. 6473) showing the intramedullary fibres of nerves 5 to 11 in a projection onto the lateral rhombencephalic wall. Some fibres of the unipolar cells forming the ventral longitudinal fasciculus are also shown in projection (*arrowhead*). The roots of the hypoglossal nerve with its extramural fibres are indicated by black dots. The accessory nerve possesses long extramural fibres that are accompanied by neural crest cells (cf Fig. 2B): those fibres begin in the spinal cord at low cervical levels. The medial and lateral longitudinal fasciculi are shown. The levels of the sections represented in Figs. 8 and 11 are indicated. **B** Reconstruction of the brain from transverse sections (No. 8066) showing the longitudinal tracts. The level of the section in Figure 8A is indicated. The height of the rhombencephalon has greatly increased in comparison with **A** (*white arrowhead*). The bar represents 0.2 mm. **C** Schematic cross-section showing the nucleus of the lateral longitudinal fasciculus opposite the sulcus limitans, with its cells forming the bundle. Unipolar neurons ventral to it begin to form the ventral longitudinal fasciculus (*arrowhead*). Some of those fibres cross to the other side, as the ventral commissure, before ascending. **D** Schematic cross-section showing the common nuclear sheet of motor neurons, which begins to separate into two cellular columns: the ventromedial column for visceral efferent nerves 5 to 11, the ventrolateral column for somatic efferent nerves 6 and 12. The fibres of nerves 5 to 11 pass along the marginal layer before leaving the neural tube at the level of the sulcus limitans

distinctive component in stage 13 (Fig. 8A). Its cells are darker and smaller. These two parts of ganglion 7/8 can be seen in more than half of the embryos. The endolymphatic duct arises in half of the specimens. A connection between otic vesicle and surface ectoderm can be seen in many embryos.

Neural crest, pharyngeal placodes, and cephalic mesenchyme. Placodal activity in stage 12 was limited to pharyngeal arches 2 to 4. No clear trigeminal placodal contribution was detected, and the surface ectoderm of pharyngeal arches 2 to 4 did not really form placodes in the sense of localized thickenings in the ventrorostral aspect of the arches. Epipharyngeal (so-called epibranchial) placodes are clearly present in stage 13 (Figs. 3B, 11D to F). The cells they give off in areas with an interrupted basement membrane (Fig. 11D, E, F) are constituent parts of the cranial ganglia. Epipharyngeal placode 1 contributes to the inferior part of the facial, placode 2 to the inferior ganglion of the glossopharyngeal, and placode 3 of arches 4 and 5 to the inferior ganglion of the vagus. Hence ganglia 7 to 10 clearly possess a proximal part formed from neural crest cells and a distal part contributed mainly by placodal cells. The neural crest component is formed in stages 10 to 13. The situation is more difficult to judge for the trigeminal ganglion. In stage 13, cells are clearly given off from the surface ectoderm (Figs. 8A, 11A) and they join the neural crest material formed in earlier stages. However, the ectoderm of the trigeminal area does not show the peculiarities of arches 2 to 4: its cells are not as high, and the area of cellular production is not a clearly restricted zone, as is the case for arches 2 to 4. The whole ectodermal surface covering the trigeminal ganglion produces cells that are added to the ganglion.

Cephalic mesenchyme. The premandibular condenstation (Fig. 3) penetrates rostrally between optic vesicle and D1. The cephalic mesenchyme is very scarce on the dorsal aspect of the brain (Figs. 5E, F), where few cells are present between surface ectoderm and brain wall. Thinning out occurs in the mesenchyme adjacent to the ventral parts of the brain (Fig. 11F), where the primary meninx is beginning to form (O'Rahilly and Müller 1986). Special areas of cephalic mesenchyme are seen in pharyngeal arches 1 and 3 in stage 13, where an especially dense core of cells is present in the ventralmost parts.

Secondary neurulation. Secondary neurulation begins at the time of closure of the caudal neuropore, which is in advanced embryos of stage 12 (Müller and O'Rahilly 1987). It involves differentiation of neural tube without the intermediate phase of a neural plate. At this stage the material of the neural tube, notochord, and hindgut forms a common, undifferentiated, mesenchymal mass best seen in horizontal (Fig. 6A, B) and sagittal (Fig. 6C, D) sections. The solid end of the neural tube, the neural cord, is well delineated towards the surface ectoderm, but possesses no basement membrane towards the mesenchyme and towards the caudalmost part of the newly-forming notochord (Fig. 4). No neural crest is present. With the exception of one embryo, the neural tube is single. An accessory lumen indicating a possible duplication seems to be present in a few sections of No. 8066. In all other cases the canalization of the newly-formed solid part of the secondary neural tube is regular, and its lumen is continuous with the central canal that had developed in stage 12. Figure 7 shows the lengthening of the neural tube with regard to the number of isolated somites. The neural tube in all cases is longer than the somitic column, although its length is not strictly proportional to that of the somitic pillar. Embryos of stage 13 possessing 34 somitic pairs display different lengths of neural tube.

