Anat Embryol (1986) 175:205–222

Anatomy and Embryology



# The development of the human brain and the closure of the rostral neuropore at stage 11*

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**Summary.** Twenty embryos of stage 11 (24 days) were studied in detail and graphic reconstructions of twelve of them were prepared. The characteristic feature of this stage is 13–20 pairs of somites.

The notochord sensu stricto appears first during this stage, and its rostral and caudal parts differ in origin. Rostrally, the notochordal plate is being transformed into the notochord in a caudorostral direction. The caudal part, however, arises from the axial condensation in the caudal eminence in a rostrocaudal direction. The caudal eminence (or end bud) represents the former primitive streak. The somites are increasing in number at a mean rate of 6.6 h per pair.

The rostral neuropore closes towards the end of stage 11. The closure is basically bidirectional, being more rapid in the roof region and producing the embryonic lamina terminalis and future commissural plate in the basal region. The caudal neuropore is constantly open. The brain comprises telencephalon medium (represented by the embryonic lamina terminalis) and a series of neuromeres: 2 for the forebrain (D1 and D2), 1 for the midbrain, and 6–7 for the hindbrain (Rh A–C; Rh D is not clearly delineated). The forebrain still occupies a small proportion of the total brain, whereas the spinal part of the neural tube is lengthening rapidly. Some occlusion of the lumen of the neural tube was noted in 4 embryos, all of which had an open rostral neuropore. Hence there is at present no evidence that occlusion plays a role in expansion of the human brain. The marginal (primordial plexiform) layer is appearing, particularly in rhombomere D and in the spinal portion of the neural tube. The neural crest is still forming from both the (open) neural groove and the (closed) neural tube, and exclusively from both neural (including optic) and (mainly) otic ectoderm.

The optic sulcus is now prominent, and its wall becomes transformed into the optic vesicle towards the end of stage 11. At this time also, an optic sheath derived from mesencephalic crest and optic crest is present. The mitotic figures of the optic neural crest are exceptional in being situated in the external part of the neural epithelium. The otic pit is becoming deeper, and its wall is giving rise to neural crest that is partly added to the faciovestibulocochlear ganglion and partly forms an otic sheath. The nasal plate does not yet give off neural crest.

**Key words:** Human embryo – Human brain – Neural tube – Rostral neuropore – Neural crest

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* Supported by research grant No. HD-16702, Institute of Child Health and Human Development, National Institutes of Health (USA)

*Abbreviations:* Figs. 1–10. *a* Endoderm caudal to neurenteric canal or its site; *B* Endoderm rostral to neurenteric canal or its site; *A-H* Primordium of adenohypophysis; *All.* Allantoic primordium; *Ao* Aorta; *C.E.* Caudal eminence (caudal bud, end bud); *Caud. lim.S.* Caudal limiting sulcus; *Ch.* Chiasmatic plate; *D* Diencephalon; *F* Foregut, pharynx; *Cl.* Cloacal membrane; *Ggl* Ganglion; *H* Hindgut; *I* Infundibulum; *L.T.* embryonic lamina terminalis; *M* Mamillary area; *Mes.* Mesencephalon, mesencephalic; *Mit.* Mitotic figure; *Nas.* Nasal plate; *N.C.* Site of neurenteric canal; *N.Cr.* Neural crest; *Not.Pl.* Notochordal plate; *Not.* Notochord; *O-Ph* Oropharyngeal membrane; *Opt.* Optic primordium; *Opt.S.* Optic sulcus; *Ot.* Otic pit; *Ot.sh.* Otic sheath; *Ph.Ar.* Pharyngeal arch: *Pr.* Mesenchyme of prechordal plate; *Pros.* Prosencephalon; *Rec.* Postoptic recess; *Resp.* Respiratory primordium; *Rh* Rhombomere; *S.V.* Sinus venosus; *s.* Somite; *Tel.* Telencephalon; *Th.* Thickening; *Thyr.* Thyroid primordium; *Trig.* Trigeminal; *X* Caudal neuropore; *Y* Rostral neuropore

## Introduction

A review of stage 11 based on 16 embryos was published by Streeter (1942). Some of the specimens had been described in monographs by previous authors and these were summarized. Details of the nervous system have not been covered adequately, however, and hence a further investigation was needed. Twenty embryos of good quality have been studied, and comparisons have been based on a standardized procedure, chiefly graphic reconstructions. An assessment of variation has also been undertaken. Attention has been devoted mainly to the brain and its associated organs, such as the optic and otic primordia, and to the notochord and caudal eminence.

Stage 11 is characterized by the presence of 13 to 20 pairs of somites and by the formation and closure of the rostral neuropore. The first and second pharyngeal arches develop during this stage, and an otic pit is present.

## Material and methods

In Table 1 are listed the 20 embryos studied, as well as the details concerning number of somites, embryonic

**Table 1.** Embryos of stage 11 studied

| Embryo | Pairs of somites | Embryonic length (mm) | Magnification of reconstruction | Stain | Thinness of section (μm) | Plane |
|---|---|---|---|---|---|---|
| 4783 | 13 | 2.3 | 200 | I.H. | 5 | Trans. |
| 4877 | 13 | 2 | | Al.coch. | 15 | Trans. |
| 6344 | 13 | 2.5 | 150 | Al.coch. | 6 | Trans. |
| 7851 | 13 | 4.3 | | H.E. | 8 | Trans. |
| 318 | 13/14 | 2.5 | 80 | Al.coch. | 25 | Trans. |
| 4529 | 14 | 2.4 | 250 | Al.coch. | 10 | Trans. |
| 8962 | 15 | 1.55 | | | | Sag. |
| 7611 | 16 | 2.4 | 150 | H.E. | 8 | Trans. |
| 7358 | 16 | ? | | H.E. | 25 | Oblique |
| 8005 | 16/17 | 3 | | | | |
| 470 | 17 | 4.3 | 200 | Al.coch. | 10 | Trans. |
| 5072 | 17 | 3 | 150 | H.E. | 10 | Trans. |
| 6784 | 17 | 5 | 150 | I.H., G. | 6 | Trans. |
| 7702 | 17 | 3.7 | 150 | Al.coch. | 10 | Trans. |
| 4315 | 17 | 4 | | I.H. | 10 | Trans. |
| 8116 | 17 | ? | | Azan | 8 | Sag. |
| 164 | 18 | 3.5 | | Al.coch. | 20 | Trans. |
| 7665 | 19 | 4.36 | 100 | | 6 | Trans. |
| 2053 | 20 | 3.1 | 100 | Al.coch | 10 | Trans. |
| 6050 | 19/21 | 3 | 100 | Al.coch. | 10 | Front. |

**Table 2.** Features that change during stage 11

| Embryo | Pairs of somites | Angle of cranial flexure | Rostral neuropore | Oro-pharyngeal membrane unruptured | Aorta paired throughout | Notochord touches spinal cord | Notochord touches digestive epithelium | Notochordal plate still present in some areas | Occlusion in neural tube |
|---|---|---|---|---|---|---|---|---|---|
| 4783 | 13 | 114 | + | + | + | + | + | + | Rh4, S4–12 |
| 6344 | 13 | 117 | + | + | + | + | + | + | – |
| 7851 | 13 | | + | ? | + | + | + | + | – |
| 318 | 13/14 | 110 | + | + | + | + | + | + | – |
| 4529 | 14 | 111 | + | + | + | + | + | + | – |
| 7611 | 16 | 120 | + | + | + | + | + | + | – |
| 470 | 17 | 91 | + | + | + | + | + | – | – |
| 5072 | 17 | 148 | + | + | + | + | + | – | – |
| 6784 | 17 | 112 | + | + | + | + | + | + | Ggl. 9 to S6 |
| 7702 | 17 | 143 | + | + | + | + | + | – | S1–15 (interrupted) |
| 4315 | 17 | 94 | + | + | + | + | + | + | – |
| 8116 | 17 | 113 | + | – | + | + | + | – | – |
| 164 | 18 | | + | – | + | + | + | – | – |
| 7665 | 19 | 118 | + | – | + | – | – | – | S1–8 |
| 2053 | 20 | 114 | – | – | – | – | – | – | – |
| 6050 | 19/21 | 130 | – | + | – | – | – | – | – |

length, stain, plane of section, and the enlargement of the graphic reconstructions. Most were fixed in formalin. The embryos belong to the Carnegie Collection. The mean length is 3.3 mm and the age is believed to be approximately 24 postovulatory days. For one embryo (No. 7702) only bromides were at hand. The last embryo (No. 6050) is transitional to the next stage in that it was found to have 21 pairs of somites on one side. Born reconstructions of 13 embryos were available. For 12 embryos new graphic reconstructions were prepared, using every or every second section from either drawings or bromides. New reconstructions were made because graphic reconstructions are generally more useful in defining details, and are particularly important for taking measurements. The transition from notochordal plate to notochord was examined carefully and the notochordal relationship to neural tube and caudal eminence was investigated. The numbers of somites were found to be different in some cases from those previously reported. The sites of mitotic figures and areas with cytoplasmic inclusions were added, and external features of the neural tube (such as sulci and protrusions) were superimposed onto median reconstructions in order to define the different parts of the brain. Differences in thickness of the surface ectoderm, especially in the head, were carefully plotted.

The neural tube was measured as a central axis from the rostral neuropore to the caudal eminence. The caudal eminence comprises the area between the end of the differentiated notochord and the cloacal membrane. The cervical flexure was defined 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.

A completely dysraphic embryo (No. 779) is not in-

cluded in Table 1. This 14-somite specimen has been described in detail by Dekaban (1963) and Dekaban and Bartelmez (1964). It is believed to be the youngest human embryo depicted with such a condition.

## Results

Some findings of general importance are illustrated in Fig. 1 and are listed in Table 2: number of somites, angle of cranial flexure, intact oropharyngeal membrane, rostral neuropore.

*General.* Photographs of the external form are available in Streeter (1942). In general the embryo follows a gentle curve but a lordosis is visible in some specimens. Its width is uniform throughout, and the caudal eminence is as wide as the head. The two pharyngeal arches as well as the otic pit are identifiable as slightly more opaque areas. The S-shaped heart is visible by transparency, as is the pharynx in lateral views. Head-on views show the rostral neuropore in most embryos. The developing neuromeres can be seen in dorsal views.

*Prechordal plate.* The prechordal plate is more difficult to recognize than in earlier stages. It lies adjacent and rostral to the notochord, and caudal and lateral to the adenohypophysial area. Its cells are not as well organized as those of the notochord (see also Figs. 5 to 8 in Gilbert 1957). Scant prechordal material is found in the median plane, most having migrated laterally to form the premandibular condensation. In one embryo (No. 4877,13 s.), the relationship between migrated prechordal cells and the mesenchyme of the heart, typical for stage 10 (Fig. 8B), can easily be seen in sections. In most embryos of stage 11, however, the prechordal material is now limited to the premandibular area.

