Congenital Malformations of the Central Nervous System in Rats Produced by Maternal Zinc Deficiency [1]

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ABSTRACT   Teratogenic effects of maternal zinc deficiency in rats have been observed, confirming previous reports. The deficient diet differed in several respects from that used by Hurley and coworkers but the results were essentially the same. Special attention was given to malformations of the central nervous system and to tissue anomalies not recognizable by gross inspection of the fetuses.

Hurley and Swenerton ('66) demonstrated that a zinc-deficient diet fed female rats throughout pregnancy results in malformations of many types in a high percentage of the young. One of the most interesting results of these experiments was that it was possible to obtain malformations by transitory deficiencies limited to a few days of gestation (Hurley et al., '71). Such short periods of zinc deprivation lead to a rapid fall of zinc levels in the maternal blood plasma (Hurley, '69) as pregnant rats cannot mobilize zinc from body tissues in amounts sufficient to supply the needs of the fetuses (Hurley and Swenerton, '71). These experiments exemplify a situation in which pregnant mammals fail to protect their embryos from a nutritional deficiency although they have stores of the nutrient in their body. This behavior differs from many other situations in which the embryos draw from the maternal tissue stores in case of dietary deprivation. Apgar ('68) found that the reproductive performance of pregnant rats fed a diet deficient in zinc can be much improved if the animals are given progesterone and estrone or if a small amount of zinc is added to their drinking water (Apgar, '70). Cox et al ('69) did not obtain malformed fetuses when pregnant rats were fed a zinc-deficient diet although the mothers' weight gain was much depressed and the fetal weights were reduced by the dietary deprivation. Mills et al. ('69), however, observed hy-drocephaly, microphthalmia, and reduced fetal weight in the offspring of female rats that received a diet low in zinc before mating, and a diet containing only 1 ppm of zinc during gestation.

In order to resolve these differences and to study further the role of essential trace metals in fetal development we are examining the effects of controlled intake of zinc and copper in drinking water of pregnant rats receiving a semipurified diet deficient in both zinc and copper. In preliminary experiments we have confirmed that malformations can be produced in fetuses of rats fed a zinc-deficient diet throughout pregnancy and have given special attention to the malformations of the central nervous system observed in some of the young. It seems significant that the experiments of Hurley and Swenerton ('66) could be confirmed although our zinc-deficient diet differed in several respects from that of the original authors.

MATERIALS AND METHODS

Female Wistar rats were given for adaptation for 4 days prior to mating a semipurified diet and drinking water containing 10 μg of zinc (as zinc acetate) and 2 or 4 μg of copper (as copper sulfate) per ml. After mating, when sperm had been found in the vagina (called the 1st day of pregnancy), the drinking water was changed

to water that contained no zinc but provided 2 or 4 μg of copper per ml.

The diet had the following percentage composition: Dried egg white 20.0, corn starch 62.5, cellulose powder 3.0, corn oil 9.0, salt mix 4.0, water-soluble vitamin mix 0.5, fat-soluble vitamin mix 1.0, and choline chloride 0.15. The salt mix given was the Wesson modification of the Osborn-Mendel mix without copper. The water-soluble vitamin mix (prepared in cornstarch) consisted of riboflavin 4.0, thiamine HCl 4.0, pyridoxine HCl 2.0, calcium pantothenate 12.0, niacinamide 20.0, folic acid 0.1, inositol 80.0, menadione 0.4, biotin 0.04, and cyanocobalamine 0.002 mg/g of mix. The fat-soluble vitamin mix (prepared in corn oil) consisted of calciferol 20.0 μg, and vitamin A palmitate 6.0, and α-tocopherol acetate 20.0 mg/g of mix.

Rats with surviving young were killed on the 20th or 21st day of pregnancy. The young were weighed, examined by inspection, and fixed in Bouin's solution for sectioning or in alcohol for clearing. A number of young were selected for histologic examination because of recognizable malformations of the head. The sections were stained with hematoxylin-eosin.

RESULTS

Thirty females were used in the preliminary experiment and 25 of these carried their young to term or near term. The 193 fetuses obtained on the 21st day of gestation had a mean weight of 2.36 g as compared to the mean weight of 3.71 g of control fetuses of the same gestational age. After inspection, clearing, or sectioning, 136 of the 193 young (70.5%) were found to be abnormal. A variety of malformations were recognized in many organs, including the central nervous system (CNS).

