

# Evidence that the caudal portion of the neural tube develops by cavitation of a neural cord in the caudal eminence of human embryos

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*The formation of the secondary neural tube was traced in serial sections of human embryos of developmental stages 13 to 17 (32–41 days after fertilisation). It was found that the secondary neural tube formation begins with cavitation of the neural cord. The minute cavities are seen in embryos at stages 13 and 15. At stages 16 and 17 the numerous cavities coalesce to form a single central canal.*

**Key words:** human neuroembryology, secondary neurulation, neural cord, cavitation, secondary neural tube

## INTRODUCTION

The central nervous system develops from a hollow structure called the neural tube, which is produced by neurulation. There are two neurulation processes, viz. primary and secondary neurulation [13, 17–20].

In the primary neurulation mechanism the neural tube arises from the neural plate by folding and midline fusion to form the primary neural tube (PNT) [1, 2]. In human embryos primary neurulation commences at stage 8 and extends to stage 12, when the posterior neuropore closes. This occurs when approximately 24–26 somites are present. The site of final closure corresponds approximately to future somite 31, the level of future sacral vertebra 2 (S2), which can be seen from stage 13 onwards [9]. This level is considered to be the junction between the primary and secondary neural tubes. The primary neural tube gives rise to the brain and spinal cord down to the S2 level.

Secondary neurulation involves the secondary neural tube (SNT) formation from the pluripotent loosely associated mesenchymal cells of the caudal eminence (the end bud of tailed species) without direct involvement of the surface ectoderm. It begins with the closure of the posterior neuropore

(stage 12) and extends to about stages 17, 18 or 20 [11, 12]. The caudal eminence mesenchyme comprises a stem-cell population derived from the regressing primitive streak and Hensen's node. It has the potential to create a variety of structures, including the caudal hind gut, coelom, blood vessels, notochord, somites and spinal cord. In the dorsal part of the caudal eminence mesenchymal cells undergo condensation and mesenchymal-neuroepithelial transformation into the neural (medullary) cord, which subsequently cavitates (i.e. hollows out) to give rise to the secondary neural tube [5]. Typically, cavitation involves the coalescence of several smaller cavities into the central cavity or secondary neurocoel [19]. The primary neurocoel is continuous with that of the secondary one. Secondary neurulation makes up the lowest portion of the spinal cord, caudal to S2 level. The slow growth of the caudal neural tube compared with the vertebrae results in the displacement of the junction area of the primary and secondary neural tubes at higher (lumbosacral) level in neonates [14].

In the literature there is some controversy regarding the development of the secondary neural tube in human embryos. Müller and O'Rahilly [9, 10] observed a direct extension of the lumen of the primary

neural tube into that of the neural cord. No cavitation of the neural cord and no overlapping zone of the primary and secondary neural tubes were observed. These findings suggest that the secondary neurulation in humans resembles that in mice [7, 12, 20]. On the other hand, some investigators have claimed that multiple cavities and overlapping zones were present in human secondary neural tube formation as occurs in chick embryos [7, 8, 16, 18].

The present study was undertaken to analyse the formation of the secondary neural tube in human embryos of stages 13–17.

### MATERIAL AND METHODS

Twelve human embryos from the Anatomy Department Collection of Poznań University of Medical Sciences ranging from 5, 0 to 14, 0 mm in crown-rump length, corresponding to the developmental stages between 13 and 17 (32 to 41 days), were examined microscopically (Table 1). All the embryos were embedded in paraffin or paraplast and serially sectioned in various planes at 5 or 10  $\mu$ m thickness. The sections were stained according to the Mallory method, with haematoxylin and eosin, cresyl violet or toluidine blue according to Nissl's method. Some of them were impregnated with Bodian's protargol. The sections were photographed using a Canon S40 digital camera and a total magnification was estimated with the aid of properly calibrated Carl Zeiss AxioVision LE Release 4.5 analytical software.

