Creation of Myelomeningocele In Utero: A Model of Functional Damage From Spinal Cord Exposure in Fetal Sheep

By Martin Meuli, Claudia Meuli-Simmen, Charles D. Yingling, Grover M. Hutchins, Kathleen McBiles Hoffman, Michael R. Harrison, and N. Scott Adzick

San Francisco, California and Baltimore, Maryland

● A recent study in human fetuses with myelomeningocele (MMC) suggested that the primary malformation is not neural but a failed closure of the posterior vertebral column and paraspinal soft tissue, which leads to exposure and secondary destruction of the spinal cord. The goal of this study was to test whether chronic exposure of the normal spinal cord to the amniotic space produces a lesion similar to human MMC. In fetal sheep at 75 days' gestation (group A) and 60 days' gestation (group B) (term = 150 days), the lumbar spinal cord was exposed to the amniotic cavity by excising skin and paraspinal soft tissues, and by performing a laminectomy. Some animals from both groups were fetectomized and assessed morphologically at 100 days' gestation. The remainder were delivered near term and assessed clinically, electrophysiologically, and morphologically. In group A, all animals showed MMC-type pathology. The exposed spinal cord was herniated out of the spinal canal and rested on the dorsal membranes of a cystic sac. The neural tissue was stretched and flattened out. Histologically, the hallmarks of the spinal cord were not discernable and the cytoarchitecture was lost. These changes were less severe at 100 days than at term. The three survivors in group A were paraplegic. In group B, the two survivors and two fetuses harvested at 100 days had healed skin wounds and near normal spinal cord histology. The other animal harvested at 100 days had a MMC-type lesion with less severe histological changes. The two survivors had a mild paraparesis. In conclusion, surgical exposure of the normal spinal cord to the amniotic space in a 75-day sheep fetus results in a MMC-type pathology at birth, which clinically and morphologically resembles human MMC. The creation of a spina bifida–type lesion in a 60-day-old fetus may result in spontaneous healing and minimal neurological deficit at birth. This "healing experiment of nature" suggests that in utero repair of MMC might prevent spinal cord damage and spare neurological function.

From The Fetal Treatment Center, the Department of Anesthesia, and the Animal Care Facility, University of California, and Microsurgical Laboratory, Davies Medical Center, San Francisco, CA; and the Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, MD.

Presented at the 1994 Annual Meeting of the Section on Surgery of the American Academy of Pediatrics, Dallas, Texas, October 21-23, 1994.

Supported by NIH HD25505, GM 27345/014, The Harold G. and Leila Y. Mathers Charitable Foundation, and the March of Dimes.

Address reprint requests to N. Scott Adzick, MD, The Fetal Treatment Center, HSW 1601, University of California San Francisco, Third & Parnassus Aves, San Francisco, CA 94143-0570, or M. Meuli, MD, Department of Surgery, University Children's Hospital, Steinwiesstr. 75, 8032 Zurich, Switzerland.

0022-3468/95/3007-0025$03.00/0

INDEX WORDS: Myelomeningocele, neural tube defect, spina bifida, spinal dysraphia, fetal surgery.

MYELOMENINGOCELE (MMC), a prominent member of the family of spinal dysraphic lesions, is a frequent congenital malformation in human beings, leading to paraplegia, urine and stool incontinence, sexual dysfunction, hydrocephalus, and skeletal deformations. The etiology and pathogenesis of MMC are unknown and probably multifactorial. The current theory is that the malformation involves a primary disorder of neurulation that occurs in the early development of the embryonic central nervous system.[1-4] However, recent studies of human fetuses with MMC suggest that the primary disorder may not be neural but rather a mesoderm-related malformation of the posterior spine, meninges, and soft tissues leading to spina bifida, dorsally nonclosed dura and pia mater, and thus open exposure of the spinal cord. Consequently, the unprotected but primarily normal neural tissue is progressively damaged mechanically and/or chemically during pregnancy, labor, and vaginal delivery.[5,6] The aim of this study was to test this hypothesis in a large fetal animal model and to specifically address the question whether chronic exposure of the normal spinal cord to the amniotic space produces a lesion and symptoms similar to those of human MMC.

MATERIALS AND METHODS

Creation of Spina Bifida–Type Lesion

For exposure of the fetal spinal cord to the intrauterine environment, a spina bifida–type lesion was surgically created, which mimicked as closely as possible the anatomical defect found in human patients with MMC.[5,6] In order to determine the impact of duration of spinal cord exposure, the spina bifida–type lesion was created at two different time points.