Among the 136 abnormal young only three had exencephaly whereas 40 had externally recognizable hydrocephaly; other anomalies were discovered after histological sectioning. Exencephalies and most hydrocephalies diagnosed by inspection showed additional lesions by microscopic examination. Of great interest were microscopic tissue malformations which were always present in grossly deformed speci-

Fig. 1 Head of a rat fetus with exencephaly.

mens but sometimes also in animals whose heads seemed externally normal. Such animals were sectioned because of an abnormal eye or palate or because of an obvious CNS malformation of a littermate. Spinal cord anomalies were found in fetuses that appeared short and had abnormal tails.

Exencephaly

A rat fetus (21st day) with exencephaly is illustrated in figure 1. The exencephaly is an extensive lesion that covers the head from its frontal to its occipital ends, sitting like a cap over the skullbase. Frontal serial sectioning revealed absence of eyeballs and optic nerves although there were rudimentary eye lids on both sides. Large portions of the forebrain were seen on the surface of the head over the nasal cavities not covered by skull bones and meninges (fig. 2). The exencephalic brain appeared much overgrown in transverse as well as in anteroposterior direction. The illustration shows a distorted open neural plate, similar to those described in specimens deformed by other teratogenic procedures. The plate, situated on top of the head, is separated from the cranial base
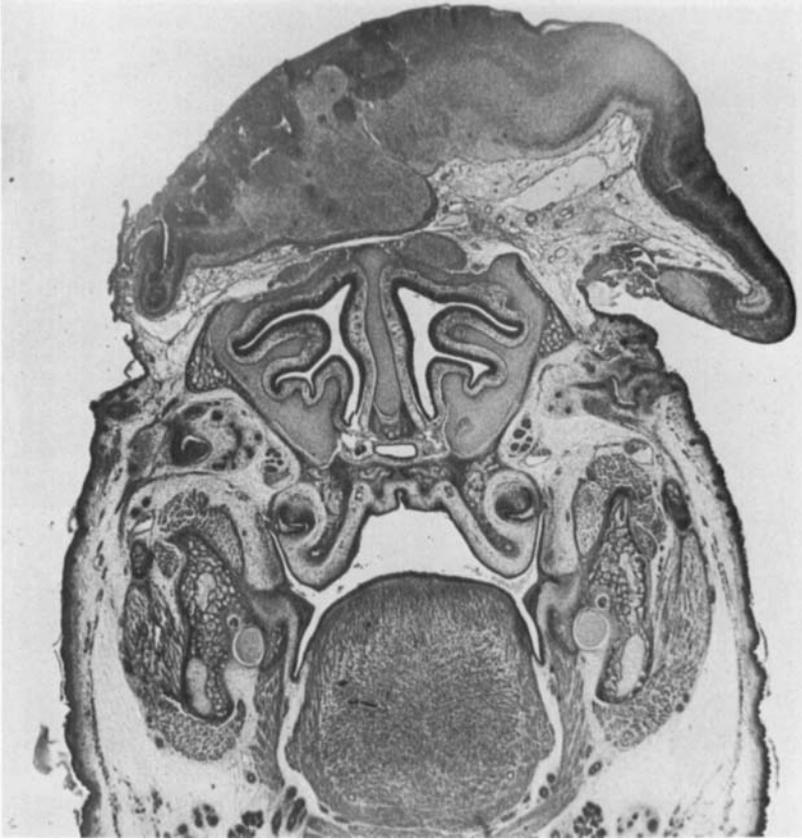
Fig. 2 Frontal section through the head of fetus with exencephaly showing prosencephalic neural plate over the nasal cavity. There are no eyes but lids can be seen on the animal's left side. The palate is high but not cleft, as indicated by the nasopharyngeal duct. × 15.