*Oropharyngeal membrane.* The oropharyngeal membrane (Table 2) has begun to rupture in 4 out of 15 embryos.

*Notochordal plate and notochord.* Embryos possessing 13 and 14 pairs of somites still show large areas of notochordal plate that form the roof area of the pharynx and/or other parts of the alimentary canal, as in earlier stages. The development into the notochord follows a distinctly longitudinal pattern from caudal to rostral. The last areas with notochordal plate are in the pharynx.

Several steps are involved in the separation of the notochordal plate from the alimentary system (Figs. 2, 3): (1) Beginning already at stage 10 the plate becomes U-shaped; (2) the vertical parts of the U begin to move medially and touch each other; (3) the adjoining digestive epithelium begins to form a cytoplasmic bridge, which can be extremely thin; (4) mitotic figures appear on both sides of that bridge, a complete cellular lining forms, and the still U-shaped notochord is entirely cut off. During all those steps, the number of notochordal cells per section remains constant: 8–10 cells. This is the number of cells found already in stage 8, when the notochordal plate first forms. The number of mitotic figures in embryos having fewer than 20 pairs of somites is 12 (average in 4 embryos). In one embryo (No. 6050), however, the number has increased to probably more than 20 (plane of section does not allow definitive

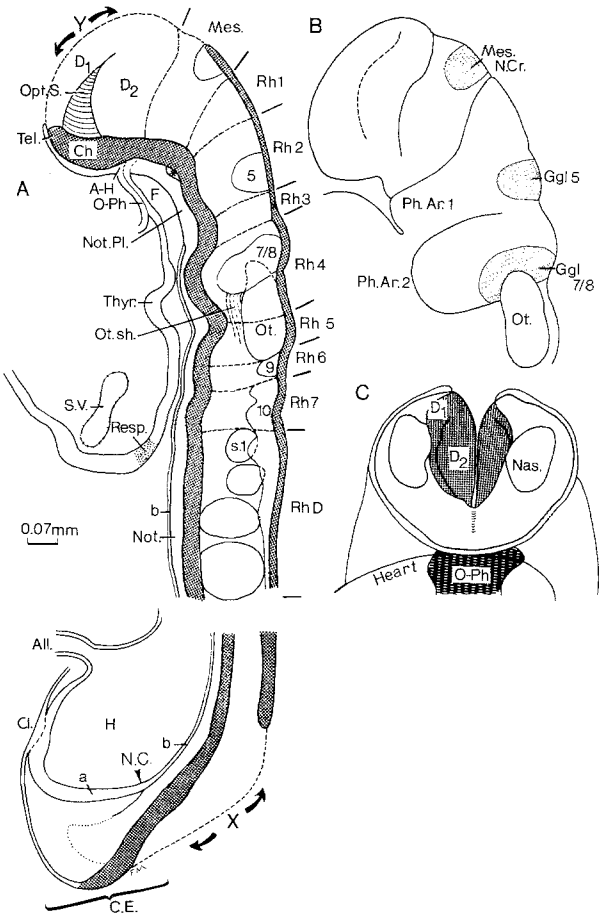


**Fig. 1 A–C.** Features present at stage 11 shown in embryo No. 7611 (16 pairs of somites). **A** A median section of the brain. The somites of the right side as well as the ganglia and neural crest of the cranial nerves are projected onto the outline. The lateral parts of the brain are indicated by *interrupted lines*. A break is shown in the neural tube at the transition from RhD to the spinal cord. Most embryos of stage 11 still show an open rostral neuropore, in many the oropharyngeal membrane is still uninterrupted, and embryos having 13 to about 16 pairs of somites still possess a notochordal plate in some areas. The notochordal plate and/or notochord in most cases touch the neural tube as well as the intestinal epithelium. The rhombencephalon now shows 6–7 rhombomeres rostral to RhD. The optic primordium is evaginating and a prominent optic sulcus represents the beginning of the optic ventricle. The *asterisk* indicates an artifactual dehiscence between the brain and the notochordal plate. The caudal eminence (*C.E.*) comprises the area of the former primitive streak. **B** Lateral view showing two prominent pharyngeal arches. The otic pit extends from the area of arch 2 to that of arch 3 (from the caudal part of Rh4 to Rh5). **C** A head-on view showing the nasal plate, which consist of thickened surface ectoderm on both sides of the widely open rostral neuropore. For abbrevations (Figs. 1–10) please see footnote on page 205

enumeration) and in another (No. 2053), 17 mitotic figures could be counted. If the two parallel parts of the U-shaped notochordal plate do not touch in the median plane, a canal can be included (Figs. 2, 5B) once the endodermal bridge is complete.

The part of the notochord that forms directly from the axial condensation in the caudal eminence is far thicker and more advanced in differentiation (Fig. 3E, F). It possesses intercellular vacuoles in some embryos (Nos. 8005,

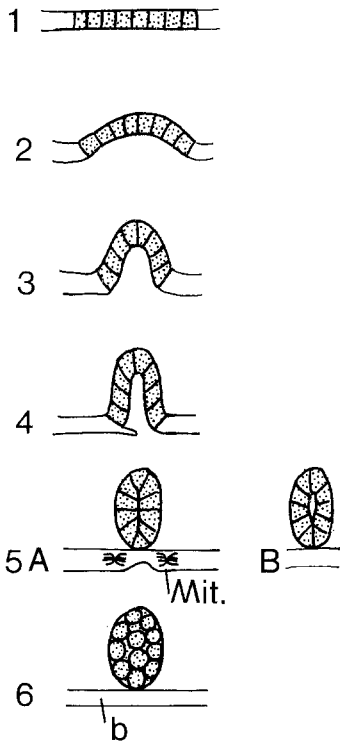


**Fig. 2.** Development of the notochord from the notochordal plate. The notochordal plate (*1, stippled*) becomes U-shaped (*2–4*) during stages 10 and 11. The adjoining digestive epithelial cells (*in white*) form protoplasmic bridges (*4*), and mitotic figures appear on one or both sides (*5*) of the "bridge," so that a complete cellular lining is formed ventral to the notochord (*5*). The number of notochordal cells per section does not change during the separation from the digestive epithelium (*6b*). Cell division in the notochord begins after this event. During the separation a small remnant of the intestinal cavity may persist (*5B*). It does not correspond, however, to a true notochordal canal

8116, 6050, 7665). A basement membrane is probably always present, and shows particularly well in azan-stained or silver-treated sections.

The notochord and notochordal plate are still intimately related to the neural tube, and the notochord to the digestive epithelium, in that no mesenchyme intervenes between the adjacent structures (Table 2). The basement membranes of the neural tube and notochord touch each other, and where, in reconstructions, the notochord and neural tube are separated by a gap, this is an artifact (Fig. 1A). Only in the most advanced embryos of stage 11 does a real separation begin (Fig. 10C) at the cranial flexure and in restricted areas of rhombencephalon D by the formation of the primordial meninges.

Interruption of the contact in more caudal areas begins to occur at the time the paired dorsal aortae fuse. The two aortae enlarge (Fig. 4A) and grow towards the median plane, so that the alimentary canal has to move ventrad. At the time fusion is completed (Fig. 4B), the aorta intervenes between the notochord and the digestive epithelium (Fig. 4B).

*Site of the neurenteric canal.* As shown for embryos of stage 10 (Müller and O'Rahilly 1985), an abrupt change is visible at this site in median sections. Rostral to the neurenteric canal or its site, the roof of the alimentary canal

**Table 3.** Length of somites at stage 11 (μm)

| Embryo | 6344 | 318 | 4529 | 7611 | 7665 | 6050 | 2053 | Mean |
|---|---|---|---|---|---|---|---|---|
| Mean of somites 5 to 7 | 143 | 120 | 73 | 82 | 126 | 100 | 133 | 111 |
| somites 10 to 12 | 126 | 87 | 91 | 111 | 102 | 90 | 133 | 106 |
| somites 17 to 19 | | | | | 103 | 70 | 55 | 76 |

was formed by the notochordal plate. Caudal to the neurenteric canal a relatively thick endoderm consisting of two rows of cells was present (Fig. 1A). Such a difference is not as readily found in embryos of stage 11, because of the caudorostral transformation of the notochordal plate into the notochord and the accompanying formation of epithelium (*b*) in the median plane. However, a difference in thickness (*a* versus *b* in Fig. 1A) seems still to indicate the former site of the neurenteric canal. It was found at somites 12/13 (No. 7665), s. 15 (No. 6344), s. 15/16 (No. 4783), s. 16/17 (No. 5072), s. 18/19 (No. 4329), and at the level of future somite 21 (No. 2053).

*Caudal eminence.* The caudal eminence (or end bud) is located between the cloacal membrane and the site of the neurenteric canal (Fig. 1A), and represents the region of the former primitive streak. It contains dense axial material (present already in advanced embryos of stage 10), from which new parts of the notochord are added, as already described (Müller and O'Rahilly 1985), and paraxial condensations from which the somites develop (Fig. 5E, F). It occupies 10–5% of the total length of the embryo. An abnormal caudal eminence was detected in one embryo (No. 470, Fig. 5D): the longitudinal median axis of the hindgut is deviated to one side with regard to the axis of the neural tube and notochord, and the paraxial condensation of that side is deviated to the other half.

The unilayered surface ectoderm is limited by a basement membrane towards the mesenchyme throughout most of its extent. Special areas include the contact regions with the endoderm of the hindgut; here the two laminae touch without a clear basement membrane between them. The ectoderm in most embryos is slightly thickened in the proximal part of the cloacal membrane (Fig. 5A–D). The thickened ectoderm contains cytoplasmic inclusions in most cases. Inclusions are present also in the epithelium of the hindgut and the caudalmost part of the notochord. Proximal to the cloacal membrane, the surface ectoderm continues into the multilayered transitional tissue towards the neural ectoderm. The caudalmost thick part of the notochord loses its basement membrane before its cellular components mix with the axial mesenchyme of the caudal eminence (Fig. 6C).