Discussion

Notochord. The undulating form of the rostral part of the notochord reported here would appear to be different from

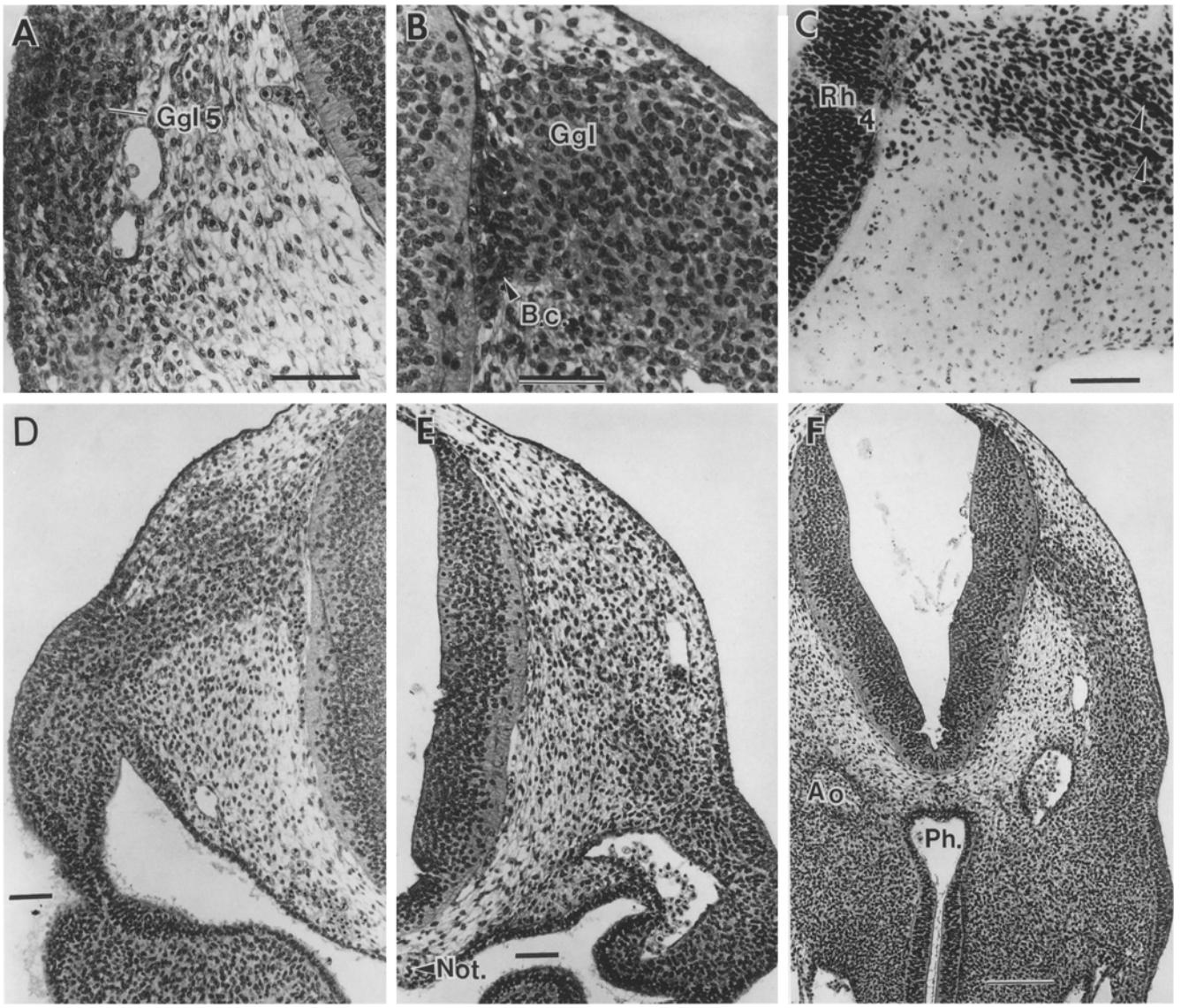

Fig. 11 A–F. Cranial nerves and pharyngeal placodes at stage 13. **A** (section 4-2-12 of No. 7889) shows the trigeminal ganglion and placodal activity in a cross-section. The marginal layer of the wall of Rh2 and adjacent blood vessels can be seen. The thinned out mesenchyme along the neural tube is evident. **B** (section 5-2-6 of the same embryo) shows the facial ganglion with its boundary cap and some of the wall of Rh4 with unipolar neurons. The boundary cap forms a dense cluster between ganglion and brain wall. **C** (section 2-6-10 of No. 6473) shows the facial ganglion with some cells possessing afferent fibres. Blood vessels along the wall are pial. The area of the nucleus of the lateral longitudinal fasciculus is clear and many nerve fibres can be seen in the marginal layer. **D** (section 5-4-12 of No. 7889) shows the two parts of the facial ganglion, the proximal consisting mainly of neural crest, the distal, denser, produced by the pharyngeal placode 1 of arch 2. The basement membranes of the surface ectoderm and of the pharyngeal epithelium are interrupted and both give off cells. **E** (section 7-3-5 of the same embryo) shows a loose proximal and a dense distal ganglion of nerve 9. The dorsal aorta is giving off aortic arch 3. The marginal layer of Rh6 reaches almost to the roof. **F** (section 7-2-8 of No. 7889) shows superior and inferior ganglia of nerve 10, pharyngeal placode of pharyngeal arch 4, and the transition from laryngopharynx to oesophagus. The dense mesenchyme in the ventral areas is in contrast to the loose zone of primary meninx around the brain. The bars represent 37.5 μm

die segmentalen Wellen der Chorda dorsalis as seen in the trunk. Claims that the latter flexures are artifactual have been denied (Verbout 1971).

