Journal of Korean Medical Science
Vol. 9, No. 2, 116-122 April 1994

Morphological Study of Surgically Induced Open Neural Tube Defects in Chick Embryos

– Postoperative 24 Hours –

Heon You, M.D., Ki-Bum Sim, M.D.,* Kyu-Chang Wang, M.D.,
Dong-Gyu Kim, M.D. and Hyun-Jib Kim, M.D.

*Department of Neurosurgery, Seoul National University College of Medicine, and *Department of Neurosurgery, Seoul Red Cross Hospital, Seoul, Korea*

For the experimental study of neural tube defect (NTD), a surgical model has advantages over other models in a few aspects. It causes less functional derangement of cells and the NTDs can be made selectively by surgery. The authors planned to use the surgical model for the experimental study of NTD. As the first step for the studies, the chronological changes of morphology during the early postoperative period were investigated using post-incubation 3-day chick embryos. The objectives of this study are (1) the morphological evaluation of the surgical model as a method for studies of open NTD, and (2) the observation of morphological changes for the first 24 hours after surgery which include 'overgrowth' appearance and the continuity between the surface ectoderm and the neuroectoderm. The morphological changes were observed by light microscope and scanning electron microscope.

Immediately after surgery, typical open NTDs were observed. Morphologically they were very similar to the appearance of spontaneous (non-surgical) open NTDs. The opened neural tubes were everted progressively and they looked rather flat at 24 hours after surgery. Cellular hyperplasia ('overgrowth' appearance) was noted within 24 hours after surgery and became more prominent during the 24 hours. There was increasing continuity between the surface ectoderm and the neural tissue until 24 hours after surgery when the continuity looked almost complete. In conclusion, surgically induced NTDs are morphologically very similar to spontaneous NTDs. Overgrowth appearance and the continuity between the surface ectoderm and the neural tissue at the site of NTD, once thought of as evidences supporting 'failure to close' theories, should not be considered as such because they were also observed in this 'reopen' model.

Key Words : *Chick embryo, Neural tube defect, Surgery.*

Address for correspondence : *Kyu-Chang Wang, M.D., Division of Pediatric Neurosurgery, Seoul National University Children's Hospital, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea. Tel : (02) 760-3489, Fax : (02) 744-8459.*
This study was partly supported by a Seoul National University Hospital Research Grant (05-92-002).

INTRODUCTION

Open neural tube defect (NTD) is a midline fusion defect involving the skin, muscle, bone and central nervous system owing to the embryological

dysgenesis of the neural tube. The incidence of this anomaly is reported as one or two in 1,000 live births. There are several hypotheses about its pathogenesis. However, no single theory is definitive.

The developmental arrest theory and the overgrowth hypothesis postulate an effect on the neural plate so early in the development that neurulation never occurs at the affected level ('failure to close' theories). The hydrodynamic theory and the neuroschisis theory hypothesize rupture of an already formed neural tube at the affected site ('reopen' theories). The continuity between the neuroectoderm and the surface ectoderm at the site of the open NTD and the rostrocaudal distribution of the lesions (The neuropores close lately at the cranial and the lumbosacral areas.) were thought of as morphological evidences supporting the 'failure to close' hypothesis (Campbell et al., 1986 ; Warkany, 1977). However, the significance of these findings in the explanation of abnormal embryogenesis is controversial (Rokos and Knowles, 1976 ; Campbell and Sohal, 1990). Overgrowth hypothesis is that the neural tube fails to close due to the overgrowth of neural tissue everting the neural folds and preventing fusion (Brocklehurst, 1971 ; Cleland, 1883 ; Patten, 1952). Though the 'overgrown' appearance of spontaneous NTDs supports this hypothesis, the interpretation is also controversial. Fowler (1953) insisted that the overgrowth phenomenon is not the cause but the result of the NTD.

Among the experimental models of NTD, the surgical model is one of the 'reopen' models and has advantages over other models in a few aspects. It causes limited functional derangement of cells compared to chemical and genetic models. Also selective lesions can be easily made by surgery. The authors planned to study various aspects of NTDs using the surgical experimental model. As the first step, the morphological changes during the early postoperative period were observed and the appropriateness of a surgical model as a method for the study of open NTDs was evaluated. The continuity of the surface ectoderm and the neuroectoderm at the site of surgery and the overgrowth appearance were observed in this study using the 'reopen' model. The significance of these findings as evidences supporting 'failure to close' theories was denied.