*Somites.* The rostrocaudal extension of the individual somites is irregular. Hence, in order to obtain a mean somitic length, three areas were chosen for calculation: somites 5 to 7, somites 10 to 12, and (where present) somites 17 to 19. Table 3 shows that (1) there is not a clear gradient in size between rostral and caudal regions: in most cases the rostral somites are larger; and (2) there is no correlation

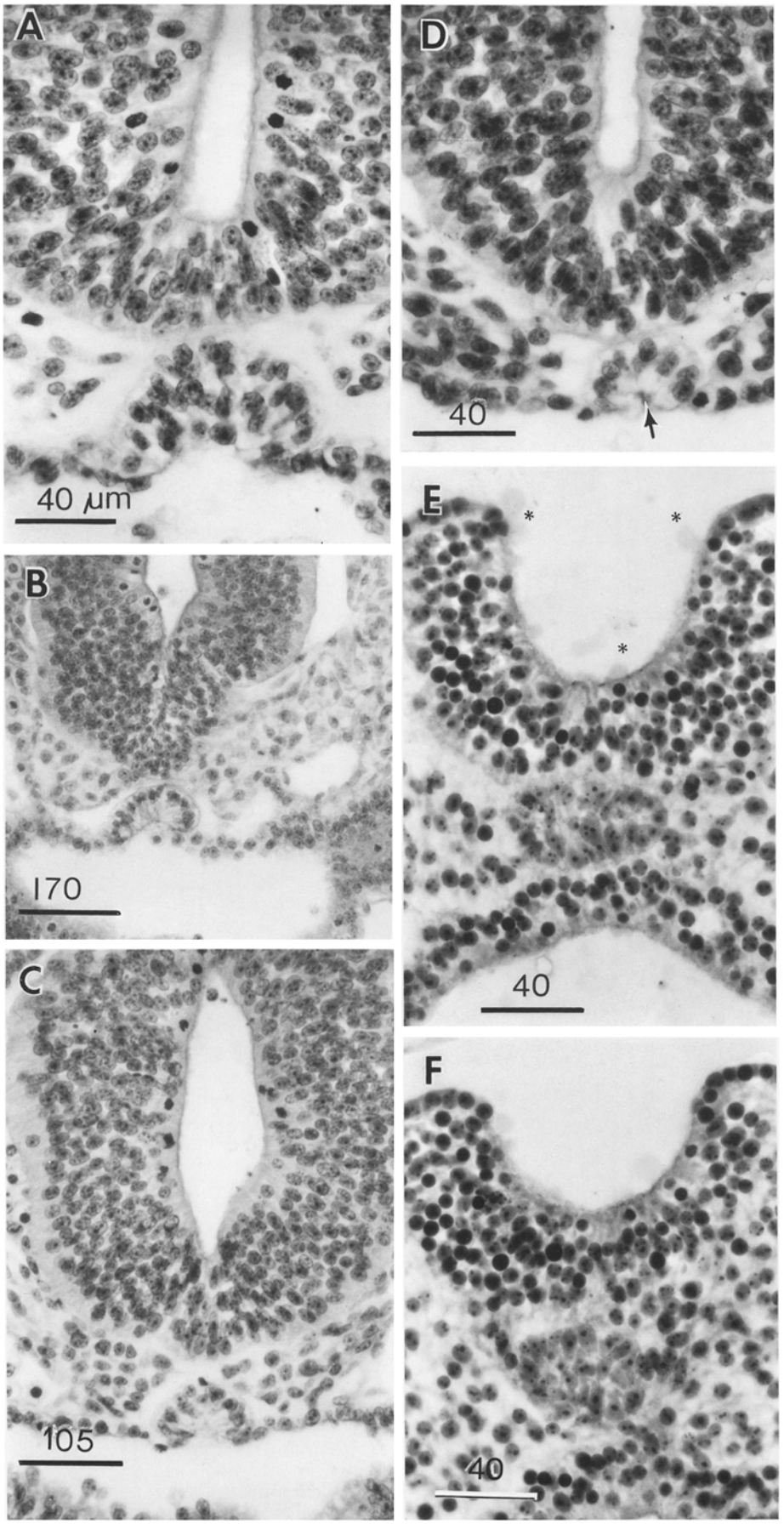


**Fig. 3A–F.** Photomicrographs of the developing notochordal plate and notochord.
**A–C** Arched notochordal plate; beginning of formation of protoplasmic bridges; mitotic figures appearing in the digestive epithelium.
**D** A small remnant of the digestive cavity is included in the forming notochord (*arrow*), but does not represent a real notochordal canal.
**E, F** Notochord caudal to the site of the neurenteric canal. The notochord in the region of the caudal eminence is rod-like from the beginning and develops from the condensed axial mesenchyme. It can be seen that the caudal part of the notochord (**E, F**) differs considerably in appearance from that further rostrally (**A–D**). Small blebs are visible on the surface of the neural groove (*asterisks*)

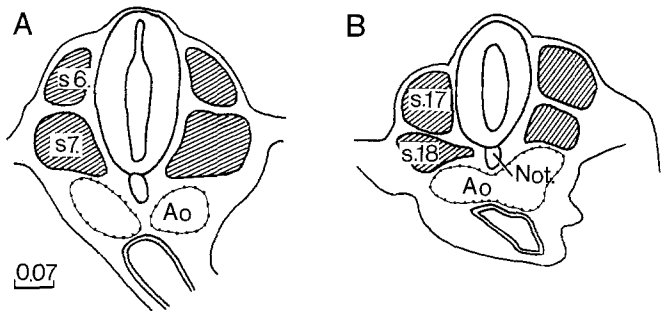


**Fig. 4A, B.** Development of the unpaired aorta and its probable influence on the notochord. In the two embryos having 20 and 19/21 pairs of somites the two dorsal aortae begin to join (**A**). They meet in the area between the notochord and the alimentary canal, thereby separating these formerly closely related structures (**B**)

with the number of somites: the means are almost the same in an embryo of 13 somites as in one of 20.

*Rostral neuropore and its closure.* The formation and closure of the rostral neuropore in stage 11 are basically a bidirectional process (Fig. 7): it continues from the rhombencephalon to the mesencephalon and proceeds from the optic chiasma towards the roof of D1, thereby forming the embryonic lamina terminalis and the future commissural plate. In embryos with 13 somites the mesencephalon is still open (No. 6344, Fig. 8D), partly closed (No. 4783), or completely closed (No. 318). The rostrocaudal closure at this time has scarcely begun, and hence the material of the future lamina terminalis still lies laterally. The only embryo with 14 somites has a partly closed mesencephalon, and the closure at the level of the lamina terminalis has begun. Embryos with 17 somites show the roof of D2 to be closing (Fig. 10A): when 19 somites are present the area medial to the optic vesicles is still open. Two embryos having 20 and 19/21 somites show a completely closed forebrain. During closure, the former headfold of the embryo begins to change from an oblong to a wider structure, caused mostly by the evagination of the optic vesicles (Fig. 8). The maxillary processes show in the "face" in embryos having approximately 17 somites.

Table 4 gives the percentage of the rostral neuropore in relation to the total length of the neural tube. The embryos with 13 and 14 somites have corresponding values; those with 16 to 19 somites show some variation.

*Caudal neuropore.* Table 4 provides the percentage of the caudal neuropore in relation to the total length of the neural tube. The average of 10% for all embryos and of 10% for the embryos having only 13 somites indicates that (1) the closure of the neural tube from stage 10 to stage 11 is a very rapid event and (2) closure and elongation of the neural tube are proceeding at the same speed.

*Brain.* The forebrain, consisting of D1 and D2 (already present in stage 10), is still relatively simple. Part D1 is largely the area of the optic primordium and, in the median plane, the chiasmatic plate. The caudal limit of the plate is sometimes indicated by a postoptic recess (Fig. 10A). The floor of D2 includes mamillary and neurohypophysial components, and the latter is recognizable indirectly by the primordium of the adenohypophysis, which is the ectodermal region at the summit of the oropharyngeal membrane (Fig. 10B). Part D2 still protrudes towards the median plane (Fig. 1C), and its right and left convexities almost touch each other in the least advanced embryos. As the optic primordia become more evaginated, the walls of D2 recede and the ventricular cavity begins to expand. The medial parts of the forebrain rostral to D1, which fuse during the closure of the rostral neuropore, are telencephalic.

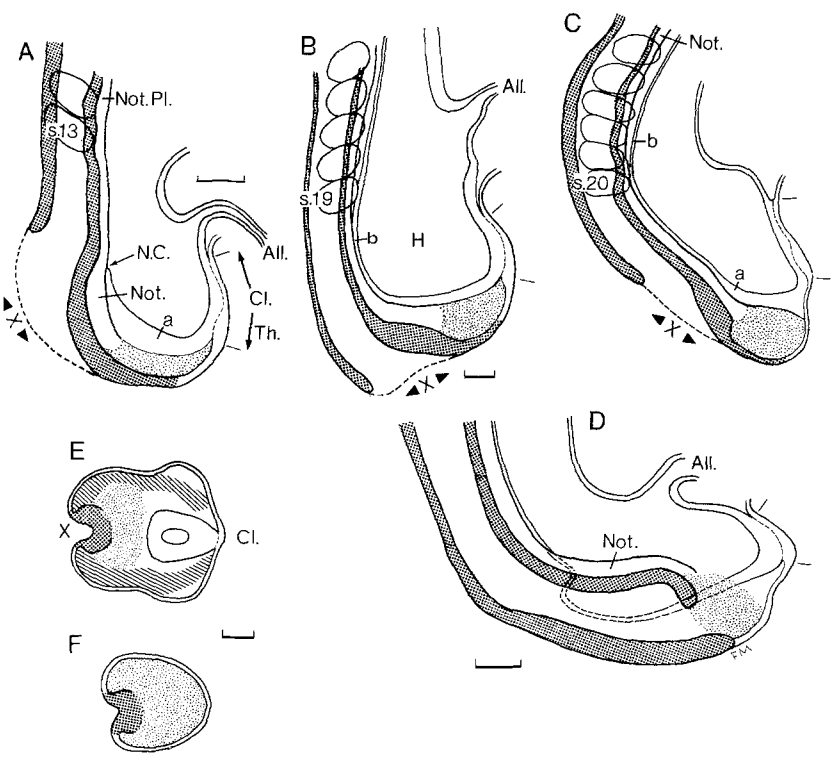


**Fig. 5A–F.** The caudal neuropore and the caudal eminence in stage 11.
**A** An embryo (No. 4783, 13 pairs of somites) in which the notochordal plate still extends through the whole embryo to the site of the former neurenteric canal, having direct contact with the digestive cavity. Caudal to the site of the neurenteric canal (*N.C.*) a thick endodermal layer separates the notochord (here thick and rod-like) from the digestive cavity.
In **B** (embryo No. 7765, 19 pairs of somites) and
**C** (Embryo No. 2053, 20 pairs of somites) epithelium (*b*) intervenes between the notochord and the digestive cavity rostral to the site of the neurenteric canal.
In **D** (embryo No. 470, 17 pairs of somites) the caudal eminence is abnormally formed.
**E** and **F** show transverse sections through the caudal eminence of another embryo (No. 7702, 17 pairs of somites). All the sections (**A–F**) show the axial condensation of mesenchyme (*stippled*) which is joined laterally by a paraxial condensation (segmental plate, *hatched*) from which the somites will form. The bar represents 0.1 mm in all cases

