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# The development of the human brain, the closure of the caudal neuropore, and the beginning of secondary neurulation at stage 12*

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**Summary.** Twenty-four embryos of stage 12 (26 days) were studied in detail and graphic reconstructions of five of them were prepared. The characteristic features of this stage are 21–29 pairs of somites, incipient or complete closure of the caudal neuropore, and the appearance of upper limb buds. The caudal neuropore closes during stage 12, generally when 25 somitite pairs are present. The site of final closure is at the level of future somite 31, which corresponds to the second sacral vertebral level. Non-closure of the neuropore may be important in the genesis of spina bifida aperta at low levels. The primitive streak probably persists until the caudal neuropore closes, when it is replaced by the caudal eminence or end-bud (*Endwulst oder Rumpfknospe*). The caudal eminence, which appears at stage 9, gives rise inter alia to hindgut, notochord, caudal somites, and the neural cord. The material for somites 30–34 (which appear in stage 13) is laid down during stage 12, and its absence would be expected to result in sacral agenesis. Aplasia of the caudal eminence results in cloacal deficiency and various degrees of symmelia.

The junction of primary and secondary development (*primäre und sekundäre Körperentwicklung*) is probably at the site of final closure of the caudal neuropore. Secondary neurulation begins during stage 12. The cavity of the already formed spinal cord extends into the neural cord, and isolated spaces are not found within the neural cord. Primary and secondary neurulation are probably coextensive with primary and secondary development of the body, respectively. The telencephalon medium has enlarged, two mesencephalic segments (M1 and M2) are distinguishable, and rhombomere 4 is reduced. The sulcus limitans is detectable in the spinal cord and hindbrain (RhD), and in the mesencephalon and diencephalon, where it extends as far rostrally as the optic sulcus in D1. A marginal layer is appearing in the rhombencephalon and mesencephalon. The first nerve fibres are differentiating, chiefly within the hindbrain (from the nucleus of the lateral longitudinal tract). Optic neural crest is at its maximum, and the otic vesicle is giving crest cells to ganglion 7/8. Neural crest continues to develop in the brain and contributes to cranial ganglia 5, 7/8, and 10/11. The spinal crest extends as far caudally as somites 18–19 but shows no subdivision into ganglia yet. Placodal contribution to the trigeminal ganglion is not certain at stage 12. Such a contribution to ganglion 7/8 is not unlikely. Involvement of neural crest in the formation of the derivatives of pharyngeal arches 1 and 2 is possible but has not yet been confirmed in the human embryo.

**Key words:** Human embryo – Human brain – Caudal neuropore – Neural crest – Secondary neurulation

## Introduction

His, who later (1904) illustrated a specimen of stage 11, described (1880) two embryos of stage 12 (M, about 26 pairs of somites, and Lr, about 28 pairs of somites), in which he distinguished telencephalon (*Hemisphärentheil*) from diencephalon (*Zwischenhirn*). It was pointed out by Streeter (1942) that His, after a careful study of the surface anatomy of embryo M, prepared serial sections, thereby "setting a new standard in human embryology." Indeed, His may be considered as the "Vesalius of human embryology" (O'Rahilly and Müller 1987a).

A review of stage 12 based on 22 embryos was published by Streeter (1942). Details of the nervous system, however, have not been covered adequately, and hence a further investigation was needed. Twenty-four embryos of good quality have now been studied, as in the previous account of stage 11 (Müller and O'Rahilly 1986a). Particular attention has been paid to the neural crest, the cephalic mesenchyme, and the ill-understood caudal region, as well as secondary neurulation.

Stage 12 is characterized by the presence of 21 to 29 somites and by the closure of the caudal neuropore.

## Material and methods

The 24 embryos studied are listed in Table 1, which gives details of the number of somites, embryonic length, stain, plane of section, and the enlargement of the graphic reconstructions. Every section or every second section was used in the preparation of the graphic reconstructions. Born reconstructions of 6 embryos were available. In one of the graphic reconstructions the mitotic figures were superimposed, and in some the areas with cytoplasmic inclusions

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**Table 1.** Embryos of stage 12 studied

| Embryo | Pairs of somites | Embryonic length (mm) | Magnification of reconstruction | Stain | Thinness of section (μm) | Plane |
|---|---|---|---|---|---|---|
| 486 | 21 | 4 | | Al. coch. | 10 | Trans. |
| 8963 | 22 | 3.8 | | I.H. | 10 | Trans. |
| 8943 | 22/23 | 3.9 | 166 | H.E. | 8 | Trans. |
| 4784 | 23 | 3 | | ? | 10 | Trans. |
| 8505b | 23 | ? | | Azan | ? | Sag. |
| 8964 | 23 | 2.8 | | I.H. | 8 | Trans. |
| 4245-7 | ca. 24 | 3.5 | | Al. coch. | 10 | Trans. |
| 8505a | 24 | ? | | H. Phlox. | ? | Trans. |
| 9145 | 24 | 5.4 | | I.H. & Phlox. | 8 & 6 | Trans. |
| 5056 | 25 | 3 | | Al. coch. | 10 | Trans. |
| 9097 | 25 | 3.4 | 150 | Al. coch. E. & Silver | 10 | Trans. |
| 7852 | 25 | 3.7 | 100 | H.E. | 10 | Trans. |
| 8942 | 25 | 3.8 | | I.H. | 5 | Cor. |
| 8944 | 25 | 4 | 150 | I.H. | 8 | Sag. |
| 4763 | 26 | 3 | | Al. coch. | 10 | Cor. |
| 6937 | 26 | 3 | | I.H., G. | 10 | Cor. |
| 6144 | 27 | 3.3 | | Al. coch. | 10 | Trans. |
| 5923 | 28 | 4 | | Al. coch. | 10 | Trans. |
| 6488 | 28 | 3.2 | | A. coch. | 10 | Trans. |
| 7999 | ca. 28 | 3.2 | | H.E. | 10 | Trans. |
| 8941 | 28 | 4.9 | | I.H. | 6 | Trans. |
| 1062 | 29 | 4.5 | 100 | Al. coch. | 20 | Trans. |
| 7724 | ca. 29 | 3.5 | | H.E. | 8 | Sag. |
| D9035 | ? | 4.5 | | Silver | 10 | Cor. |

were added (except for cranial ganglia 7/8 and 5, which are entirely filled with inclusions). In order to determine the segments of the forebrain and midbrain, sulci and protrusions were reconstructed. Measurements of the brain and/or of the whole neural tube were taken along a central axis from the site of the rostral neuropore to the caudal eminence. The angle of the mesencephalic (cranial) flexure was measured 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.

## Results

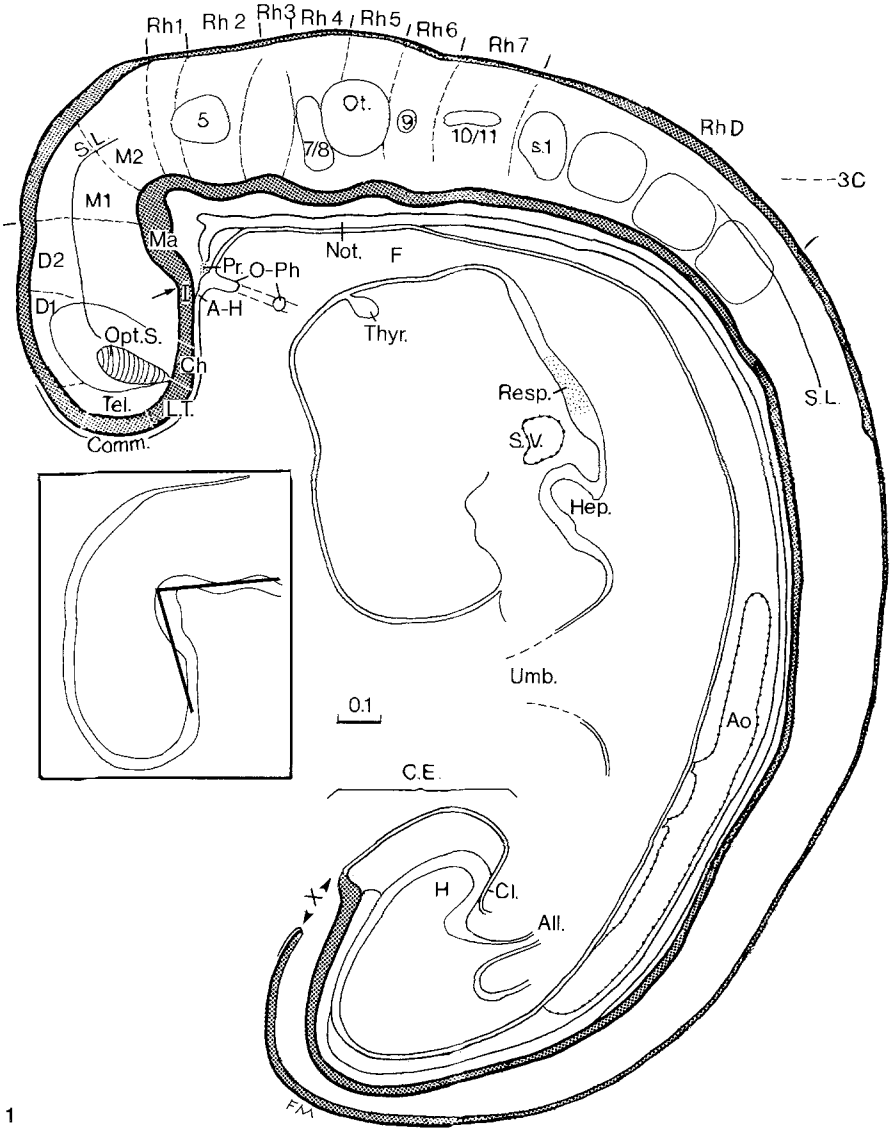
*External.* Compared with embryos of stage 11, those of stage 12 are C-shaped; the head is bent ventrally towards the heart, and the caudal part of the trunk is bent towards the ventral aspect of the body. In most embryos the caudal end deviates from the median plane (Streeter 1942, Plates 1 and 2), and this had to be allowed for in the graphic reconstructions. Three pharyngeal arches and three clefts are well visible from the exterior. The upper limb buds have appeared, and in the most advanced embryos (e.g., No. 1062) the lower limb buds are beginning to develop.

*General.* Some of the characteristic features are shown in Figs. 1 and 2. The optic vesicle is prominent in all embryos of stage 12. The rostral neuropore is always closed, and the caudal neuropore is closed in about half (13 of 22 embryos). The otic pit has developed into the otocyst, but it still opens into the amniotic cavity. The dorsal aortae fuse, and 2–3 pairs of aortic arches are present. The thyroid primordium is a solid accumulation of cells and it is still connected to the pharyngeal endoderm. The pulmonary primordium is at the level of somites 3–4. The hepatic, and sometimes the cystic, primordium is clearly visible. The notochord is fully developed in all embryos and is separating from both the neural tube and the intestine, except in special areas.