by a loose meshwork of connective tissues and blood vessels; it is relatively thick near the midline and thins out laterally. Some differentiation into cortical layers is seen on both sides and rosettes are noticeable in the external cortical layers (fig. 2). The lateral ends of the plate represent the parts that should have moved dorsad to close the forebrain vesicle. This is indicated by the displaced plexus chorioidei, seen at the lateral ends of the plate, which are directed toward the amniotic cavity instead of the ventricles. Below the nasal septum there is a narrow nasopharyngeal duct and there is no cleft palate; but the roof of the mouth is arched and in the midline there is a narrow palatine furrow directed upwards. This feature is of interest since such narrow palatine grooves are seen in anencephalic children in whom they can

be mistaken for palate clefts although they do not establish communications between the oral and nasal cavities. In a more caudal section, through the diencephalic area (fig. 3), the brain appears thicker than in the previous section although it too consists of an open neural plate. The upper (dorsal) terminations of the skin are seen on both sides of the cranial base; the cutaneous layers end abruptly and are contiguous on the animal's right with a thin layer of epithelium and on the left with choroid plexus. The connection of the diencephalon with the anterior lobe of the pituitary gland is drawn out and elongated but the pituitary appears large and well developed. This picture resembles those seen in rats with exencephaly produced by various other teratogens where the pituitary assumes balloon shape in
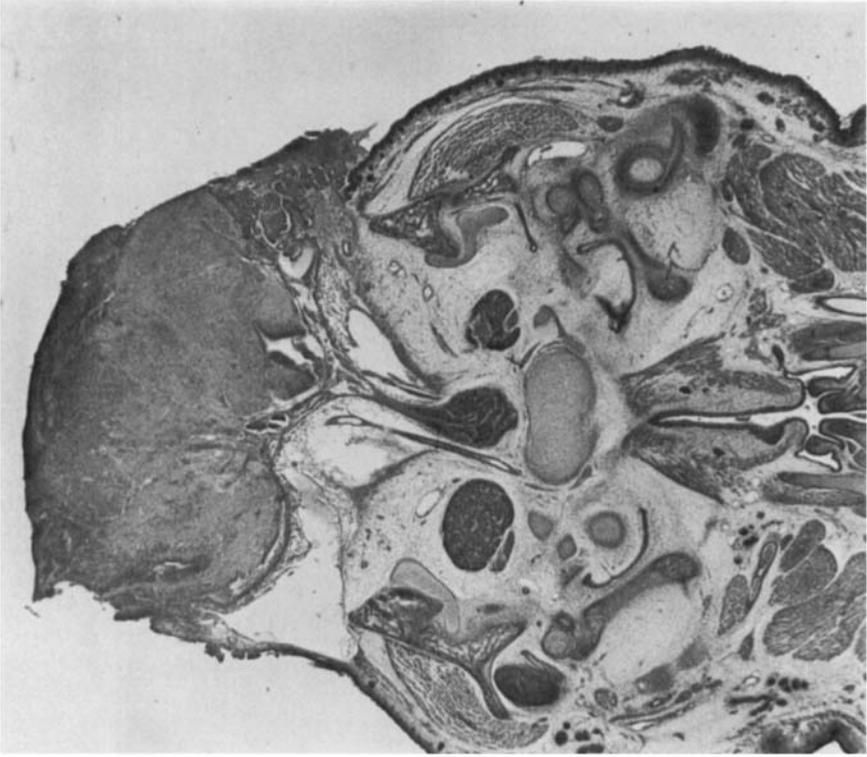
Fig. 4   Section through the head of another exencephalic fetus. The cerebral remnants are sequestered from the cranial base and the connection of the adenohypophysis with the diencephalon is extremely tenuous. × 15.
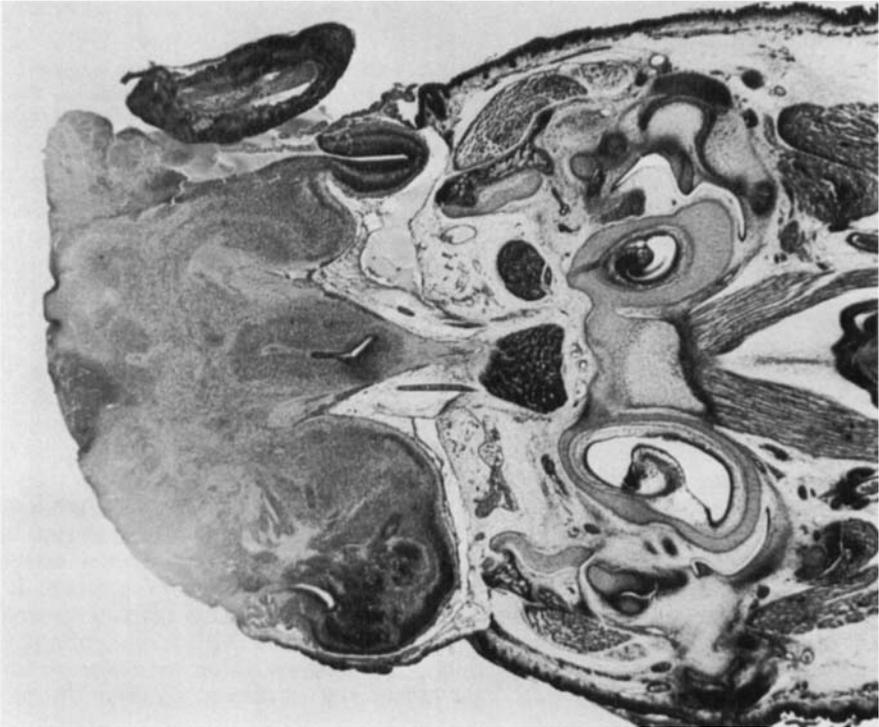