MATERIALS AND METHODS

Fertile eggs of commercial source (Poolmuwon, Korea) were incubated under constant temperature (38-39℃) and humidity (50-60%). On the third day of incubation, a window was made on the eggshell with a drill and the eggshell membrane was excised. The embryos which corresponded to stage 16-18 of Hamburger and Hamilton (1951) were stained by topical application of 1% neutral red solution under a surgical microscope. To reopen the neural tube, a midline incision of 6-8 somite length was made in the posterior roof of the central canal at the wing bud level with a 30 gauge needle.

Following the incision, each egg was re-incubated. The embryos were assigned to 3 groups according to the re-incubation period after incision ; immediate postoperative group, postoperative 5-hour group and postoperative 24-hour group (7 embryos each). Five embryos from each group were fixed in 10% neutral buffered formalin. After then, they were embedded in paraffin. Serial transverse sections of 4 μm thickness were made and stained with hematoxylin-eosin. Two embryos of each group were fixed in 2% glutaraldehyde solution for examination by a scanning electron microscope. Two embryos of the postoperative 24-hour group were observed *in ovo* with a surgical microscope just before and just after surgery, at 5 and 24 hours after surgery to investigate the sequential changes of external morphology in the same embryo. The findings of these embryo at each observation time are included in the results of the group with the same postoperative re-incubation period. For example, the findings at 5 hours after surgery are included in the description of the postoperative 5-hour group.

Two embryos of stage 18 (which were not operated on) were sectioned and stained with hematoxylin-eosin for comparison.

RESULTS

All the embryos which underwent surgical incison showed open NTDs.

Normal post-incubation 3-day chick embryos

A well-formed neural tube was abutted by somites on both sides. Both neuropores were closed. The central canal was easily shown under the sur-