### RESULTS

In embryos at stage 13 (32 days after fertilisation) the ectodermal ridge and the cloacal membrane are positioned cranially to the caudal eminence and both form a part of the ectodermal ring. This ridge has an important function in epitheliomesenchymal interaction. The neural cord reaches the tip of the embryo (Fig. 1).

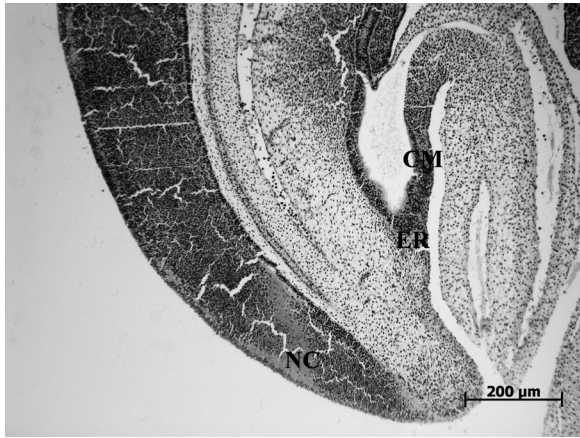
In the caudal eminence the somites, the neural cord, notochord and gut may be distinguished (Fig. 2). In the neural cord, which consists of darkly stained cells, minute cavities may be seen.

During stages 14 (33 days after fertilisation) and 15 (36 days after fertilisation) the mesenchyme within the caudal eminence is reduced and cavities in the neural cord appear (Figs. 3, 4). These are larger toward the junctional area with the primary neural tube and form a single cavity in the upper part of the neural tube (Fig. 5). The somites move to the midline.

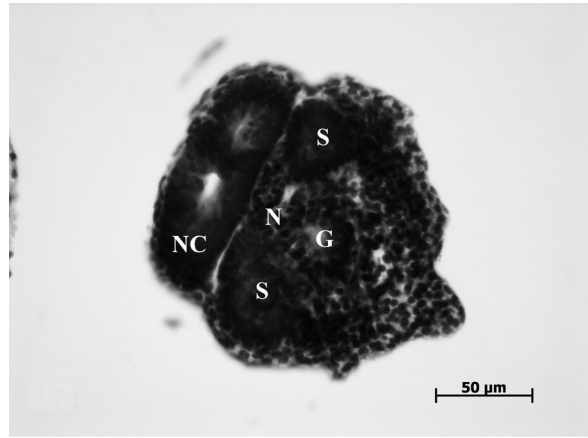
The development of the secondary neural tube by cavitation is well seen at stage 16 (39 days after fertilisation) and stage 17 (41 days after fertilisation). The neural tube is canalised by many cavities (Figs. 6, 7), which in the upper part form a single cavity (Fig. 8). Canalisation of the secondary neural tube proceeds in a cranial-to-caudal direction. A single lumen in the upper part of the secondary neural tube becomes continuous with the lumen of the primary neural tube.

**Table 1.** Developmental stage, age and plane of section of embryos investigated

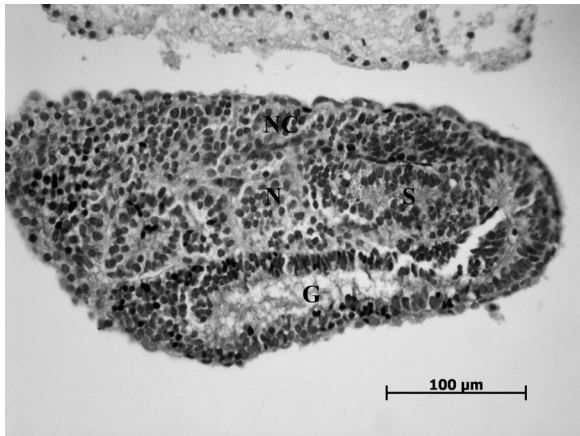
Catalogue No.	C-R length [mm]	Developmental stage	Age [postfertilizational days]	Plane of section
A	6.0	13	32	Sagittal
P-41	5.0	14	33	Horizontal
II WW	7.0	14	33	Sagittal
A-19	7.0	14	33	Frontal
A-17	7.0	14	33	Horizontal
B-175	9.0	15	36	Horizontal
A-16	9.0	15	36	Horizontal
IV	10.0	16	39	Horizontal
B-180	13.0	17	41	Sagittal
A-1	14.0	17	41	Horizontal
B-129	13.0	17	41	Sagittal
PJK-2	13.5	17	41	Horizontal



