TERATOLOGY 48:21–31 (1993)

Embryonic Development of the Mammalian Caudal Neural Tube

R.A.J. NIEVELSTEIN, N.G. HARTWIG, C. VERMEIJ-KEERS, AND J. VALK
Department of Diagnostic Radiology, Free University Hospital, Amsterdam 1007 (R.A.J.N., J.V.) and Department of Plastic and Reconstructive Surgery, University Hospital, Rotterdam (R.A.J.N., N.G.H., C.V.-K.), The Netherlands.

ABSTRACT In the literature, some controversy still exists about the embryonic developmental processes involved in the formation of the caudal neural tube. Therefore, a three-dimensional and histological study concerning the normal development of the caudal neural tube was performed on both mouse and human embryos. Three developmental processes can be distinguished in caudal neural tube development: caudal neuropore closure, secondary neurulation, and degeneration and differentiation of the secondary neural tube. Caudal neuropore closure occurs at the level of somite 32–34 in both species. Therefore, primary neurulation leads to the formation of all spinal cord segments and ganglia. Secondary neurulation involves cell deposition from a cluster of neurectodermal cells at the caudal end of the closed neural tube, directly around a lumen, the lumen always in contact with the lumen of the primary neural tube. This process leads only to the formation of the primordia of the filum terminale and ventriculus terminalis and, possibly, part of the conus medullaris. Secondary neurulation is followed by a period characterized by degeneration and differentiation of the secondary neural tube. Its lumen and neural tissue will disappear, whereas part of the secondary neurectodermal cells differentiate to a fibrous layer comparable and continuous with the marginal layer of the primary neural tube. This fibrous layer represents the future filum terminale. The embryological processes indicated above can be helpful in the interpretation of congenital anomalies affecting the caudal spinal cord and spine.

Knowledge of normal embryonic and fetal neural tube development is of great importance in understanding the pathogenesis of neural tube defects, especially in the lumbosacral region. In the literature three distinct phases are described in caudal neural tube development: caudal neuropore closure, secondary neurulation, and retrogressive differentiation (Schoenwolf, '84; O'Rahilly and Müller, '87; Lemire, '88). The closure of the caudal neuropore indicates the end of primary neurulation, according to Müller and O'Rahilly ('87), in human embryos during Carnegie stage 12 (~ day 27, 3–5 mm crown-rump length (C-RL)). At this stage the embryos have 21–29 somites, but unsegmented material for somites 30–34 is already present. These somites appear during stage 13. However, different opinions are given about the presomitic level of the final closure of the caudal neuropore. Müller and O'Rahilly ('87) describe the localization concerned in human embryos at the level of the presumptive somites 30–31, which corresponds to the first or second sacral vertebra. Schoenwolf ('84), in his study performed on mouse embryos, suggests the site of caudal neuropore closure to be situated at the level of the somites 32–34, which corresponds to the third to fifth sacral vertebrae. This is confirmed by Smits-Van Prooije ('86) in both rat and mouse embryos.

Received July 6, 1992; accepted February 10, 1993.

Address reprint requests to R.A.J. Nievelstein, Department of Diagnostic Radiology, Free University Hospital, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands.

During secondary neurulation, which takes place between Carnegie stages 12 and 20 (day 27–48 of human development, 4–21 mm C-RL), an additional part of the neural tube is formed (Müller and O'Rahilly, '87, '88). Two theories explaining this process are proposed in the literature. The first one is based on data in chick embryos and is confirmed in rats (Romanoff, '60; Bentliff and Gordon, '65; Schoenwolf and Delongo, '80). Four major events are distinguished. Dorsally in the developing tail cells of the primitive streak, (i.e., tail bud; Schoenwolf, '84) aggregate to form a solid medullary cord. Then this cord differentiates into peripheral and central cells, after which multiple, isolated lumina appear. Finally, all lumina coalesce to form a single central cavity, which makes contact with the lumen of the primary neural tube. Accessory lumina were also observed in human embryos (Lemire, '69), but they were limited in most embryos to only one.

The second theory, introduced by Schoenwolf ('84) in mouse embryos, involves two major events. Cells localized dorsally in the tail bud cluster together and form a medullary rosette or plate. In this medullary rosette cavitation takes place, the lumen always in contact with the lumen of the primary neural tube. Müller and O'Rahilly ('87, '88) suggest a similar process in human embryos.

After secondary neurulation, part of the secondary neural tube will disappear by a process indicated as "retrogressive differentiation," starting during Carnegie stage 20 (~ day 50, 21–23 mm C-RL) of human embryonic development (Lemire, '88; McLone and Naidich, '89). It is characterized by degeneration, regression, and differentiation of part of the secondary neural tube. The histological features of the "retrogressive differentiation" are not clearly given in the literature. For example, no attention has been payed to cell death, a programmed phenomenon during normal embryonic development (Vermeij-Keers et al., '83).

In view of the discrepancies indicated above, a three-dimensional and histological study concerning the normal development of the caudal neural tube is required. The point of controversy in all theories about the formation of the caudal part of the neural tube in mammalian embryos is the localization of the somitic level of final closure of the caudal neuropore. Very young human embryos, however, when available, are usually in bad cellular condition. For that reason and since neural tube development in murine embryos resembles that in humans, both human and mouse embryos were studied in order to obtain developmental information for the interpretation of congenital anomalies affecting the caudal spinal cord and spine.

MATERIALS AND METHODS

Mouse embryos

Mouse embryos (49) of the CPB-S strain (18) and AJL strain (31), aged 9.0 to 17.3 days after conception, were studied microscopically. The embryos were fixed in Carnoy's fixative and embedded in paraffin. They were cut into 10-μm transverse sections and stained in haematoxylin-eosin. Somites or vertebral bodies (in older stages) and spinal ganglia were counted for each embryo. Three-dimensional graphic reconstructions were made of caudal neural tube development in mouse embryonic tails (CPB-S strain) according to the method of Tinkelenberg ('79). Four representative stages in development were chosen; 9.8, 10.5, 11.9, and 13.7 days after conception.