Adenohypophysis. The primordium of the adenohypophysis can be located in stage 10 (Müller and O'Rahilly 1985) but its material is probably already localized in stages 8 and 9 (Couly and Le Douarin 1982, in the chick). Its development was summarized by O'Rahilly (1973, 1983a). From a comparison with stage 12 it seems that the adenohypophysial pocket is derived mostly from material that lies rostral to the oropharyngeal membrane. The term craniopharyngeal pocket, therefore, is not precise. How much, if any, pharyngeal epithelium is involved cannot be decided in a purely morphological study. Ferrand (1973) showed an exclusively ectodermal origin of the pouch in quail embryos. The contact between adenohypophysial ectoderm and D2 remains close during development, although mesoderm ac-

Table 3. Changing relationships between parts of C.N.S. at stages 9–13. Percentages of parts of C.N.S. relative to total length of neural plate/tube

Embryo	T	D1	D2	Pros.	M1	M2	Mes.	Is.	RhA	RhB	RhC	RhD	RhA–C	Total Rh	Spinal cord
Stage 9 (mean of 4 embryos)				13.5			6.3							62.5	
Stage 10 (mean of 13 embryos)				17.3			6.6		7.7	7.6	8.4	15.8	23.7	39.4	
Stage 11 (mean of 12 embryos)		4	4.4	8.4			4.5		7.7	4.6	8.2	13.4	20.2	33.6	52.2
Stage 12 (mean of 7 embryos)	2	3.2	3.2	8.4	2.6	2.9	5.5		5.4	2	7.6	12.9	15	28	58.4
Stage 13 (mean of 10 embryos)	2.8	2.3	2.3	7.4	2.9	3.2	6.2	2.5	8.8	1.7	7.2	8.7	18	26.4	59.7
588	2.9	1.8	1.8	6.5	3.3	2.6	5.9	1.3	8.4	1.6	8.7	8.2	18.7	26.9	60.8
836	3.4	2.3	2	7.7	2.3	2.5	4.8	2	9.2	1.5	5.2	9.2	15.9	25.0	61.6
1954A	2.9	1.7	1.9	6.5	3.6	3.1	6.7	1.1	9.2	2.1	5.5	9.9	17.5	26.7	60.1
5874	2.6	2.6	2.6	7.8	2.5	2.9	5.4	1.4	9	1.8	8.5	9.5	19.3	28.7	58.1
6473	1.9	2.2	1.6	5.7	2.4	3.6	6	2.2	8.5	2.3	9	5.3	19.8	25	63.2
8066	2.7	2.3	2.3	7.3	2.9	3.5	6.4	1.6	7.4	1.4	7.2	8.2	16.0	24.2	62.2
Fol (1884)	3.7	2.3	2.1	8.1	3.3	4.2	7.5		7.9	1.6	6.1	7.7	15.6	23.3	61.0
Ingalls (1907)	2.9	2.9	2.6	8.4	1.6	2.2	3.8		8.9	1.3	7.7	10.9	17.9	28.8	59.1
Holmdahl (1934)	2	2.5	3.4	7.9	4.7	5.2	9.9	2.7	11.0	2	4.9	8.4	17.9	26.4	56.0
Blechschmidt (1963)	2.5	2.7	2.7	7.9	2.7	2.9	5.6		8.6	1.8	8.8	9.7	19.2	28.9	55.6
146	2.6	2.8	3.2	8.6	2.4	2.4	4.8	4.4	10	2.8	6.4	8.2	19.2	27.4	59.2

Table 4. Growth of the brain in stages 9 to 13

Embryos	Percentage of neural tube occupied by brain	Percentages of parts of brain relative to total length of brain											Total Rh	Angle of mesenc. flex.
		T	D1	D2	Pros.	M1	M2	Mes.	Is.	RhA	RhB	RhC	RhD		
Stage 9 (mean of 4 embryos)	82.3				16			7.7						75.8	
Stage 10 (mean of 10 embryos)	63.3				27			10		12	12	13	24	61	127
Stage 11 (mean of 14 embryos)	48				19			10		15	13	21	29	73	117
Stage 12 (mean of 15 embryos)	41.5	4.8	7.9	7.6	20.2	8	6.7	14.7		15	5.9	18.9	27.5	66.5	89
Stage 13 (mean of 10 embryos)	40.3	6.8	5.8	5.7	18.3	7.2	8.1	15.3	4.0	21.8	4.3	17.8	21.5	65.5	53
588	39.2	7.4	4.5	4.5	16.4	8.2	6.6	14.8	3.3	21.4	4.1	22.2	21	68.7	56
836	38.9	8.9	5.9	5	19.8	5.9	6.3	12.2	5	23.6	3.8	13.5	23.6	64.5	72
1954A	39.9	7.1	4.3	4.8	16.2	9	7.6	16.7	2.9	22.9	5.2	13.8	24.8	66.7	45
5874	41.9	6.1	6.1	6.1	18.3	5.9	6.8	12.7	3.3	21.5	4.2	20.3	22.6	68.6	65
6473	36.8	5.3	5.9	4.3	15.5	6.6	9.9	16.5	5.9	23	6.3	24.3	14.5	68.1	35
8066	37.8	7	6	6	19	7.6	9.2	16.8	4.3	19.6	3.8	19	21.7	64.1	44
Fol (1884)	39	9.5	6	5.4	21.9	8.3	10.7	19		20	4.2	15.5	19.8	59.9	14
Ingalls (1907)	40.9	7	7	6.3	20.3	3.9	5.5	9.4		21.9	3.1	18.8	26.6	70.3	63
Holmdahl (1934)	44	4.4	5.5	7.8	17.8	10.6	11.7	22.3	6	25	4.4	11	18.9	59.4	61
Blechschmidt (1963)	44.4	5.7	6.1	6.1	17.9	6.1	6.6	12.7		19.3	3.9	19.7	21.9	64.8	75
1954B		3.8	5.1	5.1	14	10.2	8.9	19.1		19.1	3.2	27.4	17.2	66.9	59
148	40.8	6.4	6.9	7.8	21	5.9	5.9	11.8	10.8	24.5	6.9	15.7	20	67.2	64