In eight fetal lambs at 75 days' gestation (group A) and in 11 at 60 days' gestation (group B) (term = 150 days) the undamaged spinal cord between L1 and L4 was exposed to the amniotic space. For fetal sheep surgery we used standard techniques previously described in detail.[7] Briefly, the ewe underwent general halothane/oxygen anesthesia, the uterus was exteriorized through an infraumbilical midline laparotomy, and the back of the fetus was exposed via a hysterotomy. The spina bifida–type lesion was created under 3.5× loupe magnification, using microsurgical instruments. A circular skin and panniculus carnosus excision (diameter, 4 cm) was made over the lumbar spine. The posterior spine was exposed by excising the median portions of the paraspinal musculature bilaterally, and then a complete laminectomy of lumbar levels L1 to

L4 was performed. Thereafter, the dorsal portion of the dura was excised between the origins of the dorsal roots from L1 to L4. The fetus with this open defect was returned to the uterus and amniotic fluid volume was restored with warm sterile saline. The hysterotomy was closed with a TA-90 stapler (US Surgical Corporation, Norwalk, CT), the maternal laparotomy was closed in layers, and the ewe was returned to her stall. Two fetuses from group A (creation of defect at 75 days) and three from group B (creation of lesion at 60 days) were killed at 100 days gestation for morphological assessment. The remainder were delivered near term (at 145 days' gestation) by cesarean section for neurological examination, measurement of somatosensory evoked potentials (SEP), and morphological assessment.

Clinical Assessment for Neurological Deficit

The clinical evaluation of the newborn lambs was carried out on day 1 and 3 of life by a veterinarian according to a standardized protocol.[8] Briefly, general health status, mental status, and neurological functions including cranial nerves, spinal reflexes, and postural reactions, as well as gait and posture, were assessed. Pain perception of forelimbs, hindlimbs, face, and rump was assessed by pricking with a needle (superficial pain) and by pinching with a hemostat (deep pain). Finally, the animal was observed for micturition and defecation. In addition, 10 normal newborn lambs were examined analogously as controls. The clinical assessment of study and control lambs was videotaped to facilitate comparison of data points.

Measurement of Somatosensory Evoked Potentials

The SEP represents the response of the somatosensory cortex to stimulation of peripheral nerves, and requires intact neural conduction through the dorsal column pathway of the spinal cord.[9] In order to conclusively show sensory transmission to the brain, cortical SEP to bilateral forelimb and hindlimb stimulation were recorded in addition to the clinical assessment. On postnatal day 3, lambs were anesthetized with intravenous propofol (Stuart Pharmaceuticals, Wilmington, DE) and then cortical SEP were recorded to stimulation of both posterior tibial and ulnar nerves with techniques adapted from those used routinely for clinical SEP evaluation in human beings.[10]

Morphological Assessment

After SEP recording, the animals were euthanized with Beuthanasia-D (Schering, Kenilworth, NJ) and immediately thereafter perfused via the aorta with phosphate buffered saline standard solution (1.5 L/animal) for wash out and a formalin 10% solution (3 L/animal) for fixation. The entire spine en bloc and the brain were harvested. The brain was inspected for hydrocephalus, and the site of the spinal lesion was removed intact (T12 to S2), sectioned into blocks, and processed for histology. Serial sagittal and cross sections were stained with hematoxylin-eosin and Gomori's trichrome.

RESULTS

Nine fetuses (47%) suffered intrauterine demise or abortion and were lost to the study.

In group A, all animals (n = 5) showed MMC-type pathology. At term, three animals were born with a cystic, fluid-filled back lesion (Fig 1). The exposed spinal cord had lost its cylindrical shape and had a plate-like appearance. It laid on the dorso-median

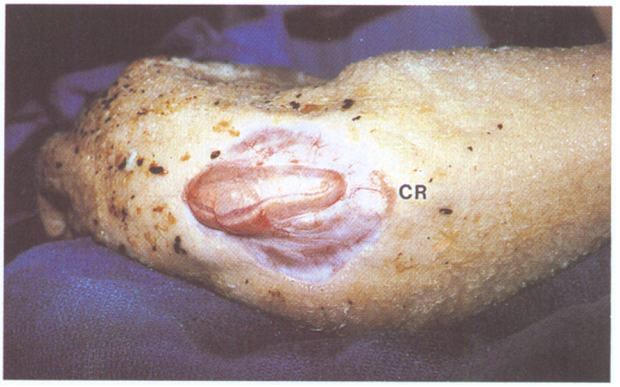


Fig 1. Overview of MMC lesion at birth (creation at 75 days' gestation). The spinal cord remnants appear as two separated and extremely flattened "hemicords." CR, cranial.