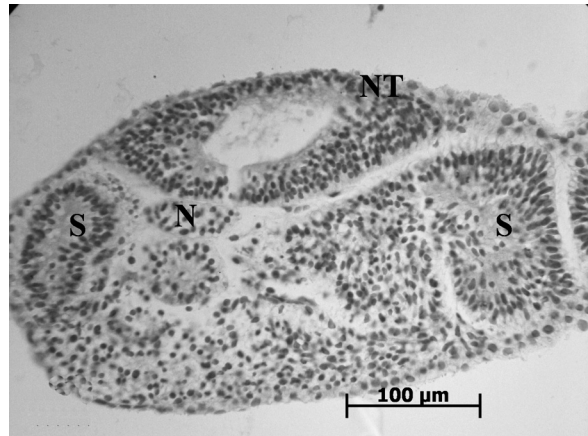
**Figure 1.** Sagittal section of embryo at stage 13; CM — cloacal membrane; ER — ectodermal ridge; NC — neural cord.



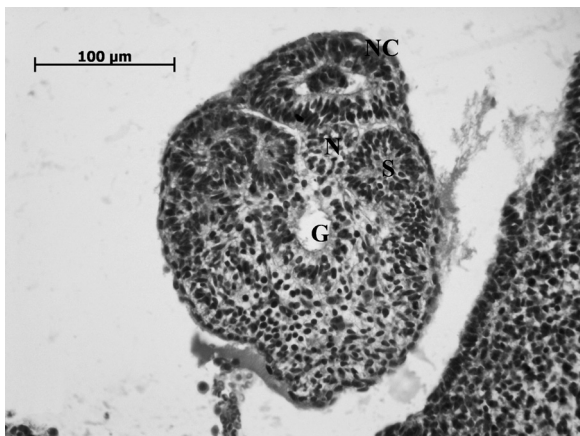
**Figure 4.** Cross-section of embryo at stage 15; NC — neural cord; N — notochord; G — gut; S — somite.



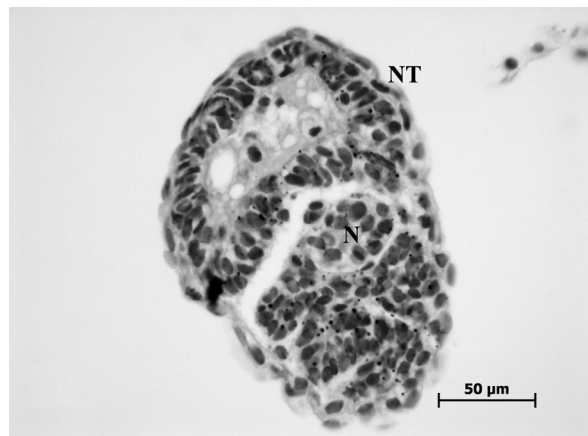
**Figure 2.** Sagittal section of embryo at stage 13; NC — neural cord; N — notochord; G — gut; S — somite.



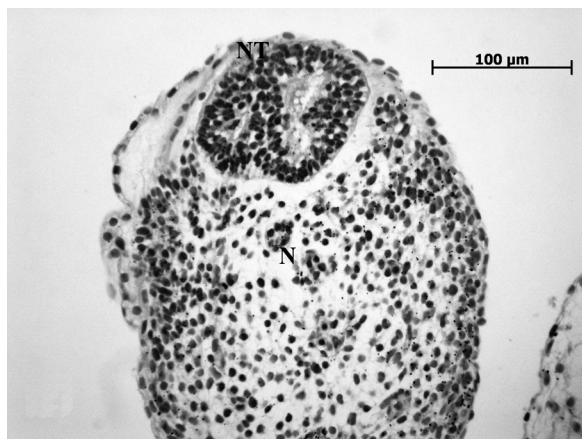
**Figure 5.** Oblique section of embryo at stage 14; NT — neural tube; N — notochord; S — somite.



