Journal of Korean Medical Science
Vol 4, No. 1, P 35-50, Mar. 1989

Experimental Exencephaly and Myeloschisis in Rats

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To elucidate the early sequential morphogenenetic progress of exencephaly and myeloschisis, rat embryos whose mothers had been treated with hypervitaminosis A were studied at l-day interval from gestation day 10.5 to 15.5.

In exposed animals sequential change was found in both exencephaly and myeloschisis as the embryos grew up. The 10.5-day old exencephalic embryos had still widely open cephalic neural tubes. Exencephalic embryos older than 13.5 days of gestation showed strikingly severe eversion and overgrowth of the cephalic neuroepithelium, thus failed in forming normal primitive brain. The convex dorsal surface of the exencephaly was covered with ependyma, which was connected directly with surrounding surface eqithelium at the periphery.

The earliest morphologically recognized myeloschisis was in the 13.5-day old embryos. In myeloschisis, divergence at the roof plate and eversion of the spinal neural tube, disorganized overgrowth of the neuroepithelium, malformed and misplaced spinal ganglia and nerve roots, and absence of the neural arch and dermal covering were characteristic.

It is suggested that exencephaly results from failure of the cephalic neural tube closure which is followed by eversion and overgrowth of the neuroepithelium. And failure in closure of the posterior neuropore and disturbance in the development of the tail bud probably play major role in the morphogenesis of myeloschisis.

Key Words: *Exencephaly, Myeloschisis, Vitamin A, Morphogenesis, Malformation, Teratology*

INTRODUCTION

Anencephaly, exencephaly, encephalocele, spinal and cranial meningoceles are congenital anomalies of the central nervous system which are found not infrequently in the newborns and stillbirths (McCormick, 1971; Chu and Chi, 1980; Chi and Park, 1982), and collectively called neural tube defects. Much valuable knowledge has been accumulated in the past on the etiology, pathogenesis, and methods of treatment and prevention of these serious malformations (Shenefelt, 1972), but there are still unanswered questions as far as the cause and pathogenesis of the neural tube defects are concerned(Seller, 1987).

Morphologic studies on the experimental exencephaly have confirmed remarkable overgrowth and eversion of the cephalic neuroepithelium as the common characteristic features (Cohlan, 1953; Kalter and Warkany, 1961; Langman and Welch, 1966; Smith et al., 1987; Smith and Huntington, 1981; Wood and Smith, 1984; Yasuda and Okamoto, 1986). And it seems generally approved that exencephaly is gradually converted to anencephaly by degeneration, hemorrhage, necrosis, and ultimate deletion of the exposed and malformed primitive brain tissue (Kalter

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and Warkany, 1961; Langman and Welch, 1966; Smith et al, 1978; Peters et al., 1979; Smith and Huntington, 1981; Smith et al., 1982; Wood and Smith, 1984). Several different theories, however, have been proposed concerning with the pathogenesis of the neural tube defects. Two hypotheses are relatively popular; one is that neural tube defects result from primary nonclosure of the neural tube, and two is the theory claiming the secondary rupture of once well-closed neural tube (Gardner, 1961; McLone et al, 1983; Caldarelli et al, 1985).

Meanwhile, for the spina bifida or myeloschisis several conflicting concepts have been proposed on its pathogenesis, and none of them can be applied to every form of the spina bifida (Patten, 1953; Warkany et al, 1986; Gardner, 1961; Marin-Padilla, 1966; Padget, 1970). Moreover, in myeloschisis and meningomyelocele their morphogenesis can be well explained by defective neural tube, but in cases of occult spina bifida, menigocele and meningocystocele the neural tubes are evidently closed and therefore their morphogenesis can be hardly understood by the defect in the neural tube (Barson, 1970; Emery and Lendon, 1973). Presently the most prevailing theory on the morphogenesis of myeloschisis seems to be the failure in the closure of the posterior neurpore (Brocklehurst, 1971; Copp, 1985).

Animal experiments are invaluable in the investigation of complicated developmental disorders, because the consecutive developmental stages of the malformations can be studied extensively. In neural tube defect disorders, it seems desirable to examine very early stages of the maldevelopment, during the period of neurogenesis when brain morphogenesis is well under way but the neural tube is still open, and to trace the sequential processes from the early changes immediately after the administration of a teratogen to the evident malformations at the end of gestation (Theodosis and Fraser, 1978).

MATERIALS AND METHODS

Inbred virgin Sprague-Dawley (S-D) female rats in estrus and weighing 200-250gm were mated with breeder males of the same strain, and housed in cages standard laboratory rat diet and tap water being allowed ad libitum. The day when sperms were found out on the vaginal smears was defined as day 0 of gestation.

To examine the time and mode of the neural tube closure in the rats, 2 normal pregnant rats per day were sacrificed by cervical dislocation on 8.5, 9.0, 9.5, 10.0, 10.5, 11.0 and 11.5 days of gestation, respectively. Their uterine horns were extirpated immediately and fixed in Bouin's solution for at least 24 hrs. Under dissecting microscope the embryos were dissected out of the decidua and examined thoroughly with emphasis on their shape and size, stage of neurulation, external shape of primitive brain and spinal cord, and somite numbers. Identical preparations were made for gross observation of growth and development of the normal control embryos aged from 10.5 to 15.5 days of gestation at one-day interval. Their general external shape and sagittal sectioned features were examined consecutively as the days of gestation increased. One embryo per each of the gestation days was paraffin embedded, step sectioned transversely, and H&E stained for examinations of the growth and development of their brain and spinal cord.

To provoke maternal hypervitaminosis A, A-mulsin high concentrate (manufactured by MUCOS Emulsionsgesellschaft mbH, 8192 Geretsried 1, West Germany. Reg No A 2065-Ministry of Health, West Germany) was administered orally with stomach tube in a single dose of 150,000 U of vitamin A palmitate (Koh et al, 1987) on 7.5, 8.0, or 9.5 days of gestation. Pregnant rats treated with hypervitaminosis A were sacrificed by cervical dislocation on the planned days of gestation, i.e. from 10.5 to 15.5 days at intervals of 24 hrs. Their embryos were prepared for gross and microscopic examination in the same manners as the control embryos.

RESULTS

1. Closure of neuropores in normal embryos

Examination of the normal embryos from 8.5 to 11.5 days of gestation under stereoscopic microscope at intervals of 12 hrs revealed that the cephalic neural tube closed between 10.0 and 11.0 days of gestation and posterior neuropore closed between 10.5 and 11.5 days. Neural tube closure started firstly at the cervical region.

2. Incidence of malformations in control and experimental groups

The control group comprised of 2 pregnant rats per each day from 10.5 to 15.5 days of gestation, which yielded 163 implantations in total. 156 implantations (95.7%) were normal, 5 (3.0%) absorbed, 2 (1.2%) with retarded growth, but none of the control group had CNS anomalies (Table 1.)

Table 1. Summary of pregnancy outcomes and results of maternal hypervitaminosis A in Sprague-Dawley rats

	Control	Maternal hypervitaminosis A group				
		7.5*	8.0*	8.5*	9.0*	Total
No. of dams	13	18	20	20	17	73
No. of implantation sites	163	208	203	208	203	822
Litter size	12.5	11.6	10.2	11.6	11.9	11.3
No. of normal embryos	156	161	87	113	35	396
(%)	(95.7)	(77.4)	(42.9)	(54.3)	(17.2)	(48.2)
No. of resorptions	5	13	52	69	85	219
(%)	(3.0)	(6.2)	(25.6)	(33.2)	(41.9)	(26.6)
No. of embryos with malformations in						
CNS	0	29	63	25	59	176
(%)	(0.0)	(13.9)	(31.0)	(12.0)	(29.1)	(21.4)
Extra-CNS** only	2	5	1	1	24	31
(%)	(1.2)	(2.4)	(0.5)	(0.5)	(11.8)	(3.8)

Note: *; the days of gestation when excess vitamin A was administered
**; including embryos with retarded growth, abnormal-shaped tails, or other externally overt malformations
% represents percent of implantation sites.