Fig. 3   Section through the same head showing third ventricle, infundibulum, and a round adenohypophysis. × 15.

contrast to the flat adenohypophysis seen in controls (Warkany and Takacs, '68). More caudal sections show a similar picture of open neural plate containing in places rosettes and ventricular ducts; hemorrhagic areas extend within and around the brain. The picture is that of a complete exencephaly, a forerunner of anencephaly, as observed in human and animal fetuses. A littermate of the animal illustrated in figures 1–3 had a similar exencephaly but in this specimen distortion and degeneration of the brain seem more progressed, the connection of the pituitary with the brain is more tenuous, and wide spaces have already formed between the cerebral remnants and the skull base (fig. 4). This separation of nervous tissues from the cranial base foreshadows shedding of the remnants of the brain and formation of a cerebrovascular area characteristic of anencephaly. There was anophthalmia on the right and microphthalmia on the left and there were no optic nerves. As in the littermate a median palatine furrow was present.

Hydrocephaly

Most hydrocephali could be recognized by their dome-shaped cranium (fig. 5) but less severe forms could not be diagnosed without sectioning. Several patterns of anomalies were found in histologic examinations.

Frontal sections through the head of the animal illustrated in figure 5 revealed not only a very severe form of hydrocephalus but also microphthalmia of the right eye and absence of the right optic nerve. The palate was cleft so that the oral cavity communicated with the nasal cavities and the nasopharyngeal duct. Sections through the rhinencephalon show abnormal stratification of the olfactory lobes and more caudally severe disorganization of the brain about the enlarged ventricles. The periventricular walls which normally are made up of densely packed, dark-staining germinal cells (figs. 17, 18) are completely disorganized. In the hydrocephalic animal (fig. 6) the germinal cell layers are overdeveloped ventrolaterally but laterally the wall is in places devoid of subependymal cell layers. The ventral walls of the ventricles are formed by large masses of differentiated brain tissue, structures not existing in control animals. Below these lighter areas rosettes and ductules are embedded in dark-staining tissue. Neither anterior commissure nor corpus callosum