cumulates in more lateral areas. Indeed, the formation of a pocket in stage 13 seems to be related to this fact (Gilbert 1935), as has been shown also in birds (Jacobson et al. 1979). The importance of the mesenchyme for the development of the adenohypophysial pocket has been demonstrated in an experimental study of the rat, testing the effects of Streptomyces hyaluronidase, which affects mainly the mesenchymal component (Morriss-Kay et al. 1986): the pocket was absent in embryos exposed during 48 h, and the distance between the pocket and the rhombencephalon was reduced in embryos exposed for 3 h. In a human embryo 4.5 mm in length, Daikoku (1958) found the walls of the adenohypophysial pocket to consist of small cells arranged in several layers. In a human embryo of 3 mm described by Waterson (1926), the tissue on both sides of the pocket was "highly vascular." McGrath (1978) suggested that the control of the pharyngeal hypophysis is mediated through factors in the blood. Mitotic activity has been studied in the mouse (Wilson 1980) and in the rhesus monkey (Wilson and Hendrickx 1981): at stage 13 the contact area between hypophysial pouch and brain "was characterized by a thick PAS-positive layer consisting of the fused basement membranes." Seinsch (1976) found that the luminal epithelium of the adenohypophysial pouch in mouse embryos of 10 and 11 ET (approximately stages 12/13 to 14) was covered with "surface-coat," which probably has some impact on the shaping of the pouch. The inductive influence of the diencephalon on the development of the adenohypophysial pouch was shown experimentally in birds by Ferrand (1972): up to embryos of 25 somites (corresponding to approximately stage 12) the adenohypophysis does not develop without the influence of the hypothalamic floor.

A bridge of mesenchyme between notochord and the adenohypophysial pocket is present also in birds (Ferrand 1972), although the relationship between notochord and adenohypophysis is quite different in the two species.

Significance of the neural tube as a closed system. Once the neural tube has closed, its walls are subject to the pressure of the contained fluid, provided that formation of fluid is greater than absorption. The fluid, which is no longer in continuity with that in the amniotic cavity, is, in the absence of choroid plexuses, presumed to be formed by the lining cells and hence may be termed "ependymal fluid." The fluid may result in rostrocaudal enlargement and widening of the brain. Such a mechanism would be expected to contribute to the shaping of the neural tube and to preserving it from collapsing, although the main contribution to growth is from mitotic activity. In an abnormal embryo (No. 1954B) of stage 13 that possessed a widely open brain (Müller and O'Rahilly 1984), for example, the organ was only slightly smaller than in its twin. The influence of mitotic activity on growth of the brain in rat embryos was investigated by Morriss-Kay (1981): up to 15 somites (corresponding approximately to stages 8 to 11) mitotic spindles in the midbrain and hindbrain were almost always arranged parallel to the long axis of the embryo, so that each mitosis contributed only to longitudinal growth; in the forebrain the spindle orientation was predominantly in a transverse plane, allowing "increase in size of the forebrain."

Closure of the neural tube eliminates direct communication with the amniotic cavity and diffusion of α-fetoprotein into the liquor, except in "spina bifida aperta or when the fluids are separated by a membrane partially permeable to AFP" (Brocklehurst 1978).

Formation and significance of the neuromeres. The addition of a new neuromere in stage 13, the isthmic segment, corresponds in general to the observation in rat embryos by Tuckett et al. (1985), that neuromeres appear as individual entities and not from subdivision of previously formed segments. The neuromeres result from transverse bands of high mitotitc activity and are thought to have little or no significance beyond that (Källen 1953). Deol (1964), however, believed that the rhombomeres, in particular, have a significance of their own: based on experimental data of different authors and the development of Kreisler mice, he concluded that "the differentiation of the (otic) vesicle into a normal labyrinth depends on the influence of the neural tube." Van de Water et al. (1980) corroborated "that the inductive interaction occurring between the otic anlage and the neural tube is essential for the continuation of orderly labyrinthine development." Tuckett and Morriss-Kay (1985) proposed an alternative hypothesis, combining the impact of mitotitc activity with cytoskeletal components in neuromeric formation. If longitudinal growth generated by cell division in the longitudinal plane is prevented, bulges form. The shape of the bulges is maintained by the cytoskeletal components, such as microtubules and microfilaments. Nevertheless, "the mechanism underlying the generation and maintenance of neuromeric periodicity is not understood."

