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Experimentally Induced Chiari-Like Malformation with Myeloschisis in Chick Embryos

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Though several pathogenetic theories concerning the frequent association of Chiari malformation and hydrocephalus with myeloschisis have been suggested, none of them explains all the aspects of the disorder. To investigate whether myeloschisis is the direct cause of Chiari malformation and hydrocephalus or these conditions are the results of another basic event, we observed the morphological changes of the posterior cranial fossa and its components in the chick embryos with surgically induced myeloschisis. To make myeloschistic lesions, we opened the neural tube for a length of 9-11 somites in Hamburger and Hamilton stage 16-19 chick embryos. They were divided into cervicothoracic (C-T) and lumbosacral(L-S) groups according to the area of incision. The embryos were re-incubated until postoperative day 11. In the control group, embryos were incubated with the eggshell window open as their experimental counterparts. The survival rates of each group were as follows; 11% (9 survivors / 85 operated embryos), 8% (7 / 83), and 17% (10 / 60) in the C-T, L-S and control groups, respectively. Myeloschisis positive rates were 100% in the operated groups and 0% in the control group. The heads of embryos were sectioned along the sagittal plane to observe the morphological changes in the posterior cranial fossa and its components. Of the survivors, five in the C-T group, two in the L-S group and six in the control group were available for light microscopic inspection. In the majority of embryos with myeloschisis, without difference between the C-T and L-S groups, the fourth ventricles were smaller than those of the control group and the subarachnoid spaces in the posterior cranial fossa were also narrower. In embryos with severe changes, the cerebellum displaced downward comparing with that of the control embryos. No evidence of hydrocephalus was present. Though not always typical, morphological changes similar to Chiari malformation were observed in chick embryos with surgically induced myeloschisis. It suggests a strong direct causal relationship between the two conditions and supports the theories of derangements in cerebrospinal fluid dynamics rather than those of primary mesenchymal or neural origin as a pathogenetic mechanism of Chiari malformation.

Key Words : *Chiari malformation, Chick embryos, Hydrocephalus, Surgically induced myeloschisis*

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INTRODUCTION

In 1891 and 1896, Chiari described four types of cerebellar and hindbrain anomaly with hydrocephalus. His type I was described as a peg-like elongation of the tonsils and the medial part of the inferior lobes of the cerebellum, which goes along the medulla into the

cervical canal. The type II anomaly was described as elongation and displacement of parts of the lower vermis, pons, medulla oblongata and the fourth ventricle into the high cervical canal. This description emphasized brainstem displacement and cerebellar dysplasia as the major components of the malformation. Whether the eponym "Arnold-Chiari" is applied properly to this malformation in a historical sense has been debated. The term has been used in the clinical setting for years, although some authors refer to the hindbrain malformation occurring in almost all infants with a meningomyelocele as the Chiari type II anomaly.

Several theories so far proposed to explain the cause of Chiari malformation and/or hydrocephalus have not satisfactorily outlined a mechanism which correlates the myelomeningocele with this total brain and skull malformation. Because it has not been possible to study the neurodevelopmental processes of these complex malformations in human *in vivo* or by explantation, the description of them, therefore, must refer to experimental studies in animals if the dynamic processes of development are to be fully understood. The experimental models so far developed include chemical models using such as trypan blue, vitamin A, or tunicamycin, and genetic models such as Spd/Spd mice. These models cause functional derangement of cells to a remarkable extent and cannot identify whether one component of the malformation occurs primarily or secondarily to another component (Gunberg, 1956 ; Warkany et al.,1958 ; Vickers,1961 ; Marin-Padilla,1966, 1980 ; Marin- Padilla and Ferm,1979 ; Marin-Padilla and Marin-Padilla, 1981 ; McLone and Knepper, 1989). In contrast, the surgical model has advantages over other models in that the primary event is limited to the neural tube opened by surgery without affecting any other portions of the nervous system (You et al.,1994 ; Sim et al., 1995). On the base of experimetal studies with a genetic model, McLone and Knepper (1989) proposed a unified theory to explain the cause of Chiari II malformation. They suggested the cerebrospinal fluid (CSF) leak through the neural tube defect and the defective occlusion of the neurocele as the developmental factors that cause the central nervous system anomalies resulting in myelodysplasia including Chiari II malformation and hydrocephalus. To date, this theory has been in the spotlight.