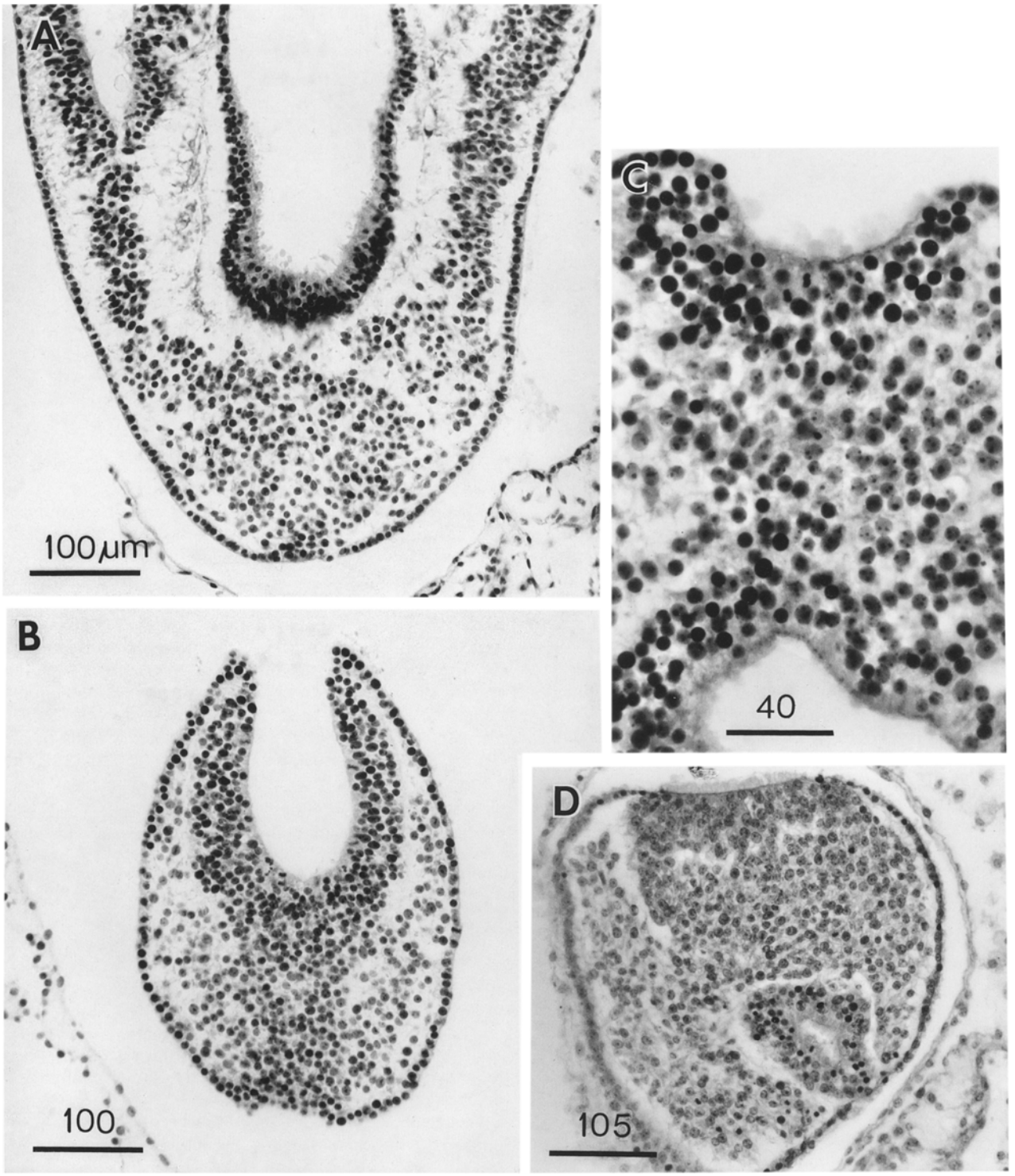


**Fig. 6 A–D.** Photomicrographs of sections of the caudal eminence. **A** An embryo (No. 4529, 14 pairs of somites) showing axial and paraxial condensations. **B** A section through the caudal neuropore and neural ectoderm of the basal plate (No. 6344, 13 s.). No basement membrane seems to be present between the neural ectoderm and the adjacent mesenchyme. **C** Axial condensation and caudal neuropore (No. 8116, 17 s.). No basement membrane separates the axial condensation from the neural ectoderm or from the digestive epithelium. Small blebs are visible on the surface of the neural groove. **D** Axial condensation (No. 8005, 16/17 s.). Neural ectoderm (here neural plate) is no longer clearly separated from the condensation

They contribute to the formation of the embryonic lamina terminalis and commissural plate.

The most uncertain part to identify precisely is the mesencephalon. Characterized in stage 10 and in the least advanced embryos of stage 11 by the presence of rostral neural crest, and in stage 11 by more or less pronounced sulci on its external surface (Fig. 1 B), its delineation towards both the diencephalon and the rhombencephalon is difficult. In the embryos studied only one segment could be found with certainty.

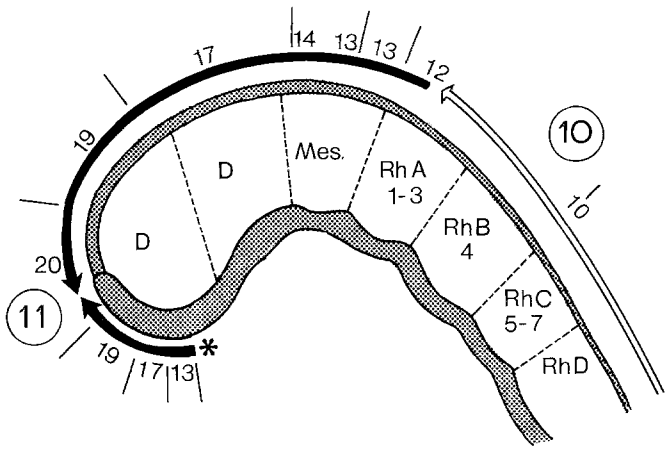


**Fig. 7.** The bidirectional closure of the rostral neuropore. The progress of closure is shown as a function of number of pairs of somites present. The opening is very large at stage 10 (*white arrow*) and becomes progressively smaller during stage 11 (*black arrows*). The *numerals along the arrows* indicate the number of pairs of somites present in various embryos. Early in stage 11 closure begins at the *asterisk* ("in front of" the chiasmatic plate). As a result, an embryonic lamina terminalis and a commissural plate become defined during stage 11. In an opposing direction, closure involves successively the roof of the mesencephalon and diencephalon. The difference in thickness in the dorsal and ventral regions is visible also in Figs. 1 and 10. The dorsal track is several times longer than the ventral path, and hence the rate of closure is much more rapid in the roof region

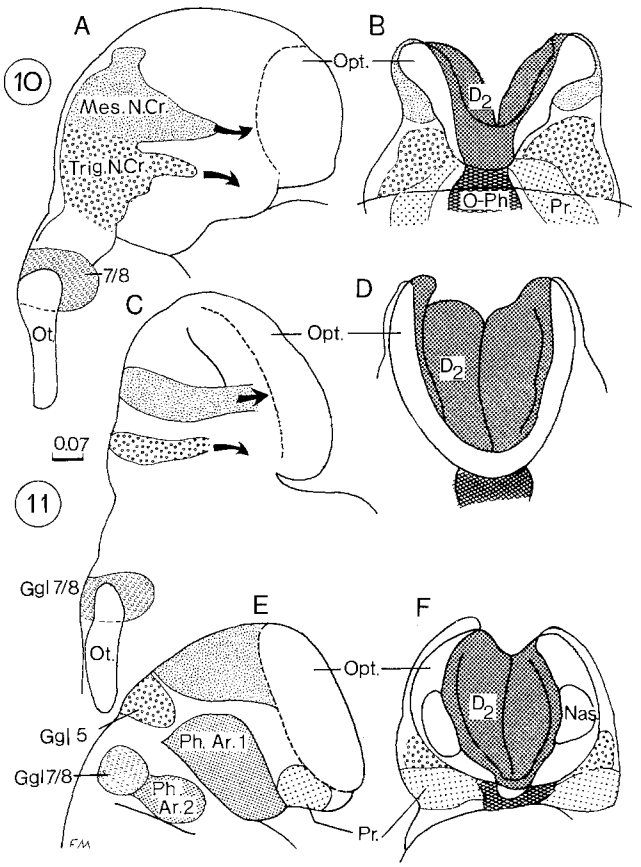


**Fig. 8A–F.** Neural crest and head mesenchyme in stages 10 and 11. **A, B** In an embryo (No. 5074, 10 s.) of stage 10, the mesencephalic neural crest cells can be seen spreading towards the optic primordium, and the trigeminal crest cells towards pharyngeal arch 1. The mesenchyme of the prechordal plate is continuous with the cardiac mesenchyme. **C–F** Two embryos of stage 11 (**C, D** No. 6344, 13 s., and **E, F** No. 4529, 14 s.). The mesenchyme derived from the prechordal plate forms the premandibular condensation in this and the following stage. Parts of the sheath of the optic primordium are now being formed by cells from the mesencephalic neural crest. The optic crest appears slightly later

In neuromeres present already in stages 9 and 10, subdivisions can be distinguished comparatively easily by the developing ganglia. The trigeminal ganglion characterizes Rh2, the faciovestibulocochlear ganglion Rh4, the glossopharyngeal Rh6, and the vagal Rh7; Rh3 and 5 appear to have no neural crest. Somites 1 to 4 enable RhD, the region of the hypoglossal nerve, to be identified.

*Optic primordium* (Figs. 8–10). Embryos with 13 pairs of somites do not possess an optic vesicle yet but only a deepened optic sulcus. Its termination in the median plane is readily visible as a postoptic recess in Fig. 10A. In head-on views the optic areas of D1 form the lateral parts of the head fold (Fig. 8D); the right and left primordia meet at the optic chiasma and together with the latter form a U-shaped rim. Part D2 is readily visible through the widely open rostral neuropore. In a 14-somite embryo the head-on view (Fig. 8F) is still very much U-shaped, and the slightly increased evagination of the optic primordia is expressed in a somewhat greater width. The connexion of the optic primordia at the optic chiasma no longer constitutes the rostral end of the forebrain, because the formation of the embryonic lamina terminalis has now begun. In 17-somite embryos (Fig. 10A, B) the caudorostral closure of the neural tube has progressed, so that little of part D2 of the diencephalon can be seen. In these embryos the first cells of the optic neural crest are beginning to form and will, together with material of the rostral (mesencephalic) neural crest, form a cellular sheath later in stage 11. The observations on the development of the optic neural crest agree completely with those of Bartelmez and Blount (1954) in that single cells or group of cells are seen leaving the wall of the vesicle (some can even be seen half-way out). The caudal limiting sulcus becomes apparent in the most developed embryos of this stage (Fig. 10D), and its location is marked by an accumulation of cells with cytoplasmic inclusions.