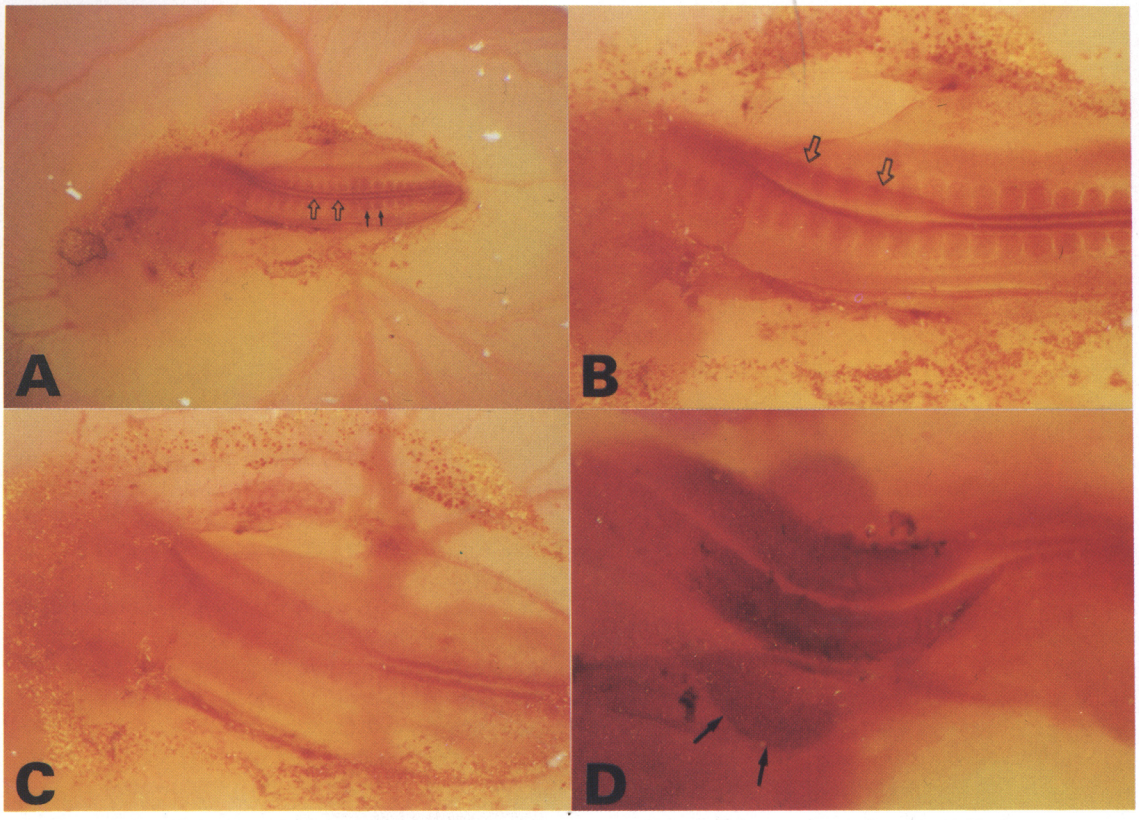

Fig. 1. Photographs showing the sequential changes of the external morphology in a postoperative 24-hour group embryo. The embryo was stained with 1% neutral red solution and observed *in ovo* with a surgical microscope just before (A) and just after (B) surgery, at 5 hours (C) and 24 hours (D) after surgery.
A : Normal appearance of a post-incubation 3-day chick embryo (Hamburger and Hamilton stage 18, ×7). The neural tube (open arrows) and the surrounding somites (small arrows) are well visualized. Both neuropores are closed. B : Immediately after surgery, the surgically induced neural tube defect (open arrows) is noted at the level of the wing bud (×15). C : At 5 hours after surgery, the lips of neural tube defect are more widely open. Neural tissue thickening has increased compared to B (×15). D : At 24 hours after surgery, neural tissue thickening is more prominent compared to B and C. The wing bud is clearly visible (arrows). The embryo is scoliotic (×15).

gical microscope (Fig. 1A). In histological sections, the neural tube was closed and was in contact with the notochord on its ventral surface. The roof of the central canal was composed of columnar cells and the surface ectoderm was intact (Fig. 2A).

Immediate postoperative group

With a surgical microscope the open and everted neural tube was observed (Fig. 1B). Histologically, the edges of incised neural tube were thickened and everted. The cut edges of the surface ectoderm had moved to the lateral area of the opened neural tube. Between the surface ectoderm and the neuroectoderm, there were small gaps recognizable under the light microscope and scanning electron microscope (Fig. 2B and Fig. 3A).

Postoperative 5-hour group

On the examination with a surgical microscope, the opened neural tubes were wider than those of the immediate postoperative group embryos (Fig. 1C). In the histological sections, the neuroectoderm was more thickened and everted, so looked 'overgrown'. It was thicker and larger than the unopened