To investigate whether myeloschisis is the direct cause of Chiari malformation and/or hydrocephalus or these conditions are the results of other basic events, we observed the morphological changes of the posterior cranial fossa and its components, the ventricular system and the subarachnoid spaces in the chick embryos with surgically induced myeloschisis. Recognition of those changes seen in association with myeloschisis would allow re-evaluation of the developmental pathogenesis of these anomalies and suggests further considerations.

MATERIALS AND METHODS

The detailed method for induction of myeloschisis was described by Sim et al. (1995) Procedures may be summarized as follows : fertile eggs were incubated under constant temperature (38-39℃) and humidity (50-60%). Eggs were assigned randomly to experimental and control groups. Experimental groups were divided into two groups, cervicothoracic (C-T) and lumbosacral (L-S) groups, according to the area of incision. The embryos of Hamburger and Hamilton (1951) stage 16-19 were used. The posterior roof of the central canal of the closed neural tube was incised longitudinally for a length of 9-11 somites (about 2.5mm) with a 30 gauge needle at the C-T or L-S area. Following surgery, they were then re-incubated *in ovo* up to a total age of 14 days(approximately Hamburger-Hamilton stage 40). Control embryos were also vitally stained with 1% neurtral red solution and re-incubated as their experimental counterparts. The survived embryos were harvested and the heads of embryos were fixed by immersion in 10% formalin solution. After fixation and decalcification, they were embedded in paraffin and serially sectioned with 10 ㎛ thickness along the sagittal plane. Light microscopic findings, especially the sizes of the fourth ventricle and the subarachnoid space of the posterior cranial fossa and presence of hydrocephalus, were investigated. The morphological changes in the posterior cranial fossa of a gestation day 16 Spd/Spd mouse embryo with a sacral open neural tube defect (permitted to study in their laboratory by Drs. McLone and Knepper) were compared with the results of the present surgical model in chick embryos to observe the differences in the extent and degree of the morphological changes.

RESULTS

The survival rate and myeloschisis positive rate of each group were as follows ; 11% (9 survivors / 85 embryos) and 100% (9 embryos with myeloschisis / 9 survivors) in the C-T group ; 8% (7/83) and 100% (7/7) in

the L-S group ; 17% (10/60) and 0% (0 /10) in the control group. Embryos which were poorly fixed or damaged during the process were discarded. So embryos available for the light microscopic study were 5 in the C-T group, 2 in the L-S group and 6 in the control group.

The morphological details of the myeloschistic lesions were previously described (Sim et al., 1995). Briefly, under the stereomicroscopic examination, the myeloschistic lesions were regular in outline. They were rather elongated in the embryos of the C-T group while ovoid in those of L-S group. There was a linear median groove terminating at the upper and lower margins of the lesion. On the histological examination of the transverse sections, the posterior column looked defective and the dorsal part of the spinal cord was open and flat. The exposed placode was continuous with the arachnoid membrane and/or the skin laterally. The primitive subarachnoid space ventral to the spinal cord was enlarged. Spinal roots and ganglia were of normal shape. In summary, the lesions were quite similar to human myeloschises.

Compared with the control group, the embryos with myeloschisis had some distinct morphological changes in the heads, though not all the embryos showed the same findings and the degree of the change was not constant; the subarachnoid spaces were narrower than those of the control embryos and the fourth ventricles were also smaller (Table 1). In Fig. 1, comparing with an embryo of the control group (A), the distance between the floor of the fourth ventricle and the ventral surface of the cerebellum was shorter, the spaces between the