Fig. 5   Head of rat fetus with hydrocephaly recognized by dome-shape of the cranium. There are also finger anomalies.
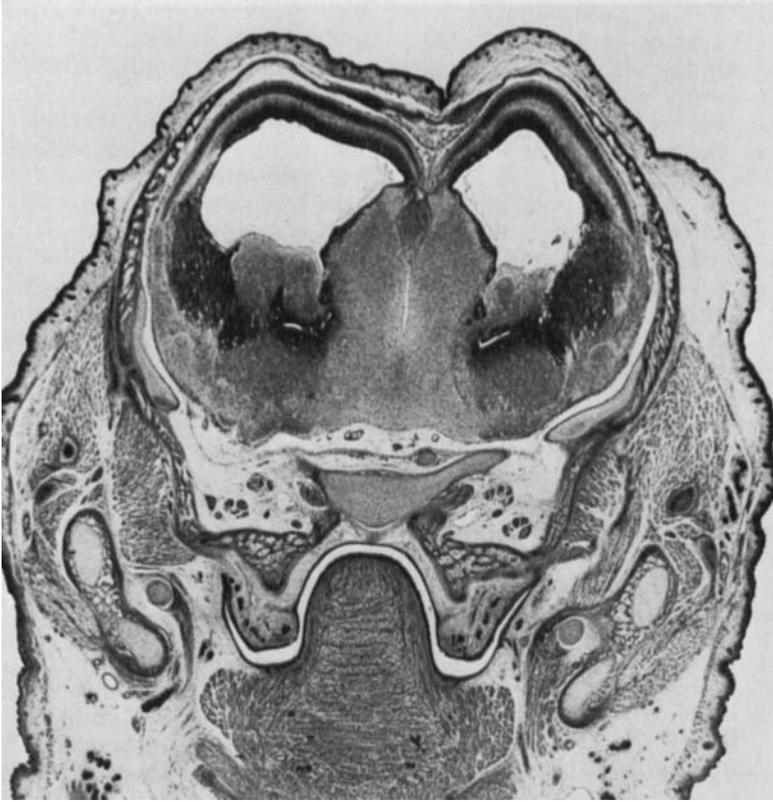
Fig. 6 Frontal section through the forebrain of a hydrocephalic fetus (21st day) revealing disorganization of the ventral walls of the lateral ventricles and thinning of cortical layers. × 15.

can be identified in this brain. The interventricular foramina are huge and the communications between the lateral and third ventricles are broad. Whereas the ventral portion of the third ventricle is not greatly enlarged its dorsal part opens up like a funnel into the wide interventricular space. At the level of the pituitary gland the interventricular communications become narrower but the thalamus remains divided by the third ventricle which reaches up to the meningeal covers and the skin of the vertex. In contrast to the normal, there are no fornix commissures and the hemispheres are not interposed between the third ventricle and the dorsal meninges since they are pushed aside by the large ventricular spaces. The lateral walls of the lateral ventricles are extremely thin; between their external and internal layers only rosettes and ducts make up the cortical tissue (fig. 7). Although the ventricular walls are very thin in places, they do enclose the ventricular system completely so that one cannot speak of hydranencephaly. In human hydranencephaly only basal ganglia and remnants of the mesencephalon are found at the cranial base and from these remnants a thin membranous sac extends forward and upward which consists of glia and pia-arachnoid; lateral and third ventricles cannot be distinguished in human hydranencephaly.

In more caudal sections of the hydrocephalic rat fetus the separation of the thalamic halves diminishes and in the mesencephalon an aqueduct which is much narrower than normal runs below the posterior commissure. Dorsal to the mesencephalon there is an enlarged and square pineal recess whose upper outline is slightly thickened by cell clusters which represent the flattened pineal gland. The section shown in figure 8 goes through a
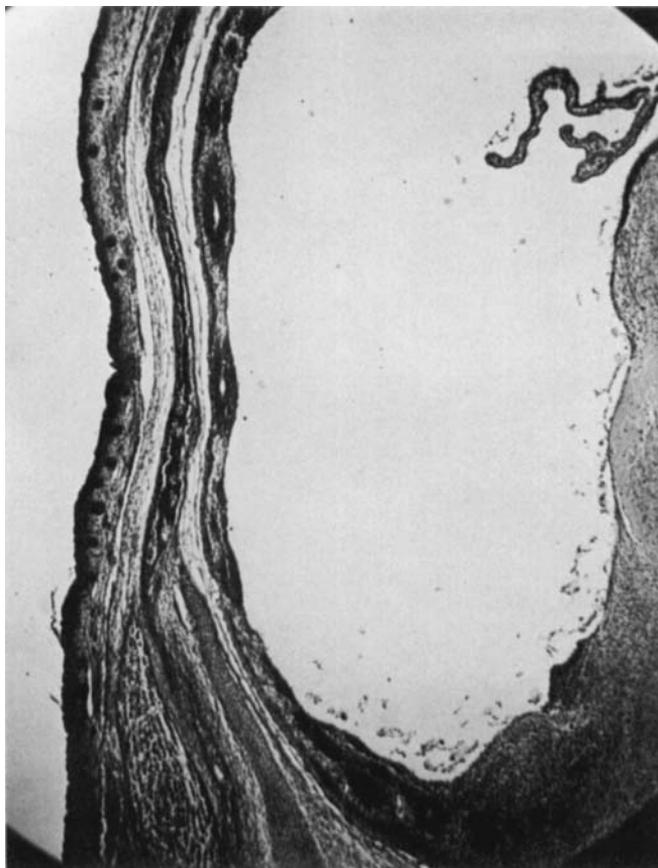
Fig. 7 Frontal section through the right lateral ventricle of hydrocephalic fetus shows extremely thin lateral wall containing ducts and rosettes. × 40.