Forebrain. The lamina terminalis referred to in this study is the "adult" lamina. The embryonic lamina of stages 12–14 includes the commissural plate, which, when it becomes defined at stage 15, is omitted from the "adult" lamina. In other words, the embryonic lamina terminalis is "l'ensemble des formations comprises entre le chiasma optique et la paraphyse" (Bossy 1966).

The borderline between telencephalon medium and D1 is at the rostral border of the chiasmatic plate, which, in stage 14, becomes the site of the preoptic recess. Hence the lamina terminalis is telencephalic. The walls of the telencephalon medium already contain the future cerebral hemispheres, which will begin to evaginate in stage 14. Von Kupffer (1906) designated the three forebrain segments as telencephalon, parencephalon, and synencephalon, which correspond to the telencephalon medium, D1, and D2 of the present study. However, the synencephalon of later stages corresponds to a caudal zone comprising only the prerubral area with the interstitial nucleus and the pretectal region. The synencephalon was labelled mesencephalon by Streeter (1945, Fig. 7xiii) and by Bartelmez and Dekaban (1962, Fig. 45), but its clear morphological delineation depends on the appearance of the posterior commissure and the habenulo-interpeduncular tract in stages 16 and 17 (O'Rahilly et al. 1987, Fig. 6). The loose material of the chiasmatic plate in D1 is in connection with the hypothalamic cell cord, which is present in some embryos of stage 13 and in all of stage 14 (O'Rahilly et al. 1984, Fig. 1). The fact that the sulcus limitans of the mesencephalon no longer extends into D1 is important for the interpretation of the hypothalamic nuclei and the hypothalamic sulcus, which will be discussed with later stages.

Mesencephalon. The formation of the nuclei of cranial nerves 3 and 4 corresponds to the time found in the rat

by Altman and Bayer (1981). The nucleus of the trochlear nerve at this time lies caudally in the mesencephalon (O'Rahilly et al. 1984); later it will be related to the isthmic segment. The medial longitudinal fasciculus (Windle 1933, in the cat) arises from the nucleus of the same side and was considered to represent "the oldest longitudinal pathway" (Mesdag 1909, cited by Windle 1932). In cat embryos corresponding to approximately stage 13 it reaches the area of the trigeminal nerve (Rhines and Windle 1941). The interstitial nucleus from which the fibres are derived "lies in the basal lamina of the midbrain and in the basal part of the diencephalic wall at the cephalic flexure" (Windle 1932). Decussating fibres will be present at stage 17, when they will traverse the posterior commissure (O'Rahilly et al. 1987). Whereas the fibres in stage 13 are purely descending, some of those present in stage 17 are ascending fibres from the vestibular area, comparable to those in the adult.

Cerebellum. The cerebellum arises from the ventricular layer of the whole alar lamina of Rh1. A rhombic lip does not appear until later stages (O'Rahilly et al. 1987). According to Miale and Sidman (1961), the formation of cells for the cerebellum of the the mouse begins in embryos comparable to stage 13. In the rhesus monkey, the neurogenesis of the deep cerebellar nuclei lasts from days 30 to 70 (Gould and Rakic 1981), corresponding approximately to stage 13 and on into the fetal period.

Rhombencephalon. The isthmic segment was already described by His (1890, 1893) as a short, narrow, and laterally somewhat flattened part of the tube: "Am embryonalen Gehirn hebt sich der Isthmus als schmales Zwischenstück zwischen Mittel- und Hinterhirn sehr scharf von seinen Nachbartheilen ab." He pointed out that the term was first used for the adult brain by Ridly in 1695. The isthmic segment was seen also by Bartelmez and Dekaban (1962), although they did not label it in the figures of their reconstructions. The isthmus rhombencephali of the BNA is a "zone of transition" (Kuhlenbeck 1973) and "contains components which can be assigned to oblongata, cerebellum and mesencephalon."

Longitudinal fibre tracts. Of the two or three fascicles forming in the rhombencephalon, the lateral longitudinal appears first. Windle (1932) believed that its dorsal part (rostral to the trigeminal nerve) presents the anlage of the mesencephalic root of the trigeminal nerve. In cat embryos, Rhines and Windle (1941) observed that the ventral longitudinal fasciculus begins to appear in caudal (occipital) areas and proceeds rostrally. Tam and Kwong (1987) observed fibres of the ventral longitudinal fasciculus and the common afferent tract in mouse embryos of 9 1/2 days. They did not refer, however, to a lateral longitudinal fasciculus. Fibres of a ventral commissure in the cervical spinal cord were described by Wentworth (1984b) in mouse embryos of 11 days, corresponding approximately to stage 14. The difference in timing could correspond to a rostrocaudal gradient, which is suggested also by the fact that the ventral roots of the hypoglossal nerve develop earlier than the ventral roots of the cervical spinal nerves. Windle (1970) described one human embryo of 4 mm (approximately stage 13) in which some corresponding features were present.