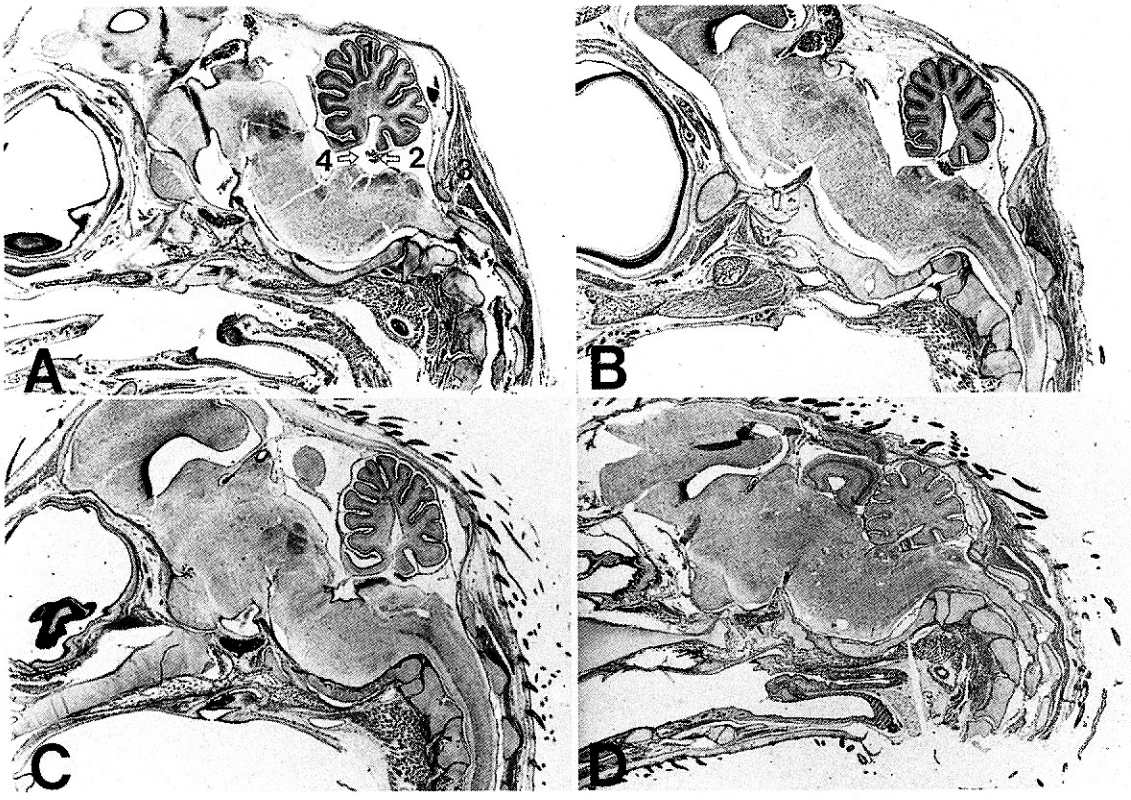

Fig. 1. Median sections of the head of 14-day old chick embryos with surgically induced myeloschisis. Photographs are taken from the slice in which the fourth ventricle is of the largest dimension. A : control group. B : (embryo No. 1, C-T group) The fourth ventricle is slightly smaller than that of control group embryos. Though not definite, cerebellar sulci are narrower. C : (embryo No. 6, L-S group) The fourth ventricle is moderately smaller than that of control group embryos. The subarachnoid space of the posterior cranial fossa are not narrower. D : (embryo No. 4, C-T group) The fourth ventricle and the subarachnoid space of the posterior cranial fossa are markedly reduced in size. The posterior cranial fossa is crowded. The lower end of the cerebellum is closer to the posterior arch of C1 than in control embryos. Changes in the shape of brainstem is absent. There is no evidence of hydrocephalus. 1 : cerebellum, 2 : choroid plexus of the fourth ventricle, 3 : posterior arch of C1, 4 : fourth ventricle (hematoxylin and eosin, original magnification × 15)

Table 1. Degree of the change in size of the fourth ventricle and the subarachnoid space of the posterior cranial fossa

No.	Site of Incision	Small Fourth Ventricle	Narrow SAS of Posterior Fossa
1.	CT	+	-/+
2.	CT	-	-
3.	CT	+++	++
4.	CT	+++	+++
5.	CT	++	-
6.	LS	++	-
7.	LS	+++	+++