Table 2. Incidence of CNS malformation, tail deformity, and growth retardation induced by maternal hypervitaminosis A in Sprague-Dawley rats

	Control	Maternal hypervitaminosis A group				
		7.5*	8.0*	8.5*	9.0*	Total
No. of implantations	163	208	203	208	203	822
No. of embryos with						
Exencephaly	0	29	63	19	43	155
(%)	(0.0)	(13.9)	(31.0)	(9.1)	(21.2)	(18.9)
Myeloschisis	0	0	0	0	5	5**
(%)	(0.0)	(0.0)	(0.0)	(0.0)	(2.5)	
Head distension	0	0	0	6	14	20**
(%)	(0.0)	(0.0)	(0.0)	(2.9)	(6.9)	
Tail deformity	0	0	0	0	38	38**
(%)	(0.0)	(0.0)	(0.0)	(0.0)	(18.7)	
Growth retardation	2	7	7	16	33	63
(%)	(1.2)	(3.4)	(3.4)	(7.7)	(16.3)	(7.7)

Note: *; the days of gestation when excess vitamin A was administered
**; The percentage of myeloschisis, head distension, and tail deformity in total is meaningless and not presented.
% represents percent of implantation sites.

Maternal hypervitaminosis group consisted of 73 pregnant rats, 822 implantation. 393 embryos (48.2%) were normal in external shape and size. In 26.6% (219 implantations) the embryos were absorbed. 176 embryos (21.4%) had CNS malformations. 31 embryos (3.8%) had extra-CNS malformations, of which 24 had malformed tails and/or abnormal posterior limb buds; all of the 24 embryos with malformed tails were offsprings of the mother rats treated with excess vitamin A on their 9th day of gestation (Table 1). Exencephaly was found out in 155 embryos (18.9%). Myeloschisis was found only in 5 embryos and all of their mother rats had been treated with vitamin A on their 9th day of gestation (Table 2).

Fig. 1. A treated 10.5-day old embryo showing widely separated neural folds with the neural tube lumen(*) open dorsally.

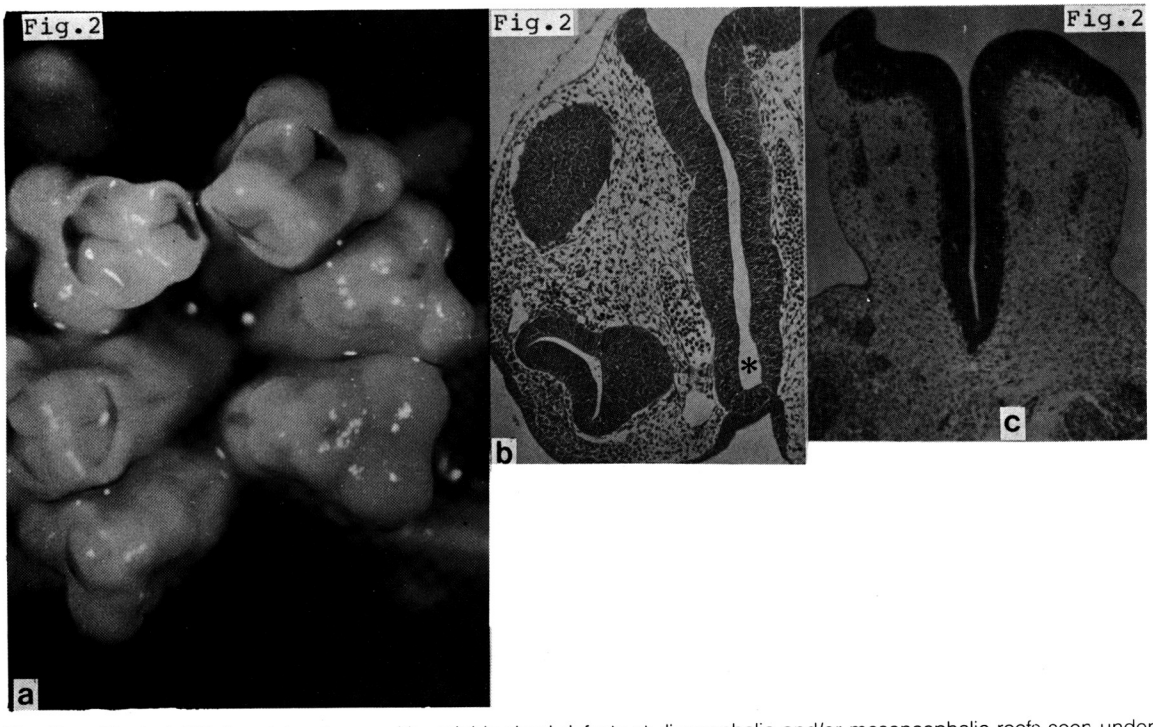


Fig. 2. a. Treated 11.5-day old embryos with variable-sized defects at diencephalic and/or mesencephalic roofs seen under stereoscopic microscope. b & c. H & E stained light microscopic features of the transverse sections at diencephalic level(b) and mesencephalic level(c) confirming that the defects are at roofs of diencephalon and mesencephalon. * in b marks the future 3rd ventricle.

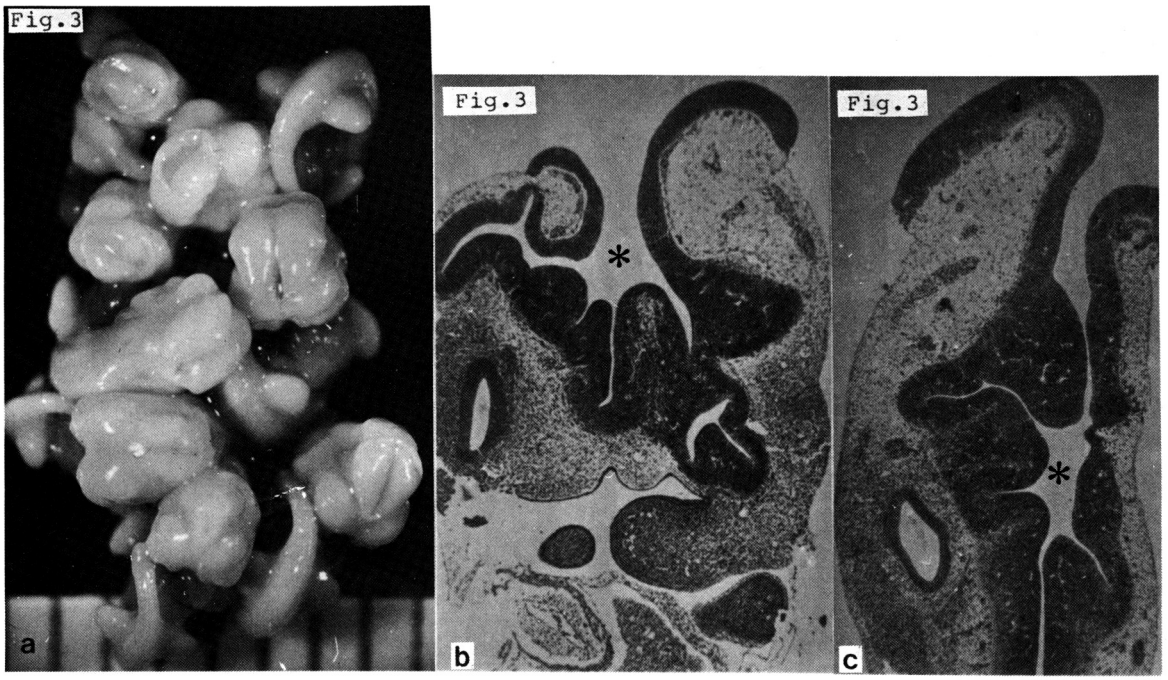


Fig. 3. Treated 12.5-day lod embryos; dissecting microscopic view (a), and H & E stained transverse sections at diencephalic (b) and mesencephalic (c) regions. The neural tube defects are located at diencephalic and mesencephalic roofs, and at rim of the defects the neuroepithelium shows mild eversion. * in b & c marks the future 3rd and 4th ventricles respectively.