portion of the membranes of the cyst and appeared to be extremely stretched, flattened, and torn into two almost-separated "hemicords." No cerebrospinal fluid leak was noted. The animals had a complete flaccid sensorimotor paraplegia, were incontinent of urine and stool, and had absent hindlimb SEP, whereas forelimb SEP were intact with normal latency (Fig 2). Lumbar gibbus formation was found. No animal had hydrocephalus. Histologically, the severe alterations of the exposed spinal cord were confirmed (Fig 3A; also see control site shown in Fig 3B). In the center of the lesions, the injury had progressed to complete absence of cord tissue in one animal and marked loss of neural tissue in the other two. Here, the neural remnants consisted of two almost separated and extremely flattened columns. The ventral meninges, the nerve roots, and the spinal nerves persisted in all specimens. The dorsal pia mater was lost along with the dorsal spinal cord, and the ventral pia was fused to the dural cut edges and the lateral margins of the surgical defect by reparative fibrous tissue. This

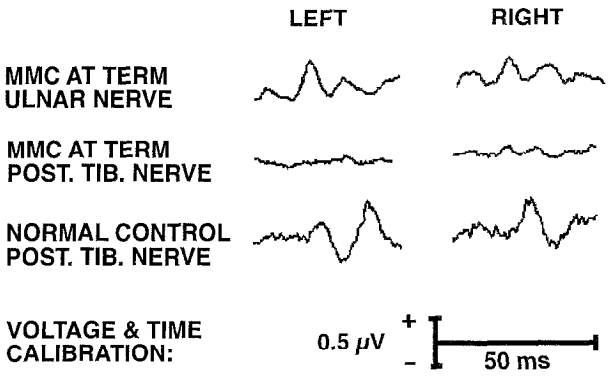
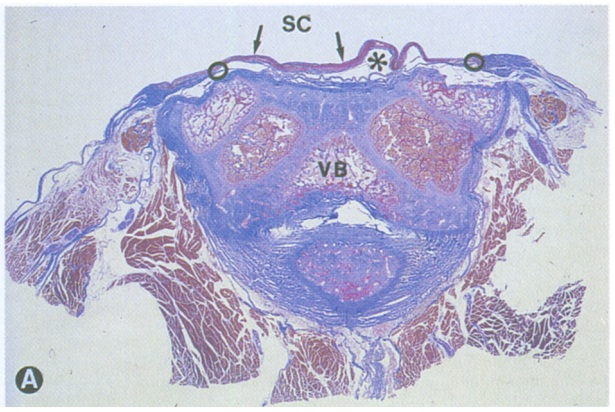


Fig 2. Cortical somatosensory evoked potentials: Normal responses from forelimbs and absent responses from hindlimbs in a neonatal MMC lamb. Hindlimb responses from a normal neonatal control lamb are shown for comparison. Post. tib. nerve = posterior tibial nerve, μV = microvolts, ms = milliseconds.