SAS=subarachnoid space
CT=cervicothoracic, LS=lumbosacral
degree of reduction in size
- ; none, + ; mild, ++ ; moderate, +++ ; marked

cerebellar cortex and the inner surface of the skull, and between the dorsal sufrace of upper brainstem and the anterior surface of cerebellar cortex were also narrower in myeloschistic embryos (B-D). The shape of skull was rather flattened in an embryo with marked morphological changes (D). No evidence of hydrocephalus was noted. In embryos with remarkable changes, the cerebellum was located downward comparing with that of control embryos, showing a shorter distance between the posterocaudal end of the cerebellum and the upper margin of the posterior arch of C1 (Fig. 1, D). Though the number of embryos studied were small, there was no distinct difference of the morphological changes in the heads between the C-T and L-S groups.

Comparing with the findings of the surgical model in chick embryos, the gestation day 16 Spd/Spd mouse embryo (mouse with homozygotic genetic defect) with a sacral open neural tube defect showed more prominent changes. The curvature of the clivus was more concave ; the brainstem looked buckled ; the ventral cervicomedullary junction moved to the upper cervical spinal canal ; the ventricles including the fourth ventricle were collapsed making the overlying neural structures rather thicker ; the subarachnoid space of the posterior cranial fossa, especially the portion behind the cerebellomedullary junction, was narrow (Fig. 2). Even in embryos with remarkable changes, only portions of the rather dramatic findings of the Spd/Spd mouse embryo were seen in the present surgical model of chick embryos.

DISCUSSION

Pathogenetic theories of Chiari malformation

The frequent association of Chiari malformation and hydrocephalus with myeloschisis has led certain workers to postulate a causal interrelation between these conditions. Putting various clinical and experimental reports together, the theories on the basic embryological defect that leads to the Chiari II malformation can generally be grouped into five categories : hydro-

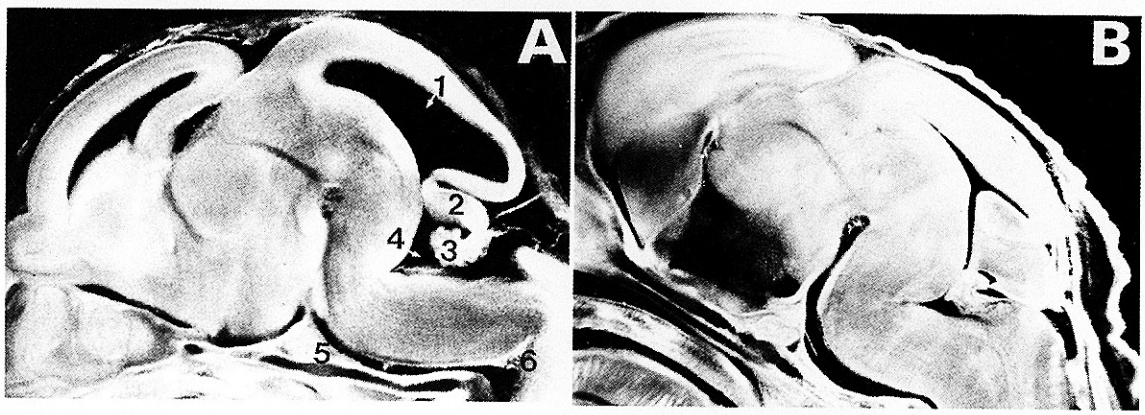

Fig. 2. Median sections of the head of gestation day 16 embryos of a normal C57 mouse (A) and a Spd/Spd mouse with a sacral open neural tube defect (ONTD) (B). Comparing with the control embryo, the embryo with a ONTD has a collapsed ventricular system. All the ventricles, especially the fourth ventricle is smaller and the subarachnoid space of the posterior cranial fossa is markedly narrower. The clivus is more curved and the lower end of the pontine curvature is displaced downward. The brainstem looks more buckled than that of the control embryo. These changes are more outstanding and similar to Chiari malformation than those in the present surgical model of chick embryos which may be partly due to the difference of animals used and partly due to model used. 1 : mesencephalic ventricle (aqueduct of Sylvius), 2 : cerebellum, 3 : choroid plexus of the fourth ventricle, 4 : fourth ventricle, 5 : clivus, 6 : cervicomedullary junction in the ventral aspect (transilluminated thick section with wet mounting, original magnification × 20)