mesometencephalic area in which there is no aqueduct and no communication between the third and fourth ventricles. The posterior portions of the enlarged and thin-walled lateral ventricles are still visible; it is noteworthy that the hemispheres do not compress or impinge upon the midbrain. Caudal to this section several aqueductal branches appear within the posterior mesencephalon; they eventually communicate with each other and with the fourth ventricle. Subsequent sections reveal no significant abnormalities. The size of the fourth ventricle is within normal limits and the cerebellum, pons, and medulla oblongata are normal in outline and relation to each other.

The hydrocephaly represented by figures 9 and 10 differs in several respects from the one just described. The ventricles are greatly distended (fig. 9) and their walls contain a few scattered light areas and fiber tracts. But the outstanding feature is the large number of rosettes and ductules in the olfactory area. The rosettes consist of cells arranged radially around a lumen and the ductules are lined by cells whose nuclei form pseudostratified patterns. Some of the ductules terminate in a neighboring ventricle with their lining contiguous with ependyma. Whereas the ventricular system of telencephalon and diencephalon is markedly enlarged its connection with the hindbrain is very tenuous as the aqueduct through the mesencephalon is extremely narrow (fig. 10). The mesencephalon itself is small and its quadrigeminal plate reduced to a single eminence. It seems worth emphasis that the arachnoid spaces about the mesencephalon
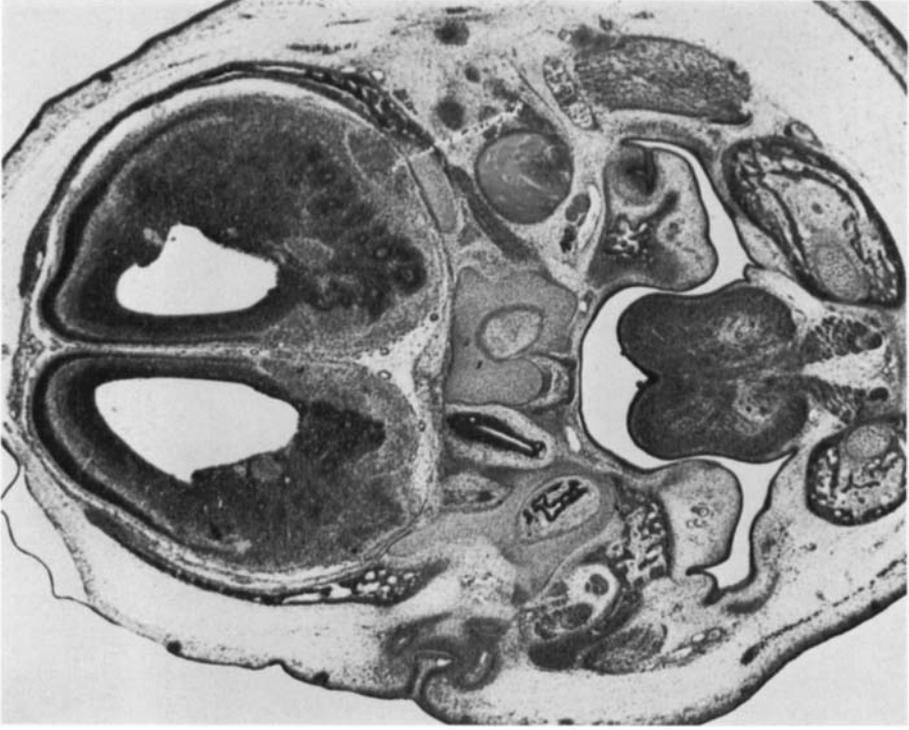
Fig. 9   Section through the forebrain of a hydrocephalic fetus (21st day). The areae olfactoriae are perforated by rosettes and ductules. × 20.
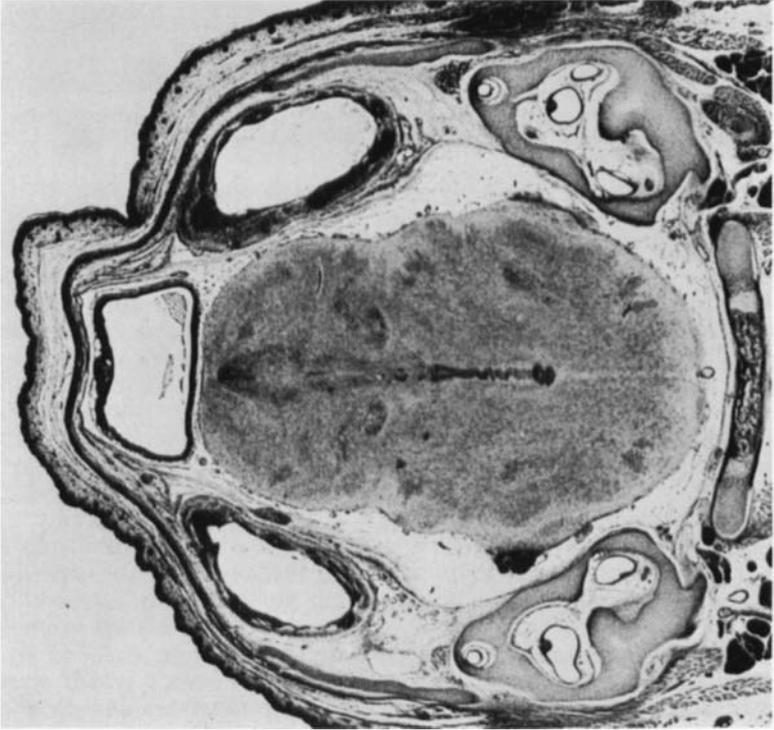