*Prechordal plate.* The prechordal plate has fully developed into the premandibular condensation (Figs. 3B, E; 6B). The two mesenchymal areas are connected with each other across the median plane by a bridge of mesenchymal cells of prechordal origin (Fig. 1). The cells are situated adjacent to the rostral end of the notochord. Slight differences in density and in arrangement of the cells are of aid in reconstructing the premandibular condensation.

*Oropharyngeal membrane.* The oropharyngeal membrane is rupturing in all embryos of stage 12 (Figs. 1, 6A). The adenohypophysial primordium is situated immediately rostral to the membrane and is in contact with the floor of the diencephalon, as is true also of stages 10 and 11. A possible indication of "Seessel's pocket" was noted in at least one specimen.

*Notochord.* The notochord is no longer continuous with the endoderm. The least advanced area (where the notochordal plate persisted longest in stage 11) is in the pharyngeal region (Fig. 1). In other places it is separated from the endoderm by intervening mesenchyme, especially in areas where the dorsal aortae fuse to form the unpaired aorta (Fig. 1). The contact with the endoderm is close again in the caudalmost parts of the embryo. Separation between the rostral part of the notochord and the brain (which began in advanced embryos of stage 11) starts at the mesencephalic flexure. By now the notochord is separated from the neural tube everywhere except in the caudalmost area.

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**Figs. 1–9.** *Abbreviations. A-H*, Primordium of adenohypophysis; *All*, Allantoic primordium; *Ao*, Aorta, aortic arches; *C.E.*, Caudal eminence (caudal bud, end-bud); *Ch.*, Chiasmatic plate; *D*, Diencephalon; *F*, Foregut, pharynx; *Cl*, Cloacal membrane; *Comm*, Commissural plate; *Ggl*, Ganglion; *H*, Hindgut; *HC*, Hypoglossal cord; *Hep.*, Hepatic primordium; *I*, Infundibulum; *L.T.*, "Adult" lamina terminalis; *M*, Mesencephalon; *Ma*, Mamillary area; *N1*, Primary neurulation; *N2*, Secondary neurulation; *Nas*, Nasal plate; *NC*, Neural cord; *N.Cr.*, Neural Crest; *Not*, Notochord; *O-Ph.*, Oropharyngeal membrane; *Opt.* Optic vesicle; *Opt.S.*, Optic sulcus; *Ot.*, Otic vesicle; *Ph.Ar.*, Pharyngeal arch; *Pr.* Mesenchyme of prechordal plate; *Resp*, Respiratory primordium; *Rh*, Rhombomere; *S.V.*, Sinus venosus; *s.*, Somite; *S.L.*, Sulcus limitans; *S.N.*, Situs neuroporicus; *Tel.*, Telencephalon; *Thyr.* Thyroid primordium; *Umb.*, Stalk of umbilical vesicle; *X*, Caudal neuropore

The plane and location of the sections in the photomicrographs of Figs. 3 and 9 are indicated in Figs. 1 and 5, respectively

**Fig. 1.** Features present at stage 12 shown in a median section of embryo No. 7852 (25 pairs of somites). Some of the laterally located structures (otic vesicle, cranial ganglia) are projected onto the median section. The typically C-shaped form of the embryo is caused by a diminishing angle (*inset*) at the mesencephalic flexure, by the rotation of the caudal end, and by a gentle curve of the trunk. The oropharyngeal membrane is interrupted (see also Fig. 6A), the notochord is fully developed, and the dorsal aortae are fusing. The thyroid gland, hepatic primordium, and respiratory area are indicated. The caudal neuropore is still open in this specimen. Seven rhombomeres can be distinguished rostral to RhD. The roof of the neural tube is thickest in forebrain and midbrain, thin in Rh 1–7, becomes thicker in RhD, and then thinner again farther caudally. The optic vesicles are fully formed. The sulcus limitans reaches from the rostral part of the mesencephalon almost to the optic sulcus. It develops also at the level of RhD and in the upper cervical region. The bar in this and in Fig. 2 represents 0.1 mm.

The shape of the rostral portion of the notochord parallels the ventral surface of the brain (Fig. 2, inset). Its prolongation towards the apex of the mesencephalic flexure still indicates the formerly close relationship. The tip of the rostral portion is separated from the adenohypophysial primordium by some remnants of the former prechordal plate. In the caudalmost area, the notochord, together with the developing neural primordium, forms what appears to be a unity. In well-preserved embryos a change in density differentiates notochordal from neural tissue. Mitotic figures are scarce and their configuration is not as clear-cut as in those of the brain. Embryo No. 8943 has only two mi-

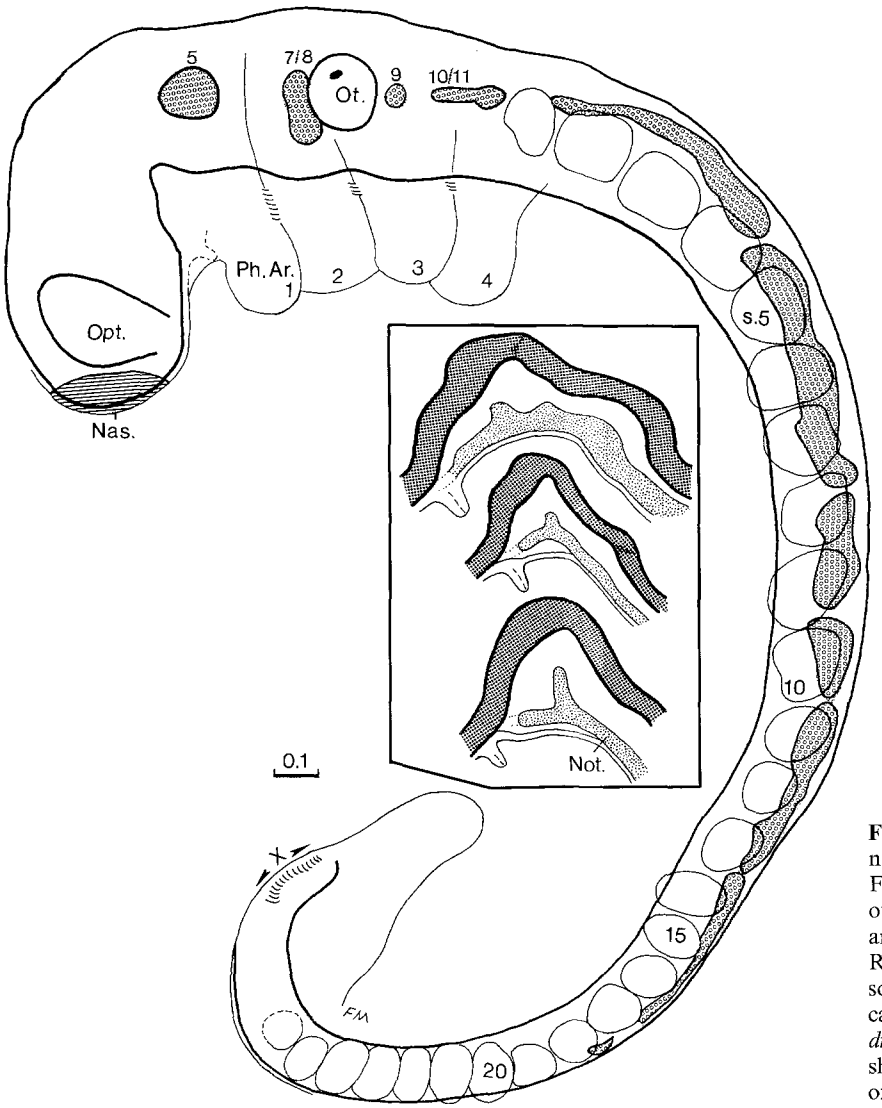
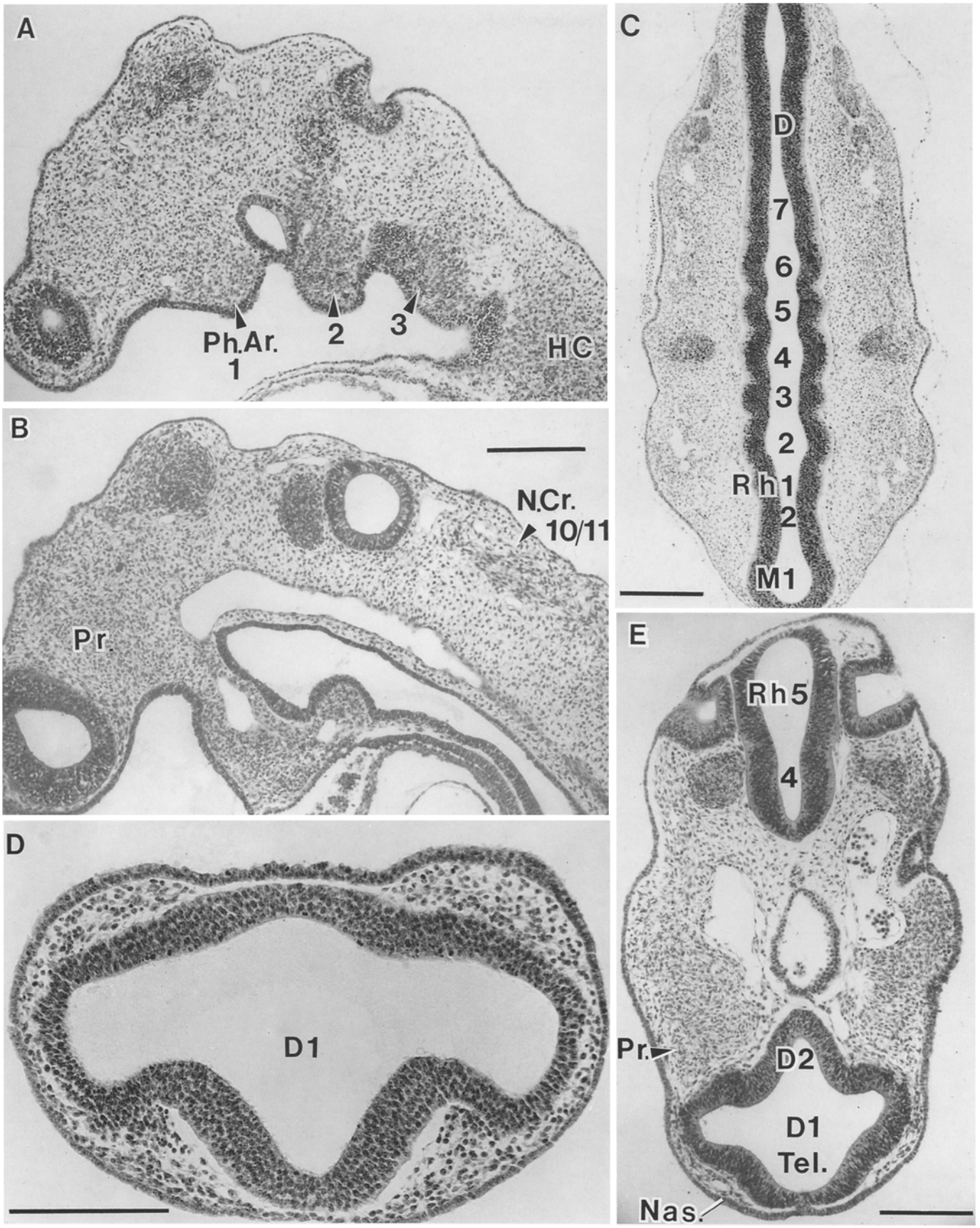



**Fig. 2.** Left lateral view of No. 7852. The nasal plate and the optic vesicle are shown. Four pharyngeal arches are demarcated. The otic vesicle is now related to pharyngeal arch 3 and to Rh 5. The neural crest for RhD and for the spinal cord (medial to the somites) is more or less continuous. The caudal neuropore is a narrow gap. The *inset drawing* shows the relationship between the shape of the notochord and that of the floor of the brain in Nos. 8943, 7852, and 6097

totic figures in the cephalic portion of the notochord, and a total of 10 more in the caudal part. The number of cells constituing the notochord in the same specimen is 8–12 per section, except in the caudalmost region, where the number is 24 cells or more. The thickest part of the notochord forms in the caudal eminence.