Motor nuclei of the rhombencephalon. Several authors describe a common cellular sheet of motor cells (Bok 1915; Tello 1923; Windle 1932; Windle and Baxter 1936), which gives rise to two cellular columns in later stages (Windle 1933, 1970). In stage 13 of the human embryo, the two columns still form a common area, but the fibres are already typical for the phase when the two cell groups are clearly separated from each other in that the exit of the somatic efferent nerve fibres is more medial than that of the visceral efferent nerves. The nerve fibres of the somatic motor hypoglossal nerve in stage 13 have a short, those of the visceral efferent nerves a long, way to their point of exit (Fig. 10D). The two cell columns are quite distinct in stages 14 and 15 (O'Rahilly et al. 1984, Fig. 2). The presence of two different cellular columns at approximately stage 13 was shown by Tam and Kwong (1987) in mouse embryos of 9 1/2 days. The nuclei of the somatic efferent nerves (6 and 12) show a high alkaline phosphatase activity, those of the visceral efferent cranial nerves, located more medially, do not. The longest extramural fibres of cranial nerves are those of the accessory nerve. As already observed by Streeter (1904), the fibres begin in the cervical area (C. 4–6) and end at the level of the superior ganglion of the vagus. The fibres are enclosed in neural crest material "as in a sleeve" and represent "a well-defined bundle." Those fibres are motor: sensory components seem to be absent in stage 13. According to the scheme given by Altman and Bayer (1984) for the rat at embryonic day 13, the hypoglossal nucleus of human embryos at stage 13 corresponds to their phase 3. Wentworth (1984a) found in mouse embryos of 10 days, corresponding approximately to stage 13, that the majority of motor neurons in the cervical cord were unipolar and secondary bipolar cells. Neurons of rat embryos of 11.3 days, corresponding approximately to stage 13, have not developed in general beyond the unipolar phase (Windle and Baxter 1936).

Optic vesicle. The optic vesicle of stage 13 was described in detail by O'Rahilly (1966, 1983b).

Mesencephalic flexure. Altman and Bayer (1982) pointed out the adaptive advantage of the flexure. The spatial approximation of forebrain and hindbrain that it brings about favours the "outgrowing trigeminal fibres and their target structures." Blechschmidt and Gasser (1978) stressed the impact on the mesenchyme in the area between diencephalon and rhombencephalon. This mesenchyme forms a transverse or submesencephalic septum, the *Mittelhirnpolster* (Hochstetter 1939), which will develop into the medial part of the tentorium cerebelli (O'Rahilly and Müller 1986). Blechschmidt and Gasser (ibid.) emphasized that the bending process has an impact also on the formation of neuromeres. Jacobson et al. (1979) showed experimentally in the chick that the mesencephalic flexure is responsible for the formation of the adenohypophysial pouch. The flexure itself is caused by greater growth in the dorsal than in the ventral mesencephalon (Goodrum 1977).

Ganglia of the cranial nerves. The morphology and histology of the human trigeminal ganglion in stage 13 is comparable to that of rats of embryonic day 12, corresponding to Carnegie stages 13/14 (Altman and Bayer 1982, Fig. 7), which shows activity of the surface ectoderm and the presence of a boundary cap. The same authors (1984) describe the

boundary cap as a transient structure of neural crest origin and having a "guiding influence on the central growth of motor fibers." Contrary to the data given by Altman and Bayer for the rat, however, a boundary cap for the hypoglossal nerve was not observed in the present study. In addition, observations of human embryos of stage 13 do not support the interpretation that the whole of the neural crest of the cranial ganglia would be absorbed in the formation of the boundary caps and that the remainder of the ganglia would consist of placodal cells. In the human embryo, ganglion 7/8 also forms a boundary cap during stage 13. Altman and Bayer (1982) found the peak for the formation of cells for this ganglion in embryos of embryonic day 12 (Carnegie stages 13/14), and they have experimental evidence that the vestibular cells of the complex form earlier than the cochlear part. Davies and Lindsay (1985) recorded a marked difference in the response of neural crest and placode-derived sensory neurons to nerve growth factor in the chick; NGF promotes survival and growth of sensory ganglionic neurons of neural crest origin but not of placodal origin.

The first, although very thin, sensory fibres in ganglia 5 and 7 were seen already in stage 12 (Müller and O'Rahilly 1987). Peach and Koch (1977) observed the formation of neural processes in mouse embryos of 10ET and 31 somites (corresponding approximately to stage 13).

Froriep's (1882) ganglion, an occipital ganglion at the level of somite 4, was not encountered in the present study. Neural crest material related to so-called dorsal roots of the hypoglossal nerve belongs to the accessory nerve, as discussed previously (O'Rahilly and Müller 1984a). So far, the only well-documented Froriep's ganglion in the human was referred to by Pearson et al. (1964) in a fetus of 46 mm: "This is the only human embryo in our collection and in those which we studied at the Carnegie Institution which possessed a ganglion of this type connected to the hypoglossal nerve."

Areas of newly formed neural crest are still recognizable at the level of the brain (Figs. 5B, 9C). They seem to have more connection with the formation of the meninges (O'Rahilly and Müller 1986) than with the development of the nucleus of the mesencephalic root of nerve 5. It cannot be excluded, however, that in mesencephalic areas the nucleus could arise via leptomeningeal tissue, as it does in birds (Narayanan and Narayanan 1978). Another origin was proposed by Windle (1932), who believed that the mesencephalic root arises from the dorsalmost cells of the nucleus of the lateral longitudinal fasciculus.