Fig. 8   Section through the mesometencephalic area of the same hydrocephalic fetus. There is no aqueduct. The pineal recess is huge, the subcommissural organ rudimentary. × 15.

Fig. 10 Frontal section through the small and deformed midbrain of the fetus illustrated in figure 9. The aqueduct is barely noticeable. × 40.

are well developed and that there are no signs of compression of the mesencephalon by the enlarged lateral ventricles.

Figure 11 shows a section through the brain of a hydrocephalic fetus removed on the 20th day of gestation. The huge lateral ventricles surround the triangular mesencephalon. The posterior commissure, the subcommissural organ, and the aqueduct are rudimentary. The communication between forebrain and hindbrain is not completely interrupted but obviously insufficient.

Tissue malformations

Central nervous system lesions were recognized in some fetuses whose heads were not grossly deformed. Some of these animals were sectioned because a littermate had a gross malformation or a cleft palate; or an eye anomaly suggested involvement of the brain.

The sections shown in figures 12 and 13 are from a littermate of the animal illus-

trated in figures 5–8. In contrast to its littermate, this fetus did not appear hydrocephalic but a cleft palate was present. Frontal sections disclosed only slight enlargement of the lateral ventricles but there were severe changes in the architecture of the forebrain. The outstanding anomalies of the brain are shown in figure 12 which illustrates disorganization of the periventricular walls similar to those of its hydrocephalic littermate (fig. 6). In place of the densely packed cell aggregates seen in control animals (figs. 17, 18) the periventricular tissues are in disorder as darkly stained areas are intermingled with better differentiated and lighter cell formations. Some of these less intensely stained areas protrude into the ventricles where they form eminences or ridges (fig. 12). There are also sagittally oriented fiber tracts within these lighter areas that are not present in controls. In some sections abnormal ductules and rosettes are scattered through the dense aggregates of

Fig. 12 Section through the brain of a littermate of the fetus illustrated in figures 5–8. Although the ventricles of this brain are only slightly distended the periventricular walls are disorganized like those of its hydrocephalic littermate. × 18.
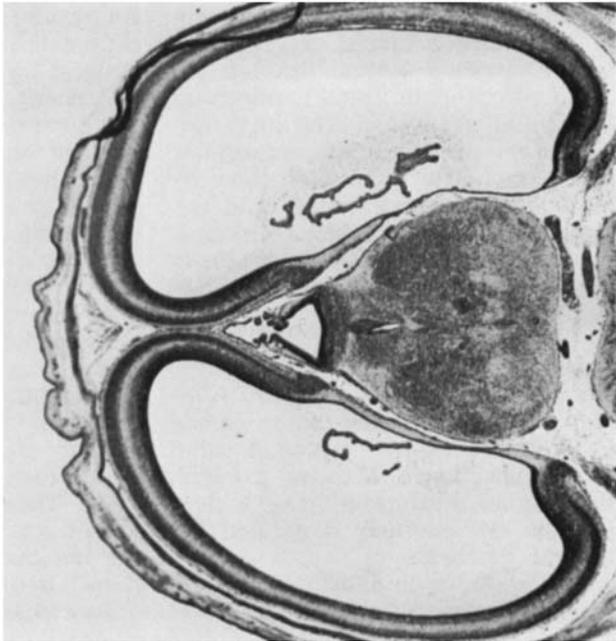