*Growth of the neural tube.* The measurements of 12 Carnegie specimens and of 2 embryos described in the literature (Pfannenstiel III described by Low 1908, and Eternod Delaf. reported on by Bujard 1914) are presented in Table 4. Compared with the average of measurements of 4 embryos of stage 9 and 13 embryos of stage 10, it can be concluded that (1) the prosencephalon grows less in stage 11 than it did in stages 9 and 10; (2) the mesencephalon shows almost constant values. An increase in growth in the midbrain is to be expected in embryos of stage 12, where two mesencephalic segments will be present as a characteristic feature; (3) the rhombencephalon shows a slight decrease in growth from stage 10 to stage 11. RhA takes up the same percentage as it did in stage 10, RhB diminishes slightly, RhC stays the same, and RhD diminishes; (4) an increase in growth is clearly present in the spinal cord; (5) the caudal eminence, representing mainly the primitive streak, although still producing material for the formation of new

**Table 4.** Changing interrelationships between parts of C.N.S. at stages 9, 10, and 11. Percentages of parts of C.N.S. relative to total length of neural plate/tube

| Embryos | Rostral neuro-pore | $D_1$ | $D_2$ | Pros. | Mes. | RhA | RhB | RhC | RhD | RhA–C | Total Rh | E (Spinal cord) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | closed part | open part |
| *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 | 42 | 11.5 |
| 318 | 12 | 7 | 5 | 12 | 4 | 7 | 7 | 7 | 17 | 21 | 38 | 40 | 6 |
| 4783 | 11 | | | 10 | 4 | 8 | 4 | 10 | 15 | 22 | 37 | 35 | 14 |
| 6344 | 11 | 3 | 5 | 8 | 5 | 6 | 4 | 12 | 18 | 22 | 40 | 38 | 9 |
| 4529 | 11 | 5 | 5 | 10 | 4 | 5 | 7 | 9 | 10 | 20 | 30 | 42 | 13 |
| E. Pfannenstiel III | | | | 12.5 | 4.5 | 9 | 3 | 3 | 12 | 21 | 33 | 34 | 16 |
| 7611 | 9 | 3 | 4 | 7 | 3 | 10 | 5 | 8 | 14 | 23 | 37 | 40 | 13 |
| 7702 | 4 | 4 | 4.5 | 8.5 | 7 | 14 | 6 | 5 | 16.5 | 25 | 41.5 | 32 | 11 |
| 470 | 1 | 5.7 | 4.4 | 10 | 4 | 7 | 4 | 11.5 | 13 | 22.5 | 30 | 50 | 9 |
| 5072 | 1 | | | 6 | 4 | 10 | 5 | 11 | 15 | 26 | 41 | 35 | 14 |
| 6784 | 8 | 2 | 4 | 6 | 6 | 5 | 4 | 5 | 10 | 14 | 24 | 50 | 10 |
| 7665 | 4.5 | 4.5 | 4.5 | 9 | 3 | 8 | 3 | 3 | 11 | 14 | 25 | 50 | 6 |
| E. Watt | 0.5 | 9 | 6 | 15 | 3 | 10 | 5 | 8 | 8 | 23 | 31 | 38 | 13 |
| 2053 | | 3 | 4 | 7 | 4 | 9 | 4 | 6 | 13 | 19 | 32 | 46 | 10 |
| 6050 | | 5 | 4 | 9 | 3 | 9 | 3 | 8 | 11 | 17 | 28 | 49 | 8 |
| E. Eternod-Del. | | | | 10 | 3.5 | 4 | 6 | 12.5 | 12.5 | 22.5 | 35 | 47 | 4 |

**Table 5.** Growth of the brain in stages 9, 10, and 11

| Embryos | Percentage of neural tube occupied by brain | Percentages of parts of brain relative to total length of brain | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $D_1$ | $D_2$ | Pros. | Mes. | RhA | RhB | RhC | RhD | Total Rh |
| *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 |
| *Stage 11* (mean of 14 embryos) | 48 | | | 19 | 10 | 15 | 13 | 21 | 29 | 73 |
| 4783 | 51 | | | 20 | 9 | 15 | 7.5 | 19 | 30 | 71.5 |
| 6344 | 53 | 6 | 8.6 | 14.6 | 9 | 12 | 8 | 22 | 35 | 77 |
| 318 | 54 | 13.5 | 9.6 | 23 | 7 | 12 | 13 | 12 | 32 | 70 |
| 4529 | 44 | 11 | 10 | 21 | 9 | 11 | 16 | 19.7 | 23 | 70 |
| Pfannenstiel III | 50 | | | 25 | 9 | 18.6 | 6 | 15.5 | 23.7 | 67 |
| 7611 | 47 | 6.5 | 9 | 15.8 | 7 | 20 | 10 | 17 | 29.6 | 77 |
| 7702 | 57 | 6.5 | 8 | 14.5 | 12.5 | 23 | 8 | 12.5 | 28.5 | 73 |
| 470 | | 12 | 9 | 21 | 9 | 15 | 9 | 24 | | |
| 5072 | 52 | | | 10.6 | 9 | 19 | 10 | 22 | 29 | 80 |
| 6784 | 39 | 4.5 | 8.7 | 13 | 11 | 12 | 11 | 22 | 25.6 | 71 |
| 7665 | 41 | 6.5 | 8 | 14.7 | 19 | | 37 | | 29 | 66 |
| 2053 | 44 | 8 | 10 | 18 | 8.6 | | 43 | | 20 | 73 |
| 6050 | 40 | 12 | 9.6 | 21 | 7 | 22 | 8.8 | 20.6 | 27 | 79 |
| Eternod-Delafield | 48 | | | 20.7 | 7 | 7.6 | 13 | 26 | 26 | 72 |

somites and of neural ectoderm decreases more than 50% from stage 10 to stage 11.

In view of the fact that the spinal cord has a higher rate of growth in stage 11 than in stages 9 and 10, a comparison of the different parts of the brain was made separately (Table 5). Even then, however, the prosencephalon occupies a smaller percentage of the total brain than in stage 10 and is more comparable to that of stage 9. The mesencephalon is stable. The rhombencephalon is more similar to that of stage 9 than 10, in that it occupies a greater percentage than in stage 10.

*Mitotic activity* (Table 6). This does not differ significantly from the values found in stage 10 with regard to the prosen-

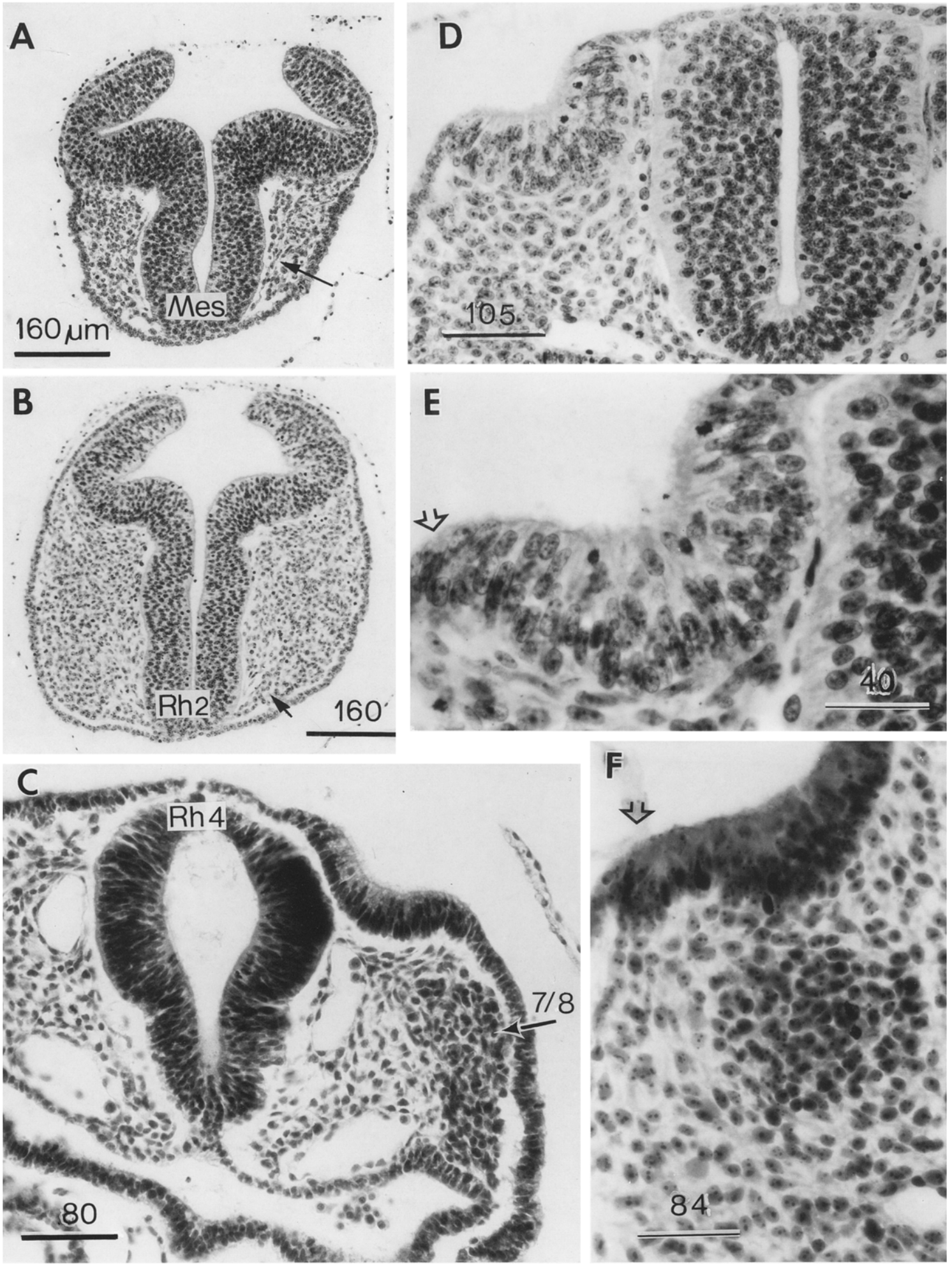
A
Mes
160 µm
B
Rh2
160
C
Rh4
7/8
80
D
105
E
40
F
84

**Table 6.** Percentages of mitotic figures in parts of C.N.S. and notochord

| Embryo | Pairs of somites | Pros. | Mes. | Total Rh | Sp.cord closed | Sp.cord open | Notochord |
|---|---|---|---|---|---|---|---|
| *Stage 10* (mean of 4 embryos) | – | 21 | 7 | 25 | 38 | | 8 |
| *Stage 11* | | | | | | | 12 (mean of 4 embryos) |
| 7611 | 16 | 20 | 7 | 31 | 37 | 7 | – |
| 7665 | 19 | 14 | 9 | 36 | 39 | 2 | – |
| 2053 | 20 | – | – | – | – | – | 17 |
| 6050 | 19/21 | – | – | – | – | – | 9 |

cephalon and the mesencephalon. Some increase is found for the rhombencephalon and the spinal cord. Mitotic activity is still present in the primordia of the cranial ganglia. A slight tendency towards overcrowding and sub-surface mitotic figures is present in the area of the optic sulci.