*Caudal eminence.* The caudal eminence in stage 11 is the area between the cloacal membrane and the site of the neurenteric canal. In stage 12, however, this site can no longer be determined, and the eminence is now the undifferentiated caudal area that lies between the caudal border of the caudal neuropore (or its site) and the cloacal membrane (Fig. 1). In those embryos in which the caudal eminence was cut transversely and in which the mitotic configurations were clear-cut, the figures were counted for the surface ectoderm, presumptive neural cord, digestive tube, and somitic plate. Most of the cell divisions are in the surface and in

**Fig. 3A–E.** Parts of the brain and cranial ganglia in stage 12. **A, B** Sagittal sections (4-1-6 and 2-6-6) of No. 8944, showing optic and otic vesicles, trigeminal and facio-vestibular ganglia, and (in **B**) neural crest for ganglia 10/11. Pharyngeal arches 2, 3, and 4 in **A** contain dense mesenchyme. The left dorsal aorta, as well as parts of aortic arches 1 and 2, are visible. **C** Horizontal section (1-3-7) of No. 7852, showing the neuromeres of the mesencephalon and rhombencephalon at stage 12, from rostral to caudal, the rostralmost part being M1. The ganglion on both sides of Rh4 is 7/8; the caudalmost condensations (*top*) are somites. **D** Optic vesicles and neuromere D1 of No. 6097 (section 1-6-9). A mesenchymal sheath separates the optic vesicles from the surface ectoderm. Very little or no mesenchyme is present between telencephalon medium and surface ectoderm. Optic neural crest is evident at the rostroventral part of the optic vesicles on both sides. **E** Forebrain of No. 8943, showing site of rostral neuropore, telencephalon medium, neuromeres D1 and D2, and optic vesicles showing optic neural crest. Rostral and caudal limiting sulci are evident. The nasal plate on both sides of the situs neuroporicus can be seen as an ectodermal thickening. The rhombencephalon at the level of Rh4 with Ggl 7/8, and Rh5 with the otic vesicle are visible. The thickened surface ectoderm of the first and second pharyngeal arches is evident. The mesenchyme caudal to the optic vesicle is the premandibular condensation (*left-hand side* of photograph); the mesenchyme of the pharyngeal arches is clearly more compact. In **C**, **D**, and **E**, the dorsal aspect is orientated towards the bottom of the page. The level of **D** is indicated in Fig. 5A, that of **D** in Fig. 5B. The bar represents 200 μm


A
Ph.Ar.
1
2
3
HC
B
N.Cr.
10/11
Pr.
C
D
7
6
5
4
3
2
Rh1
2
M1
D
D1
E
Rh5
4
Pr.
D2
D1
Tel.
Nas.

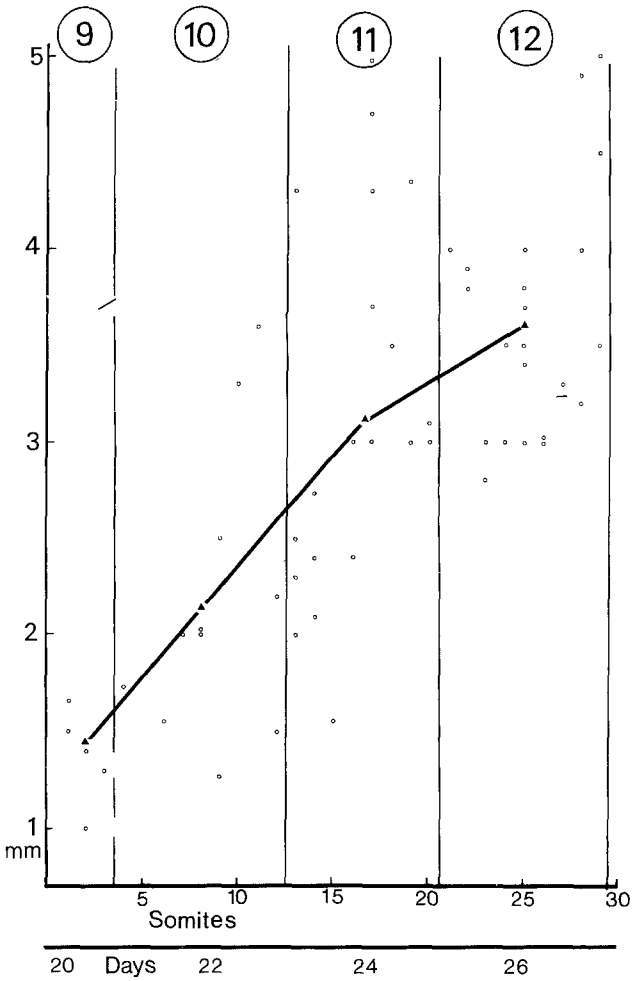



**Fig. 4.** Graph showing the number of pairs of somites plotted against embryonic length in stages 9–12. The mean position at each stage is indicated by a solid triangle. The line connecting the means demonstrates that a more or less steady increase in somitic count accompanies increase in body length. Although the age in postovulatory days has been added, it is only approximate and should not be correlated rigidly with the other parameters

the somitic plate; very seldom can figures be observed in the notochord; those of the spinal cord are arranged around the central canal.

*Somites.* The rostroventral part of somites 1 to 4 is being transformed into the hypoglossal cord (O'Rahilly and Müller 1984). The first somite, often smaller, seems to undergo degeneration, but this is not the case. In well-preserved specimens, mitotic figures are identifiable, and indicate not degeneration but transformation. The average height of one somite is 0.15 mm. That is an increase of 50% compared with the average in stage 11. The number of somites and the greatest length of a given embryo show some variation (Fig. 4). Embryos of 25 paired somites, for example, have lengths of 3.0, 3.4, 3.5, 3.7, 3.8, and 4 mm. At least somite No. 32 and the following somites develop from that part of the caudal eminence where, after closure of the caudal neuropore, secondary neurulation is occurring.

*Rostral neuropore.* Figures 3E, 5A, and 6A show the site of the rostral neuropore in an embryo transitional to stage 12. The surface ectoderm is connected to the neural ectoderm of the telencephalon (commissural plate) by two rows, side-by-side, of inclusion-containing cells derived from the surface ectoderm. These rows extend over a relatively large rostrocaudal distance (Fig. 6A). The site of the rostral neuropore in more differentiated embryos of stage 12 no longer shows this bridge.

*Caudal neuropore.* No comparable ectodermal bridge is present at the site where the caudal neuropore closes. In an embryo in which closure is almost complete (No. 6097, Fig. 7B) and in another with a recently closed neuropore (No. 1062, Fig. 7C), the site of the caudal border of the former neuropore is indicated by a pit. The level of final closure of the caudal neuropore can be estimated by allowing space for additional somites in embryos of 25 pairs. Thus, in No. 7852 the neuropore is opposite the site of future somites 30 and 31 (Fig. 7A), and in No. 6097 it is opposite future somite 31 (Fig. 7B). In a 29-somite embryo (No. 1062), the site of the closed neuropore is opposite future somite 31 (Fig. 7C). Hence the level of final closure appears to be somites 30/31.

*Brain.* The forebrain contains three segments: the telencephalon and diencephalon 1 and 2 (Figs. 1–3, 5). The rostrocaudal expansion of the telencephalon begins only now, although its formation dates back to stages 10 and 11, when, during closure of the rostral neuropore, the "adult" lamina terminalis and commissural plate become distinguishable. The commissural plate is the area of the telencephalic wall that lies medial to the nasal plates (Müller and O'Rahilly 1984). D1 is the region of the optic vesicle, and its floor contains the chiasmatic plate. The floor of D2 contains the future infundibular area (with an indication of a recess, arrow in Fig. 1; see also Fig. 5A) in contact with the adenohypophysial primordium. The floor between the infundibular region and the mesencephalon is the primordium of the mamillary body. The mamillary recess is barely identifiable. The roof of the diencephalon is slightly thinner than that of the mesencephalon (Figs. 1, 5A). The mesencephalon contains two segments which, however, are difficult to delineate. A sulcus limitans is present (Fig. 1) and reaches almost as far as the optic sulcus (although it does not do so in later stages). The parts of the rhombencephalon are characterized by distinct features, such as expansions of the floor at the level of Rh2, 4 and 6 (Figs. 1, 5A), the ganglia of the cranial nerves, and somites 1–4. A sulcus limitans is forming in RhD and reaches into the cervical part of the neural tube.

As seen in one of two silver-impregnated specimens, the first nerve fibres are differentiating (Fig. 5B). Apart from short fibres in cranial ganglia 5 and 7/8, they are related to cells that lie approximately at the level of Rh2, 4, 6, and 7, at the site where the sulcus limitans will develop in stage 13. The cells represent the first neurons of the nucleus of the lateral longitudinal tract. Fibres of the future ventral longitudinal tract are seen in Rh7. The first intramedullary fibres of nerve 12 (Fig. 5B, black circles) do not yet leave the neural wall, but fine, cellular, extramural strands are directed towards the somites. Two more (not silver-impregnated) embryos show this first sign of the hypoglossal nerve.