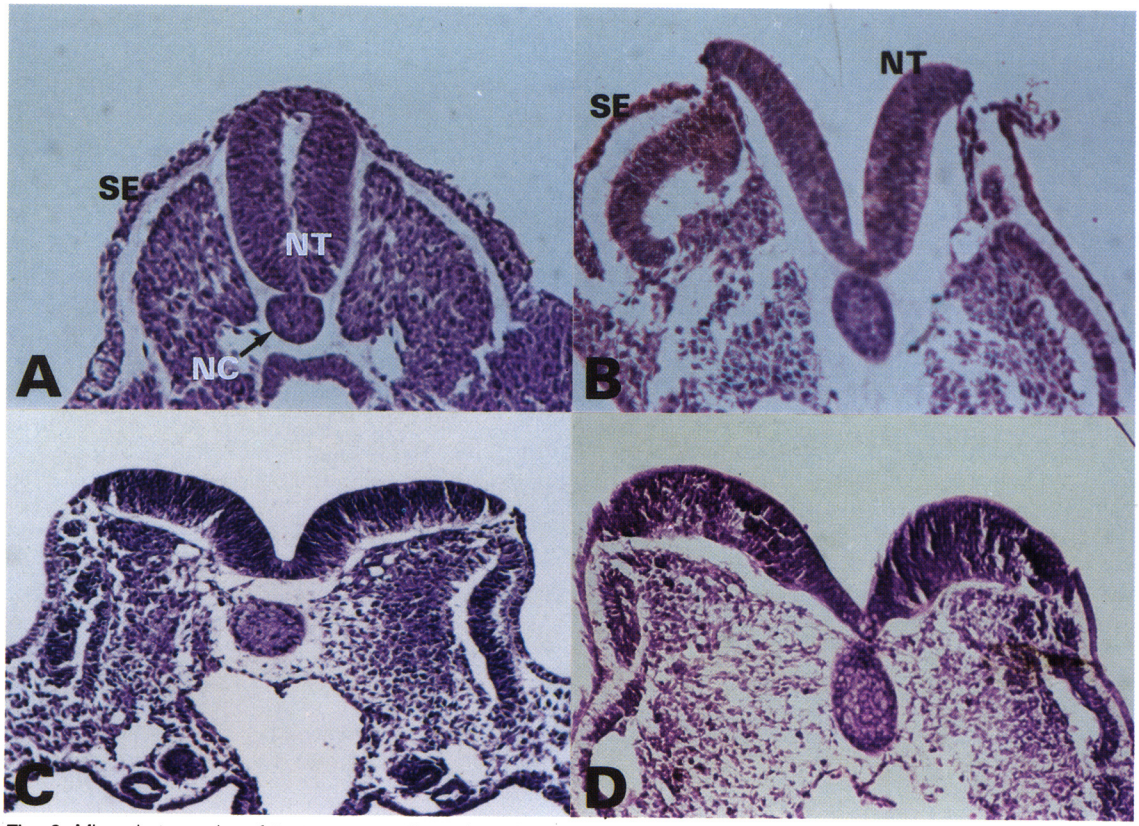

Fig. 2. Microphotographs of transverse sections through the neural tube defects. Each embryo is a representative one of each group. (H & E, ×40)
A : A control embryo of 3-day incubation (Hamburger and Hamilton stage 18). Surface ectoderm (SE), mesenchymal tissue and neural tube (NT) are well visualized. Notochord (NC) is in contact with the ventral surface of the neural tube. B : An embryo of the immediate postoperative group. The roof of the central canal is open and everted. The cut edges of the overlying surface ectoderm (SE) are displaced to the lateral side of the opened neural tube (NT). C : An embryo of the postoperative 5-hour group. There is increasing continuity between the neural tissue and the surface ectoderm. The neural tube is more widely open. D : An embryo of the postoperative 24-hour group. The neural tube appears rather flat. Neural tissue hyperplasia was noted. The continuity between the surface ectoderm and the neural tissue looks complete.

portion of the neural tube just distal to the incision site. There was an increasing continuity between the surface ectoderm and the neuroectoderm comparing to the immediate postoperative embryos (Fig. 2C and Fig. 3B).

Postoperative 24-hour group

The opened neural tubes were wider than those of the postoperative 5-hour group. They looked rather flat (Fig. 1D). The overgrowth phenomenon had definitely increased. The continuity between the surface ectoderm and the neuroectoderm looked complete. The appearance of open NTD was very similar to the spontaneous or chemically induced NTDs (Fig. 2D, Fig. 3C and Fig. 4). Both of the embryos observed serially showed progressive eversion and hyperplasia of the opened neural tube.

DISCUSSION

Experimental model

For the experimental studies of NTD, various models are used. Chemical models using, for ex-