Mature murine tails

To get acquainted with the mature morphology of the caudal spinal cord and spine, two adult murine tails were studied macroscopically by dissection using an operation microscope.

Human Embryos

Human embryos and fetus (36), ranging from 2.5 to 80 mm C-RL (corresponding the Carnegie stages 11–23 and the early fetal period), were studied microscopically. The specimens were fixed in formalin or Bouin's fixative and embedded in paraffin. They were cut in 10-μm transverse or sagittal sections and subsequently stained in haematoxylin-eosin.

RESULTS

Mouse embryos

The developmental processes involved in the caudal neural tube development are described using three-dimensional graphic reconstructions (Figs. 1–4) and histological sections of the four representative stages.

Closure of the caudal neuropore (9.0–9.8 days; Fig. 1)

Approximately 27–29 somites have been formed around 9.8 days, followed by a column of presomitic tissue. The caudal neuropore is situated at about the level of the presumptive somites 32–34, and closure takes place from a cranial as well as caudal direction (Fig. 1). About 28 spinal ganglia have been formed, corresponding to their somitic level. The primitive streak is situated at the caudal end of the neuropore, as a mass of undifferentiated cells. The cloacal membrane can be identified ventrally of approximately somite 29. Caudal from the cloacal membrane the tail gut develops. The notochord is situated between gut and neural tube. During this period, physiological cell death is observed in the fusion zone during closure of the caudal neural tube and in the primitive streak (Fig. 5).

Secondary neurulation (9.9–10.5 days; Fig. 2)

The neural tube is closed (9.9 days), and the tail develops, including the somites, secondary neural tube, notochord, and tail gut out of cells deposited by the primitive streak, i.e., the tail bud. Approximately 47 somites have been formed at 10.5 days, but there is still a column of presomitic tissue. At ~ 10.0 days, 34–35 spinal ganglia can be observed, corresponding to their somitic level. After closure of the caudal neuropore at ~ day 9.9, spinal ganglia are not formed anymore. At the caudal end of the neural tube, a cluster of neurectodermal cells is observed (Fig. 6). In slightly more cranial sections, these neurectodermal cells appear to have a more columnar character with small intercellular spaces and a radial orientation, surrounding a small round lumen (Figs. 6, 7A). This lumen is always directly continuous with the lumen of the formed secondary neural tube and likewise also with the lumen of the primary neural tube. Cell death is not observed at the caudal end of the just formed neural tube lumen (Fig. 6). The tail gut has been elongated by a similar kind of developmental process. From ~ day 10.3 on, the tail gut subsequently disappears by a process of cell death (Fig. 7). Cell death is seen throughout the whole length of the tail gut, resulting in disappearance of its lumen and breakthrough of the endoderm at various places along the whole tract in the tail. Notochord can be identified between the degenerating tail gut and the secondary neural tube. It develops also out of the undifferentiated cell mass of the primitive streak.

Secondary neurulation (10.6–11.9 days; Fig. 3)

Around day 11.9, ~34 sclerotomes, i.e., the somitic parts that give rise to the vertebral bodies, are observed, followed by a column of sclerotomic tissue in which the succeeding sclerotomes cannot be identified yet; 34–35 spinal ganglia are observed. The cluster of neurectodermal cells is still present at the caudal end of the developing neural tube, and further elongation of the secondary neural tube has taken place. As observed in transverse sections, the secondary neural tube consists of a pseudostratified epithelium, surrounding a small round lumen, with the nuclei found aligned at two levels (Fig. 8). Caudal in the tail, a tail gut rudiment is seen in which cell death is observed. The notochord can be followed almost unto the end of the tail.