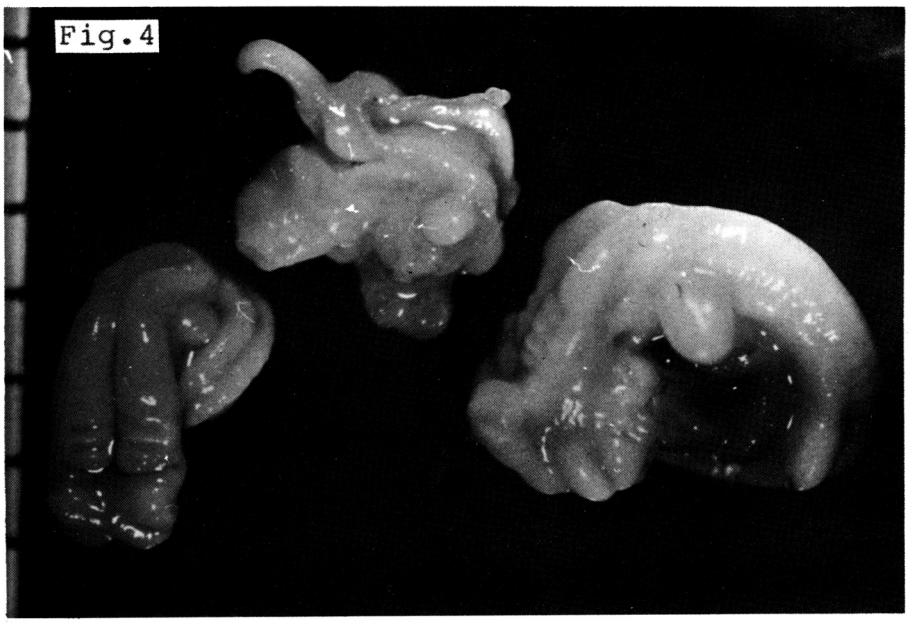


Fig. 4. Treated 12.5-day old embryos affected by craniorachischisis totalis.

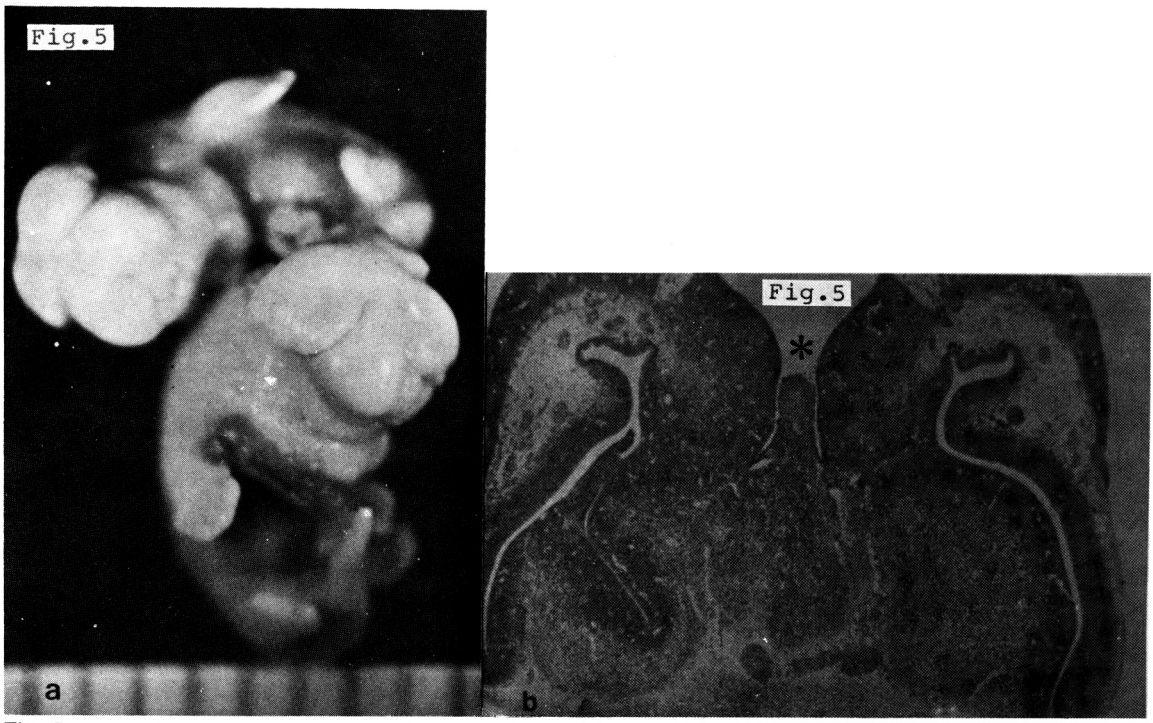
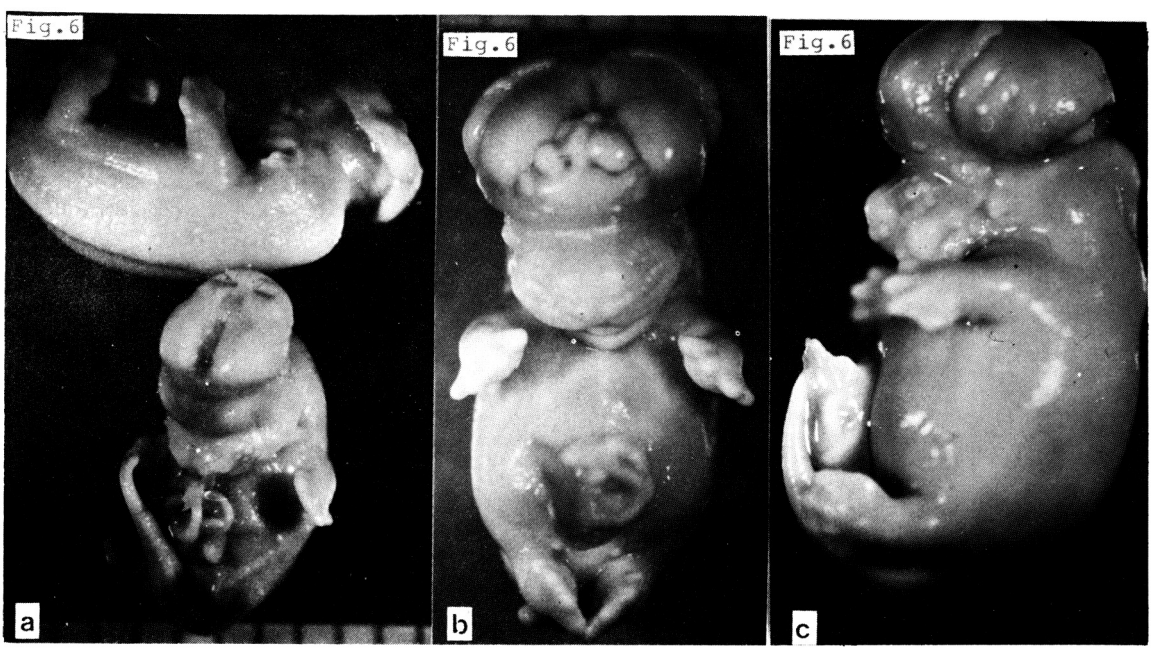


Fig. 5. Treated 13.5-day old embryos showing the typical of exencephaly. a. stereoscopic microscopic view. b. morphology H & E stained transverse section at diencephalic level. *points the dorsally open future 3rd ventricle; bilateral lateral ventricles look normally developing.