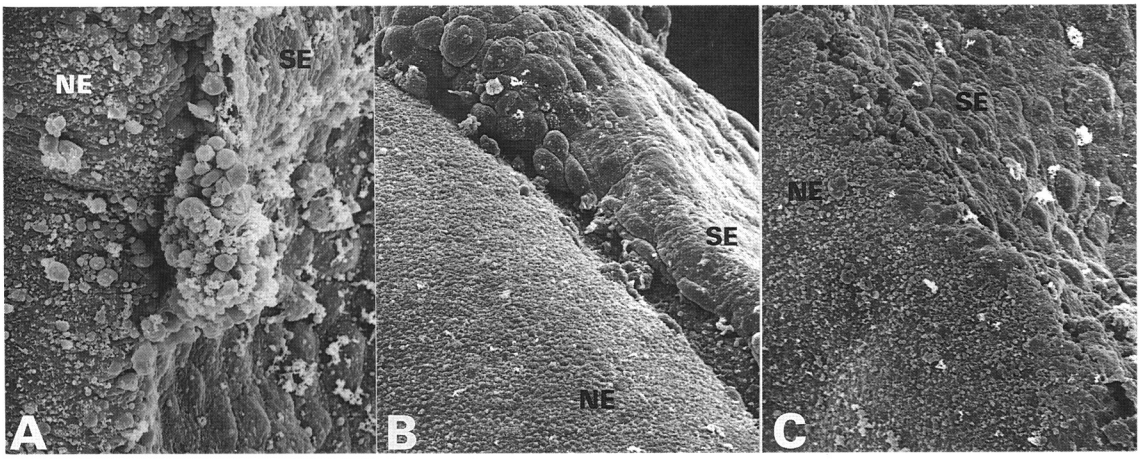

Fig. 3. Scanning electron microscopic findings of an immediate postoperative group embryo (A), a postoperative 5-hour group embryo (B) and a postoperative 24-hour group embryo (C) (×1,000).
A : In the immediate postoperative group embryo, a gap and incision artifacts are seen at the junctional area between the opened neural tube (NE=neuroectoderm) and the surface ectoderm (SE). The margin of the gap is rough. B : At 5 hours after surgery, the junction looks rather smooth compared to A. C : At 24 hours after surgery, the continuity between the neural tissue and the surface ectoderm is complete.

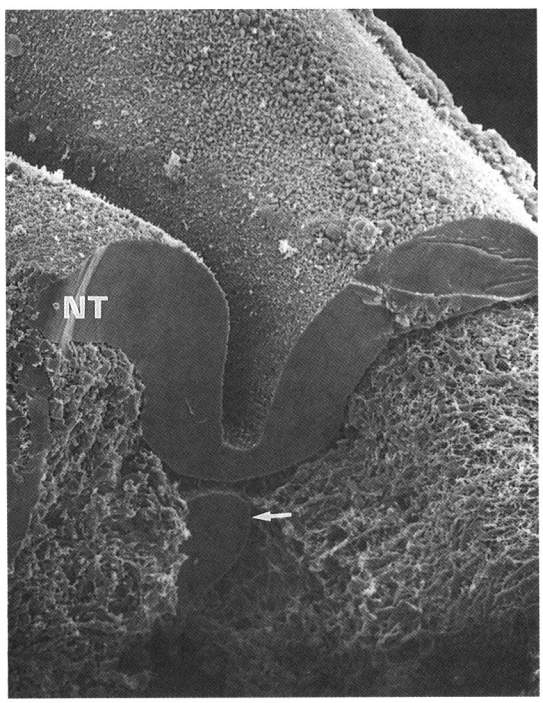

Fig. 4. Scanning electron microscopic findings of a transected postoperative 24-hour group embryo (×500). In cross sectional oblique view, the open neural tube (NT) and the notochord (arrow) are demonstrated.

ample, trypan blue, vitamin A or tunicamycin and genetic models cause functional derangement of cells to a remarkable extent and the location and the size of the induced NTDs are variable and can not be predicted (Caldarelli et al., 1985 ; Chong-Hiyo et al., 1989 ; McLone et al., 1983). On the other hand, the surgical model has advantages over these models in the sense that it causes a lesser degree of functional derangement of cells and the location and the size of the NTDs can be easily modified by the surgical technique. For example, the studies of regeneration processes are the cases in which the surgical model is better. As shown in this study, surgically induced open NTDs were morphologically very similar to the open NTDs of non-surgical origins. So this experimental model seemed to be suitable for studies of open NTD.

Though the chick does not belong to the mammals, chick embryos have several advantages. The observation of young embryos is easy. In mammals the thickness of the uterus in the early gestation period precludes gross observation of embryos *in utero*. The developmental period of chick embryo, especially the early stages of development, is short. The surgical manipulation in chick embryo is easy. Eggs are cheap and easily available. Also, com-

pared to rodents, the secondary neurulation process of the distal spinal cord of chick embryo is closer to that of human. This can be an advantage for the experiments on the effect of the primary neurulation defect on the secondary neurulation.