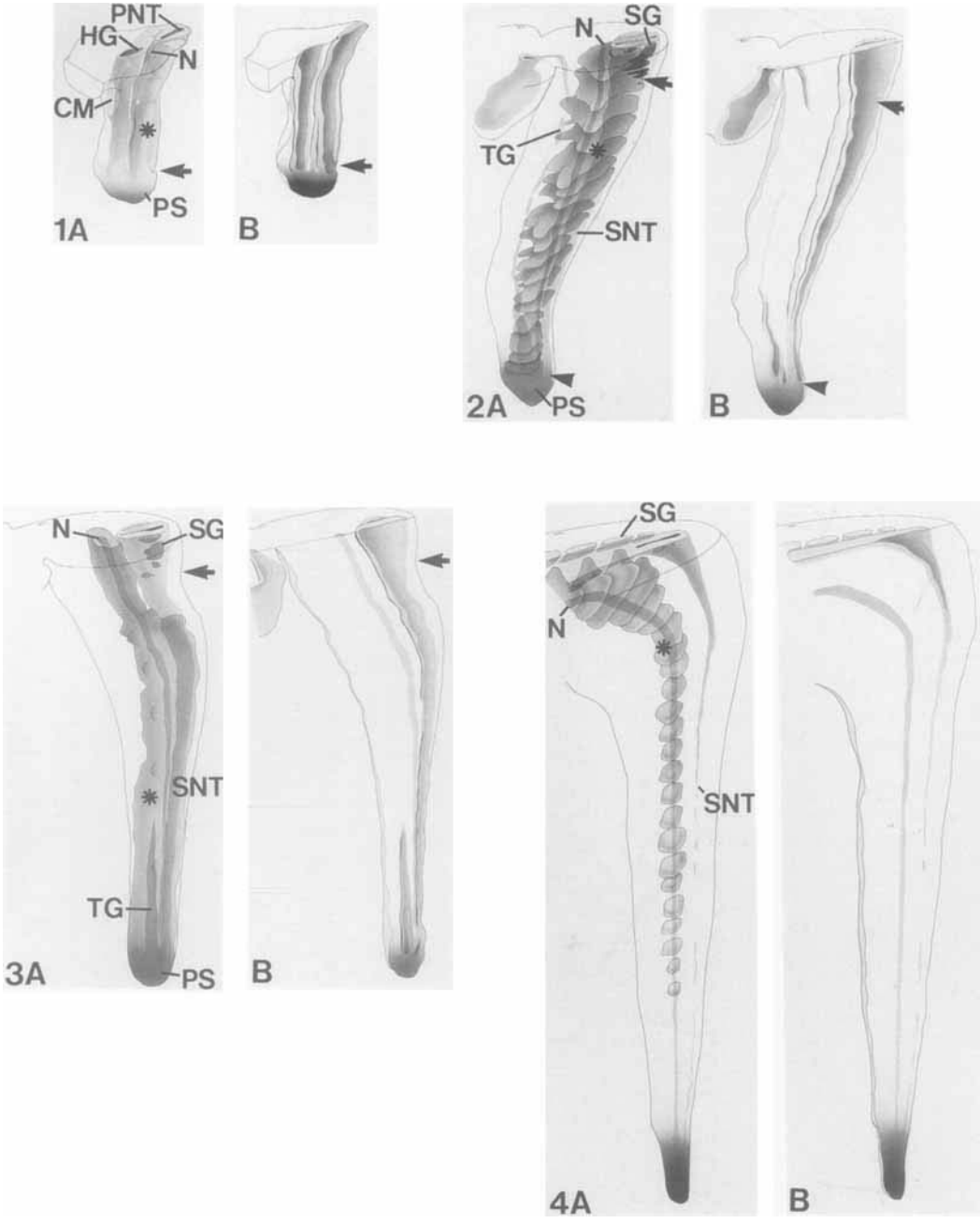
Degeneration and differentiation of the secondary neural tube (12.0–17.3 days; Fig. 4)

The process of secondary neurulation has stopped; 51 developing vertebral bodies and 34–35 spinal ganglia can be identified. From ~ day 12.0 on, part of the secondary neural tube disappears by a process of physiological cell death (Fig. 9). The cell death can be observed almost throughout the whole length of the secondary neural tube, leading to disappearance of the lumen and breakthrough of the neural tube tissue at various levels. In the most cranial part of the secondary neural tube, formation of a thin fibrous layer is observed just adjacent to the degenerating secondary neural tube. This fibrous layer is cranially directly continuous with the marginal layer of the primary neural tube. No tail gut is seen. Notochord still can be followed almost unto the end of the tail.

Mature murine tails

Macroscopical observations

After laminectomy of the lumbar and sacral vertebrae, the following structures can be observed: the caudal end of the spinal



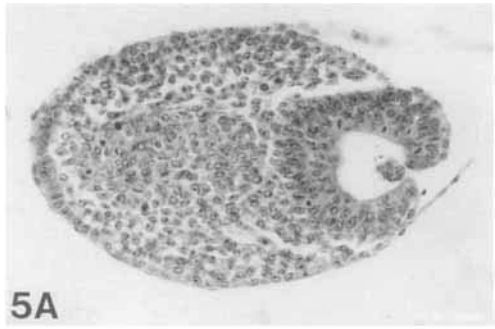
Figs. 1–4.

cord, i.e., the conus medullaris, followed by the filum terminale, i.e., a thin fibrous strand surrounded by pia mater. The filum terminale is surrounded by the roots of the cauda equina and inserts caudally into the dura mater.

Human embryos

Closure of the caudal neuropore (Carnegie stage 12; day 27, 3–5 mm C-RL)

Due to the bad cellular condition of the human embryos available concerning this developmental process, it was not possible to determine the exact level of caudal neuropore closure.



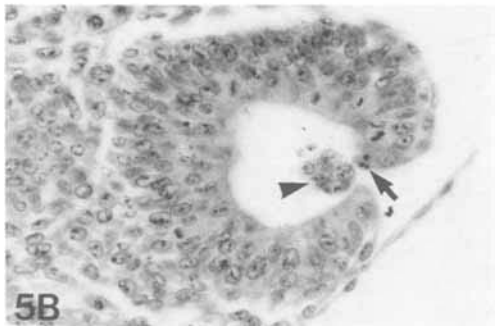


Fig. 5. **A.** Light micrograph of a 10-μm transverse section at the level of the closing caudal neuropore, in the 9.8-day-old mouse embryo of Figure 1. **B.** Detail; cell death (arrow), and detached and partially apoptotic neurectodermal cells (arrowhead) can be identified in the closing neuropore.

Secondary neurulation (Carnegie stages 12–19; days 27–48, ± 4–21 mm C-RL)

This process starts at ~ day 27 of development (Carnegie stage 12), after closure of the caudal neuropore. Caudal to the level of approximately somite 32, a morphologically different part of the neural tube can be identified (Fig. 10). This part of the neural tube is formed by a pseudostratified epithelium, surrounding a small round lumen, with the nuclei found aligned at two levels. The lumen of this secondary neural tube is always continuous with the lumen of the primary neural tube. In the caudal region another 5 somites are formed, leading to a total of ~ 37 somites. About 34 spinal ganglia are identified corresponding to their somitic levels, and in the region of secondary neurulation spinal ganglia are not observed. In one of the human embryos (± 14 mm C-RL, Carnegie stages 16–17), a gut rudiment was seen in the caudal region (Fig. 11). In older embryos it was not present anymore.