cephalus / hydrodynamic theory (Chiari, 1891, 1896 ; Cameron, 1957 ; Peach, 1965a, b ; Gardner, 1977), traction theory (Penfield and Coburn, 1938 ; Lichtenstein, 1942), dysgenesis of the hindbrain/developmental arrest theory (Cleland, 1883 ; Daniel and Strich, 1958 ; Peach, 1965a), small posterior fossa/overgrowth theory (Barry et al., 1957 ; Padget, 1972 ; Padget and Lindenberg, 1972 ; Marin-Padilla and Marin-Padilla, 1981) and unified theory (McLone and Knepper, 1989). Although each of these theories explains certain elements of the Chiari malformation, none of them fully explains all the nature of the disorder or its developmental processes.

The study of the development of human Chiari malformation has been based on the observations of individual specimens or some collections of embryos and fetuses. While each specimen represents an important aspect of the malformation, it does not allow us to understand the dynamic processes. However, it has not been possible to study human neurodevelopmental dynamic processes either *in vivo* or by explantation. The description of the human Chiari malformation, therefore, must refer to important experimental studies in animals if the dynamic processes of development are to be fully understood. There have been a few experimental models for this complex malformation. The experimental models so far developed have used chemicals such as trypan blue and vitamin A, or viruses like reovirus type I, as the teratogen and experimental animals such as mouse, rat and hamster (Gunberg, 1956 ; Warkany et al.,1958 ; Vickers,1961 ; Marin-Padilla,1966,1980 ; Margolis and Kilham,1969 ; Marin-Padilla and Ferm,1979 ; Marin-Padilla and Marin-Padilla, 1981). Another is a genetic mutant of abnormal neurulation which includes the delayed Splotch (Spd/Spd) mouse embryo with a sacral neural tube defect (McLone and Knepper, 1989). The basic neurological anomalies which characterize Chiari type II malformation, such as downward displacement of the hindbrain and cerebellum, reduction of the pontine flexure and caudal myelocele (spina bifida), have been all induced in the experimental models using trypan blue in rats, or vitamin A in hamsters. However, cerebellar herniation into the cervical canal has not been described in any of these experimental models for reasons which will be mentioned later. It has been pointed out that experimentally induced Chiari malformation is not present in young affected embryos, but that the malformation could develop at later embryonic stages. A prerequisite for the development of this late anomaly is the presence of a small posterior cranial fossa which is overfilled during the postnatal growth spurt of the cerebellum which is then forced to grow downward into the upper cervical spinal canal and upward into the supratentorial space (Marin-Padilla and Marin-Padilla, 1981 ; McLone and Knepper, 1989). It seems that neither the neurological abnormalities nor the complications will manifest in Chiari malformation until the developing cerebellum overfills its skeletal encasement, which is small due to underdevelopment of the occipital bone. Only under the circumstances in which the compression of the medulla, the development of the secondary obstructive hydrocephalus, or so-called herniation of the cerebellum into the upper cervical canal occurs, will the neurological symptoms progressively develop and the complications begin to appear. In the severe form of Chiari malformation, the complete clinical features of the disease can occur even prenatally (Duckett, 1966 ; Bell et al., 1980). Margolis and Kilham (1969) observed Chiari malformation in the suckling hamster with neonatal hydrocephalus induced by reovirus type I. The marked cerebellar herniation into the cervical canal and, to a lesser degree, that of the hindbrain, observed in this type of experimental Chiari malformation was indistinguishable from that found in the human Chiari malformation. They offered an argument against the concepts which propose that Chiari malformation and hydrocephalus have their common origin in the same noxious influence. Their studies demonstrated that Chiari malformation can be induced in the absence of spina bifida, and supported the postulate that Chiari malformation is the result of hydrocephalus. However, their experiment did not explain a causal interrelation between Chiari malformation and myelomeningocele. Moreover, it is not an appropriate model because the hydrocephalus occurs later in the embryos with Chiari malformation and myelomeningocele. Marin-Padilla and Marin-Padilla (1981) proposed a small posterior fossa theory on the base of the observation in vitamin A-treated hamster embryos. They suggested that various neural anomalies which are closely related with Chiari malformation are secondary in nature, and that each anomaly reflects the particular stage in the closure of the neural folds affected by the primary mesodermal insufficiency (Marin-Padilla and Marin-Padilla, 1981). However, their model has weak points ; no occurrence of cerebellar herniation into the cervical canal, the absence of other manifestation of Chiari II malformation and no proof of the assumption that the mesenchymal defects precede neural defects (McLone and Knepper, 1989).