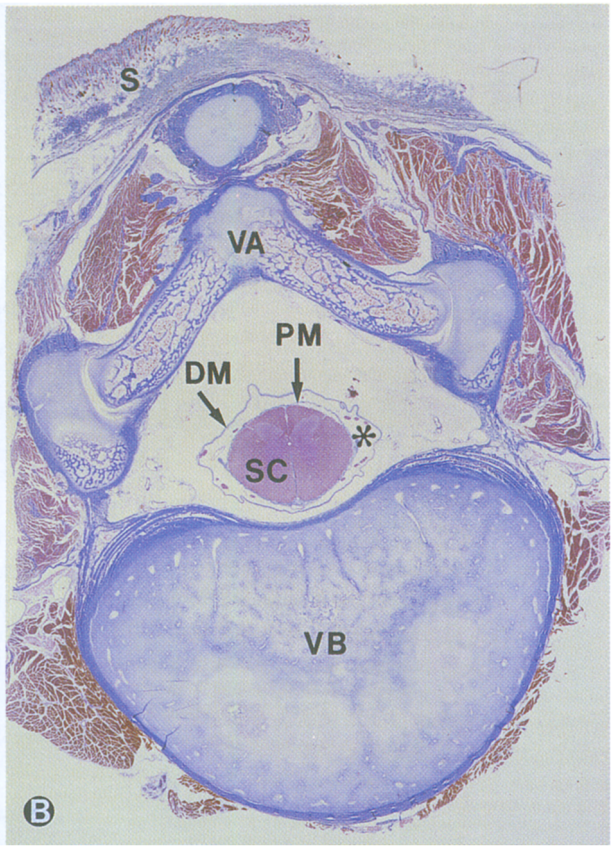


Fig 3. (A) Transverse section through the center of a MMC lesion at term (creation at 75 days). The spinal cord remnants (SC, each arrow points at one "hemicord") are exposed on the surface and rest on the ventral part of the pia mater. Pia mater and dura mater are both fused to the connective tissue at the margin of the surgical defect (encircled areas), thereby enclosing the subarachnoid space (asterisk). The subarachnoid space is deflated, whereas in vivo it is distended by cerebrospinal fluid leading to a cystic sac. (Gomori trichrome stain.) (B) Cross section through lumbar spinal cord (SC) in an normal newborn lamb. S = skin, VA = vertebral arch, DM = dura mater, PM = pia mater, asterisk = subarachnoid space, VB = vertebral body. (Gomori trichrome stain.)

fusion closed the subarachnoid space underneath the exposed neural tissue and explained both the absent leakage of cerebrospinal fluid and the cystic fluid-filled appearance shown in Fig 1. A thin layer of

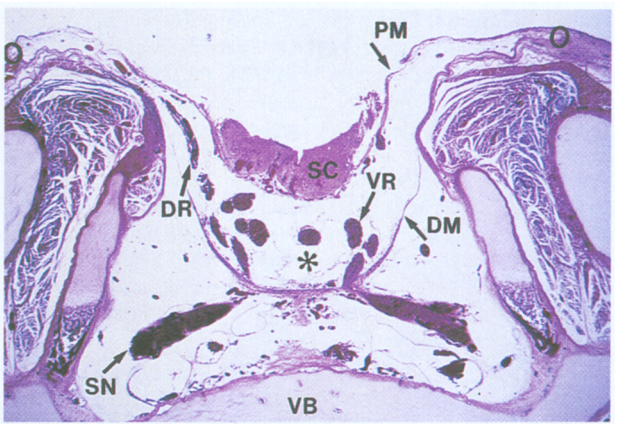


Fig 4. Transverse section through the center of a human fetal MMC (22 weeks' gestation). The vertebral canal is open, the dura mater (DM) is open and fused to the deep dermis, the pia mater (PM) is open and fused to the superficial dermis and epidermis (area of fusion is encircled). The cystic sac is created by accumulation of cerebrospinal fluid in the subarachnoid space (asterisk) between pia and dura. The dorsally destroyed spinal cord (SC) is exposed on the dorsal surface of the sac without any meningeal covering. DR = dorsal nerve root, VR = ventral nerve root, SN = spinal nerve, VB = vertebral body. (H&E stain.)

reparative collagenous connective tissue covered all surgically exposed surfaces including the neural remnants. The spinal cord and its coverings proximal and distal to the lesion were normal. Note the similarities with a comparable human lumbar MMC (Fig 4). At 100 days, the MMC lesion was macroscopically and histologically smaller and the destructive changes in the spinal cord were less severe than at term. These specimens showed a pronounced widening of the anterior median fissure that finally progresses to nearly complete separation of the spinal cord remnants into the two flat columns found at term.

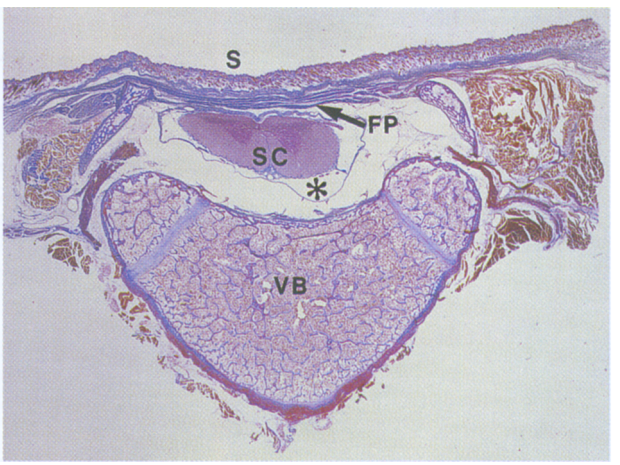


Fig 5. Transverse section through the center of a spontaneously healed defect at term (creation at 60 days). Underneath the skin (S) there is a thick and robust fibrous peel (FP) which must have formed very soon after wounding so as to stop MMC development. The spinal cord (SC) is flattened, but the cytoarchitecture is intact. Asterisk = subarachnoid space, VB = vertebral body. (Gomori trichrome stain.)