Figs. 1–4. **A.** Artist impressions of the three-dimensional graphic reconstructions of the caudal region of mouse embryos, in a frontolateral view (in proportion). **B.** The midsagittal sections demonstrate the extend of the lumen of both the neural tube and tail gut. CM = cloacal membrane, HG = hindgut, N = notochord, PNT = primary neural tube, PS = primitive streak, SG = spinal ganglion, SNT = secondary neural tube, TG = tail gut.

Fig. 1. 9.8 days p.c. This developmental stage represents the period of caudal neuropore closure. The 28th and 29th somites are visualized, followed by a column of presomitic tissue (asterisk). Closure of the caudal neuropore is situated at the level of the presumptive 32–34 somite (arrow).

Fig. 2. 10.5 days p.c. This stage represents the period of secondary neurulation. The tail somites (asterisk) and last spinal ganglia (SG) are visualized. The level of closure of the caudal neuropore is marked by an arrow; it is the level of last spinal ganglion or approximately the 34th somite. Elongation of the secondary neural tube (SNT) has taken place (between arrow and arrowhead), which is the result of cell deposition from a cluster of neurectodermal cells (arrowhead) localized in the primitive streak (PS). The tail gut (TG) is disappearing by cell death.

Fig. 3. 11.9 days p.c. It still represents the period of secondary neurulation. The 34th sclerotome is followed by a column of sclerotomic tissue in which the subsequent sclerotomes cannot be identified yet (asterisk). The level of closure of the caudal neuropore is marked by an arrow. In the tail gut rudiment (TG) cell death is still observed.

Fig. 4. 13.7 days p.c. This stage concerns the period of degeneration and differentiation of the secondary neural tube. The developing vertebral bodies of the tail can be identified (asterisk). Cell death in the secondary neural tube (SNT) results in disappearance of the lumen and breakthrough of the neural tube tissue at various levels. The tail gut is not present anymore.

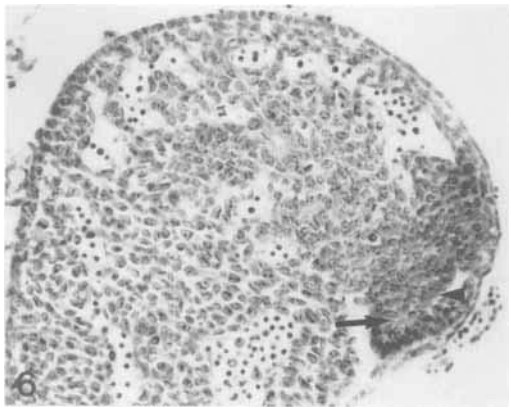
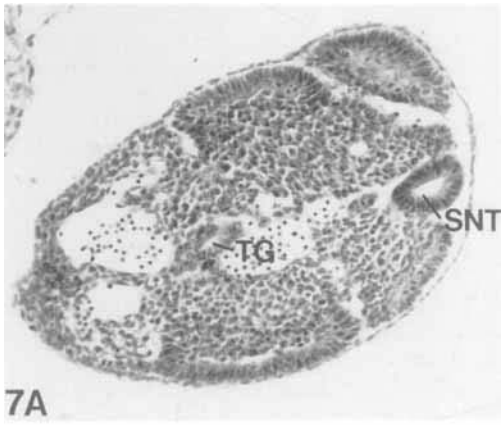


Fig. 6. Light micrograph of a 10-μm near transverse section at the caudal end of the secondary neural tube in the 10.5-day-old mouse embryo of Figure 2 (see level of the arrowhead). It demonstrates the neurectodermal cell cluster at the caudal end of the neural tube (arrowhead) and the just formed neural tube lumen (arrow). No cell death can be identified.



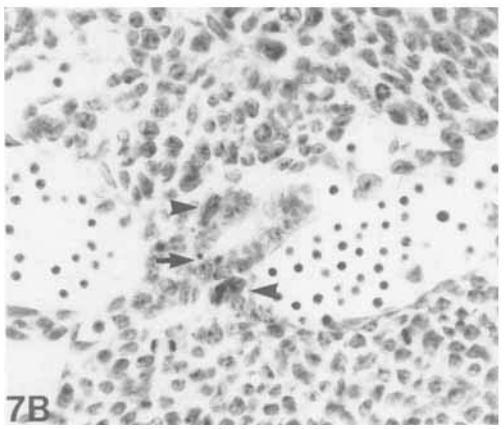


Fig. 7. **A.** Light micrograph of a 10-μm transverse section through the tail of the 10.5-day-old mouse embryo of Figure 2. It demonstrates the secondary neural tube (SNT), and the degenerating tail gut (TG). **B.** Detail of the degenerating tail gut, with cell death (arrow) and apoptotic cells (arrowheads).

Degeneration and differentiation of the secondary neural tube from Carnegie stage 20 onward (± day 50, 21–23 mm C-RL)

In the oldest human embryos and fetus (21–80 mm C-RL), the secondary neural tube is disappearing, whereas just adjacent to the degenerating neural tube, formation of a fibrous layer is observed (Fig. 12). This fibrous layer is cranially directly continuous with the marginal layer of the primary neural tube. Just adjacent to the fibrous tissue, a primitive meningeal layer develops out of the mesenchyme surrounding the neural tube. This meningeal layer is cranially continuous with the primitive pia mater surrounding the primary neural tube. The so-formed structure of fibrous tissue and primitive pia mater is surrounded by spinal roots and ganglia and inserts caudally into the primitive dura mater.