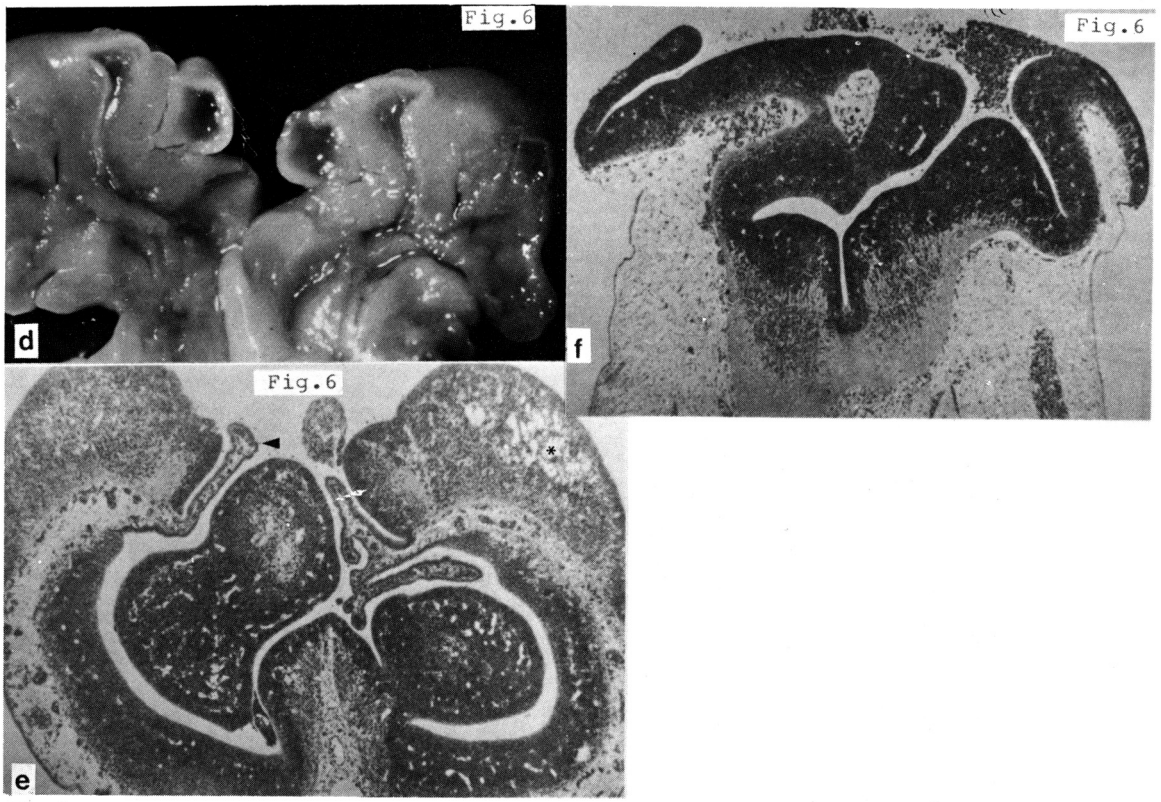


Fig. 6. a, b, and c. Stereoscopic microscopicviews of 14.5 (a), and 15.5 (b & c)-day old exencephalic embryos. d. A sagittal section of the exencephalic head reveals that the everted area is diencephalon and mesencephalon. e & f. H & E stained transverse sections at diencephalic (e) and mesencephalic (f) regions; in e which was sectioned through inter ventricular foramina, the arrow head indicates choroid plexus and * marks widened intercellular spaces within the exposed brain tissue.

3. Morphologic observation of the experimental group

Morphologic study of the exencephaly and myeloschisis in the sequence of age of the rat embryos revealed the following results. Cephalic neural tubes of the 10.5-day old exencephalic embryos remained unclosed; their cephalic neural folds were widely separated and the neural tube lumina were open dorsally from the otic placode to the anterior neuropore (Fig.1). The 11.5-day old exencephalic embryos had variable-sized defects at diencephalic and/or mesencephalic roofs. Light microscopic exam confirmed that the defects were located at diencephalic (3rd ventrucle) and/or mesencephalic roofs (Fig.2). The 12.5-day old exencephalic embryos showed mild eversion of the neuroepithelium at the rim of the neural tube defects. The roofs of the 3rd ventricle and/or mesencephalon were open (Fig. 3). Some had craniorachischisis totalis (Fig.4). In the 13.5-day old exencephalic embryos, eversion and overgrowth of the neuroepithelium constituting lateral walls of diencephalon and/or mesencephalon were so prominent that the superior surface of the head was topped with the exencephalic brain tissue resembling a cap. The 3rd ventricle was opened at the roof and the lateral walls, i.e diencephalon was severaly deformed, while lateral ventricles were normally developing (Fig. 5). The 14.5 and 15.5-day old embryos showed a typical exencephaly (Fig. 6). On both stereoscopic exam of sagittal sections and light microscopic exam of transversely sectioned specimens, the everted portion of the exencephalic tissue were absent, but microscopically the exencephalic brain tissues of the 15.5-day old embryos contained multiple foci of widened intercellular spaces and hemorrhage (Fig. 6-e, 7).

In every exencephalic embryo the dorsal surface of the exposed brain was covered with the ependymal lining which was connected directly to the surface epithelium at the peripheral border of the exencephaly

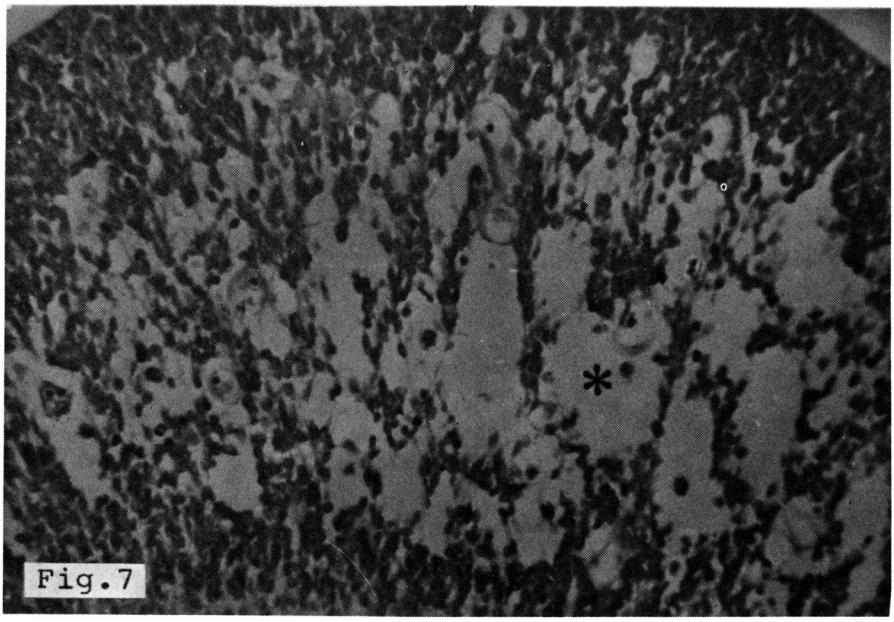


Fig. 7. Widened intercellular spaces (*) within the exposed brain tissue of a 15.5-day old embryo with exencephaly.

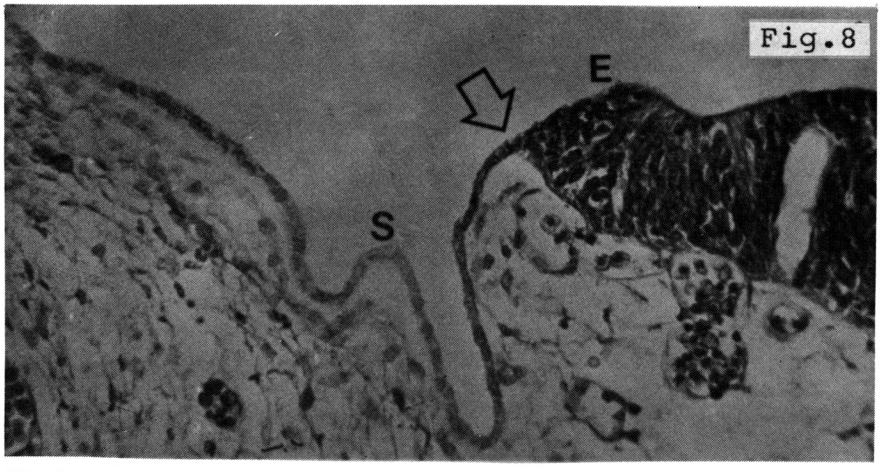


Fig. 8. Direct continuation of ependyma (E) andsurface epithelium (S) at the periphery of an exencephaly.