*Optic vesicle.* The optic primordium is entirely evaginated and forms the optic vesicle. In addition to the caudal limit-

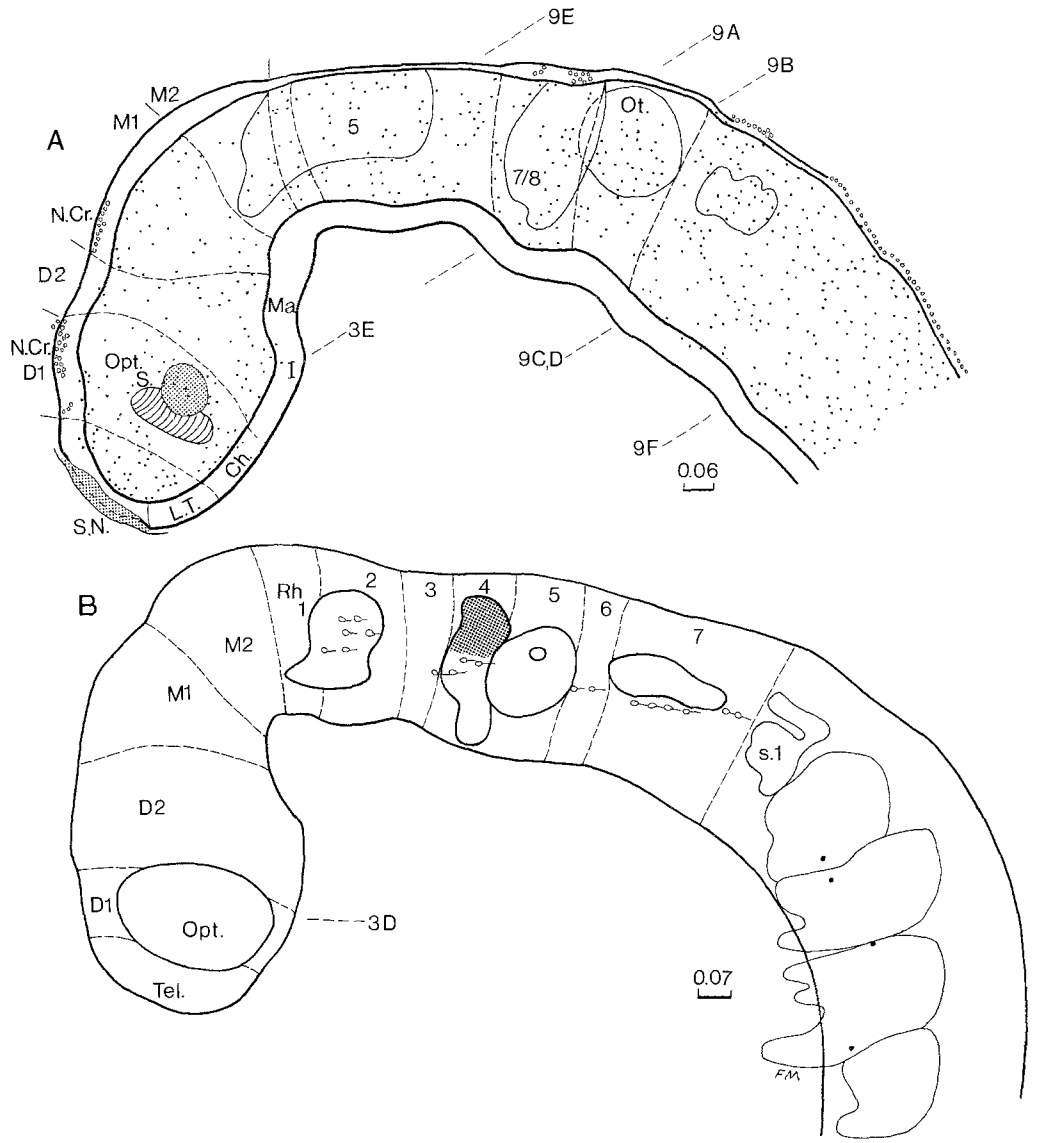



**Fig. 5. A** Median section of embryo No. 8943, which is transitional from stage 11 to stage 12. The angle at the mesencephalic flexure is still more than a right angle. The rostral neuropore has recently closed and the cells in the area around it (*fine stippling*) contain numerous inclusions. Cellular inclusions are also present in a clearly circumscribed (*stippled*) area of D1. The mitotic figures, which show a fairly even distribution, have been added as black dots. **B** Neuromeres and nerve fibres in the brain of No. 6097. In this silver-impregnated embryo, cells with nerve fibres are plotted onto the lateral wall of the rhombencephalon, and the roots of the hypoglossal nerve are indicated at the level of somites 2, 3 and 4 in RhD. Most of the plotted cells probably represent the nucleus of the lateral longitudinal tract. They are concentrated in Rh2, 4 and 7, in the areas where ganglionic formation is under way. Some short and thin silver-impregnated fibres can be found also in those ganglia. Somites 1 to 4 show a characteristic transformation of the rostral part, which participates in the formation of the hypoglossal cord. The bars represent 0.06 and 0.07 mm, respectively

ing sulcus, which appears in advanced embryos of stage 11, a rostral limiting sulcus is now present (Figs. 3E, 6A). The vesicle grows caudally during stage 12 and gradually develops a medial surface. Optic neural crest is still being formed (Fig. 3D) and the whole surface of the vesicle is surrounded by mesenchyme. The first artery (a branch of the internal carotid) appears on the ventrolateral part of the vesicle (Figs. 3E, 6B). Cytoplasmic inclusions, found predominantly in D1 at stage 10, are still present at the caudal part of the optic sulcus (Fig. 5A).

*Growth of the neural tube.* The whole neural tube was measured in four Carnegie specimens, as well as in three embryos described in the literature (Table 2). Compared with the means for stages 9, 10, and 11, the differential rostro-caudal growth changes little from stage 11 to stage 12. A constant decrease in the percentage of the rhombencephalon from stage 9 on and a relatively small percentage of the forebrain compared with that of stage 10 are obvious. The brain occupies 42% of the neural tube. Table 3 shows the percentages of the different parts of the brain, now entirely related to the length of the brain, for 8 Carnegie embryos and for 7 embryos described in the literature. The prosencephalon does not extend more than in stage 11; the telencephalon of stage 12, therefore, has lengthened at the expense of the diencephalon. The mesencephalon with its two segments has enlarged slightly. Rhombomere B (Rh4) is reduced to half of the percentage that it occupied during stage 11. All the other rhombencephalic parts are comparable.

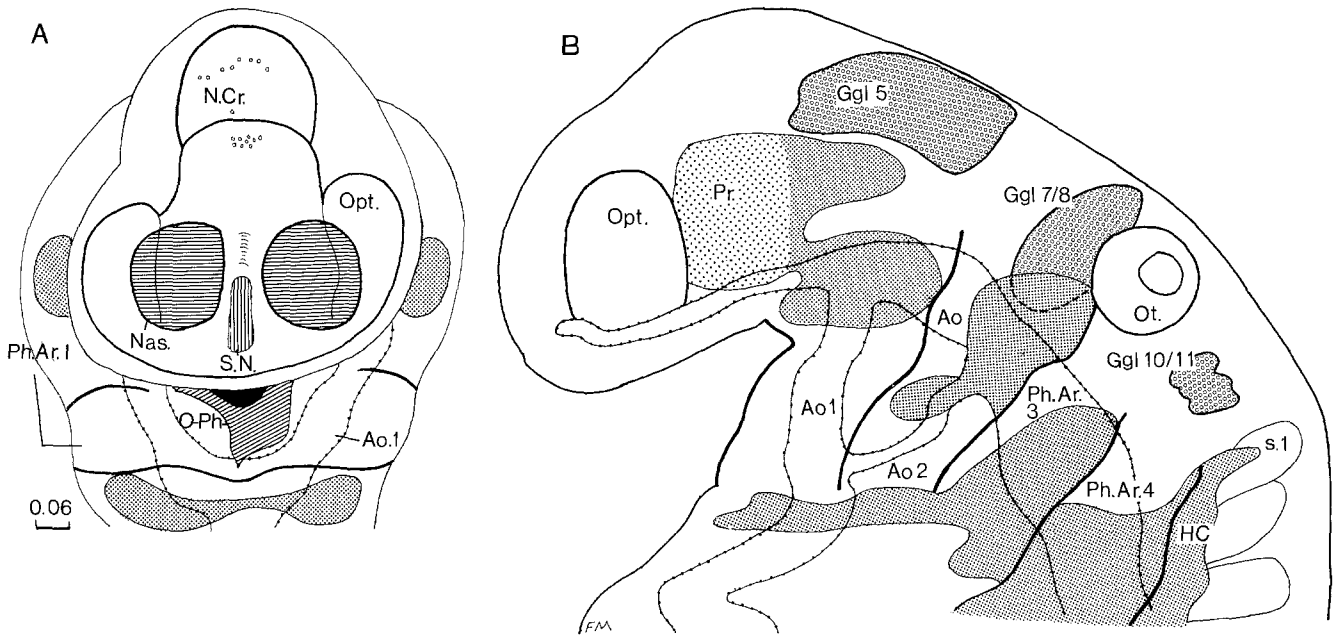



**Fig. 6.** **A** Head mesenchyme (*stippled*) in No. 8943, head-on view, showing the site of the rostral neuropore, the nasal plates, optic vesicles, diencephalon and mesencephalon (each with some neural crest material), pharyngeal arch 1, and disintegrating oropharyngeal membrane. Aortic arches 1 unite at the truncus arteriosus. The premandibular condensation cannot be seen in this view, because it is covered by the optic vesicles; mesenchyme of pharyngeal arch 1 is visible, as well as the rostrally-reaching mesenchyme (at the *bottom* of the drawing) derived from arches 3. **B** Cranial ganglia, pharyngeal arches and mesenchyme of the same specimen from lateral. Although four pharyngeal arches can be plotted, only two aortic arches are visible. Different kinds of head mesenchyme are plotted: the derivative of the prechordal plate (the premandibular condensation), the mesenchyme forming in the pharyngeal arches (with contributions from the thickened surface ectoderm), and finally the material derived from somites 1 to 4 (the hypoglossal cord). Neural crest seems to mix with arch-mesenchyme at the level of pharyngeal arch 2, and the material of somites 1–4 mingles with arch-mesenchyme of arch 4. The mesenchyme of arch 3 reaches the heart; that of arch 4, together with the hypoglossal cord, invades the laryngeal area and later the tongue. The bar represents 0.06 mm

**Table 2.** Changing relationships between parts of C.N.S. at stages 9–12. Percentages of parts of C.N.S. relative to total length of neural plate/tube

| Embryo | T | D1 | D2 | Pros. | M1 | M2 | Mes. | 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 | 52.5 |
| *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 |
| 6097[25] | 1.8 | 3.6 | 3.4 | 8.8 | 3.5 | 3.8 | 7.3 | 5.5 | 2.5 | 8.8 | 13.8 | 16.8 | 30.6 | 53.2 |
| 7852[25] | 2 | 3.5 | 3.7 | 9.2 | 2.6 | 3 | 5.6 | 5.9 | 1.1 | 8.3 | 13 | 15.3 | 28.3 | 56.8 |
| 5923[28] | 2.5 | 3.4 | 2.5 | 8.4 | 2.4 | 2.5 | 4.9 | 5.7 | 2.3 | 6.2 | 10.8 | 14.2 | 25 | 61.5 |
| 1062[29] | 1.9 | 2.6 | 3.8 | 8.3 | | | 7 | 3.8 | 2.4 | 8.7 | 11.9 | 14.9 | 26.8 | 57.9 |
| E. Girgis[22] | 0.6 | 2.3 | 3.9 | 6.8 | 1.9 | 2.1 | 4 | 4.3 | 2.1 | 7.8 | 17.9 | 14.2 | 32.1 | 57.4 |
| E. Thompson[23] | 1.9 | 3.5 | 3.5 | 8.9 | 2.6 | 2.9 | 5.5 | 5.8 | 1.8 | 5.8 | 11.1 | 13.4 | 24.5 | 61 |
| E. West[28] | 3 | 3.5 | 1.9 | 8.4 | | | 3.7 | 6.5 | 2.2 | 7.8 | 11.7 | 16.5 | 28.2 | 59.6 |

Note: The *superscripts* in the first column are somitic counts

*Mitotic activity in the brain.* The mitotic activity was recorded for embryo No. 8943 (Fig. 5A). The percentages of the mitotic figures in the various parts of the brain are listed in Table 3. These percentages parallel closely those that indicate the lengths of the same parts of the brain.