Theories of NTD and the early postoperative findings of surgically induced NTDs

There are many debates about the pathogenesis of open NTD. It is still unknown whether this abnormality of fusion is a failure of initial neural tube closure or a secondary reopening of a closed neural tube.

Developmental arrest (von Recklinghausen, 1886) and overgrowth hypotheses (Lebedeff, 1881 ; Cleland, 1883 ; Patten, 1952) belong to the idea of 'failure of neural tube to close'. In 1886, von Recklinghausen suggested that myeloschisis begins as a simple failure of the embryonic neural plate to close due to an arrest of normal development with subsequent secondary changes in surrounding tissues. Lebedeff in 1881 and Cleland in 1883 suggested that the neural tube failed to close owing to overgrowth of the neural tissue everting the neural folds and preventing fusion. Patten (1952) reported overgrowth in human dysraphic embryos. He interpreted the overgrowth as a primary phenomenon interfering with normal closure of the neural tube.

There were two features of NTDs that point to failure of closure as the more reasonable pathogenesis. The first is continuity between the neuroectoderm and the surface ectoderm and cellular hyperplasia (Campbell et al., 1986). The second is the rostrocaudal distribution of the open NTDs (Warkany, 1977). Chemical teratogens such as trypan blue and excessive vitamin A administered to pregnant rats can produce open NTD showing 'overgrowth' (Brocklehurst, 1976). However, Fowler (1953) observed overgrowth appearance of the neural tissue in NTDs experimentally induced by mechanical opening of the closed neural tube in chick embryos which suggested that the overgrowth phenomenon is not the cause but the result of NTD.

The reopening of a closed neural tube hypothesis includes hydrodynamic theory (Virchow, 1863 ; Gardner, 1960) and neuroschisis theory (Padget, 1968 and 1970). In 1863, Virchow suggested that excessive fluid within the central canal could lead to a cystic dilatation of the lower spinal cord (Brocklehurst, 1976). Gardner (1960 and 1973) suggested that inadequate escape of cerebrospinal fluid from the neural tube in early development leads to overdistension and bulging of the neural tube, followed by rupture to produce open NTD. He presumed that inadequate escape of cerebrospinal fluid was due to impermeability of rhombencephalic roof plate. Also this theory has several weak points. Experimental animal models suggest that the severe forms of open NTDs result from failure of the neural tube to close rather than from reopening of the closed tube, because initial point of rupture should decompress the neural tube and limit the extent of the lesion (Lemire et al., 1965). Moreover, myeloschisis has been found before the choroid plexus has formed (Osaka et al, 1978). Padget (1968 and 1970) observed a cleft in the dorsal midline of the neural tube of monkey and human embryos. Padget suggested that proteinaceous fluid within the neural tube can leak into the surrounding mesoderm and the surface ectoderm, forming 'neuroschistic bleb', and rupture of neuroschistic bleb may produce open NTD. Padget also observed continuity between the neuroectoderm and the surface ectoderm, overgrowth phenomenon, and reparative process. However, no etiology for the cleft in the neural tissue has been suggested. In 1978, Osaka et al. argued that neuroschistic blebs are artifacts of the experimental process.

In this study, the authors observed morphological changes of the surgically induced open neural tube defect during the first 24 hours. Whether 'overgrowth' phenomenon is real and a part of the regeneration process is not certain. Otherwise the regeneration process (healing process) was not detected during the 24 hours. Actually, according to the scanning electon microscopic findings (not shown), the extents of neural tube defects were longer than those of the surface ectoderm defects. Cellular hyperplasia (overgrowth phenomenon) became prominent during the first 24 hours. The observation of 'overgrowth' appearance in a reopened NTDs support the idea that the 'overgrowth' phenomenon is not the cause but the result of NTD. To be certain whether the neural tube becomes really hyperplastic and to know the sequential pattern of the cellular hyperplasia, experiments using cell kinetic methods will be needed.