DISCUSSION

During the development of the caudal neural tube, the following events are important: the closure of the caudal neuropore, the formation of the secondary neural tube, and finally, its degeneration and differentiation. Thorough knowledge of these developmental events is essential in understanding the variety of neural tube defects observed in the caudal region. However, different views are still expressed in the literature concerning the part of the neural tube formed by secondary neurulation. Often the whole lumbosacral region is thought to be the result of secondary neurulation (Lemire et al., '75; Dryden, '80). Müller and O'Rahilly ('87) note that secondary neurulation in human embryos only leads to the formation of most of the sacral and all coccygeal segments. To differentiate which part of the neural tube is formed by primary and which part by secondary neurulation, it is important to know the exact level of closure of the caudal neuropore.

Caudal neuropore closure occurs during Carnegie stage 12 (day 27, 3–5 mm C-RL) and determines the end of the primary neu-

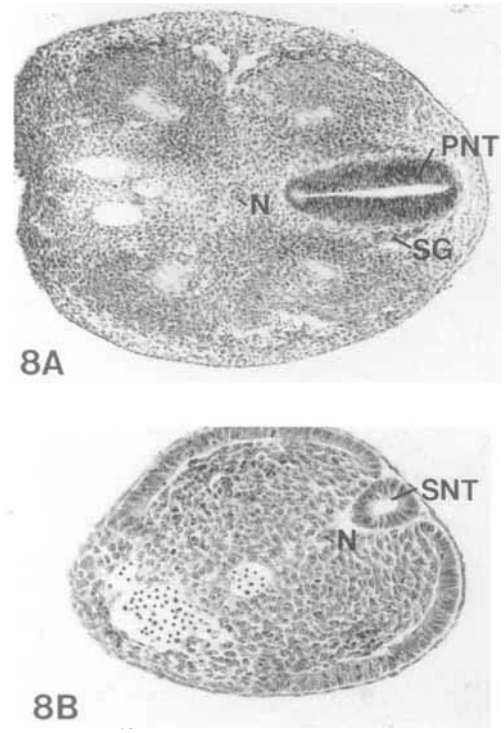


Fig. 8. Light micrographs of 10-μm transverse sections through the tail of the 11.9-day-old mouse embryo of Figure 3. **A.** demonstrates the primary neural tube (PNT), consisting of a pseudostratified epithelium with the nuclei found aligned at least 5–6 levels. The last spinal ganglion (SG) can be identified. N = notochord. **B.** demonstrates the secondary neural tube (SNT), consisting of a pseudostratified epithelium with the nuclei found aligned at maximal two levels, surrounding a small round lumen. N = notochord.

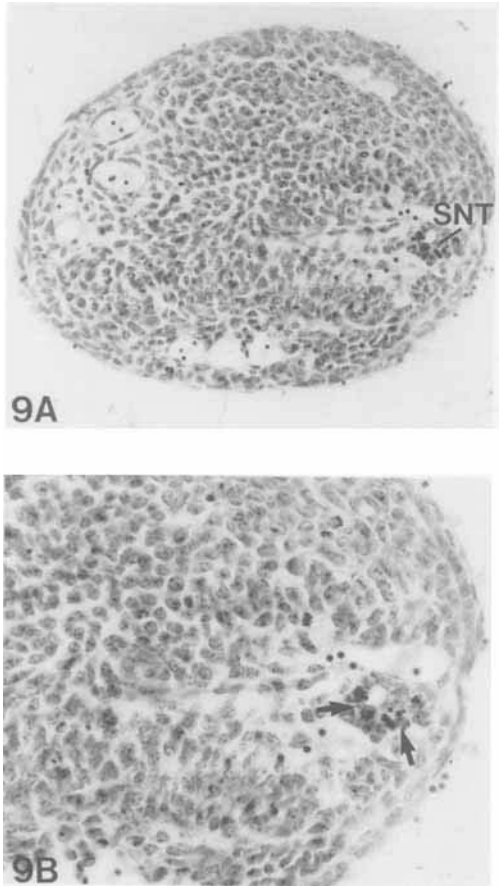
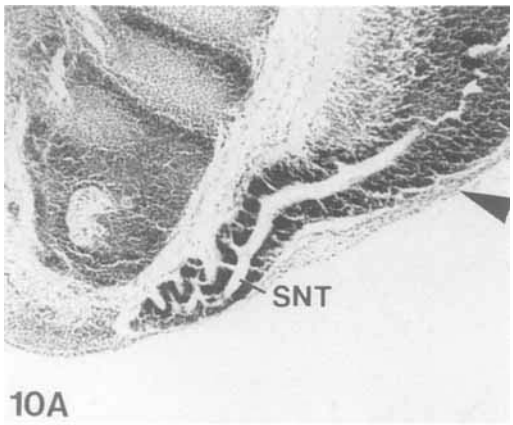


Fig. 9. **A.** Light micrograph of a 10-μm transverse section through the tail of the 13.7-day-old mouse embryo of Figure 4, demonstrating the degeneration of the secondary neural tube (SNT). **B.** Detail; the degenerating secondary neural tube, in which cell death is clearly visible (arrows).

rulation (Müller and O'Rahilly, '87, '88). In mouse embryos, caudal neuropore closure is situated at the level of the somites 32–34 (Schoenwolf, '84), and in contrast according to Müller and O'Rahilly ('87) in human embryos at the level of the 30–31 somite.