(Fig.2-b & c, 3-b & c, 5-b, 6-e & f, 8). No remarkable difference in the basis morphology of the exencephaly was recognized among the affected embryos. Exencephalic embryos were commonly accompanied by severe growth retardation or even degeneration or even degeneration of their body. Some other anomalies such as looked nearly absorbed, and these embryos were often associated with anophthalmia, microphthalmia, microtia, malformed mandibles, macroglossia and kindred facial anomalies (Fig.6,9,10). Amniotic fluid of the exencephalic embryos appeared turbid than that of normal embryos, containing debris. It was also noted that the exencephaly was almost always associated with depression of the 4th ventricle roof and shrinkage of the forebrain (Fig. 9), which was probably caused by leakage of CSF out of the ventricles via defects of the neural tube.

4. Observation on myeloschisis

All of the myeloschisis were located at the lum-

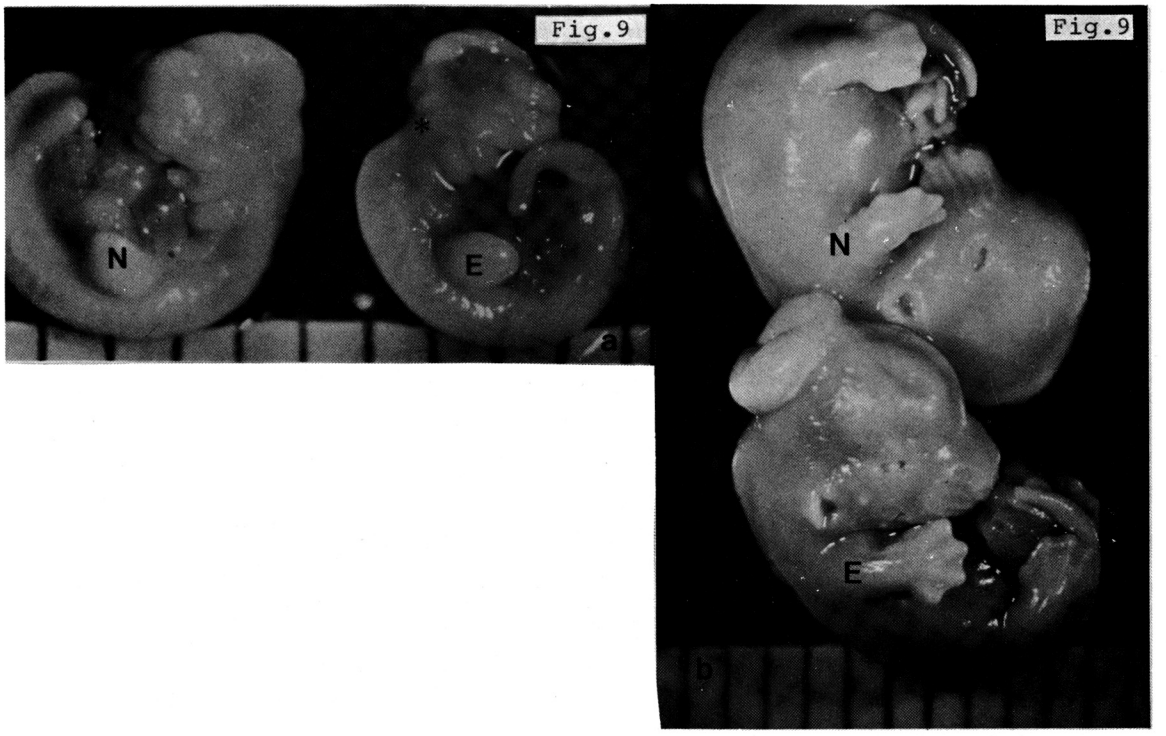


Fig. 9. In comparison with normal embryos (N),exencephalic embryos (E) had depression at 4th ventricle roof (a, *) and shrinkage at forebrain (b). It is probably caused by CSF leakage out ofthe ventricle via exencephaly. Embryos in figure b have microphthalmia.

bosacral region. Three of them were associated with exencephaly (so-called double neural tube defects) (Fig.10). The earliest embryos found to have myeloschisis were the 13.5-day old ones, and many of the 11.5 and 12.5-day old embryos whose mothers had been treated with excess vitamin A on their 9th day of gestation had such malformed tails as short, blunt-tipped, or curved ones (Fig.11). Myeloschisis in the 13.5, 14.5 and 15.5-day old embryos was similar in shape, becoming gradually larger and more prominent as the age of the embryos increased (Fig. 10,12,13). Grossly the myeloschisis looked like a mushroom like protrusion at lower back with irregular pits and furrows on its dorsal surface (Fig.10). Distal to the myeloschisis tails were either absent or malformed (Fig.10,12). On microscopic exam the myeloschisis was formed by focal over growth of the primitive spinal cord neuroepithelium(Fig. 13, 16). The primitive spinal cord architecture was quite irregular and disorderly. Two or three neural tube lumina were often found in a myeloschisis, which were diverted, everted and open dorsally. The primitive white matter, groups of primitive nerve cells that would presumably develop to form anterior horns, spinal ganglia and nerve roots were all abnormal in shape and location. Dorsal surface of the myeloschisis was not covered with the surface epithelium but with the ependyma, and the ependymal cells and the surface epithelial cells were connected directly with each other at the periphery of the myeloschisis as in the case of the exencephaly (Fig.13,16).

Proximal to the myeloschisis where the dorsal aspect was covered with intact epithelium and mesenchymal structures was located malformed spinal cord consisting of remarkable neuroepithelial overgrowth and deformed central canal, which transited more proximally into normal cord (Fig.14). In a 13.5-day old embryo with a curved tail, a midline streaky depression was identified at lumbosacral region under stereoscopic microscope (Fig.12-c); on light microscopy the spinal neural tube had a defect at its roof plate although the overlying mesodermal and epithelial coverings were intact (Fig.15). The everted and overgrown neuroepithelium in the myeloschisis of the 14.5 and 15.5-day old embryos contained multifocal widened intercellular spaces and hemorrhage (Fig.16). The primordia of the vertebral bodies and notochord were normal, but the neural arches had