McLone and Knepper(1989) have recently proposed

a unified theory for the embryogenesis of Chiari malformation, which incorporates elements of each of the preceding theories. To date, this theory has been in the spotlight. They used the delayed splotch (Spd/Spd) mice with neural tube defects on gestation days 9 through 18 to study the initial developmental defects of the Chiari II malformation and examined the morphological and biochemical events. In their model, the neural tube defects are known to occur genetically and there are not marked alterations in the posterior cranial fossa until gestation day 18 (16 or less by authors' results). According to this genetic model, a neurulation defect is a prior feature of Chiari II malformation, through which CSF escapes from the central canal of the neural tube. In addition, defective transient occlusion of central canal (which, in normal animals and in humans, precedes and is responsible for rapid brain enlargement) was identified as another developmental factor that causes Chiari II malformation and the associated cerebral and skull anomalies. They stressed that the persistent venting of CSF interferes with proper ventricular enlargement and eventually results in multiple anomalies of the nervous system. Finally, they emphasized the important role of distention of the primitive cranial ventricular system in the normal cerebral development and subsequently in the calcification and bone formation of calvarium during the prenatal period. Most importantly, impaired ventricular enlargement has effects on the development of the chondrocranium; this is most evident in the development of the small posterior cranial fossa. This small posterior cranial fossa becomes fixed and incapable of accommodating the later explosive growth of the cerebellum. As a result, the posterior cranial fossa contents are displaced both cephalad through the tentorial incisura and caudad through the foramen magnum. Thus, they concluded that Chiari II malformation is a result of a series of interrelated time-dependent defects in the development of the ventricular system, which leads to multiple anomalies in the brain development. Though their speculation is based on the chronological changes of the nervous system, however, it is still possible that the associated morphological changes are the results of a basic genetic defect rather than results of a single initial lesion, myeloschisis. In other words, because the genetic defect affects all parts of nervous system, each of the anomalies of nervous system can be destined to occur as a separate event in a separate time schedule not by precedingly formed anomalies but by the gene defect.

Significance of the surgical model in the study of pathogenesis of Chiari malformation

To investigate whether myeloschisis is the direct cause of Chiari malformation or the two conditions are the results of another basic event (for example, gene defect in the Spd/Spd mouse), we studied the relationship between the two in chick embryos with surgically induced myeloschisis. To comprehend this complex malformation, it is absolutely necessary to understand some developmental aspects which are directly and indirectly involved in its morphogenesis. We thought this study would be very helpful to analyze the effect of CSF dynamics on the development of Chiari malformation and various myeloschisis and/or Chiari malformation-associated anomalies because there are not systemic effects (Chemical or genetic models may have systemic effects.) but local effects as an initial event in this surgical model of myeloschisis. To make myeloschisis we opened the closed neural tube of chick embryo at total age 3 days (Hamburger-Hamilton stage 16-19) by surgery without affecting any other portion of the nervous system. Even if certain changes occur at the distant area from the incision, it will be the result of the local event no matter it is direct or indirect. The most probable mechanism by which a local event can make changes at the distant area of nervous system is hydrodynamics.