*Cranial flexure* (Table 2). The angle changed from 150 to 104 degrees in stage 10. In stage 11 angles of 148 and 143 seem to be the maximum. More frequent values lie between 120 and 110 degrees. The minimum noted was 90 degrees.

*Cytoplasmic inclusions.* These were found at the same sites as in stage 10, but were not as frequent and were more limited in location. An accumulation of inclusions is recognizable at the site of the neural folds immediately prior to the closing of the rostral neuropore. In some embryos elimination of parts of cell nuclei was observed also in areas without inclusions. Midbodies were especially well seen in the neural cavity of one embryo (No. 7611).

*Marginal layer.* A cell-free layer is distinct in the lateral walls of RhD and of the spinal cord, but is less developed in RhA to C. In the mesencephalic area it is only beginning to form, and it is not yet present in the forebrain.

*Occlusion of the neural tube* (Table 2). Where the faciovestibulocochlear ganglion forms, the neural tube at this level of Rh4 is compressed because of the large amount of cells given off by the neural crest. This is the case already in stage 10. In some embryos of stage 11 the cavity in Rh4 becomes almost completely obliterated. The same embryos show obliteration of the ventricular space in more caudal areas. Of the 20 embryos, 4 show occlusion to some degree, and some more or less completely. (In many instances only half or three-quarters of the ventricular lumen is occluded.) It is to be stressed that all 4 embryos show an open rostral neuropore.

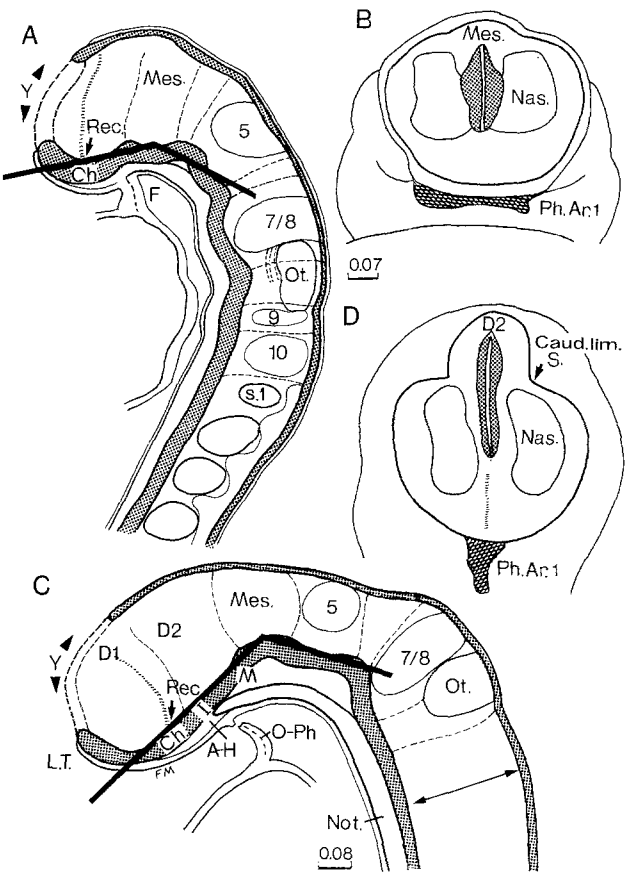


**Fig. 10A–D.** Decrease of the angle at the cranial flexure and flexion of the head. **A, B** Embryo No. 7702, 17 s. and **C, D** embryo No. 7665, 19 s., showing that the angle of the cranial flexure diminishes. The caudal limiting sulcus is developing. The rhombencephalon is widening. Mesenchyme is developing in the area between the notochord and the brain (**C**), and the oropharyngeal membrane is beginning to rupture (**C**)

*Neural crest.* Neural crest cells are still being given off at the site of Rh4, Rh6, and Rh7 (as described in stage 10 by Müller and O'Rahilly 1985), all of which are now closed. Therefore, the same areas, open in stage 10 and producing neural crest already at this former stage, have two different modes of production of neural crest material. What is the same in both modes is the fact that the neural crest cells are clearly derived from the neural ectoderm. Rostral (mesencephalic) neural crest was still seen in the least advanced embryos of stage 11; in the most advanced, newly formed rostral crest cells could no longer be detected. Optic neural

**Fig. 9A–F.** Photomicrographs of neural crest, optic primordium, and otic pit. **A, B** Transverse sections (No. 7611, 16 s.) showing the optic evaginations and the still open rostral neuropore. In **A** the optic sheath is beginning to form. Mesencephalic neural crest is marked by an *arrow*. **B** Shows the trigeminal neural crest (*arrow*). **C** Otic pit (No. 4529, 14 s.) showing cells of the otic sheath. **D, E** Otic sheath (7611, 16 s.). No basement membrane is visible between the epithelium of the otic pit and the cells for the otic sheath. **F** Otic pit (No. 8116, 17 s.). Cells from the edge of the otic pit (*arrow*) join ganglion 7/8

crest has been mentioned with the optic primordium. Some crest material is present at the level of Rh1 in two embryos (Nos. 7611 and 7702, 16 and 17 pairs of somites respectively). The material for the future glossopharnygeal and vagal ganglia is generally scarce. The spinal neural crest in the least advanced embryos reaches up to somite 9; in the most advanced to somites 13/14. Parts Rh3 and Rh5 are crest-free.

*Nasal plate and neural crest.* The nasal plate is one of the ectodermal areas giving rise to neural crest. Although it does so only in later stages, the plate becomes apparent in stage 11. It is shown in the head-on views of embryos having 14 or more pairs of somites (Figs. 1, 8, 10). It lies on both sides of the neuropore, and, when this closes, it occupies the lateral aspect of the area that is the last to become closed.

*Otic pit and neural crest.* The otic plate, present already in stage 9, begins to be invaginated in the most advanced embryos of stage 10. Various degrees of indentation are present in the embryos of stage 11, in which the formation of an otic vesicle does not yet take place. Numerous mitotic figures are present (Fig. 9C–F). It is possible to see cells leaving the otic epithelium even more clearly than in stage 10 (Fig. 9E, F, arrows). The basement membrane is disrupted at those sites, and the cells in some embryos form strands, especially on the lateral aspect (Fig. 9D) and migrating ventrally. Ventral to the otic pit they form a characteristic cellular sheath (Figs. 1A, 9C–E, 10A), and they still have the appearance of neural crest cells. Already in stage 10 the otic plate was expanding from RhB into RhC, and in embryos of stage 11 the wall of the otic pit still occupies both rhombomeres. The caudal area of RhB, which is now Rh4, and the rostral part of Rh5 are its site now. In the most advanced embryos of stage 11 the otic pit is almost completely related to Rh5, and to the area of the future pharnygeal arch 3. The faciovestibulocochlear ganglion, however, retains its relation to Rh4 and to the hyoid arch.

*Neural crest and head mesoderm.* A comparison with an excellent embryo of stage 10 helps to elucidate the progress in stage 11 (Fig. 8). In the most advanced embryos of stage 10 the areas of two pharyngeal arches become delineated on the surface by changes in the thickness of the surface ectoderm. The mesencephalic neural crest is spreading out on the lateral surface of the head mainly parallel to the mesencephalon and towards the optic primordium (Fig. 8A, B). The number of trigeminal crest cells seems slightly more limited, but this material is also migrating. Ganglion 7/8 is closely related to the area of pharyngeal arch 2. The material of the prechordal plate seen in a head-on view is limited in its migration by the chiasmatic plate rostrally and by the dorsal aortae caudally. It is spreading out laterally into the premandibular area and ventrally to the heart.

The pharyngeal arches are particularly well outlined in one embryo (No. 4529, 14 pairs of somites: Fig. 8E). The mesencephalic neural crest is still being produced and spreads towards the optic evagination, paralleling the mesencephalon and diencephalon 2 laterally. In more advanced embryos mesencephalic neural crest mixes with optic crest and forms the sheath of the optic vesicle. The trigeminal neural crest is more condensed than in stage 10. A condensation of cells is visible inside the mandibular arch, but it is not possible to decide whether it is neural crest material deposited there during stage 10 or paraxial mesenchyme or both. Also in pharnygeal arch 2, two different groups of cells seem to be present, the first clearly representing ganglion 7/8, and the second, also slightly more condensed, forming a prolongation within the arch. A continuation from one group of cells to the other is clear also in other embryos. The prechordal mesenchyme is limited to the premandibular area.

*Secretory phenomena.* In at least 3 embryos of stage 11 the open caudal part of the nervous system seems to secrete a material that resembles protoplasm in consistency and forms small droplets inside the wall of the developing neural tube, especially at the transition to the surface ectoderm. The larger droplets contain more hyaline (instead of granular) material (Fig. 3E, asterisks: see also Figs. 3F and 6C). Some droplets, at the same level, are visible in the hindgut.

## Discussion

The arbitrary but convenient distinction between stages 10, 11, and 12 depends primarily on the number of pairs of somites present: 13–20 pairs for stage 11. The age at stage 11 is generally taken to be $24 \pm 1$ days, which is in agreement with v. Hayek (1931), who listed 23–27 in his description of a 16-somite embryo recovered surgically 25 days after cohabitation.

*Prechordal plate.* Gilbert (1957) correctly described the cells deriving from the prechordal plate in stage 11 as "a diffuse mass of mesenchymal cells, situated just beneath the surface ectoderm at the upper end of the mandibular arch and maxillary process."

*Notochord, notochordal plate, and axial condensation.* Already in advanced embryos of stage 10 a dual mode of origin of the notochord is recognizable (Müller and O'Rahilly 1985): (1) rostral to the neurenteric canal or its site, the notochord develops from the notochordal plate, whereas (2) caudally it arises from the axial condensation.

*Rostral part of notochord.* Cell counts in the notochordal plate indicate clearly that the number of cells present in stage 8 (O'Rahilly and Müller 1981) does not change in stages 9 and 10. In advanced specimens of stage 11, in which the notochordal plate has been transformed into a notochord, mitotic activity begins to increase. In a very advanced embryo (No. 2053) the notochord is thicker and has changed from a rather slender to a robust, rod-like structure. The relationship between the notochordal plate and the alimentary canal, the roof of which it forms, was well understood by Keibel (1889), Low (1908). Wallin (1913), Watt (1915), Bartelmez and Evans (1926), Wen (1928), Orts Llorca et al. (1958), and Mori (1959). The presence of a "canal" in the newly formed notochord in embryos of stage 11 (explained in Fig. 2) was illustrated by Bartelmez and Evans (1926) in their Fig. 17 (No. 470). However, this is not a real notochordal canal, which appears much earlier in embryos of stage 8 (O'Rahilly and Müller 1981) in the notochordal process, and disappears shortly thereafter when the notochordal plate develops. The

complicated and variable development of a notochordal canal was summarized by O'Rahilly (1973).