Unfortunately, young human embryos are often in a bad cellular condition, which makes it difficult to study the exact side and period of caudal neural tube closure. The microscopical observations of the caudal neural tube in human embryos indicate, however, that caudal neuropore closure in human embryos occurs at about the level of the 32nd somite. First, caudal to this level a morphologically different part of the neural tube is seen, showing great similarity with the secondary neural tube in mouse embryos, i.e., a pseudostratified epithelium with the nuclei found aligned at two levels, surrounding a small round lumen. Second, during primary neurulation deposition of cells into the mesodermal compartment takes place, out of the transition zone surface-ectoderm-neurectoderm, called the neural crest (Smits-Van Prooije, '86; Smits-Van Prooije et al., '88). These neural crest cells will give rise to the spinal ganglia and vertebral arches. In this way, ~ 34 spinal ganglia are formed in human embryos, corresponding to their somitic level. After closure of the caudal neuropore, i. e., during secondary neurulation, formation of spinal ganglia has stopped both in mouse and human embryos. From this and the observa-



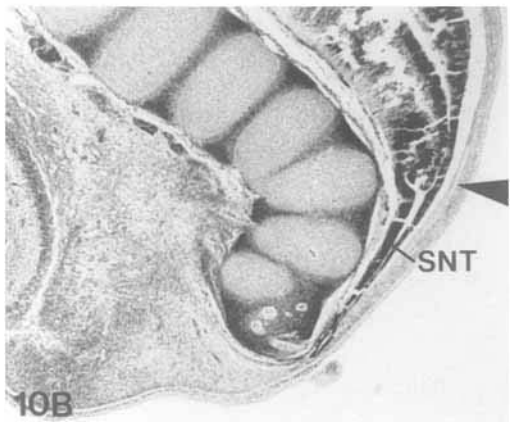


Fig. 10. Light micrographs of 10-μm sagittal sections through the caudal region of human embryos, demonstrating the morphologically different part of the neural tube, i.e., the secondary neural tube (SNT), caudal to approximately the 32nd somite (arrowheads). **A.** 17 mm human embryo (Carnegie stage 18). **B.** 28 mm human embryo (Carnegie stage 23).

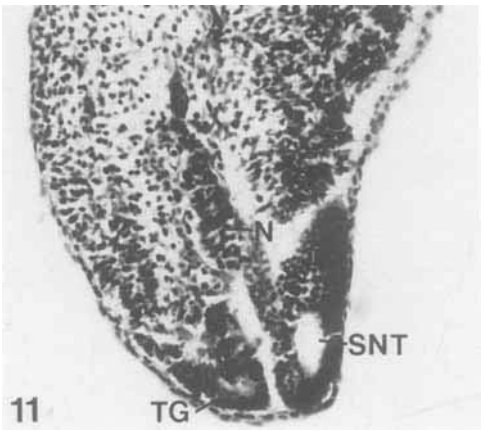


Fig. 11. Light micrograph of a 10-μm near sagittal section through the caudal region of a human embryo of ~ 14 mm C-RL (Carnegie stage 16–17), demonstrating a tail gut rudiment (TG). SNT = secondary neural tube, N = notochord.

tion of a morphologically different part of the neural tube in the caudal region, it seems likely to conclude that secondary neurulation in human embryos also starts below the level of the 32–34 somite, which corresponds to the future third to fifth sacral vertebrae. This is supported by the observations of myelomeningoceles with and without skin defects in the lower sacral region (Naidich et al., '83; Copp and Brook, '89). Since these anomalies are best explained as defects in the fusion process of the neural walls during primary neurulation (Copp and Brook, '89; Hoving, '90), the caudal neuropore is most likely situated at the level of the lower sacral vertebrae. Primary neurulation leads, therefore, to the formation of all spinal cord segments and spinal ganglia in human embryos. Secondary neurulation involves only the formation of the primordia of the ventriculus terminalis and filum terminale and, possibly, part of the conus medullaris. The conus medullaris is defined as that part of the spinal cord just caudal to the first coccygeal segment.

These findings not only have implications for the understanding of sacral myelomeningoceles, but also of other neural tube defects in the sacral region, e.g., the agenesis of the caudal part of spinal cord and spine in cases with the caudal regression syndrome (Duhamel, '61; Banta, '78). It is still believed that the normal and likewise abnormal development of the lumbosacral spinal cord and spine takes place during secondary neurulation (Ignelzi and Lehman, '74; Banta, '78; Van Der Knaap and Valk, '88). However, the results of the present study suggest that the agenesis of spinal cord, spinal ganglia, and vertebrae observed in this syndrome more likely results from a disturbance in the formation and/or differentiation of the caudal part of primary neural tube, spinal ganglia, and somitic tissues. A disturbance in secondary neurulation would only result in an abnormal or absent filum terminale and ventriculus terminalis in combination with minor (only coccygeal) vertebral defects.