*Mesencephalic flexure.* The angles formed by the mesencephalic flexure (shown in Fig. 1 (inset) for example) are listed in Table 3. The angle changes from a mean of 127° in stage 10, and 117° in stage 11, to 89° in stage 12. Less advanced embryos, such as No. 8943 with 23 paired somites

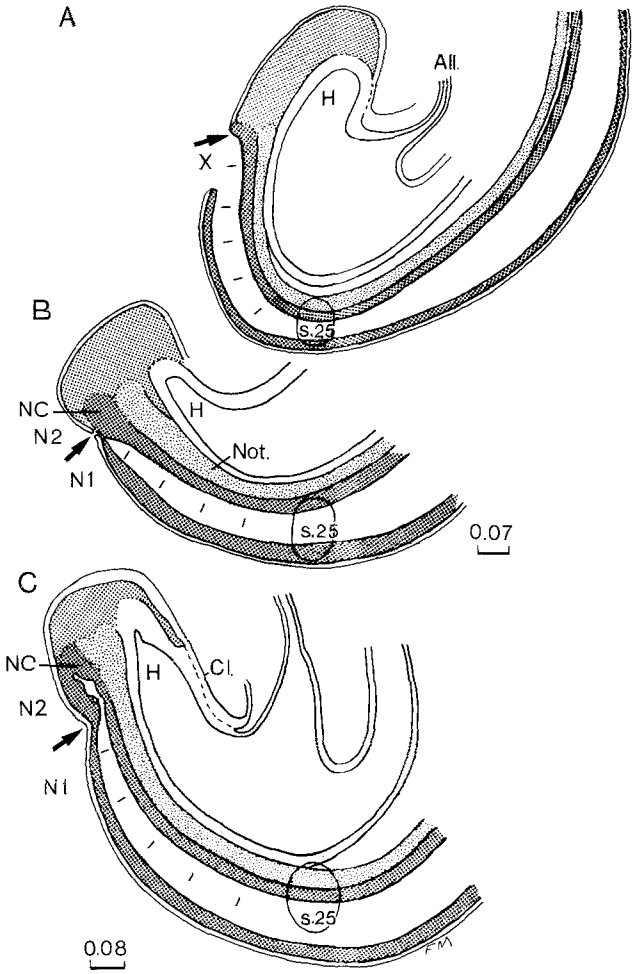



**Fig. 7A–C.** Caudal end of embryos of stage 12. **A** No. 7852 with still open caudal neuropore. **B** No. 6097 with just closing caudal neuropore. **C** No. 1062 with already closed neuropore. The graphic reconstructions are oriented with somite 25 as a reference landmark. The site where the caudal neuropore (X) closes last is marked by an arrow. The bars represent 0.07 and 0.08 mm

and an open caudal neuropore, have a greater angle than more advanced specimens. The diminishing angle results in the bending of the head towards the cardiac area and contributes to the C-shape of the whole embryo.

*Closure of the caudal neuropore and beginning of secondary neurulation.* The caudal neuropore closes in a rostrocaudal direction. As seen in Fig. 7, the caudalmost point of closure seems quite regular with regard to the site of the future somites. No filling material is present between the surface ectoderm and the closing neural tube. Neural crest cannot be detected at the site of closure. In specimens in which the neuropore is still open, the hindgut reaches more caudally than the neural plate and notochord (Fig. 7A). In embryos with beginning secondary neurulation (Fig. 7B), spinal cord, notochord, and hindgut extend caudally to approximately a comparable level. The formation of the neural tube and the notochord in embryos with an open caudal neuropore follows the steps described for embryos of stage 11 (Müller and O'Rahilly 1986a). Once the caudal neuropore is closed, the whole caudal area of the embryo is covered by surface ectoderm. The continuing formation of the future spinal cord takes place without involvement of the ectoderm and is called secondary neurulation (Fig. 8). The phenomenon is best investigated in cross-sections. Because the caudal area of the embryo is often curled, however, relatively few specimens can be studied adequately. The caudal eminence contains two chief kinds of tissue, and they probably differ mostly in their concentration of cells. The more concentrated tissue is present particularly in the middle part. A small opening (Fig. 8B) indicates the end of the neural canal; a small distance apart (ca. 40 μm), the cells of the dense tissue are arranged radially around the canal. The tissue in other areas of the condensation does not yet show an orderly arrangement. The concentration at the ventral aspect of the caudal eminence that represents the material of the hindgut becomes more distinct in Fig. 8C. The cavity of the hindgut in Fig. 8E is connected without interruption with that in Fig. 8F. The neural canal is also continuous from Fig. 8B to 8F and farther. The notochord, which becomes visible in Fig. 8F, is not yet separated from the neural tube by a basement membrane at this level. The cells of the neural tube now tend to be arranged in rows, and the same holds for those of the hindgut. The loose mesenchyme in the lateral areas does not yet contain the primordia of somites at this level.

*Cytoplasmic inclusions.* These are found where they were present in stages 10 and 11: at the site of the rostral neuropore, at the level of D1, in the caudal eminence at the cloacal membrane, and in the newly developing notochord. Cytoplasmic inclusions are increasingly present in the ganglia of the cranial nerves (Fig. 9A, B, E).

*Marginal layer.* A marginal layer of the brain is present in the rhombencephalic wall (Fig. 3C, E). It extends from the floor as far towards the roof as the dorsal limit of the ganglia. A distinct marginal layer begins to appear in mesencephalon 2. No marginal layer, however, is present in the forebrain (Fig. 3D, E).

*Neural crest.* Formation of neural crest continues in the brain at the levels of Rh2, 4, 5, and 7. Mesencephalic neural crest is present in No. 8943, and the same embryo shows crest material in the roof of D1 (Figs. 5A, 6A). The trigeminal ganglion still receives cells from the roof of Rh2 (Fig. 9E): the photomicrographs show, in addition, crest cells spreading from the ganglion towards the surface ectoderm, and rostrally approximately to the mesodiencephalic sulcus (arrow). Ganglion 7/8 receives cells from the roof of Rh4 (Fig. 5A) but also from its lateral walls (Fig. 9A). Whole strands of cells extend from the ventricular layer to the ganglion, thereby interrupting the marginal layer and the basement membrane at this level. The area where those strands are present is specifically marked in Fig. 5B; it is present in the dorsal half of the ganglion also in No. 8943. The arrangement of neural crest 10/11 is equally special. The cells lie with their longitudinal axes parallel to the long axis of the rhombencephalon and form loose strands at this stage (Fig. 3B). The neural crest of the spinal cord extends farther caudally and is present as far as somite 18 (Fig. 2) in No. 7852, and somite 19 in No. 6097 (Müller and O'Rahilly 1980, their Fig. 7). The material is not yet organized into ganglia.

*Otic vesicle and neural crest.* The otic vesicle is now clearly related to Rh5 (Fig. 3E). In most embryos, cells forming

**Table 3.** Growth of the brain in stages 9 to 12

| Embryos | Percentage of neural tube occupied by brain | Percentages of parts of brain relative to total length of brain | | | | | | | | | | | Angle of mesencephalic flexure |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | D1 | D2 | Pros. | M1 | M2 | 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 | mean:127 |
| *Stage 11* (mean of 14 embryos) | 48 | | | | 19 | | | 10 | 15 | 13 | 21 | 29 | 73 | mean: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 | mean: 89 |
| 8943[23] | | 6.3 | 11.7 | 4.3 | 22.3 | 10.3 | 2.6 | 12.9 | 16.6 | 7.2 | 21.5 | 19.5 | 64.8 | 112 |
| mitot. fig. | | 6.3 | 11.3 | 6.3 | 23.9 | 6.4 | 3.2 | 9.6 | 14.5 | 6.8 | 24.7 | 20.6 | 66.6 | |
| 4784[23] | | 3.6 | 6.6 | 8.3 | 18.5 | 10.3 | 9.6 | 19.9 | 15.6 | 6.8 | 18.2 | 20.9 | 61.5 | 88 |
| 6097[25] | 46.8 | 3.2 | 7.4 | 7.8 | 18.4 | 6.1 | 6.4 | 12.5 | 13.5 | 3.7 | 18.9 | 33 | 69.1 | 89 |
| 7852[25] | 43 | 4.7 | 8 | 8.5 | 21.2 | 6 | 7.2 | 13.2 | 13.6 | 2.6 | 19 | 30.2 | 65.4 | 77 |
| 8944[25] | | 4.6 | 8.3 | 4.8 | 17.7 | 9.9 | 5.7 | 15.6 | 15.2 | 5.1 | 15.3 | 30.9 | 66.5 | 82 |
| 5923[28] | 38.4 | 6.5 | 8.9 | 6.5 | 21.9 | 6.3 | 6.5 | 12.8 | 14.9 | 6.0 | 16 | 28 | 64.9 | 77 |
| 1062[29] | 42.1 | 4.5 | 6 | 9.5 | 20 | | | 16.7 | 9 | 5.7 | 20.5 | 28 | 63.2 | 75 |
| 7724 | | 5.3 | 5.8 | 8.9 | 20 | 8.4 | 8 | 16.4 | 15.8 | 5.8 | 14.7 | 27 | 63.3 | 70 |
| E. Girgis[22] | 42.8 | 2.1 | 5.4 | 11.3 | 18.8 | | | 7.1 | 13.7 | 5.4 | 21.4 | 33.3 | 73.8 | 119 |
| E. Thompson[23] | 39 | 4.9 | 9 | 9 | 22.9 | 6.7 | 7.5 | 14.2 | 15 | 4.5 | 15 | 28.5 | 63 | 134 |
| E. Rosenbauer[24] | | 2.2 | 11.2 | 8.7 | 22.1 | | | 7.9 | 15.9 | 5.8 | 25.3 | 23.1 | 70.1 | 87 |
| E. West[25] | 40.4 | 7.5 | 8.6 | 4.8 | 20.9 | | | 9.1 | 16.1 | 5.4 | 19.4 | 29 | 69.9 | |
| E. Sternberg[28] | | 5.5 | 6 | 6 | 17.5 | | | 13.7 | 19.8 | 4.4 | 20.3 | 23.6 | 68.1 | 78 |
| E. Reiter[28] | | 6 | 7.3 | 7.7 | 21 | | | 13.7 | 10.3 | 5.1 | 21.4 | 28.2 | 65 | 78 |
| E. Reiter[29] | | 3.7 | 8.2 | 7 | 18.9 | | | 13.9 | 18.9 | 4.1 | 15.6 | 28.7 | 67.3 | 74 |

Note: The *superscripts* in the first column are somitite counts

the otic sheath in stage 11 can still be observed (Figs. 3E, 9A, B).