The relationship between neural crest material and the formation of cranial and spinal ganglia was discussed by Holmdahl (1934), who stated that no morphological continuity exists between the two: "No more can be said than that the spinal or the cranial ganglia respectively are formed in a loose mesenchyme lateral to the neural tube in a tissue in which some cells originated from the neural crest." This comment is particularly interesting in relation to a recently formulated hypothesis of Altman and Bayer (1984). Based on descriptive evidence in rat embryos, they concluded that, because neural crest material is scarce after the closure of the neural tube, and because the spinal ganglia appear suddenly in conjunction with the dissolution of the medial somitic shell, and because the spinal ganglia are segmental from the beginning, therefore the ganglia form from the somites. Neural crest material is used for the boundary caps of the spinal ganglia. These authors stressed, however, that they do not yet have experimental data. One of the questions that arises is why no spinal ganglia form in the area of the occipital somites. Streeter's (1904) description of the spinal neural crest in human embryos of stage 13 indicates a clear relationship between neural crest and formation of the spinal ganglia.

Ganglia of cranial nerves and placodes. Although cells are given off from the thickened surface ectoderm already in human embryos of stages 11 and 12 (Müller and O'Rahilly 1986a, 1987), the typical placodes as described in the mouse (Verwoerd and Oostrom 1979) and in birds (D'Amico-Martel and Noden 1983) are present only in stage 13. Before this stage the material for placodes is incorporated in the ectodermal ring (O'Rahilly and Müller 1985, Figs. 3, 5). The placodes are defined as "some small areas dorsal to the pharyngeal grooves" (Verwoerd and van Oostrom 1979). These areas may be comparable to the epibranchial placodes of the lower vertebrates. Whereas there is little doubt about the existence and activity of nasal and otic plates, the pharyngeal placodes of arches 2 to 4 in human embryos needed more careful examination. This was clearly acknowledged already by Verwoerd and Oostrom, who stated that the "relationship between placodes and the larger regions of thick ectoderm has scarcely been studied in human embryos" (ibid.). The development of the pharyngeal placodes in human embryos follows the same pattern as in the mouse (Verwoerd and Oostrom, ibid.). The thick ectoderm covering the pharyngeal arches in early stages begins to become thin gradually, and "as a result only small separate areas of thick ectoderm remain." In general the relationship of placodal material to neural crest cells in stage 13 for nerves 7 to 10 is comparable to that described in the chick: the proximal part of the ganglia is formed by neural crest, the distal by placodal material. The trigeminal region was found difficult to interpret by Smits-van Prooije (1986). She found a continuous basal lamina in ultrathin sections of mouse and rat embryos (corresponding approximately to stages 10 to 14) at the level of pharyngeal arch(es) 1 (and 2), although she did not exclude placodal activity, because it "is so scarce" in this area. Van Campenhout (1948) found in Carnegie embryo No. 8066 (compare Fig. 8A of the present study) great placodal activity at the level of the trigeminal ganglion: "On peut réellement parler d'un enfoncement des éléments d'origine placodique dans la crête." The description of the trigeminal ganglion and its "placode" by Ingalls (1907) is in total agreement with the results here. In the chick, too, the placodal activity of pharyngeal arch 1 seems to be different from that in arches 2 to 4. D'Amico-Martel and Noden (1983) found numerous small foci scattered throughout the surface ectoderm of the trigeminal area, and the greatest activity occurred between HH stages 14 and 16, which, according to Butler and Juurlink (1987), correspond approximately to Carnegie stages 12–13. On the whole, the contribution of placodal cells to the trigeminal ganglion from the surface ectoderm seems to be quite different from the addition of material distal to the neural crest component in the case of nerves 7, 9, and 10.

In contrast to animals, where the two cell populations can be marked experimentally, exact time schedules cannot be provided in the human. It is feasible only in a restricted way to summarize the development of neural crest and pla-

codal material in human embryos. Vagal placodal material seems to begin to form already in stage 10 but is more limited than the formation of neural crest material in that stage (Müller and O'Rahilly 1985, Fig. 6). Evidence of placodal material in stage 11 is present at the level of pharyngeal arches 2 and 3. Therefore, it seems that neural crest components of ganglia 5 and 7 are formed earlier than placodal material for the same ganglia, and placodal material for nerves 9 and 10 earlier than the neural crest material of the corresponding ganglia. In the chick, thymidine-marking has shown that placodal cells are earlier than neural crest cells for all ganglia (D'Amico-Martel 1982).

Otic vesicle. The otic vesicle was described in detail by O'Rahilly (1963, 1983). Topographically it is related to Rh4 in stage 9 (Müller and O'Rahilly 1983), to Rh4 and 5 in stages 10 and 11 (Müller and O'Rahilly 1985, 1986), and is in its almost definitive place opposite Rh5 in stage 12 (Müller and O'Rahilly 1987). The relationship between otic plate/vesicle and the proper rhombomere has a bearing on its development, as was shown by Deol (1964) and stressed by Van de Water et al. (1980). Wilson (1983), who studied an exencephalic mutant of the mouse, implies that "there could be a common factor simultaneously affecting both" rhombencephalon and otic structures, because of the early and concurrent existence of defects already in embryos of 8 1/2 days (corresponding approximately to stage 10 in human embryos). Cells given off from a rostrolateral area of the otic vesicle were observed by Politzer (1956) and described as an acoustic ganglion. Comparison with slightly older stages shows that the material is probably purely vestibular because, already in stage 14, it sends nerve fibres to a zone corresponding to the utricle (O'Rahilly et al. 1984, Fig. 3). *In vitro* culture of mouse otocysts (Cheuk et al. 1978) has shown that the area in question develops later into the utricular and saccular maculae. The mesenchymal otic sheath that begins to form in stage 11 from certain regions of the otic plate (Müller and O'Rahilly 1986) surrounds most of the otic vesicle in embryos of stage 13 and was also observed by Streeter (1918a) at this stage. It is not possible, however, to decide whether the whole mesenchymal sheath originates from the otic vesicle. Noden (1986) termed the otic capsule a problematic structure, which is "largely formed by paraxial mesoderm but receives several minor contributions from crest cells in birds." McPhee and van de Water (1986) found in the mouse that between days 11 and 13 the otic vesicle acts as an inductor of chondrogenesis of periotic mesenchyme.