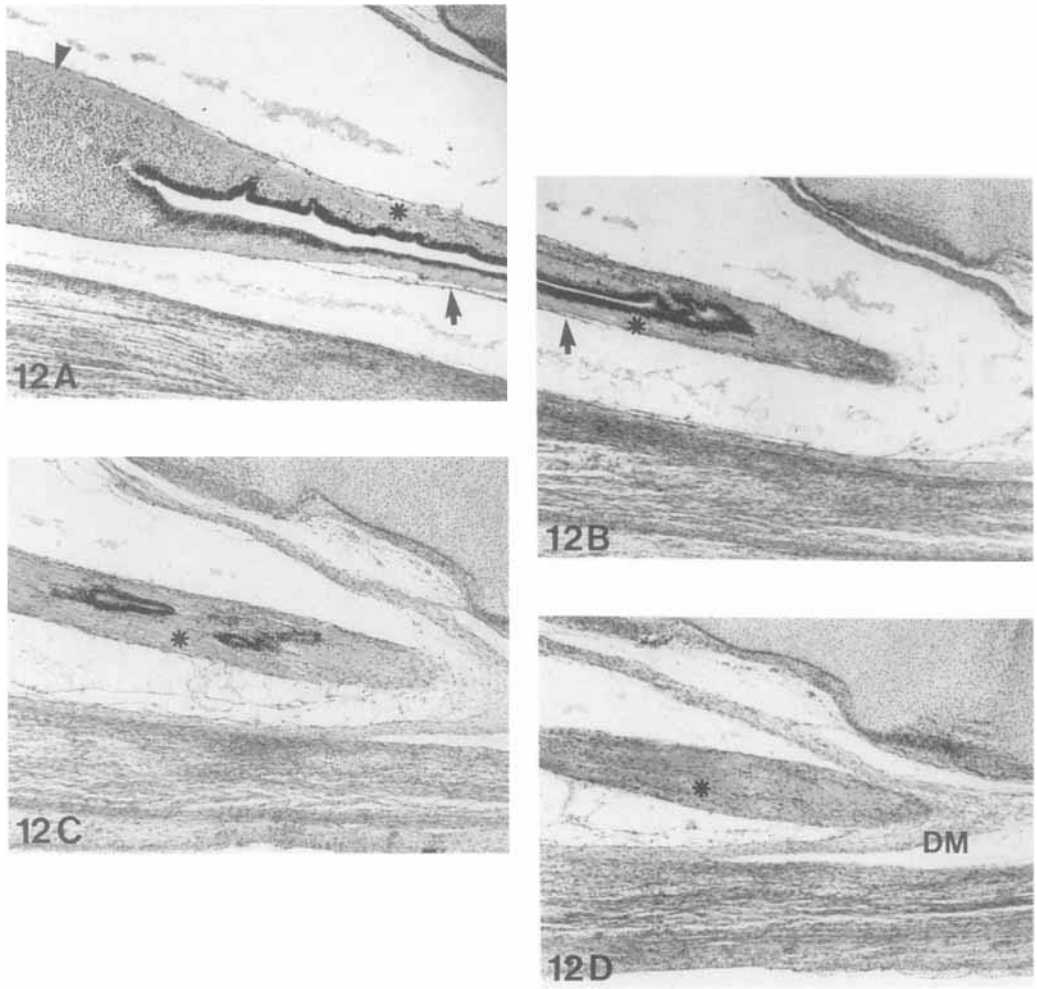


Fig. 12. Light micrographs of 10 μm near sagittal sections through the caudal region of a 80 mm human embryo, demonstrating the process of degeneration and differentiation. Disappearance of the secondary neural tube is observed (**B** and **C**), besides formation of a fibrous layer just adjacent to the neural tube (asterisk, **A–D**). This fibrous layer is more cranially continuous with the marginal layer of the primary neural tube (arrowhead, A) and is surrounded by one layer of meningeal cells, i.e., the primitive pia mater (arrows, **A** and **B**). This structure of fibrous tissue surrounded by pia mater represents the primitive filum terminale, which caudally inserts into the primitive dura mater (DM).

The process of secondary neurulation described in the present study differs much from the findings in chick and rat embryos (Romanoff, '60; Bentliff and Gordon, '65; Schoenwolf and Delongo, '80) but shows great similarity with the observations of Schoenwolf ('84) performed in mouse embryos. Slightly cranial to the cluster of neurectodermal cells, situated at the caudal end of the developing neural tube, the neurectodermal cells become radially orientated, surrounding a small round lumen. During this luminization process, cell death is not observed. Probably the deposition of cells from the cluster of neurectodermal cells around a lumen is responsible for the ultimate form. According to Schoenwolf ('84), the lumen of the secondary neural tube is passively formed as a result of polarization of the surrounding cells so that the apical and basal ends of these cells become different. The lumen formed by this luminization

process is always directly continuous with the lumen of the fully formed neural tube. Isolated or accessory lumina, as seen in the tail bud of chick and rat embryos (Romanoff, '60; Bentliff and Gordon, '65; Schoenwolf and Delongo, '80) and in the caudal region of human embryos (Lemire, '69), were not observed by Schoenwolf ('84) and in the present study concerning both mouse and human embryos. The discrepancies in the different studies concerning the luminization of the secondary neural tube could be based on spatial misinterpretations due to the folding of the caudal end of the embryo. Therefore, three-dimensional reconstructions of serially sectioned embryos are used to elucidate the morphology of this region.

The next step in caudal neural tube development is the process of degeneration and differentiation, in the literature called "retrogressive differentiation" (Lemire, '88; McLone and Naidich, '89). Although never well documented, it is usually described as a combination of regression, degeneration, and differentiation. Physiological or programmed cell death, i.e., apoptosis, plays a major role during this process as shown in the present study. It is noteworthy that cell death is often described as an important factor in human embryonic development (Vermeij-Keers et al., '83; Vermeij-Keers, '90). The degeneration of the caudal neural tube results in disappearance of the secondary neural tube, whereas just adjacent to the degenerating neural tube a fibrous layer is formed that is directly continuous with the marginal layer of the primary neural tube. The marginal layer of the primary neural tube is a fibrous layer formed by neuroglial cells, which are formed by differentiation from the primitive neurectodermal cells (Arey, '65). It is most likely that the fibrous layer surrounding the secondary neural tube is also formed by differentiation from the secondary neurectodermal cells. The mesenchyme just adjacent to this fibrous layer differentiates to form a meningeal structure, the primitive pia mater. This corresponds to the observations by Sensenig ('51) in his study concerning the development of the meninges. The structure of fibrous tissue surrounded by primitive pia mater will become the filum terminale.