In group B (n = 5), the two survivors and two fetuses harvested at 100 days had healed lumbar wounds with near normal histology of the somewhat flattened spinal cord (Fig 5). There was no significant loss of neural tissue, and the site of former dura excision was repaired by connective tissue so that the subarachnoid space around the spinal cord was reconstituted. The other animal harvested at 100 days had a small MMC-type lesion with changes very similar to those seen in the animals of group A taken at 100 days. Clinically, the two survivors had a mild paraparesis, but they could ambulate and had sensation in their hindlimbs. No signs of urine or stool incontinence were detected. Hindlimb SEP were present and of normal latency compared with a control group of normal neonatal lambs.

DISCUSSION

Our results show that exposure of the normal and undamaged spinal cord to the intrauterine environment for the second half of gestation leads to a pathology that has striking clinical and morphological similarities to human MMC. In severe cases of MMC, the patient is paraplegic, and so were our animals. Typically, the human lesion is cystic, fluid-filled, and the protruded, plate-like neural remnants lie exposed on the dorsal aspect of the cystic sac; often, the bifid vertebral column shows gibbus formation.[11] We found exactly the same characteristics in the experimental lesions. Gibbus formation is probably caused by an imbalance of extensor and flexor forces along the spine, caused by the large dorsal tissue defect that locally leaves the flexor forces unopposed. In contrast, other conditions often associated with MMC such as hydrocephalus, Arnold-Chiari malformation, and clubfoot deformity were not present.[12]

Histologically, the configuration of the experimental (Fig 3) and human lesion (Fig 4) is very similar. Pia and dura in our model were fused to the margins of the surgical defect by fibrous tissue (as a result of the reparative response), thereby reclosing the subarachnoid space. In human beings, the dorsally open pia is fused to the epidermis and superficial dermis, and the open dura is fused to the deep dermis (as a developmental anomaly) thereby closing the subarachnoid space. Thus, in both, the cystic sac is located only underneath (and not around as normally present) the exposed neural tissue and formed by pial and dural membranes and the cerebrospinal fluid contained within the subarachnoid space. In both, the neural remnants are divested of their dorsal meningeal coverings and exposed on the dorsal surface of this sac. In both, the nerve roots and the spinal nerves persisted.

There may be both chemical and mechanical factors that progressively damage the spinal cord. A conceivable chain of pathophysiological events may include initial damage to the unprotected neural tissue by amniotic fluid. Its composition, however, is not yet very different from either extracellular fluid or cerebrospinal fluid.[13] Thus, the damage is probably minor. Later, reparative processes produce a thin fibrous membrane that covers the entire surface of the surgical defect. Also, the reparative response leads to closure of the formerly open subarachnoid space via fusion of ventral pia and dura (see above). Consequently, the leakage of cerebrospinal fluid into the amniotic space stops and cerebrospinal fluid reaccumulates in the subarachnoid space ventral to the exposed neural tissue. Because the posterior spinal elements are absent, the spinal cord tissue and its membranes herniate out of the spinal canal and eventually form a cystic MMC. The ballooning forces stretch the neural tissue longitudinally and transversely. The stretching forces thin out and tear the spinal cord, and on a cellular level, there is disruption of neuronal connections and neuronal death. Toward the end of the pregnancy the extremely friable and barely protected neural tissue suffers additional "chemical" damage from the amniotic fluid, which now contains much more urea and creatinine than during the first half of gestation.[13] Also, because the amount of amniotic fluid significantly decreases late in gestation, the likelihood of mechanical trauma increases, caused by the tight contact with the uterine wall. Finally, the forces of labor and vaginal delivery cause additional injury to the exposed neural tissue.[14] The sum effect of these factors is anatomical destruction and complete functional loss of the spinal cord.