There are a few variables which may have effects on the development of myeloschisis and/or Chiari malformation. These include the location and size of the myeloschisis, the embryonic stage at surgery, and the period of re-incubation after surgery. There seemed to be no difference in morphological changes between the C-T and L-S groups though the number of embryos studied were small. We are studying the effects of the size of myeloschisis. Preliminary results of the ongiong study have shown that the shorter the lenghth of the incision on the neural tube is, the higher the rate of the healing of the lesion is (unpublished data). However, if the unified theory is applied, we are expecting that the difference of the final size of myeloschisis will make relatively little difference in the development of Chiari malformation as long as the lesion is open because, once the myeloschisis is made, CSF leak continues until the open neural tube defect is occluded. Further study is needed to determine whether the morphological changes are affected by the stage when the neural tube is surgically opened. In the genetic model, the escape of proteinaceous material or CSF from the neural tube

is assumed to start at the earlier embryonic stage than in our model. To compare the effect of timing of CSF leak, the neural tube should be opened or prevented from closure at different stages in our model. The period of re-incubation after surgery will also play a critical role to develop the Chiari malformation which will be further analyzed and discussed. We sacrificed the embryos with the surgically induced myeloschisis at total age 14 days (approximately Hamburger-Hamilton stage 40). The short postoperative re-incubation period may limit the magnitude of the deformity in the present study. Under these circumstances, only small fourth ventricles, narrow subarachnoid spaces were developed. It seems reasonable that compression of brainstem, cerebellar herniation into the cervical spinal canal do not manifest until the developing cerebellum overfills its small posterior cranial fossa. The developing cerebellum which grows mainly at the late *in ovo* or post-hatch period will be displaced to an anomalous position later. In the genetic model, there are not marked alterations in the posterior fossa until late gestation days, like our results. In addition, the defective occlusion of the spinal central canal is not considered to be present in the early embryonic stages in our model. It may contribute to the lesser degree of deformity in our study than that in the genetic model. However, the differences of animal used in both studies prohibit direct comparison between the results of the two studies.

The study of this experimentally induced Chiari-like malformation has emphasized the developmental aspects of the lesion. Though not always typical, the direction of deformation in this study is identical to that seen in the Chiari malformation in human and in the genetic mouse model. It allows the assumtion that CSF leak through the myeloschisis can be a cause of Chiari malformation and induces various myeloschisis-associated anomalies. It suggests a strong direct causal relationship between the two conditions and supports the theories which involves CSF dynamics rather than those of primary mesenchymal or neural origin.

Hydrocephalus in Chiari malformation

The exact cause of hydrocephalus in Chiari II malformation is still uncertain. Chiari (1896) considered hydrocephalus as a causal mechanism speculating that the expanding intracranial lesion pushes the cerebellum into the foramen magnum. According to the literature, congenital hydrocephalus has been attributed to blockage in CSF circulation due to stenosis of the aqueduct of Sylvius (Lichtenstein, 1942), to abnormalities of the fourth ventricle (Gardner, 1977) or meninges (Weed, 1920). Padget (1972) suggested that hydrocephalus was the result of folding and fusion of the neural wall at the mesencephalon (i.e., causing aqueduct stenosis and forking) or at the metencephalo-myelencephalic junction (i.e., causing a failure of opening of the foramen of Magendie and/or Luschka of the fourth ventricle). McLone and Knepper (1989) considered that hydrocephalus is secondary to the maldevelopment of the CSF pathway in the posterior cranial fossa. Conclusively, obstruction of the outlets of the fourth ventricle, stenosis at the cerebral aqueduct, obliteration of the subarachnoid space at the level of the foramen magnum by the herniated hindbrain with caudal displacement of the outlets of the fourth ventricle, and/or obstruction at the level of the dysplastic tentorium may be the causes of the blockage of CSF outflow and, consequently, may contribute to the development of hydrocephalus. However, the hydrocephalic changes are not evident until late gestational or *in ovo* life both in the genetic model and in our study. The morphological changes of Chiari malformation precedes the development of hydrocephalus in the genetic model and possibly in the present study, which support the idea that the Chiari malformation may be the cause, not the result of the hydrocephalus. It seems likely the late growth spurt of the cerebellum and the associated disturbance in the CSF flow causes hydrocephalus in late embryonic life. Whether hydrocephalus will be accompanied in later life as a secondary phenomenon of maldevelopment of the CSF pathway in the posterior cranial fossa in our experiment, in spite of the difference of the animal used from that used by McLone and Knepper (1989), remained to be studied.

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