In conclusion, three developmental processes can be distinguished in caudal neural tube development: caudal neuropore closure, secondary neurulation, and degeneration and differentiation of the secondary neural tube. Caudal neuropore closure in human embryos is situated at about the level of the somites 32–34 (i.e., the future third to fifth sacral vertebrae) and, therefore, primary neurulation leads to the formation of all spinal cord segments and spinal ganglia. Only the primordia for the ventriculus terminalis and filum terminale and, possibly, part of the conus medullaris are formed during secondary neurulation. After secondary neurulation, degeneration (by cell death) and differentiation of the secondary neural tube takes place, resulting in the development of the filum terminale. The embryological processes indicated above are of great importance for the understanding of the pathogenesis of congenital anomalies affecting the caudal spinal cord and spine. In general, anomalies such as the spina bifida aperta or (myelo-) meningoceles are best explained as defects of the primary neurulation, even in the lower sacral region, whereas anomalies such as the tethered cord syndrome, fibrolipoma of the filum terminale, and congenital tumors are more likely the result of a disturbance during secondary neurulation and/or the period of degeneration and differentiation. A more detailed discussion of the pathogenesis of these anomalies, however, falls beyond the scope of this work.

ACKNOWLEDGMENTS

The authors thank S.B. Blankevoort, medical illustrator, and J. Lens, photographer, for their support and contributions.

LITERATURE CITED

Arey, L.B. (1965) Developmental Anatomy: A Textbook and Laboratory Manual of Embryology, 7th ed. Saunders, Philadelphia.

Banta, J.V. (1978) Caudal aplasia syndrome, Chap. 12. In: Handbook of Clinical Neurology, part 32. P.J. Vinken, and G.W. Bruyn, eds. North Holland, pp. 347–354.

Bentliff, S., and L.H. Gordon (1965) Spinal cord formation distal to the posterior neuropore. Abst. Terat. Soc., *5*:4 (abst.).

Copp, A.J., and F.A. Brook (1989) Does lumbosacral spinal bifida arise by failure of neural folding or by defective canalisation? J. Med. Genet., *26*:160–166.

Dryden, R.J. (1980) Duplication of the spinal cord: A discussion of the possible embryogenesis of diplomyelia. Develop. Med. Child Neurol., *22*:234–243.

Duhamel, B. (1961) From the mermaid to anal imperforation: The syndrome of caudal regression. Arch. Dis. Child, *36*:152–155.

Hoving, E.W., C. Vermeij-Keers, A.M. Mommaas-Kienhuis, and N.G. Hartwig (1990) Separation of neural

and surface ectoderm after closure of the rostral neuropore. Anat. Embryol., *182*:455–463.
Ignelzi, R.J., and R.A.W. Lehman (1974) Lumbosacral agenesis: Management and embryological implications. J. Neurol. Neurosurg. Psychiatry, *37*:1273–1276.
Lemire, R.J. (1969) Variations in development of the caudal neural tube in human embryos (Horizons XIV–XXI). Teratology, *2*:361–370.
Lemire, R.J. (1988) Neural tube defects. JAMA, *259*: 558–562.
Lemire, R.J., J.D. Loeser, R.W. Leech, and E.C. Alvord (1975) Normal and Abnormal Development of the Human Nervous System. Harper, Hagerstown, MD, pp. 54–83.
McLone, D.G., and T.P. Naidich (1989) The tethered spinal cord (ch5). In: Pediatric Neurosurgery, 2nd ed. R.L. McLaurin, L. Shut, J.L. Venes, and F. Epstein, eds. WB Saunders, Philadelphia, pp. 71–75.
Müller, F., and R. O'Rahilly (1987) The development of the human brain, the closure of the caudal neuropore, and the beginning of secondary neurulation at stage 12. Anat. Embryol., *176*:413–430.
Müller, F., and R. O'Rahilly (1988) The development of the human brain from a closed neural tube at stage 13. Anat. Embryol., *177*:203–224.
Naidich, T.P., D.G. McLone, and D.C. Harwoord-Nash (1983) Spinal Dysraphism (ch17). In: Computed Tomography of the Spine and Spinal cord, Vol. I. T.H. Newton, and D.G. Potts, eds. Clavadel Press, San Anselmo, CA.
O'Rahilly, R., and F. Müller, eds. (1987) Developmental Stages in Human Embryos. Carnegie Institution, Washington, DC.
Romanoff, A.L. (1960) The Avian Embryo. Structural and Functional Development. Macmillan, New York.
Schoenwolf, G.C., and J. Delongo (1980) Ultrastructure of secondary neurulation in chick embryo. Am. J. Anat., *158*:43–63.
Schoenwolf, G.C. (1984) Histological and ultrastructural studies of secondary neurulation in mouse embryos. Am. J. Anat., *169*:361–374.
Sensenig, E.C. (1951) The early development of the meninges of the spinal cord in human embryos. Contrib. Embryol., *24*:147–157.
Smits-van Prooije, A.E. (1986) Processes involved in normal and abnormal fusion of the neural walls in murine embryos. Thesis, State University of Leiden.
Smits-Van Prooije, A.E., C. Vermeij-Keers, R.E. Poelman, M.M.T. Mentink, and J.A. Dubbeldam (1988) The formation of mesoderm and mesectoderm in 5- to 41-somite rat embryos cultured in vitro, using WGA-Au as a marker. Anat. Embryol., *177*:245–256.
Tinkelenberg, J. (1979) Graphic reconstructions, microanatomy with a pencil. J. Audiov. Media Med., *2*:102–106.
Van Der Knaap, M.S., and J. Valk (1988) Classification of congenital abnormalities of the CNS. A.J.N.R., *9*:315–326.
Vermeij-Keers, C. (1990) Craniofacial embryology and morphogenesis; normal and abnormal (ch3). In: Craniofacial Malformations. M. Stricker, J. van der Meulen, B. Raphael, and R. Mazzola, eds. Churchill Livingstone, New York.
Vermeij-Keers, C., R.F. Mazzola, J.C. van der Meulen, and M. Strickler (1983) Cerebro-craniofacial and craniofacial malformations: An embryological analysis. Cleft Palate J, *20*:128–145.