Our findings are consistent with the hypothesis that some forms of human MMC evolve consequent to a failed closure of the posterior spine.[5,6] Other evidence supports this theory. The striking morphological similarities between fetal human lesions (Fig 4) and our experimental lesions (Fig 3) suggest a similar pathway: Nonclosure of the posterior spine followed by progressive damage to the exposed spinal cord. This mechanism was also suggested in postnatal MMC patients where closure of the neural tube apparently had occurred.[15] Some investigators reported on human fetuses and postnatal patients with MMC who showed differentiated remnants of the spinal cord, ventral and dorsal rootlets, and spinal ganglia.[5,6,15,16] These findings are compatible with a secondary alteration of a primarily normal spinal cord. Alternatively, the unfolded neural plate could differentiate into these structures without undergoing previous neurulation. Human spina bifida occulta,

spina bifida with meningocele, and spina bifida with MMC all have nonclosure of the posterior spine in common, but only the most severe forms involve the spinal cord. Conversely, there is no MMC-type malformation of the spinal cord with an intact vertebral column. This constellation strongly suggests that the primary problem is inherent to the mesodermally derived musculoskeletal elements. Finally, previous experiments in monkeys,[17] rats,[18] and pigs[19] have shown that surgical exposure of the fetal spinal cord damages the developing neural tissue. Because there is convincing clinical and experimental evidence that another subset of MMC arises from failed neurulation,[1-2,20-21] it is reasonable to propose that both failed closure of the posterior spine and failed neurulation are tenable pathophysiological features.

As shown in some animals of group B, creation of MMC in a 60-day-old fetus may result in spontaneous healing because the amazing healing potential at this early gestation overrides the MMC formation. Most strikingly, these animals had minimal neurological deficit at birth. If the exposed spinal cord is sufficiently covered at a relatively early time point, progressive spinal cord destruction and loss of function is stopped. A similar mechanism might be involved in the pathogenesis of spina bifida occulta. More importantly, this "healing experiment of nature" suggests that in utero repair may prevent neurological damage and potentially spare function. We are currently testing whether delayed in utero repair of evolving MMC lesions results in a better neurological outcome at birth.

In conclusion, we have developed a new fetal MMC model that closely resembles human MMC. Our results support the concept that some phenotypes of human MMC might benefit from fetal surgery that is currently being performed for life-threatening malformations.[22]

ACKNOWLEDGMENT

The technical assistance of Lora Mesiano, Vicente Lapiz, Ilse Sauerwald, and Liz Montgomery is gratefully acknowledged.

REFERENCES

1. Copp AJ: Neural tube defects. Trends Neurosci 16:381-383, 1993

2. Copp AJ, Brook FA, Estibeiro JP, et al: The embryonic development of mammalian neural tube defects. Progr Neurobiol 35:363-403, 1990

3. Lemire RJ: Neural tube defects. JAMA 259:558-562, 1988

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

5. Hutchins GM, McGowan KD, Blakemore KJ: Spinal dysraphia: Not a neural tube defect? Am J Hum Genet 51:A319, 1992 (abstr)

6. Jordan MA, Heffez DS, Hutchins GM: The relationships of the spinal cord and meninges in meningocele, meningomyelocele, and iniencephaly. Teratology 43:472, 1991 (abstr)

7. Harrison MR, Jester JA, Ross NA: Correction of congenital diaphragmatic hernia in utero. I. The model: Intrathoracic balloon produces fatal pulmonary hypoplasia. Surgery 88:174-182, 1980

8. DeLahunta A (ed): Veterinary Neuroanatomy and Clinical Neurology. Philadelphia, Saunders, 1983

9. Cusick JF, Myklebust J, Larson SJ, et al: Spinal evoked potentials in the primate: Neural substrate. J Neurosurg 49:551-557, 1978

10. Nuwer MR, Aminoff M, Desmedt J, et al: IFCN recommended standards for short latency somatosensory evoked potentials. Report of an IFCN committee. Electroenceph Clin Neurophysiol 91:6-11, 1994

11. Shurtleff DB, Lemire RJ, Warkany J: Embryology, etiology, epidemiology, in Shurtleff DB (ed): Myelodysplasias and Exstrophies: Significance, Prevention, and Treatment. Orlando, FL, Grune & Stratton, 1986, pp 39-64