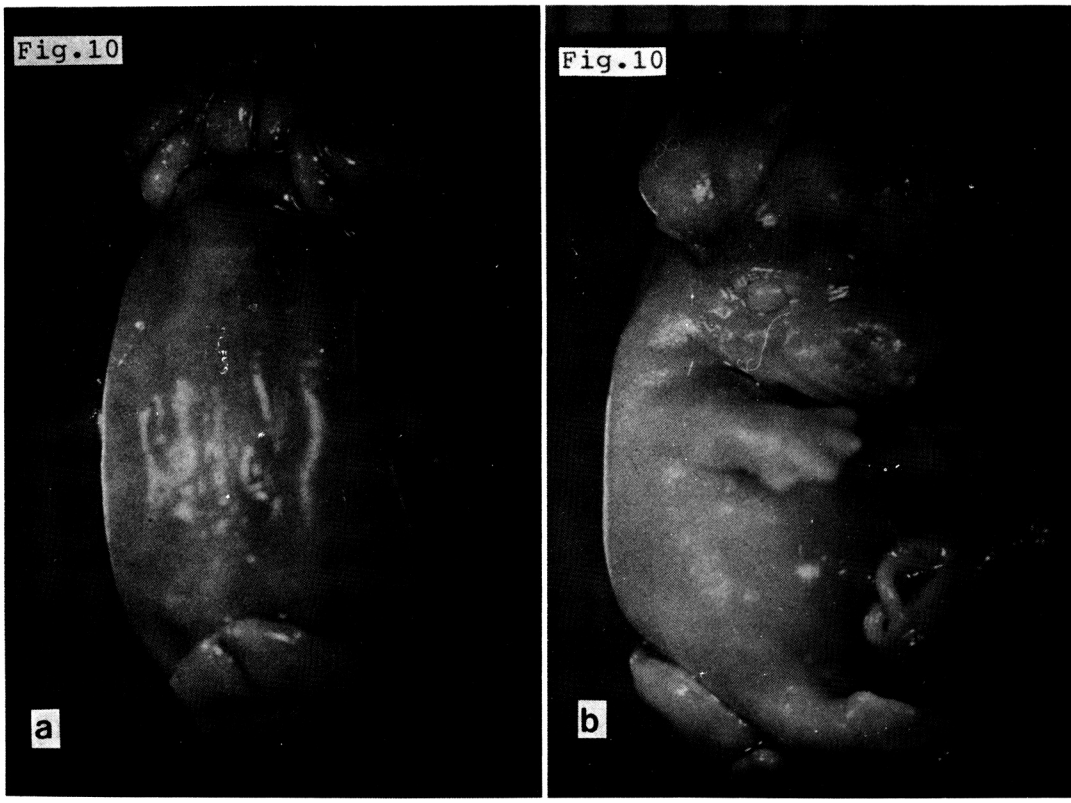


Fig. 10. 15.5-day old embryos with double neural tube defects.

failed both in fusing dorsally and in encircling the myeloschitic cord (Fig.13,16). A wide loosely cellular space was found in a 15.5-day old myeloschitic embryo between the myeloschitic cord and the vertebral body primordium (Fig.16).

DISCUSSION

The animal experimental model of inducing CNS malformations in the rat offsprings with maternal hypervitaminosis A was firstly introduced by Cohlan. Biochemical basis and morphogenetic mechanism of the fetal malformations induced by maternal hypervitaminosis A are unknown (Marin-Padilla & Ferm, 1965; Morriss & Steele, 1974; Morriss & Thompson, 1974; Irving et al, 1986). Teratogenic action of maternal hypervitaminosis A is not confined to either any species or any organs, but depends on the dose of vitamin A administered and the developmental stage of the embryos at the time they are exposed to the drug (Kalter & Warkany, 1961; Langman & Welch, 1966; Morriss, 1972; Shenefelt, 1972; Kistler, 1981). In this experiment embryonic neural tube defect was induced by single p.o. administration of 150,000 U of vitamin A palmitate to pregnant rats at the critical period. Embryonic absorption rate was 26.8%, and CNS malformations was induced in 21.4%. Although this method is easy to perform and well-known as a consistently effective measure of inducing neural tube defects in experimental animals, it is recommended to administer smaller doses of vitamin A for 2-3 days during the critical period in order to increase CNS malformation rate (Langman & Welch, 1966; Smith et al, 1978). The dosage that we have tried is far higher than the conventional therapeutic dose of vitamin A.

Neural tube closure is a very important stage of the neurulation, but much remains to be elucidated about the process (Gordon, 1985). In rodents neural tube closure starts initially at the cervical region and the anterior neuropore and mesencephalic roof close lastly in the cephalic neural tube. The posterior neuropore closes a little later than the cephalic region, and is the lastly closing are of the whole neural tube (Geelen & Langman, 1977; Kaufman, 1979; Schoenwolf, 1979; Jacobson & Tam, 1982; Steele et al, 1983; Irving et al, 1986; Yasuda et al, 1987). Examination of the control embryos in this study from 8.5 to 11.5 days of gestation under stereoscopic microscope at

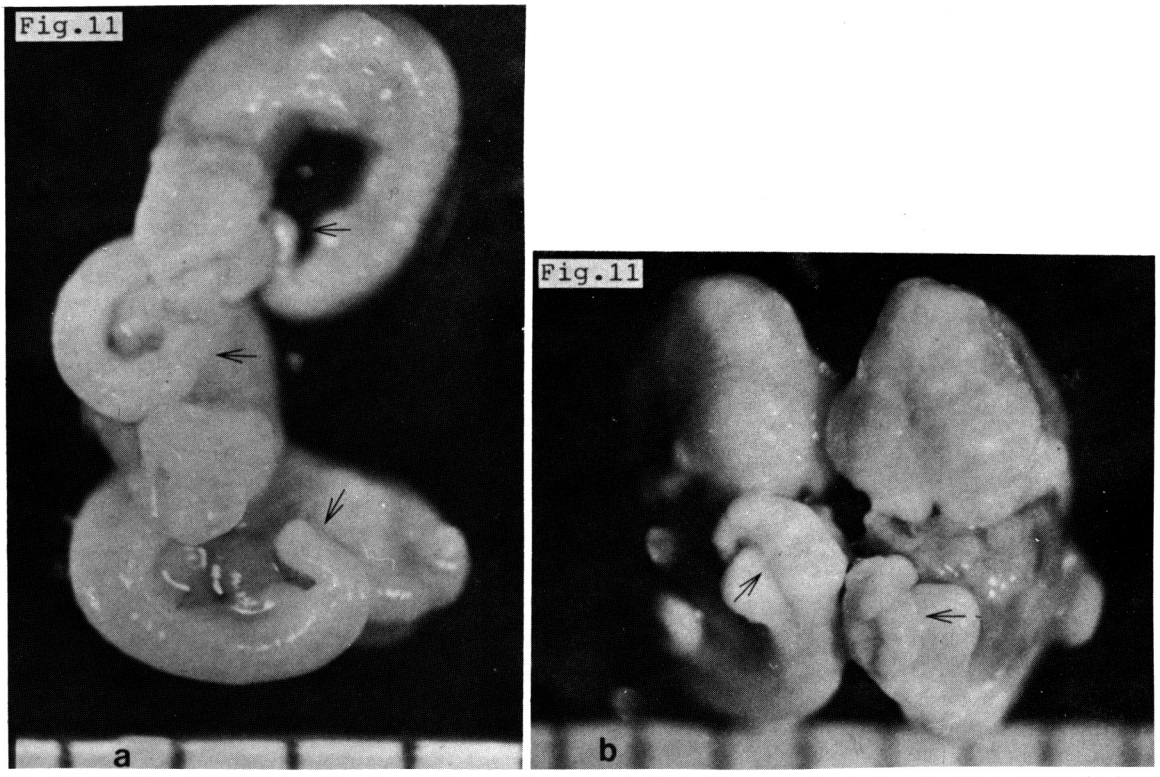


Fig. 11. 11.5 (a) and 12.5 (b)-day old embryos whose mothers were treated with excess vitamin A on the 9th gestation day have blunt malformed tails (arrows).

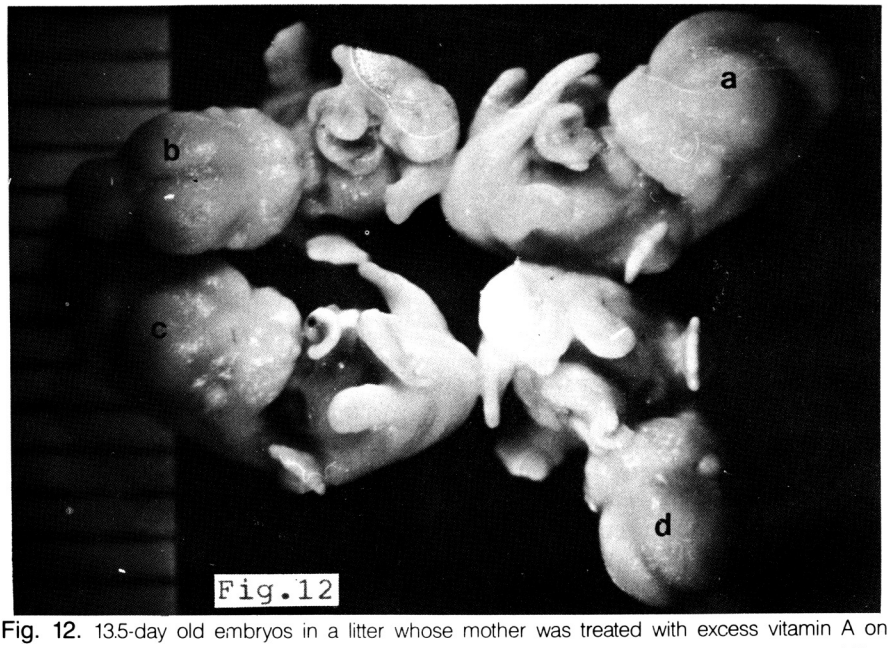


Fig. 12. 13.5-day old embryos in a litter whose mother was treated with excess vitamin A on the 9th gestation day. a. normal tail. b. myeloschisis and malformed tails. c. curved tail; a midline streaky depression is seen at lumbosacral region. d. malformed tail.