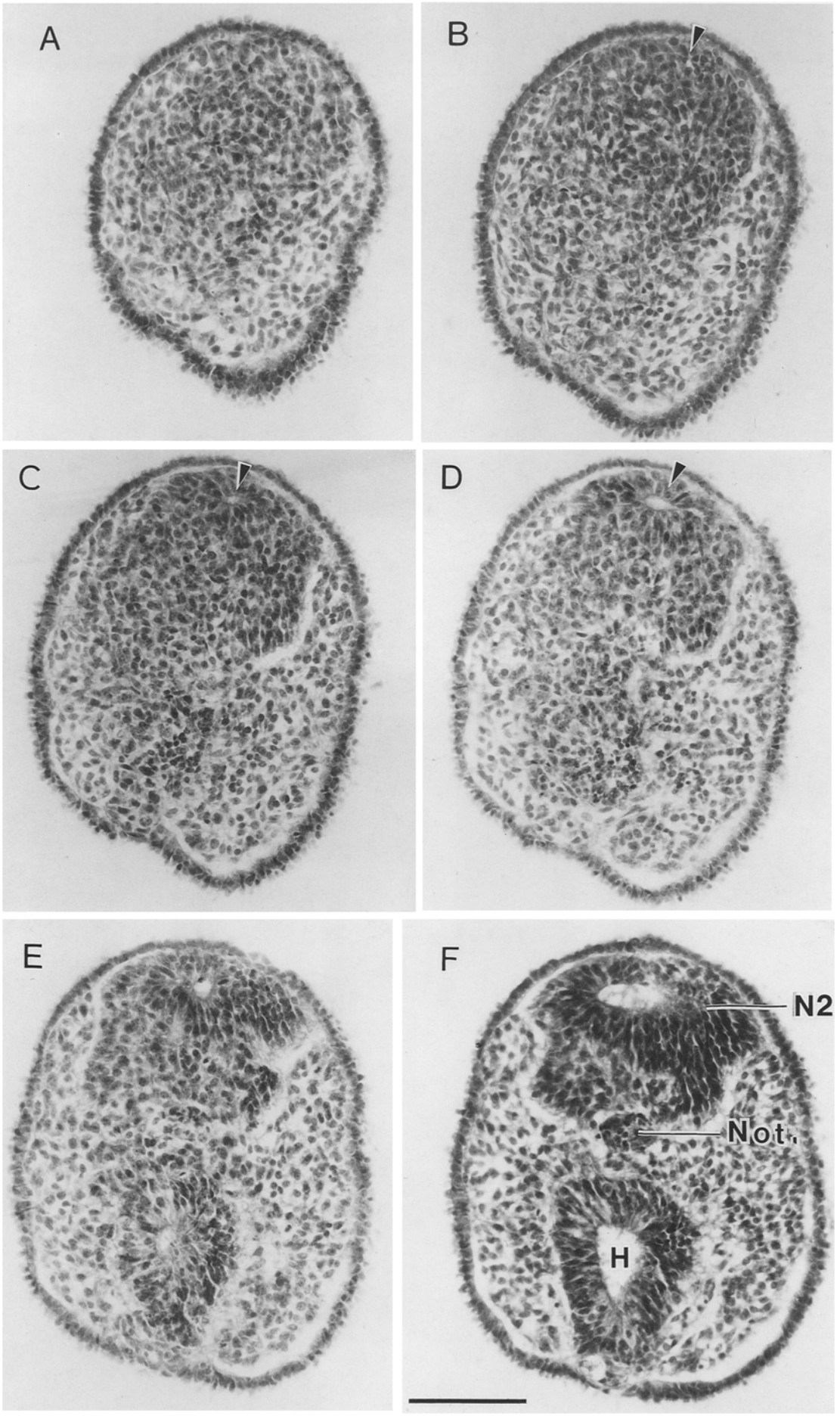
*Primary meninx.* The cells of the neural crest of RhD continue to form pia mater (Fig. 9F), as they did already in advanced embryos of stage 11. An area of loosly dispersed mesenchymal cells, especially at the apex of the mesencephalic flexure, is the precursor of the primary meninx (O'Rahilly and Müller 1986).

*Neural crest and cephalic mesenchyme.* Differences in density make it possible to plot the cranial ganglia and the cephalic mesenchyme as separate entities (Fig. 6). No overlap or mingling seems to be present between the trigeminal ganglion and the arch mesenchyme of pharyngeal arch 1 (Fig. 3A). In pharyngeal arch 2, however, the ganglionic cells penetrate the arch mesenchyme (Figs. 3A, E; 9B). In pharyngeal arch 3, the ganglionic material is well separated from the arch mesenchyme (Fig. 3A, B). Mesenchyme from pharyngeal arch 3 reaches the heart. Arch 4 is occupied by the hypoglossal cord and the mesenchyme of arch 4. The arch mesenchyme is derived partly from ectodermal thickenings (so-called placodes), particularly in pharyngeal arches 2 (Fig. 9B) and 3 (Fig. 9C, D). The interruption of the basement membrane in arches 2 and 3, where cells are given off to the mesenchyme, is especially well visible in a silver-impregnated embryo (No. D9035). Direct contribution of cells to the ganglia of the cranial nerves, however, is a very rare event.

## Discussion

*Features distinguishing stage 12 from stage 11.* Closure of the caudal neuropore occurs during stage 12, and 21 to 29 pairs of somites are present. The upper limb buds appear. Embryos having more than 29 somitic pairs and a caudal neuropore that is still open are considered to be abnormal.

**Fig. 8A–F.** Serial sections through the caudal eminence of No. 8505a, from caudal to rostral. The dorsal surface is shown uppermost in each case. **A** A thickening of the tissue is present, especially in the dorsal part, which in **B** contains a small opening (*arrowhead*). In **C** and **D**, it is surrounded by radially arranged cells ("medullary rosette"), representing spinal cord. A condensation in the ventral part of the section represents the beginning of the hindgut, which is not yet separated from the mesenchyme by a basement membrane. The cavity of the hindgut becomes visible in **E**, the notochord only in **F**. In all the sections the surface ectoderm is complete: the newly-forming spinal cord no longer develops from a superficially-located neural ectoderm. The bar represents 100 μm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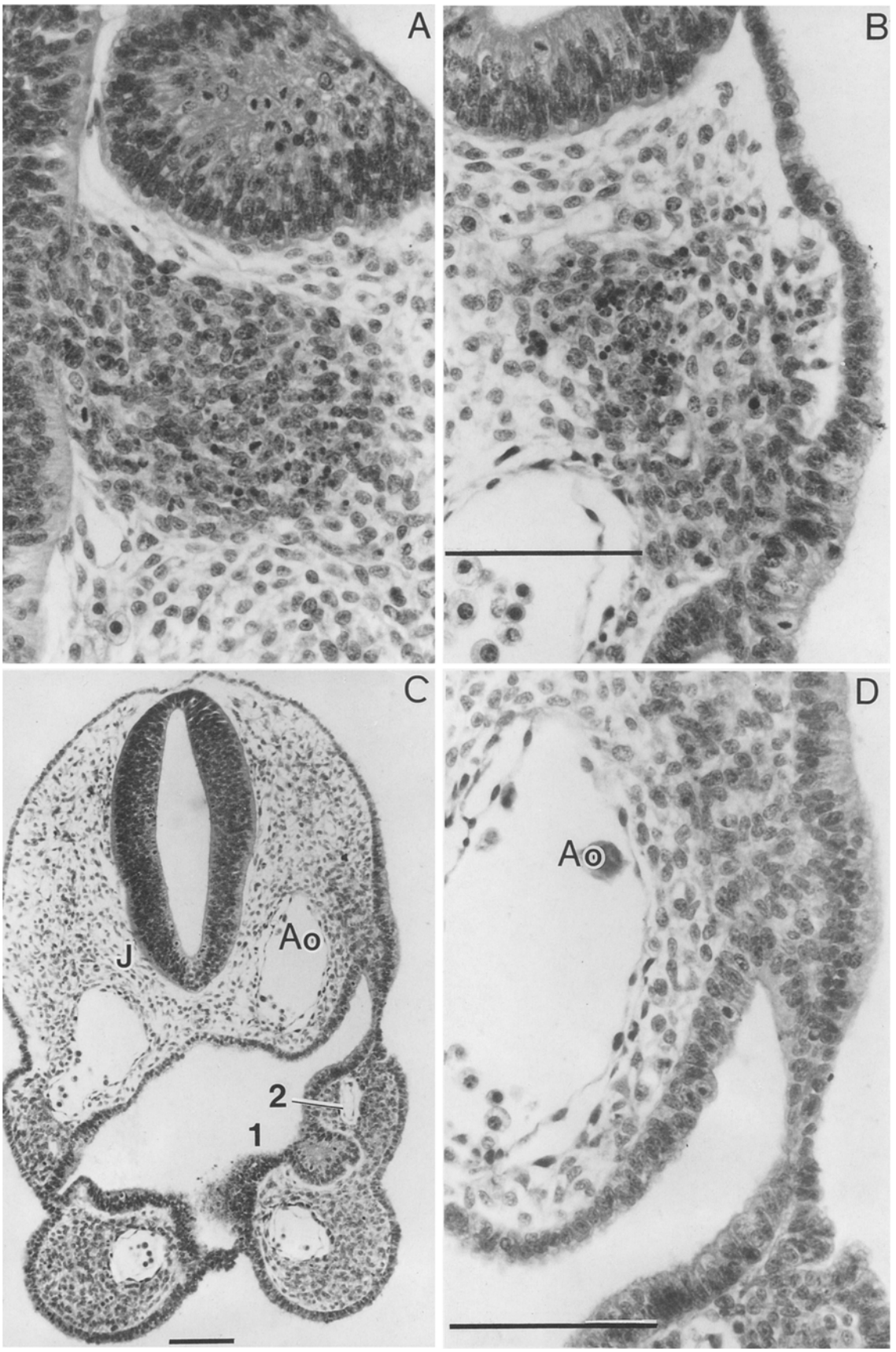
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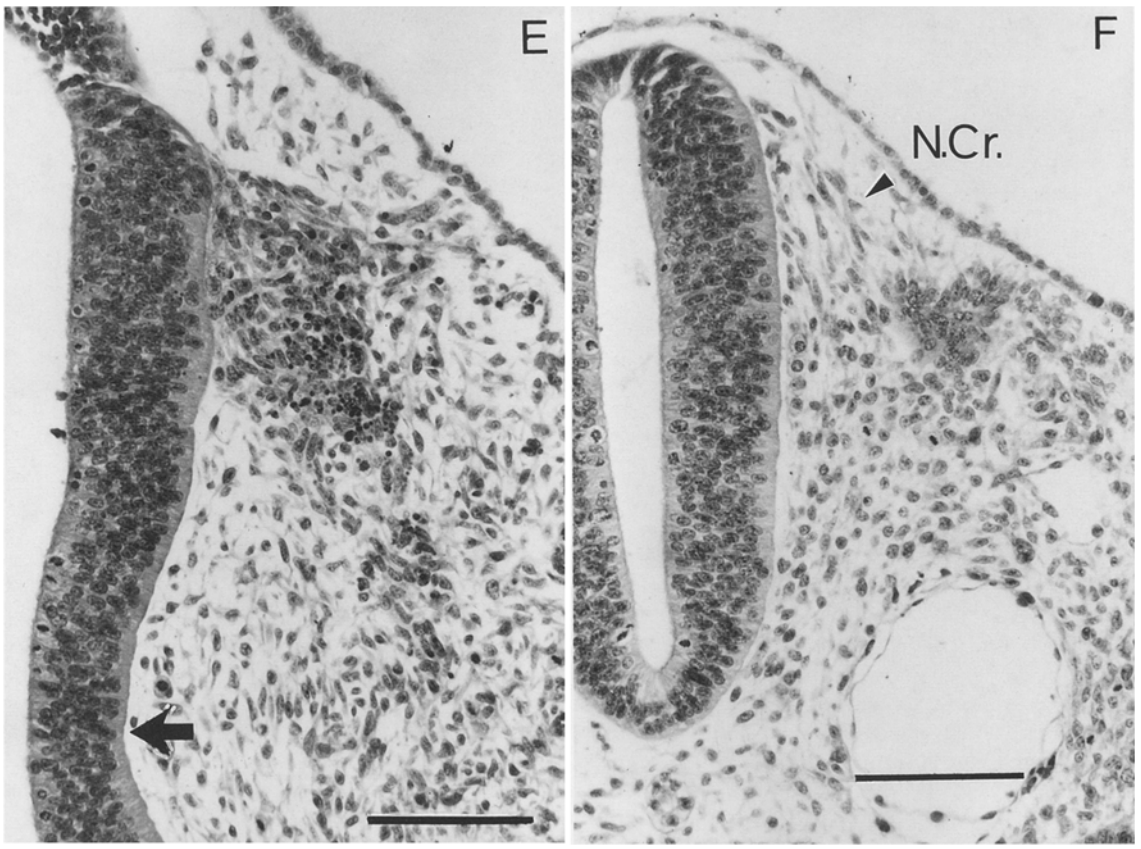