*Caudal part of notochord.* The caudal portion of the notochord was recognized as being different from the rostral by Wallin (1913) and by Watt (1915). It shows a more marked reaction for alkaline phosphatase "until it reached nearly the same intensity of the caudal mass of the mesoblasts and the primitive streak" (Mori 1959). The present authors, however, are probably the first to point out the difference in development between the two parts, the rostral being derived from the notochordal plate, the caudal from the axial condensation (Müller and O'Rahilly 1985). This development in the caudal eminence in human embryos corresponds with the findings of Butcher (1929), who found in the rat that the end bud contributes to the formation of the notochord. Similarly, in the mouse, the data of Tam (1984) would seem to imply the formation of the notochord from the material of the caudal eminence (his Fig. 4). The chick seems to be different in that "the tail bud contributes cells to the posterior part of the neural tube, but not to the notochord" (Schoenwolf 1978; Schoenwolf et al. 1985).

A very close relationship still exists between the notochord/notochordal plate and the neural tube/plate in stage 11. Therefore, induction, if ever present, could still take place along almost the whole length of the neural axis. (A space appearing between notochord and neural tube in reconstructions of stage 11 embryos at the level of the spinal cord is an artifact.)

Schoenwolf (1982) mentions another function of the notochord, namely in bilateral elevation of the neural plate and stretching of the neural tube dorsoventrally. Applying this to the human embryo, it would seem that this adhesive force would be all the greater in areas where the notochord is still plate-like, in the least advanced embryos of stage 11. A relatively extensive contact area exists also in the caudal region of all embryos of this stage, where the notochord from the onset of its formation in the caudal eminence is more voluminous. How the mechanism postulated by Schoenwolf would work in the forebrain region, however, is more difficult to interpret.

*Neurenteric canal.* The site of the neurenteric canal is moving further caudad from the site of somite 18 in stage 10 (cf. Fig. 2 in Müller and O'Rahilly 1985) to the site of somite 21 in stage 11. The discrepancies in the values of some of the embryos of stage 11 are probably caused by the inadequacy of endodermal thickness as a criterion for determining its exact site. Watt's (1915) claim of a neurenteric canal "immediately succeeding the caudal edge of the cloacal membrane" in a 19-somite embryo (his Pl. 1, Fig. 1) is unconvincing and appears to be erroneous.

*Caudal eminence (or end bud).* Mori (1959) found a very intense reaction for alkaline phosphatase in an embryo of stage 11 (13/14 somites). The caudal eminence "remains an active seat of proliferation of neural tube, mesoderm, and chorda dorsalis, with a continuation of somites from paraxial mesoderm." Svajger et al. (1985) "suggest the formation of the tail gut in the rat embryo by a mechanism analogous to the secondary neurulation in the mouse."

Bartelmez and Evans (1926) pointed out that asymmetry is very characteristic in early embryos "so long as the nervous system dominates the external view." They referred especially to the left and right neural folds in the forebrain. Surprisingly, however, they made no mention of an abnormal asymmetry relative to the caudal eminence in embryo No. 470, but remarked that "interpretation is difficult in our 16-somite embryo C.C.470." The only percentage for the primitive streak they gave for a stage 11 embryo (No. 4783) was 9–11 per cent.

Tam (1984) studied experimentally the histogenetic capacity of tissues in the caudal end of the mouse embryo. He demonstrated that the caudal eminence in its pluripotentiality takes over the function of the primitive streak. He believes with other authors "that the transition from a streak contribution to a purely bud contribution occurred at the lumbosacral level of the embryo." From this viewpoint, the caudal eminence of embryos of stage 11 that show insufficient material as yet for 25 pairs of somites (not all of them already individualized) would still possess a primitive streak.

A slight thickening of the surface ectoderm in the proximal part of the cloacal membrane cannot represent a precursor of the *Schwanzleiste* (O'Rahilly and Müller 1985) because the latter develops distal (caudal) to the cloacal membrane.

*Surface ectoderm.* The surface ectoderm at stage 11 possesses areas of at least three different thickness (O'Rahilly and Müller 1985). Similar findings were recorded by Steiner (1929) in an 18-somite embryo. He found unilayered flat ectoderm over the neural tube and heart; multilayered, cylindrical epithelium over the pharyngeal arches; and cuboidal over the remainder of the body. His description, "Von der Kiemenbogengegend aus erstreckt sich ein Streifen zwei- bis dreireihig angeordneter, prismatischer Epithelzellen an der Seite des Rumpfes eine größere Strecke kaudalwärts fort," is interpreted by the present authors as the forerunner of the occipitocervical part of the ectodermal ring.

*Somites.* The mean time for the formation of one pair of somites during stages 9–12 inclusive is 6.6 h. Goedbloed and Smits-van-Proije (1986) found 1.7 h in the mouse and 2–3 h in the rat. Somites first appear at $20 \pm 1$ days in the human and (according to the above-mentioned authors) at 7.75 days in the mouse and 8.95 days in the rat. Hence, if it could be assumed that the rate is constant and variation is minimal, the somitic count might provide further precision in estimating the age of an embryo.

Bellairs (1985) pointed out that in the chick the "newly formed tail bud somites are relatively small" because less material is present at the time of formation.

*Closure of rostral neuropore.* Bidirectional closure of the rostral neuropore, first described by Johnston (1909) in lower vertebrates, has been discussed by Müller and O'Rahilly (1984). It seems that closure may occur in several places independently (Müller and O'Rahilly 1985).

*Neuromeres in brain.* Neuromeres are transverse swellings in the developing neural tube. They can best be assessed by evaluating the three main planes of section. Reconstructions of sagittal sections show swellings and grooves best, whereas transverse sections are preferable in determining the relationship to ganglia. Indications of neuromeres were already evident at stages 9 (Müller and O'Rahilly 1983) and

10. At stage 10 deepenings in the floor plate of the mesencephalon, RhA and RhB are evident (Müller and O'Rahilly 1985, Fig. 7). Those deepenings, especially at the level of the rhombomeres, become apparent very clearly only in stage 12 and are visible still in stage 17. A combination of the various data shows 2 neuromeres in the forebrain (D1 and D2), 1 in the midbrain, and 6–7 in RhA to C. Part RhD, the area of s. 1–4 (the hypoglossal region), is clearly delineated neither by a groove nor by a deepening. D1 and D2 correspond to neuromeres a and b, M to proneuromere B, RhA, B, C to proneuromeres C, D, E of Bergquist and Källén (personal communication, 1969). Their inventory contains no equivalent for RhD of the present authors. RhA–C, as described by Bartelmez (1923) for stage 9, were termed proneuromeres by Bergquist (1952), because of their existence in the still open neural groove. Three-dimensional reconstructions demonstrate the neuromeres clearly as serial swellings of the brain. In that of Politzer (1928, his Fig. 13), showing an 18-somite embryo, his M represents D2, Ir is M, and Rh6 is Rh5.

*Optic primordium.* The development of the optic primordium and the appearance of a caudal limiting sulcus in stage 11 were described and illustrated by O'Rahilly (1966).

*Midbrain.* Whether the midbrain contains one or two segments in stage 11 is considered to be an open question by Wen (1928), and "a considerable degree of confusion still exists" (Bartelmez and Dekaban 1962). Sternberg (1927) and Davis (1923) described only one segment. In Bartelmez and Dekaban's reconstruction of No. 7665 and Bartelmez and Evans' (1926) reconstruction of No. 470, two segments are shown. The second midbrain segment corresponds to what in this study is proposed as midbrain; their first segment of the midbrain, however, represents approximatively pretectum and prerubrum of D2 according to the present interpretation. Only in stage 13 can a part of D2 clearly be identified as the so called synencephalon (O'Rahilly and Müller, unpublished). A distinct morphological delineation of the synencephalon depends on the appearance of the posterior commissure and the fasciculus retroflexus (habenulopeduncular tract), and hence has to await stages 16 and 17.

*Growth of neural tube.* Desmond and O'Rahilly (1981) included embryos of stages 11 to 23 in their study, but because embryos of younger stages were not measured, the real beginning of enlargement could not be determined. Tuckett et al. (1985) showed that there is no growth of the mesencephalon in rat embryos in stages comparable to 10–12 in the human. A differential growth pattern was described for the chick by Jacobson (1980). Elongation of closed areas is faster than elongation of open ones. During 4 h the closed neural tube grew 29% more rapidly compared with the open tube rostrally (3–4%) and caudally (7%). A greater increase in length of the closed parts of the neural tube is also indicated by the percentages given in Table 4. Hence the validity of Schoenwolf's (1982) statement that "it is obvious that a direct relationship exists between stretching of the neuroepithelium and closure of the neural groove" but experiments are necessary to determine "how this elongation occurs and whether this intriguing relationship is causal or merely coincidental."

*Mitotic activity.* Mitotic figures could be counted in only two advanced embryos of stage 11, so that the percentages given may not be entirely representative for the whole group. Despite that, the values are not very different from those for the average in stage 10.

Tuckett et al. (1985) suggest that "cells generated in the midbrain migrate rostrally to populate and augment the expanding forebrain." They found a non-increasing midbrain in rat embryos beside a rapidly increasing forebrain, a relatively too large number of newly produced cells in the midbrain, and not enough in the forebrain. Smart (1965) distinguished non-surface (without reaching the ventricular surface) mitotic figures from surface mitotic figures, where the rate of proliferation is very high in an "ependymal choke."

Probably unique in the whole nervous system is the localization of the mitotic figures giving rise to the optic neural crest in that they lie on the external surface of the neural ectoderm. These "external" mitotic figures were observed also by Bartelmez and Blount (1954).

*Marginal layer.* The marginal layer (primordial plexiform layer) seems to play an essential role in the establishment of an early functional organization of the brain. Such ideas were first expressed (1971) and recently summarized by Marin-Padilla (1984), who pointed out that the layer "is considered functionally active since primitive synaptic contacts have been demonstrated between its fibres and the neurons." The marginal layer is "one of the oldest cerebral formations" in the phylogeny of the vertebrates (except fish) (Ramón y Cajal, cited by Marin-Padilla 1984). The presence of the marginal layer in the rhombencephalon in stage 11 presages the early differentiation of motor nuclei and tracts in stages 12 and 13 (O'Rahilly et al. 1984).