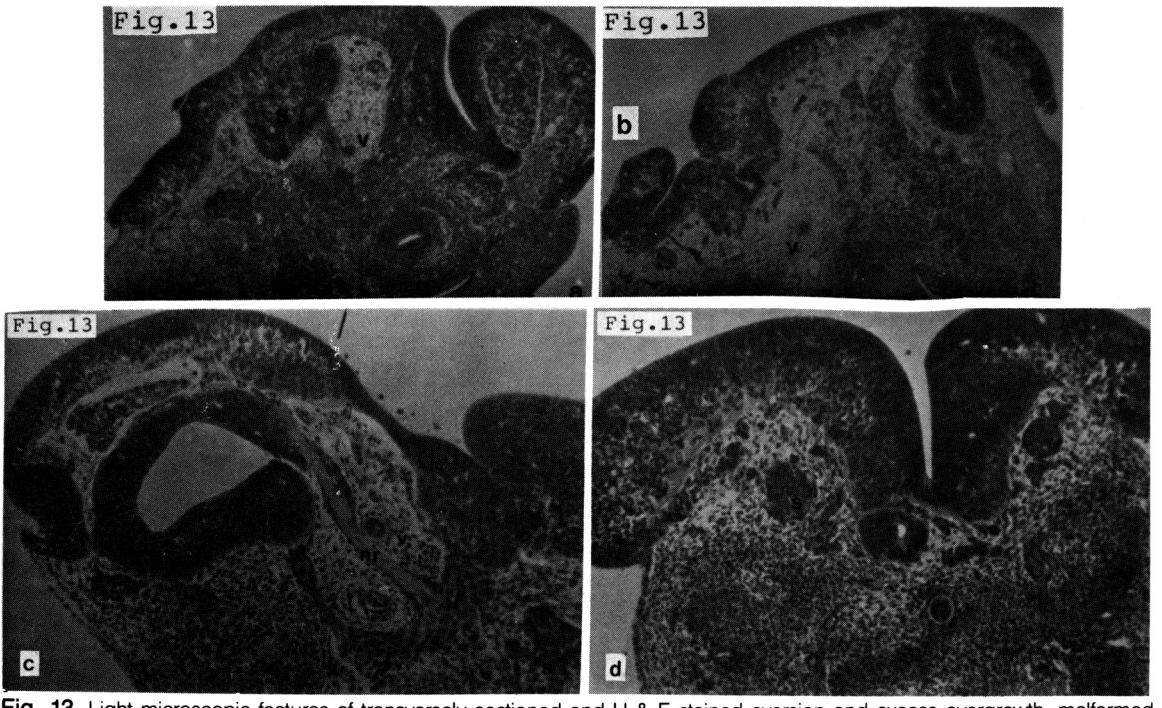


Fig. 13. Light microscopic features of transversely sectioned and H & E stained eversion and excess overgrowth, malformed and mislocated spinal ganglia(s), nerve roots(nr) and vertebral body primordia(v) made a strikingly disorganized architecture.

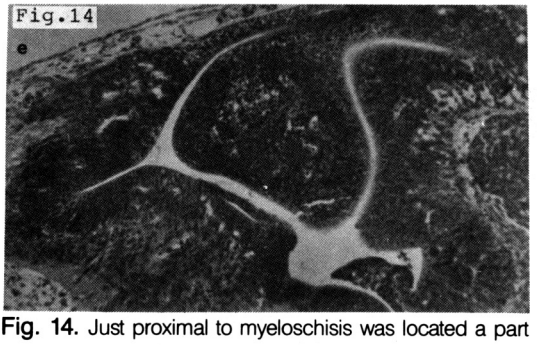


Fig. 14. Just proximal to myeloschisis was located a part of spinal cord with remarkable neuroepithelial overgrowth and irregularly branched central canal; overlying mesodermal and cutaneous ectodermal coverings were intact.

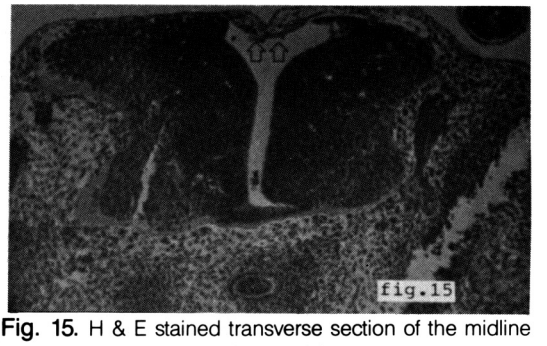


Fig. 15. H & E stained transverse section of the midline streaky depression at lumbosacral region of the embryo presented in figure 12-c. Underneath intact epithelial and mesodermal coverings a defect was seen at the roof plate of the primitive spinal cord(arrows).

intervals of 12 hrs disclosed findings consistent with above-mentioned previous reports.

It is agreed that the teratogenic action of the agent can be exerted only when it is delivered at the critical period (Shenefelt, 1972). In this experiment the neural tube closed in gestation day 10.0-11.5; cephalic neural tube closed in 10.0-11.0 days of gestation and posterior neuropore shut in 10.5-11.5 days. Vitamin A was administered once transorally during 7.5-9.0 days of gestation. Therefore there was an evident discrepancy between the time of vitamin A adminstration and the time of morphologically visible neural tube closure. It is unclear why such a gap exists. It might be explained by the possibility that vitamin A exerted prolonged effect within the body of treated pregnant rats (Langman & Welch, 1966) or that the vitamin acted upon the process of the embryonic neural tube closure preceding and therefore impossible to recognize by observing external morphology as in this experiment.