**Fig. 9A–F.** Cranial ganglia and neural crest of No. 8943. **A** Section 2-2-2, showing ganglion 7/8 and otic vesicle. Strands of cells penetrate the lateral wall of Rh4, no basement membrane is present at this site, and the marginal layer is also interrupted. Cell inclusions are numerous. **B** Section 2-3-7, taken more ventrally. Ganglionic material and cells of pharyngeal arch 2 seem to be continuous. The thick ectoderm of pharyngeal arch 2 with interrupted basement membrane seems to give off cells. In **A** and **B**, cells of the otic sheath (with their axes tangential to the surface of the otic vesicle) can be seen. Those cells separate the otic vesicle from the ganglion. **C** Section 3-1-7, and **D** enlargement of the same section. The thick surface ectoderm of arch 3 seems to give off cells to the mesenchyme. The dorsal aortae and two aortic arches can be seen, as well as some material from the ventral part of the oropharyngeal membrane. **E** Section 1-4-10, showing the trigeminal ganglion with its rostral division, which reaches the isthmus rhombencephali (*arrow*). Some cells of the trigeminal ganglion seem to be spreading towards the surface ectoderm. **F** Section 3-4-7, at the level of somite 2. Neural crest cells can be seen between RhD and the somite; some of the cells undergoing mitosis and adjacent to the neural tube represent pia mater. The levels of Fig. 9A–E are indicated in Fig. 5B. The bar represent 100 μm

*Notochord.* The paucity of mitotic figures is noteworthy. Rostrally, the number of figures is similar to the average found in stage 11. Caudally, newly formed notochord contains almost twice as many cells as the notochord at more rostral levels. It would seem therefore, that the thick caudal part of the notochord of earlier stages is being transformed into a more slender structure, which lengthens as the embryo continues to grow. Notochord and spinal cord are closely related in the caudal region. Ferner (1939) wrote of a "gemeinsame Anlage von Nervensystem und Chorda" at the level of the caudal eminence. In embryos of good qualtity a difference of density is present, and the notochord contains cell inclusions. In the chick embryo, Wallace (1982) found a small band of intense formaldehyde-induced fluorescence in the caudal end of the notochord. This zone moved down the body as the embryo lengthened and, after closure of the caudal neuropore, was located in an area corresponding to the tail-bud (his Fig. 6).

*Primitive streak.* The primitive streak appears during stage 6. It persists until the caudal neuropore has closed (Tam 1984), i.e., until the middle of stage 12, at which time it becomes replaced by the caudal eminence.

Brewer's (1938) criteria for the primitive streak, as stated in his account of an embryo of stage 6, are "the active proliferation of the cells, the loss of the basement membrane separating the primitive ectoderm [epiblast] and entoderm, the migration of the ectodermal cells, and the intermingling of the cells of the two layers of the [embryonic] disc at the caudal pole". By stages 11 and 12, however, if not earlier, the separation between the surface ectoderm and the underlying mesenchyme seems to be considerably sharper (Fig. 7) than in the initial stages.

*Caudal eminence.* The terms caudal eminence, end-bud, *Endwulst*, *Rumpfknospe*, *Schwanzknospe*, and *Rumpfschwanzknospe* are generally used synonymously. The caudal eminence is an ectoderm-covered mass of pluripotent tissue that takes the place of the primitive streak and provides structures comparable to those derived more rostrally from the three germ layers. The eminence arises as a continuation of the primitive streak (and/or node). It can be distinguished already in stage 9 (Müller and O'Rahilly 1983), is prominent in stages 10 to 12 (Fig. 7), and gradually replaces the primitive streak during stages 12 and 13. Thereafter it becomes less noticeable, although its activity as a

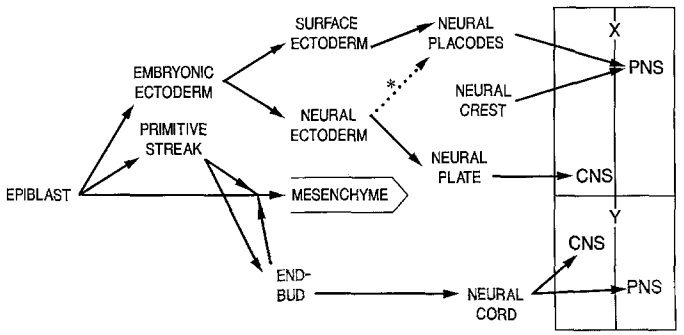


**Fig. 10.** Chart to show the relevant derivatives of the epiblast, which appears at stage 3 (4 postovulatory days). Notochord and endoderm are not included. The neural placodes appear in the surface ectoderm but were regarded by Streeter (*asterisk*) as islands of neural ectoderm. *X*, the region of primary development of the body and of primary neurulation, which is associated with the formation of the neural plate. *Y*, the region of secondary development and of secondary neurulation, which is associated with the end-bud (caudal eminence) and neural cord

bud (*Knospe*) continues for at least several more stages. In the region of secondary neurulation, the eminence during stage 12 gives rise to a solid cellular mass known as the neural cord (Fig. 7B, C), which forms the nervous system of the caudal part of the body. That the neural cord, notochord, and hindgut are not initially limited by a basement membrane is suggested by Holmdahl's (1926) illustrations (shown enlarged by Hamersma 1966) of the caudal end of two embryos of stage 12.

The relevant derivatives of the epiblast are summarized in Fig. 10.

*Primary and secondary development.* It was pointed out by Keibel (1910) that a sharp distinction cannot be drawn between the primitive streak and the caudal eminence (or "trunk bud", to use the term that he proposed). Keibel's *Rumpfknospe* consists of indifferent cellular material, which circumstance may throw light on the occurrence of coccygeal tumours (ibid.). Politzer (1951) agreed with neither Keibel nor Holmdahl and suggested replacing the term *Rumpfschwanzknospe* again by *Schwanzknospe*.

The idea of a *primäre Körperentwicklung* and a *sekundäre Körperentwicklung*, which dates from the end of the nineteenth century, was elaborated by Holmdahl in the 1920s. In the primary development, various organs are established from the germ layers, whereas, in the secondary mode, an indifferent cellular mass (or blastema) gives rise to various features in the caudal part of the body (Holmdahl 1935), including certain somites, notochord, alimentary canal, coelomic cavity, and blood vessels. The boundary between the primary and secondary processes is unclear but was believed by Holmdahl to be in the lumbosacral region in the chick embryo. In the human embryo, the level of the boundary remains uncertain but, when the primitive streak is defined as ending with closure of the caudal neuropore, the site of final closure could be considered as the junctional zone. It would correspond to the upper sacral region.

*Somites.* In experimental studies of the chick, it has been shown that somites 2 to 5 participate in the formation of the tongue, and somites 1 and 2 in the formation of the intrinsic laryngeal musculature (Noden 1986). In the human, the hypoglossal cord is derived from somites 1 to 4, and contributes to the formation of the tongue (O'Rahilly and Müller 1984). From its position in stage 12, part of the material could also be distributed to the laryngeal area.

**Table 4.** Neurulation in the human embryo

| Stage | Days | *Primary neurulation* | Reference |
|---|---|---|---|
| 8 | 18 | Neural groove and folds may appear | O'Rahilly and Müller 1981 |
| 9 | 20 | Neural groove deepens | Müller and O'Rahilly 1983 |
| 10 | 22 | Neural folds begin to fuse | Müller and O'Rahilly 1985 |
| 11 | 24 | Rostral neuropore closes | Müller and O'Rahilly 1986a |
| 12 | 26 | Caudal neuropore closes *Secondary neurulation* begins | Present study |

The absence of a tail in birds, as in the human, has been thought to be associated with "a special liability to the various degrees of malformation of the hinder regions of the spinal cord and of the sacral region" (Hughes and Freeman 1974). Such malformations can frequently be related to defective formation of sacral and coccygeal somites (ibid.). On the other hand, the possibility has also been raised that canalization during secondary neurulation may be involved in the origin of such malformations (Lemire et al. 1975).

*Caudal aplasia.* A complete deficiency of the caudal eminence (end-bud) results in cloacal deficiency and a consequent proximity of the two lower limb buds. The most characteristic of these anomalies is symmelia, in which various degrees of so-called "fusion" of the lower limbs are found. Such conditions are, to a certain extent, comparable to cyclopia.

More localized defects within the caudal eminence may account for sacral agenesis (Töndury 1944). The mesenchyme for somites 30–34, i.e., the material for sacral vertebrae 1–5, is laid down during stage 12, and the somites themselves appear during stage 13, mostly in the region of secondary neurulation. However, sacral agenesis has also been attributed to an exaggeration of the normal process of caudal regression that occurs later in the embryonic period (Duhamel 1966).

*Secondary neurulation.* The principle of secondary neurulation was clarified by Keibel in 1908: „Das caudale Ende der Medullaranlage entsteht nicht durch die Herausbildung und den Verschluss von Medullarfalten, sondern differenziert sich mit Chorda und Schwanzdarm aus der indifferenten Zellenmasse, welche wir nach Schwund des Primitivstreifens am kaudalen Embryonalende entstehen sehen und als Schwanzknospe bezeichnen können" (cited by Holmdahl 1933).

Once the caudal neuropore has closed, neural material is laid down not as a superficial plate but as a neural cord. In all the cases studied, the cavity of the already formed spinal cord extends into the neural cord in continuity, i.e., isolated spaces that would later become confluent with each

other and with the already existing cavity of the spinal cord were not observed.