In the present study, continuity between the surface ectoderm and the neuroectoderm was observed during 24-hours after surgery, so the continuity at the site of the open NTD does not exclude

the possibility that a previously closed neural tube has reopened.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to professor Je Geun Chi, M.D., professor Jeong Wook Seo, M.D., Mrs. Eul Kyung Kim and Miss Kee Jin Kim for their assistance in the preparation of the pathological specimens.

REFERENCES

Brocklehurst G : *The pathogenesis of spina bifida : A study of the relationship between observation, hypothesis, and surgical incentive. Dev Med Child neurol 13 : 147-163, 1971.*

Chong-Hiyo TP, Pruitt JH, Bennett D : *A mouse model for neural tube defects : The curtailed (Tc) mutation produces spina bifida occulta in Tc/+ animals and spina bifida with myelomeningocele in Tc/t. Teratology 39 : 303-312, 1989.*

Caldarelli M, McLone DG, Collins JA, Suwa J, Knepper PA : *Vitamin A induced neural tube defects in the mouse. Concepts Pediatr Neurosurg 6 : 161-171, 1985.*

Campbell LR, Dayton DH, Sohal GS : *Neural tube defects ; A review of human and animal studies on the eitology of neural tube defects. Teratology 34 : 171-187, 1986.*

Campbell LR, Sohal GS : *The pattern of Neural tube defects created by secondary reopening of the neural tube. J Child Neurol 5 : 336-340, 1990.*

Cleland J : *Contribution to the study of spina bifida, encephalocele, and anencephalus, J Anat Physiol 17 : 257-292, 1883.*

Fowler I : *Responses of the chick neural tube in mechanically produced spina bifida. J Exp Zool 23 : 115-151, 1953.*

Gardner WJ : *Myelomeningocele, the result of rupture of the embryonic neural tube. Cleveland Clin Quart 27 : 88-100, 1960.*

Gardner WJ : *The dysraphic states from syringomyelia to anencephaly. Excerpta Medica, Amsterdam. 1973.*

Hamburger V, Hamilton HL : *A series of normal stages in the development of the chick embryo. J Morphol 88 : 49-92, 1951.*

Lebedeff A : *Uber die Entstehurg der Anencephalie mit Spina Bifida bei Vogeln und Menschen. Virchows Arch Pathol Anat Physiol 86 : 263-298, 1881.*

Lemire RJ, Shepared TH, Alvord EC : *Caudal myeloschisis (lumbosacral spinal bifida cystica) in a five millimeter (HorizonXIV) human embryo. Anat Rec 152 : 9-16, 1965.*

McLone DG, Suwa J, Collins JA, Poznanski S, Knepper PA : *Neurulation : Biochemical and Morphological studies on primary and secondary neural tube defects. Concepts Pediat Neurosurg 4 : 15-29, 1983.*

Osaka K, Tanimura T, Hirayama A, Matsumoto S : *Myelomeningocele before birth. J Neurosurg 49 : 711-724, 1978.*

Padget DH : *Spina bifida and embryonic neuroschisis-a causal relationship : definition of postnatal confirmations involving a bifid spine. Johns Hopkins Med J 128 : 233-252, 1968.*

Padget DH : *Neuroschisis and human embryonic maldevelopment : New evidence on anencephaly, spina bifida and diverse mammalian defects. J Neuropathol Exp Neurol 29 : 192-216, 1970.*

Patten BM : *Overgrowth of the neural tube in young human embryos. Anat Rec 113 : 381-393, 1952.*

Rokos J, Knowles J : *An experimental contribution to the .pathogenesis of spina bifida. J path 118 : 21-24, 1976.*

Virchow RLK : *Die krankhaften Geschwulste. Hirschwald, Berlin. vol 1, p188, 1863.*

Von Recklinghausen F : *Untersuchungen uber die Spina bifida. Teil 2 : Uber die Art und die Entstehung der Spina bifida ihre Beziehung zur Ruckenmarks- und Darmspalte. Virchows Arch Pathol Anat Physiol 105 : 296-332, 1886.*

Warkany J : *Morphogenesis of spina bifida. In McLaurin RL ed. : Myelomeningocele. New York, Grune & Stratton, 31-39, 1977.*