Cephalic mesenchyme. The premandibular condensation of stage 13 was described as forming the primordia of the lateral and superior recti (Gilbert 1957). Holmdahl (1934) termed the mesenchyme between neural tube and surface ectoderm *membrana reuniens*. Blechschmidt and Gasser (1978) and O'Rahilly and Müller (1985) pointed out the thinness of the ectoderm on the dorsal side of the brain and the paucity of mesenchyme in this area. "The mesenchyme adjacent to the vertex of the brain becomes extremely flattened in the second month and is stretched to such a degree that its surface growth is retarded" (Blechschmidt and Gasser 1978). Most of the cephalic mesenchyme is accumulating in the basal and lateral areas, especially in the pharyngeal arches. The zones directly adjacent to the lateral walls of the rhombencephalon are thinning out, and an indication of the future primary meninx is clearly present in stage 13 (Fig. 11F). Some of those mesenchymal features can be seen nicely in a series of photomicrographs of No. 836 reproduced by Bartelmez and Dekaban (1962, Figs. 54 to 59).

Blood vessels. Streeter (1918b) reconstructed the blood channels for the brain from stage 13 onwards. His figures in Plate 1 and 2 depict embryo No. 588. Padget (1948, 1957) went into more detail and also referred to No. 588. She described a "primordial hindbrain channel," which is visible in Fig. 8A. It is a transitory blood vessel, which is "fundamentally the proliferative endothelial material from which both arteries and veins are soon derived." During stage 13 longitudinal neural arteries are in formation. They are supplied by the trigeminal and otic arteries, which represent branches of the paired dorsal aortae. Blood vessels on the roof of the rhombencephalon are related to the development of the meninges (O'Rahilly and Müller 1986).

Somites. The development of the caudal somites beyond the cloacal membrane is special in that the somitic plates join in the median plane (Figs. 4B, C, and 6E) in the more advanced embryos. In this way the somitic material forms a kind of belt, and traces of its ventral parts can be found in later stages after the somites have long been individualized and formation of vertebrae is under way (stage 18). Embryos of stage 13 have not yet the full complement of somites needed for the development of the complete vertebral column. If 34 somites are considered to be the average number for this stage, then 4–5 more units have to be formed.

Caudal eminence. The position of the cloacal membrane relative to the somites was discussed by Holmdahl (1926). The cloacal membrane of the one human embryo corresponding to stage 13 (his Figs. 53 and 85d) lies opposite s. 34 (level of future S.V.5), and the definitive cloaca is present at this time. Because all the elements of the caudal eminence, and not merely neural tube and notochord, are developing from its undifferentiated mesenchyme, it follows that the cloaca develops not from endoderm but from the *Rumpfknospe*. An illustration of the cloaca at stage 13 and its relationship to the postanal gut and the renal system was published by O'Rahilly and Muecke (1972, Fig. 1). As is the case in the human, the hindgut in rat embryos of 10 and 11 days originates from the mesenchyme of the caudal eminence by a mechanism analogous to secondary neurulation (Svaiger et al. 1985), which is not in agreement with the findings in the chick (Schoenwolf 1977). Tam (1984) showed experimentally in the mouse (including embryos that correspond approximately to stage 13) that "tissues associated with the caudal fragments were developmentally pleuripotential (pluripotential) in nature." The caudal eminence in stage 13 is different from that in advanced embryos of stage 12 in that fully individualized somites are present in it and that the paired caudal aortae begin to invade it (Streeter 1918b, Figs. 22, 23).

Secondary neurulation. Changes such as occur in mouse embryos of 11 days (Schoenwolf 1982) and that concern the shape of the newly-forming neural cord are not present. Formation of neural crest was observed in mouse embryos of 10 days (approximately stage 13) by Schoenwolf and Ni-

chols (1984). Bolli (1966) found no secondary lumina in the neural tube of human embryos up to 4 mm in length. Accessory lumina were increasingly present in embryos of 4 to 31 mm, and were most frequent in embryos of 8–9 mm (approximately stage 16). It seems, therefore, that up to stage 13 malformations in the caudal area (future lumbosacral levels) would not evolve from faulty development of the neural tube but rather, as pointed out for stage 12 (Müller and O'Rahilly 1987), from non-closure of the caudal neuropore. The section of the neural tube developed by secondary neurulation lies caudal to somite 30 and corresponds to the sacral region. It would certainly be interesting to know how much material of secondary neurulation will be integrated into the adult spinal cord. To judge from spinal nerves and ganglia, it is surely the material forming along with somites up to 35 (corresponding to coccygeal segment 1). From Fig. 7 it seems already that too much relative length is present in stage 13.

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