Secondary neurulation begins during stage 12 (Table 4).

The observations presented here are in almost full agreement with those of Schoenwolf (1984) on the mouse. No overlapping zone, as in the chick, is found in either mouse or human. Isolated lumina do not appear during cavitation of the neural cord, and it seems plausible that the cavitation requires fluid pressure because "the incipient lumen of the secondary neural tube always seems to be directly continuous with that of the more cranial, fully formed neural tube". The site of last closure differs only slightly, being at the level of somites 32–34 in the mouse, and at about somite 31 in the human.

Primary and secondary neurulation, when based on the criterion of *offene und geschlossene Rückenmarksbildung* (Holmdahl 1933), would be more or less coextensive with *primäre und sekundäre Körperentwicklung*.

*Rostral neuropore.* Cellular strands at the site of the rostral neuropore were described by Sternberg (1927) in embryos of 25 and 28 somites. He already recognized that „die Stelle des vorderen Neuroporus später im Bereiche der Kommissurenplatte liegt". Irregularities in both the neural ectoderm (Bartelmez and Blount 1954, Fig. 17) and the surface ectoderm (Bartelmez and Dekaban 1962, Fig. 62) have been recorded. A "cell cord" comparable to what was described for No. 8943 was described by Mori (1965) "interposing between, and adjoining to the skin ectoderm and the neuroepithelium of the forebrain".

*Caudal neuropore.* Although the time of closure may vary slightly in relation to somitic count (e.g., it is closed in No. 8963 with seemingly only 22 completely isolated somites: Wen 1928, Fig. 19), the opening is generally closing or closed when 25 somitic pairs are present, and invariably from 27 pairs onwards (O'Rahilly and Gardner 1979). Non-closure, however, may be found abnormally in embryos of stages 13 or 14. Such a specimen, at stage 14, has been described by Lemire et al. (1965). Non-closure of the caudal neuropore may be important in the genesis of spina bifida aperta at low levels.

The level of final closure of the caudal neuropore is at the level of approximately future somite 31, which corresponds to the second sacral vertebral level (Müller and O'Rahilly 1986b).

*Brain.* Relatively good agreement exists between the reconstruction of the brain of No. 8943 by Bartelmez and Dekaban (1962, their Fig. 60) and the present results. They indicate the mamillary, but not the infundibular, recess. What is really different is their caudal limit of the mesencephalon. Bartelmez and Dekaban stress that the rostral part of the mesencephalon develops a ventral thickening, foreshadowing the tegmentum. Wen's reconstruction of No. 8963 (1928, his Fig. 7C) indicates some relatively advanced features for an embryo of apparently only 22 fully isolated somites: the mesencephalic angle measures 52° (119° in the Girgis embryo with 22 somites), and the caudal neuropore is closed. Because either the somitic count may not be clear or the specimen may be abnormal, it was considered wiser not to include this embryo in Table 3. Streeter (1945) labelled some quite advanced features in his reconstruction of No. 5923 (Fig. 7, xiii), and these have been altered in the revision of the Carnegie Stages (O'Rahilly and Müller 1987b).

Few changes occur in the differential growth of the brain. Morphologically the most obvious are the enlargement of the telencephalon medium, the formation of two mesencephalic segments, and the reduction of rhombomere 4. The most striking feature is the appearance of the first silver-impregnated cells and fibres. Windle (1970) saw fibres of the ventral longitudinal tract at the level of cranial nerves 10 and 12; and, as in the present study, he found, with regard to the hypoglossal nerve, that "neurofibrillation is initiated in primary efferent neuroblasts" before 30 somites. The nucleus of the lateral longitudinal tract was described by Windle and Fitzgerald (1942) as a column of early neurons "paralleling the sulcus limitans and extending rostrally as far as the isthmus rhombencephali". Motor neurons in the cervical spinal cord of mouse embryos of 9 and 10 days, corresponding approximately to stage 12, were demonstrated by Wentworth (1984).

The relative mitotic activity of various parts of the brain parallels closely the lengths of those same parts. In an interesting study of the chick brain, Frank (1925) found a "vague parallelism" between the number of mitotic figures in certain areas of the forebrain and the later expansion (*Entfaltungsmass*) of those areas.

*Mesencephalic neural crest.* Although this was found in an excellent specimen of 23 somites, its depiction in other embryos was unclear. If neural crest cells contribute to the formation of the mesencephalic nucleus of the trigeminal nerve in the human embryo, what was demonstrated in birds may perhaps appear in later stages as neurons "in the leptomeningeal tissue overlying the midbrain region" (Narayanan and Narayanan 1978).

*Diencephalic neural crest.* This was observed by Mori (1969) but somewhat nearer the area of the closed rostral neuropore.

*Optic neural crest.* This is basically similar to that found in stage 11 (Müller and O'Rahilly 1986a). At stage 12 the optic neural crest reaches its maximum extent and the optic vesicle becomes covered by a complete sheath, giving the appearance of a "frightened hedgehog" (Bartelmez and Blount 1954). A three-dimensional reconstruction of the optic neural creast of an embryo of stage 12 was depicted by Bartelmez and Dekaban (1962, their Fig. 65).

*Nasal discs.* The nasal discs form a portion of the ectodermal ring on both sides of the closed neuropore (O'Rahilly and Müller 1985). The olfactory area of the brain is also becoming thicker and mitotic figures become more numerous (Bossy 1980).

*Otic vesicle and neural crest.* The contribution of neural crest cells from the ventral wall of the otic vesicle to ganglion 7/8 was described by O'Rahilly (1963, Plate 1, Fig. D).

*Ganglia of the cranial nerves.* Ectodermal thickenings at the surface of the pharyngeal arches are referred to as pharyngeal placodes. Contributions from pharyngeal placodes are a distinctive feature of cranial as opposed to spinal ganglia, as pointed out by Theiler (1949). Wen (1928), in regard

to an embryo of stage 11 (No. 4315), noted some indications of "ectodermal contribution to the future" trigeminal ganglion. Van Campenhout (1948) observed budding from pharyngeal placodes in three Carnegie embryos of stage 12 (Nos. 7999, 6097, and 5923) "sans qu'on puisse parler d'un enfoncement direct des bourgeons cellulaires dans la crête ganglionnaire proprement dite". Theiler (1949) observed a loose connection between the trigeminal placode and the neural crest (*rostrale Leiste*) at stage 12 (25 somites). Mori (1965), however, in human embryos of stage 12, was unable to confirm the existence of a trigeminal placode in his histochemical study. In the present investigation, definite placodal contributions for the trigeminal ganglion were not observed at stage 12 but such indications are now being studied at stage 13.

In the mouse, a "trigeminal placode" contributes to the trigeminal ganglion (Nichols 1986a, b). In the rat, "a lateral (possibly of placodal origin) and a medial (possibly of neural crest derivation)" anlage of the trigeminal ganglion have been distinguished (Altman and Bayer 1982). These latter authors went so far as to state that "all cranial nerve ganglia derive from placodes, with no contributions made to the neuronal population by the neural crest".

The relationship of the surface ectoderm to the formation of cranial ganglion 7/8 seems to be much closer than in the case of the trigeminal. Theiler (1949) noted close contact between the *Acusticofacialisleiste* and the *epibranchiale Placode* of the hyoid arch at stage 12. Ectodermal and endodermal thickenings in contact with ganglion 7/8 "showed an enhanced activity of the enzyme" (Mori 1965). "Their basement membrane [sic] were apparently complete [sic] lacking", a point of evidence for "active spreading out of the epthelial cells". Cellular strands reaching from the lateral wall of rhombomere 4 to ganglion 7/8 were not noted in other studies. (The "boundary cap" described by Altman and Bayer (1982) in the rat is a transitory aggregate of neural crest cells lying outside the neural tube and traversed by efferent fibres). In the present study, it was found that cells from the surface ectoderm of pharyngeal arch 2 and neural crest material seem to mingle, and hence it is possible that placodal cells contribute to ganglion 7/8 at stage 12.

It was noted in this investigation that the neural crest for ganglia 9 to 11 is not yet well differentiated at stage 12. The surface ectoderm of pharyngeal arches 3 and 4 gives off cells, but it is not yet possible to state whether or not these cells contribute to cranial ganglia. Mori (1965) found a high activity of alkaline phosphatase in the thickened surface area of pharyngeal arch 3 in embryos of stage 12. The development of the ganglia of cranial nerves 7, 9, 10, and 11 was studied in quail-chick chimeras by Ayer-Le Lièvre and Douarin (1982), who found a large contribution from the surface ectoderm. It appears that the neural crest cells "give rise to the satellite cells of the geniculate, petrous, and nodose ganglia, while the large sensory neurons originate from the placodes". In the root ganglia, that is the superior ganglia of the glossopharyngeal and vagus nerves, however, both satellite and neuronal cells arise entirely from the neural crest in the chick (Narayanen and Narayanen 1980).

Theiler (1949) saw inclusions in the cranial ganglia of developing human embryos of a comparable stage and called them *Kerntrümmer*. He saw them disappear in later stages, at the time that nerve fibres began to form.

*Neural crest and cephalic mesenchyme.* The problem of migration of the neural crest was summarized by Noden (1986), who stated that "cephalic crest cells are not moving alone through a static matrix but are part of an integrated set of tissue displacements". It has been shown experimentally in the chick that the cephalic neural crest contributes to the mesenchyme of pharyngeal arches 1 to 4 (Le Lièvre 1974). Moreover, "cartilage developed only from neural crest cells of head levels. No skeletal muscle was ever observed to develop from the neural crest" (Wachtler 1984). More recently, Le Douarin (1986) pointed out that the migrating neural crest cell population "is not composed of a homogeneous population of cells that are all endowed with the entire range of developmental capacities". Restriction of their capacities "are probably acquired through the successive cleavages of neural epithelium-derived cells".

Although migration as such cannot be shown in the human embryo, the result of it can be plotted as long as tissue densities and cellular shapes differ sufficiently. As pointed out by Johnston and Listgarten (1972, cited by Tosney 1982), "mesodermal cells do not intermingle with crest cells during early migration". Extrapolating data from the chick to the human newborn, Noden (1986) postulated the interphase between paraxial mesoderm and neural crest in his Fig. 4. According to this hypothetical representation, neural crest would be involved in the formation of the derivatives of pharyngeal arches 1 and 2. This would mean that neural crest material would have to migrate completely into these pharyngeal arches. The cellular material forming the frontonasal and maxillary prominences arises from the neural crest of the rostral midbrain and diencephalic region, according to Noden and Evans (1986). Apart from a few isolated crest cells, the medial areas of the forebrain in human embryos of stage 12 are still in direct contact with the surface ectoderm. Here again, studies of older embryos would be required to give more information about neural crest participation in the mesenchyme of the face.

The behaviour of neural crest cells in the trunk of the chick is such that neural crest migration along the neural tube towards the notochord is limited to intersomitic levels in 25-somite embryos (Thiery et al. 1982).

*Other systems.* The digestive and vascular systems have been summarized by Streeter (1942), and a detailed account of the heart and blood vessels has been provided by Rosenbauer (1955).

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