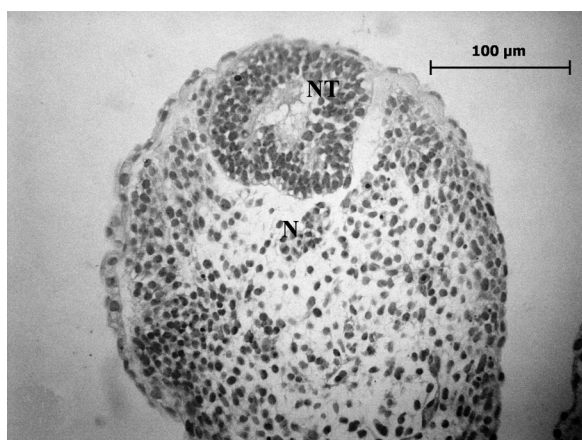
**Figure 3.** Cross-section of embryo at stage 14; NC — neural cord; N — notochord; G — gut; S — somite.



**Figure 6.** Cross-section of embryo at stage 16; NT — neural tube; N — notochord.



**Figure 7.** Cross-section of embryo at stage 17; NT — neural tube; N — notochord.



**Figure 8.** Cross-section of embryo at stage 17; NT — neural tube; N — notochord.

## DISCUSSION

The development of the secondary neural tube in human embryos is still controversial [4, 12, 16]. Knowledge of normal embryonic development of the caudal neural tube is of great importance in understanding the pathogenesis of neural tube defects in the lumbosacral region [1, 6].

Disturbances of secondary neurulation may lead to myelodysplasia, which may be manifest as spinal dysraphism or spina bifida occulta. A common abnormality is tethered cord syndrome, in which the conus medullaris and the filum terminale are abnormally fixed to the defective vertebral column. The sustained traction damages the cord, leading to loss of sensations from the legs and feet and problems with bladder control [3]. Myelodysplasia is a part of

caudal dysgenesis, the different forms of which may be discovered later in life [15].

The issue of whether secondary neurulation in humans more closely resembles that in the chick or the mouse is unresolved. In the chick embryo, secondary neurulation results in the formation of a medullary cord from dorsal cells of the caudal cell mass. Within this medullary cord cavitation creates multiple tubules, the outer cells surrounding a central lumen, within which are the inner cells. Multiple smaller lumina coalesce to form larger cavities and a single lumen is formed [2]. Secondary neurulation in the mouse begins with the formation of a medullary rosette, a cluster of caudal mass cells radially arranged about a central lumen formed by cavitation. The cells of the medullary rosette are thought to be the homologue of the outer cell group in the chick embryo [2]. Caudal growth of the secondary neural tube occurs by additional cavitation. In the mouse the secondary neural tube is directly continuous with the primary neural tube. The secondary neural tube in the chick develops independently and only later fuses with the primary neural tube. According to Lemire [8] and Hughes and Freeman [7], cavitation of the neural cord occurs in a manner similar to that in chickens. Müller and O'Rahilly [9, 10] did not find multiple cavities in their specimens and argued that secondary neurulation involves extension of the primary neural canal into the neural cord.

Saitou et al. [16] divided the secondary neural tube into three parts: 1) the most rostral region, which corresponds to the posterior part of the primary neural tube, 2) the junctional region of the primary and secondary neural tubes and 3) the caudal region, which emerges from the neural cord. In the first two parts they found a single cavity and in the third part of the secondary neural tube they frequently observed isolated cavities.

The controversy over the development of the secondary neural tube and the multiple cavities noted in the present study is evidence that this part of the neuraxis needs further investigation on extensive embryonic material in which exact ages are established for the specimens investigated.

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