12. Gilbert JN, Jones KL, Rorke LB, et al: Central nervous system anomalies associated with meningomyelocele, hydrocephalus, and the Arnold-Chiari malformation: Reappraisal of theories regarding the pathogenesis of posterior neural tube closure defects. Neurosurgery 18:559-564, 1986

13. Lind T: The biochemistry of amniotic fluid, in Fairweather DVI, Eskes TKAB (ed): Amniotic Fluid-Research and Clinical Application. Amsterdam, Elsevier Excerpta Medica, 1978, pp 59-80

14. Luthy DA, Wardinsky T, Shurtleff DB, et al: Cesarean section before the onset of labor and subsequent motor function in infants with meningomyelocele diagnosed antenatally. N Engl J Med 324:662-666, 1991

15. Emery JL, Lendon RG: The local cord lesion in neurospinal dysraphism (meningomyelocele). J Pathol 110:83-96, 1973

16. Pattern BM: Embryological stages in the establishing of myeloschisis with spina bifida. Am J Anat 93:365-395, 1953

17. Michejda M: Intrauterine treatment of spina bifida: Primate model. Z Kinderchir 39:259-261, 1984

18. Heffez DS, Aryanpur J, Hutchins GM, et al: The paralysis associated with myelomeningocele: Clinical and experimental data implicating a preventable spinal cord injury. Neurosurgery 26:987-992, 1990

19. Heffez DS, Aryanpur J, Cuello Rotellini NA, et al: Intrauterine repair of experimental surgically created dysraphism. Neurosurgery 32:1005-1010, 1993

20. Osaka K, Tanimura T, Hirayama A, et al: Myelomeningocele before birth. J Neurosurg 49:711-724, 1978

21. Lemire RJ, Shepard TH, Alvord EC: Caudal myeloschisis (lumbo-sacral spina bifida cystica) in a five millimeter (Horizon XIV) human embryo. Anat Rec 152:9-16, 1964

22. Adzick NS, Harrison MR: Fetal surgical therapy. Lancet 343:897-902, 1994

Discussion

U.G. Stauffer (Zurich, Switzerland): I thank the authors for the opportunity to discuss this paper. Its interest is twofold. The first is on embryogenesis of MMC. The authors present convincing experimental

evidence that at least some forms of myelomeningocele may evolve consequent to a failed closure of the posterior spine followed by progressive damage to the exposed, primarily normal spinal cord during pregnancy, labor, and vaginal delivery.

Would it be possible to distinguish two clinical forms of MMC according to their different embryogenesis? I mention for instance the lack or presence of hydrocephalus or the lack or presence of a tethered cord. Are there any histological criteria?

The second interest concerns possible fetal treatment in the future. The authors are at present testing in their experimental model whether delayed in-utero repair could result in a better neurological outcome at birth. Could they comment on their preliminary results?

If these preliminary experimental results are positive, do they think that an eventual in utero repair could possibly also be effective in forms of failed neurulation? If not, how could we possibly select those fetuses that could profit from an intrauterine repair?

In my country and other European countries, many pediatric surgeons are in charge of these unfortunate children. This paper seems to start a new dynamic approach to this complex malformation. It was a pleasure for me to discuss this paper.

M. Meuli (response): Thank you, Dr Stauffer, for your very interesting points. As a matter of fact, I think in the United States too it is believed that failed neurulation is the main cause for myelomeningocele and the other one is a new hypothesis that needs further examination.

The paper that you have cited from Emery that was published in 1973 in *The Journal of Pathology* says that about 80% of the human cases studied were most likely caused by failed neurulation. But he also showed in that paper a very nice histology from a case that is certainly caused by the pathogenic mechanism suggested here, because it was very clear that neurulation had occurred and there was dorsal damage to the fully neurulated cord.

You also asked whether there are criteria to distinguish between different causes. As of today, this is not possible clinically. We are working on criteria, and we are actually doing a study in aborted fetuses to try to find out. Hydrocephalus is not a criterion because it tends to develop late in gestation in all of these cases.

We have indeed done some preliminary animal studies in which we created spina bifida in the way we have described at 75 days, then we went back at 100 days, found a miniature myelomeningocele lesion, covered this with a latissimus dorsi flap, and let the animal go to term, and the animals were neurologically near normal. They were very similar to the spontaneously healed animals. So in other words, within our experimental setting the idea and our actions were successful. Whether this can be translated directly to the human situation is very questionable, and we are many steps away from envisioning such an enterprise clinically today.