*Occlusion of the brain.* In the chick occlusion occurs "near the time that the neural walls become apposed in the dorsal midline" (Schoenwolf and Desmond 1984a). The possible role in brain enlargment was discussed by Schoenwolf and Desmond (1984b), Desmond and Schoenwolf (1985), and again by Pacheco et al. (1986). "The brains of chick and human embryos begin to enlarge dramatically concomitantly with occlusion" (Desmond and Schoenwolf 1985). Furthermore, "one must assume" that a "positive intralumenal [luminal] pressure" is generated, and that a pinching together of the upper portions of the spinal cord is necessary "to initiate brain expansion" (Desmond 1982).

Desmond (1982) stated that, in the human "occlusion first occurs at stage 11 and is most prominent during stages 11 and 12" and "there is occlusion of the lumen of the spinal cord in human embryos, subsequent to the formation of a closed tube." Of the 16 embryos of stage 11 of the Carnegie Collection that she studied, 25% showed occlusion by a "conservative assessment" and 75% by a "more liberal assessment." The latter category, merely "slits" that "would be regarded as questionable occlusion," is not considered as occlusion by the present authors. Desmond found that "in all cases examined, the neural tube demonstrates uninterrupted occlusion from the level of somite number three to at least somite nine." In the present study, occlusion was found in only 4 out of 20 embryos (in 20%) with a still open rostral neuropore. The extent of the occlusion is from somite 4–12, rhombomere 4 to somite 6, somite 1–15, and somite 1–8 respectively.

No occlusion was found in the other embryos. Moreover, in the two embryos with a closed rostral neuropore (Nos. 2053 and 6050) there is no occlusion (Table 2). As shown in Table 5, longitudinal expansion of the brain and especially of the forebrain does not start at stage 11, as it should were an occlusion present comparable to that in the chick. On the contrary, from stage 10 to stage 11 the forebrain shows less growth, the mesencephalon stays the same, and the rhombencephalon decreases in relative length; most of the growth during stage 11 is achieved by the spinal cord (Table 4). Whereas the experiments on occlusion in chick embryos are convincing, the existence of the phenomenon in human embryos is not. The statement that brains of "human embryos begin to enlarge dramatically concomitantly with occlusion" (Desmond and Schoenwolf 1985) is incorrect both in relation to occlusion and to growth of the brain. Occlusion combined with a closed rostral neuropore is not present in stage 11, and brain growth in stage 11 is clearly at a standstill.

*Neural crest.* The neural crest of the human embryo was classified by O'Rahilly (1965). Mesencephalic neural crest is still forming in the least advanced embryos with an open and in those with a closed mesencephalon. Neural crest cells as seen in experimental studies are in general morphologically distinguishable from mesodermal mesenchymal cells, the former being larger and more rounded, the latter smaller and irregularly shaped (Nichols 1986). Tan and Morris-Kay (1985) refer to production of mesencephalic neural crest from open mesencephalic neural folds in the rat embryo. This material and neural crest cells from rhombomere 2 migrate into the first pharyngeal arch. A contribution to the hyoid arch observed in the chick (Le Lièvre 1974; Noden 1975) was not seen in the human. However, mesencephalic neural crest in the mouse has been reported to migrate dorsocaudally to the otic pit (Nichols 1981). Migration of neural crest from the midbrain could no longer be seen in rat embryos with 9 somites; in those with 11 somites, migration of neural crest cells was confined to the postotic area (Tan and Morriss-Kay 1985). Compared with the human embryo, migration of neural crest cells in the rat is therefore more limited in time.

Mori (1959) found an alkaline phosphatase reaction in the human in cells of the optic crest as well as in the rostral neural crest lateral to the midbrain and extending to the optic vesicle. He found neural crest cells (undoubtedly of rhombomere 2) extending into the mandibular process in his embryo with 13/14 somites. A shift of neural crest cells into the pharyngeal arches was demonstrated in mouse and rat by Mulnard (1955) by using alkaline phosphatase as a selective reaction.

The origin of neural crest cells in the rat was strictly limited to the neural ectoderm "from the 6-somite stage onwards" (Tan and Morriss-Kay 1985). Discrepancies in opinion as to whether surface ectoderm would also be involved are partly caused by technical limitations of light-microscopy. SEM pictures clearly show an exclusive origin from the neural ectoderm in the rat (ibid.). Origin from the neural ectoderm and a continuing formation of neural crest after closure of the neural tube in human embryos was already observed by Streeter (1942, his Fig. 5, xi); in the mouse it has been reported by Nichols (1981).

Neural crest-free zones were found in rhombomeres 3 and 5 in the rat and were interpreted as a demarcation of the future pharyngeal grooves (Tan and Morris-Kay 1985). Contributions to the ganglia of cranial nerves from the ectoderm of the pharyngeal arches was discussed by Müller and O'Rahilly (1980).

*Nasal plate and neural crest.* The thickening on both sides of the open and then closed rostral neuropore is part of the ectodermal ring described by O'Rahilly and Müller (1985). Hinrichsen (1985) provided an SEM picture of an embryo of stage 12, but the nasal plate was not identifiable with this method. Couly and Le Douarin (1985) showed experimentally the presence of a very early nasal plate and adenohypophysial primordium in quail-chick chimeras in stages comparable to stages 8 (presomitic) and 9 (3 somites) in the human. More surprising than the close relationship between the nasal plate and the floor of the telencephalon which was also present at this early stage, was the fact that the anterolateral neural ridge yields also the "ectodermal cells lining the nasal cavity forming the conchae and the ectoderm covering the upper beak."

*Otic pit and neural crest.* At stage 12 the otic vesicle is located at the level of rhombomere 5 (Müller and O'Rahilly 1980). Its shift from Rh4 to Rh5 was already observed by Bartelmez and Evans (1926). A contribution of cells from the otic plate, pit, and vesicle to the facial or faciovestibular ganglion in the human was observed by O'Rahilly (1963), in mice by Deol (1967, 1970) and Verwoerd and van Oostrom (1979). Mori (1959), however, found no direct connexion between the neural crest of ganglion 7/8 and the otic pit. (His embryo of 13/14 s. was probably not sufficiently advanced to show enough cells for a histochemical reaction.) In mouse embryos corresponding to stage 11, Bretos (1979) found a higher density of RNA in the cytoplasm of the otic pit, in the wall touching the neural tube, and in the adjoining neural tube itself. Also, during the invagination of the pit, she found increased alkaline phosphatase activity in the ciliated cells lining it. She noted otic cells leaving the epithelium in embryos having 20 somites, particularly ventrally and posteriorly. "In this early primordium we found two portions, one medial and the other lateral. The latter is characterized by its alkaline phosphatase reaction and by its location dorsal to the geniculate ganglion primordium." She insists, however, that these two parts do not correspond to the future vestibular and cochlear ganglia.

No reference to a formation called otic sheath by the present authors was found in the literature. The otic sheath, consisting of neural crest cells derived from the otic pit but not participating in the formation of ganglion 7/8, has to be followed carefully in consecutive stages. It is possible that it represents material for the future skeleton of the labyrinth.

*Optic neural crest.* Cells arising from the optic primordia and forming the optic crest are clearly derived from the external surface of the brain. External mitotic figures were observed also by Bartelmez and Blount (1954). As already discussed for stage 10 (Müller and O'Rahilly 1985), the eyes develop earlier in the mouse than in human embryos, and optic neural crest also is produced earlier. Nichols (1981) described optic crest (called forebrain crest) in mouse embryos clearly belonging to stage 10 (5–7 somites). In rat

embryos, production of optic neural crest reaches its height in 11- to 14-somite embryos (Bartelmez 1962).

*Derivatives of neural crest.* Derivatives of particular relevance here include meninges, head mesenchyme, and the optic sheath.

Sensenig (1951) described a single layer of cells (his Fig. 1 of embryo No. 2053) and apparently deriving from the neural crest as the "first indication of the pia mater." Its exact location around the caudalmost part of the medulla oblongata was described by O'Rahilly and Müller (1986).

The neural crest in the chick that corresponds approximately to the mesencephalic crest in the human embryo ("forebrain" crest, Noden 1978) produces peri-ocular skeletal and connective tissues, and is the only source of mesenchyme in the areas rostral to the mesencephalon. Entirely of neural crest origin are maxillary, premaxillary, and nasal bones. The "hindbrain" neural crest (caudal mesencephalic and metencephalic crest, Noden 1978) forms structures in the mandibular process.

The optic sheath has a double origin, as described by Bartelmez and Blount (1954). Strands from the mesencephalic neural crest are clearly visible in human embryos with 13 somites, reaching from the mesencephalon to the optic evagination (cf. Fig. 2 in Bartelmez and Blount 1954). No optic neural crest is being produced at this time. In an embryo with 19 somites (No. 7665) two different kinds of cells are present: (1) long and slender ones possessing two long cytoplasmic processes and most probably representing mesencephalic neural crest cells; and (2) more rounded cells from the optic crest.

*Teratology.* In order to interpret some of the findings of experimental embryology and to see them in relationship to developmental processes in the human embryo, two procedures are desirable: (1) adoption of an acceptable terminology, and (2) the use of staging.

Kirby and Bockman (1984), for example, speak of cephalic neural crest in the chick where they mean the crest from the otic primordium to somite 6. Ablation of this crest leads to conotruncal abnormalities. Perhaps even more important than the source of the crest is the question of when the pathway for the migration of neural crest to the heart is still open. In the human it seems that only in embryos of stage 10 and the least developed embryos of stage 11 does a direct pathway still exist, and it is possible that prechordal mesenchyme and perhaps mesencephalic neural crest use such a pathway.

Several laboratory mammals have been staged according to the Carnegie system, e.g., the macaque (*Macaca mulatta*) by Hendrickx and Sawyer (1975) and by Gribnau and Geijsberts (1981). The importance of staging in the rat and mouse was stressed by Müller and O'Rahilly (1984) in the study of anencephaly, where two tables were provided. Problems arise not only in comparing species but even in strains within a species. For example, although views of median sections of rat embryos by Tuckett et al. (1985) resemble the graphic reconstructions presented here, a fundamental difference is that the brain of the rat embryo develops more rapidly in relation to somitic count. This includes a more speedy evagination of the optic primordia, more rapid diminution of the cranial flexure, earlier closure of the rostral neuropore (when 16 pairs of somites have appeared versus 20 in the human), and earlier subdivision of the mesencephalon. Hence, in the rat, somitic count appears to be an inadequate basis for comparison with the human. This applies also to the mouse, in which the rostral neuropore closes when 17 pairs of somites have appeared (Verwoerd and van Oostrom 1979). Moreover, "different inbred mouse strains and hybrid crosses develop at different rates prenatally" (Wahlsten 1981), so that morphological staging is necessary if valid comparisons are to be made.

*Other systems.* The sinus venosus appears, trabeculation may be present in the ventricles, and, in a few embryos, an interventricular septum has been recorded (McBride et al. 1980). The increasing complexity of embryonic development makes it impracticable to discuss other systems in this limited account.

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Accepted August 23, 1986