Geelen et al reported that in mouse embryos treated

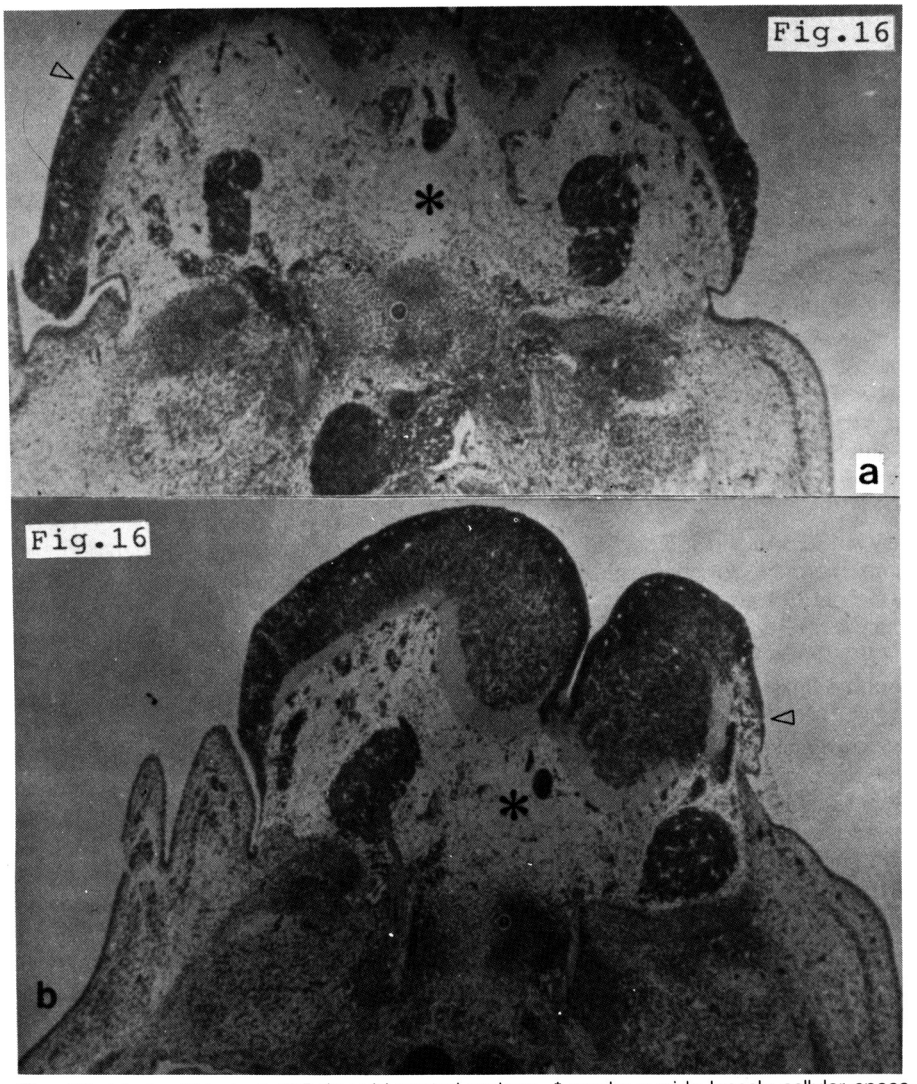


Fig. 16. Myeloschisis in a 15.5-day old treated embryo. * marks a wide loosely cellular space between the diverted spinal cord and the vertebral body primordium in amyeloschisis. The arrow head indicates widened intercellular spaces withIn the exposed spinal neuroepithelial tissue. The neural arch failed both in fusing dorsally and in encircling the everted and overgrown spinal cord.

with maternal hypervitaminosis A the neural walls folded later ally and became widely separated at 18-24 hrs after treatment and proposed increased intercellular spaces at the apical side of the neuroepithelium as the major cause of failure in neural tube closure (Geelen et al, 1980). Irving et al investigated hamster embryos whose mothers had been treated with p.o. isotretinoin once on gestation day 8. They found marked delay in neural fold apposition and also failure in neural tube closure at midbrain and hindbrain regions at 8 hrs. At 12 hrs failure in anterior neural tube closure and collapse of the forebrain could be seen, and persistent schisis at midbrain and forebrain at 24 hrs after vitamin A treatment (Irving et al, 1986). According to Wood & Smith (1984) filopodia and lamellopoida, the predominant mode of initial neural fold contact in the controls, never made contact above the cervical region and exencephaly resulted in S-D rats.

Langman & Welch (1966) reported slight eversion of the lateral wall of diencephalon and mesencephalon in 12-day old rat embryos, and concluded that large

doses of vitamin A prevented the neural groove from closing possibly by interfering with the mitotic activity of the neuroepithlial cells.

In this experiment the major findings in 10.5 and 11.5-day old treated embryos were focal neural tube defects or nonclosure at diencephalic and/or mesencephalic regions largely in accordance with the above-mentioned authors. In addition, though the defects sized variable, the ependyma and epithelial cells were clearly in direct and orderly contact with each other at the margin of the defect in every case.

In the 12.5-day old embryos neural tube defects were found also at the roofs of diencephalon and/or mesencephalon. The defects sized also variable, but their size was smaller than that in younger embryos. It deserves emphasis that the rim of the defects showed slight eversion at this age (Langman & Welch, 1966).

Exencephaly in 13.5, 14.5 and 15.5-day old embryos and the same morphologic characteristics were described by authors (Cohlan, 1953; Kalter 7 Warkany, 1961; Langman & Welch, 1966; Smith et al, 1978; Peters et al, 1979; Smith & Huntington, 1981; Smith et al, 1982; Wood & Smith, 1984; Yasuda et al, 1986; Koh et al, 1987). Though macroscopic necrosis or hemorrhage were not found in the exposed neuroepithelium of exencephaly and myeloschisis of 14.5 and 15.5-day old embryos, microscopic exam disclosed multifocal widened intercelluar spaces. This is probably an early change preceding the future degeneration, necrosis and deletion of the exposed neuroepithelium. Peters et al (1979) reported perivascular edema and widened intercellular spaces progressing to hemorrhage in the tissues of exencephaly and myeloschisis at gestation day 15 and degeneration and necrosis of neuroblasts on gestation day 17, and proposed that degeneration and necrosis might be caused by disturbance in vascular circulation.

Wood & Smith (1966) described incread extracellular space in the exposed brain tissue on the 15th gestation day and asserted that the cause of the deletion of the malformed brain tissue occurring on gestation day 19-21 was still unclear.

It is suggested by the above discussion that failure in the closure of the cephalic neural tube, i.e. roofs of diencephalon and/or mesencephalon, is the primary lesion of the exencephaly. The following eversion and overgrowth of the neuroepithelium and lack of mesodermal and epithelial coverings at the region with neural tube nonclosure progress to form the eventual morphology of the exencephaly. The 10.5-day old embryos were too small to make well-oriented tissue sections and to investigate the early microscopic changes concerned with nonclosure of the neural tube. It is recommended to study the early neuroepithelial changes with scanning electron microscopy from several hrs after a teratogen is adminstered till the known time of complete neural tube closure in the animal, in order to investigate the earlier morphological events involved in noclosure of the neural tube.

In this experiment myeloschisis was found only in the embryos whose mothers had been treated with excess vitamin A on their 9th day of gestation, and none whose mothers had been treated on their gestation day 7.5, 8.0, or 8.5 had myeloschisis. This results once again show that types of the CNS malformations determined by the developmental stage of the embryos when the teratogen is administered.

Myeloschisis was not identified by exam under stereoscopic microscope in 10.5, 11.5 and 12.5-day old embryos in this experiment. Warkany et al reported branched central canal and irregularly-shaped spinal neural tube as an early change in 11-day old treated embryos (Warkany et al, 1958). Many of 11.5 and 12.5-day old embryos treated with maternal hypervitaminosis A on the 9th gestation day had malformed tails, and tails of the myeloschitic embryos were also malformed in this experiment, but the authors failed in identifying embryos with significant delay in the closure of posterior neuropore. It seems interesting that the location of embryonic posterior neuropore and the site where secondary neurulation starts are the future lumbosacral region, the area frequently involved by the spina bifida in human and in animal experiments (Barson, 1970; Schoenwolf, 1979; Copp, 1985).

Gross and microscopic features of the myeloschisis found in this experiment are similar to those described by other authors (Warkany, 1985; Peters et al, 1979; Wiley, 1983). Grossly myeloschisis appeared a mushroom-shaped protrusion at the lower back. Representative microscopic features could be summarized as focal diversion and eversion of the primitive spinal cord at its dorsal portion associated with remarkable neuroepithelial overgrowth. The histologic architecture of the primitive spinal cord at the myeloschitic area was strikingly disorganized. Based on these morphologic findings myeloschisis in this experiment seemed less likely to have been caused by secondary rupture of already closed spinal neural tube, but more likely to have been formed by primary nonclosure of the spinal neural tube whatever the initial mechanism had been.

In 15.5-day old embryos of myeloschisis a wide loosely cellular space was located between the dorsally diverted spinal cord and the vertebral body primordia. Warkany et al described similar spaces to this, which they defined as widened subarachnoid space progressing to the large CSF-filled cavity fo meningomyelocele in fetus (Warkany et al, 1958). Multifocal widened intercellular spaces and hemorrhage were found within the myeloschitic cord tissues of 15.5-day old embryo, which are probably an early change of the future degeneration as in the case of exencephaly.

It is suggested that failure in posterior neuropore closure and the following disturbance in the tail bud formation and the secondary neurulation are the initial morphogenetic abnormalities of the myeloschisis.

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