Fig. 11 Frontal section through a hydrocephalic fetus (20th day) at the level of the subcommissural organ. A narrow aqueduct can be seen within the pyramid-shaped midbrain. × 15.
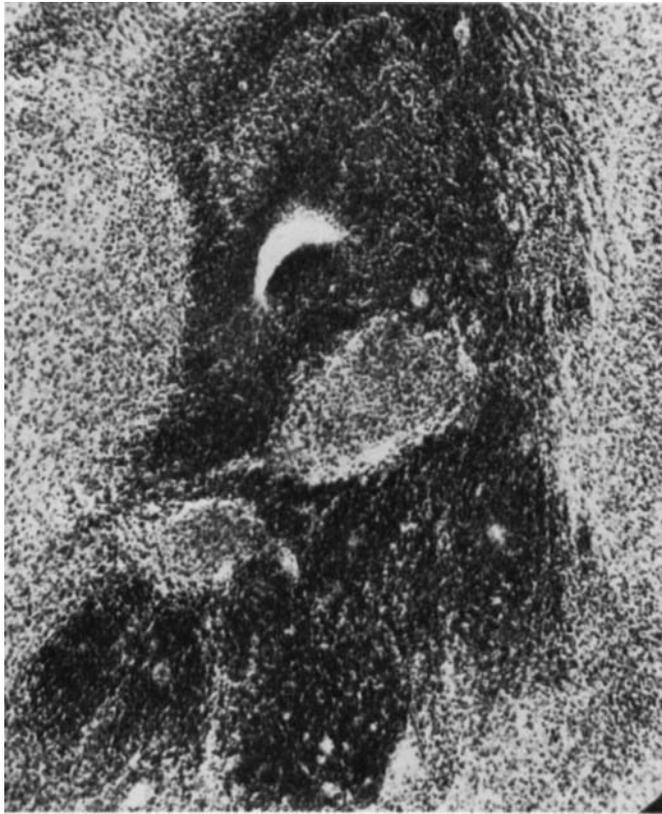
Fig. 13 High magnification of periventricular area about the rostral end of the left lateral ventricle of the fetus whose brain is shown in figure 12. × 80.

germinal cells. This fetus has no anterior commissure or corpus callosum and the commissura fornicis is rudimentary; but the columnae fornicis are descending within the well-developed septa (fig. 12). Both diencephalon and mesencephalon are reduced in their transverse diameters but the third ventricle is not distended and the aqueduct is open (fig. 14). A peculiar compartmentalization of the left lateral ventricle is produced by infolding and rosette formation of the cerebral cortex.

Other anomalies revealed by histologic sections were noted in organs connected with the CNS. There were many abnormalities of the eyes such as anophthalmia, microphthalmia, cataract, coloboma, and absence or interruption of the optic nerve. The chiasma opticum was absent when both optic nerves were missing or the chiasma was asymmetrical when only one optic nerve was connected to the brain.

The pituitary and pineal glands were deformed in some of the specimens.

At the caudal end of the CNS, malformations of the spinal cord (myelodysplasias) were revealed by histologic sections (figs. 15, 16). We did not observe myeloschisis or other forms of externally recognizable spina bifida.

The zinc-deficient diet employed by us for only 6 days resulted in severe malformations of some of the young. Since these experiments are still in progress they will be reported later.

COMMENTS

Maternal nutritional deficiencies have been used as experimental teratogenic tools for decades. Vitamin A, riboflavin, and vitamin E deficiencies were among the first procedures available for regular production of congenital malformations in mammals. Deficiency of folic acid, vitamin
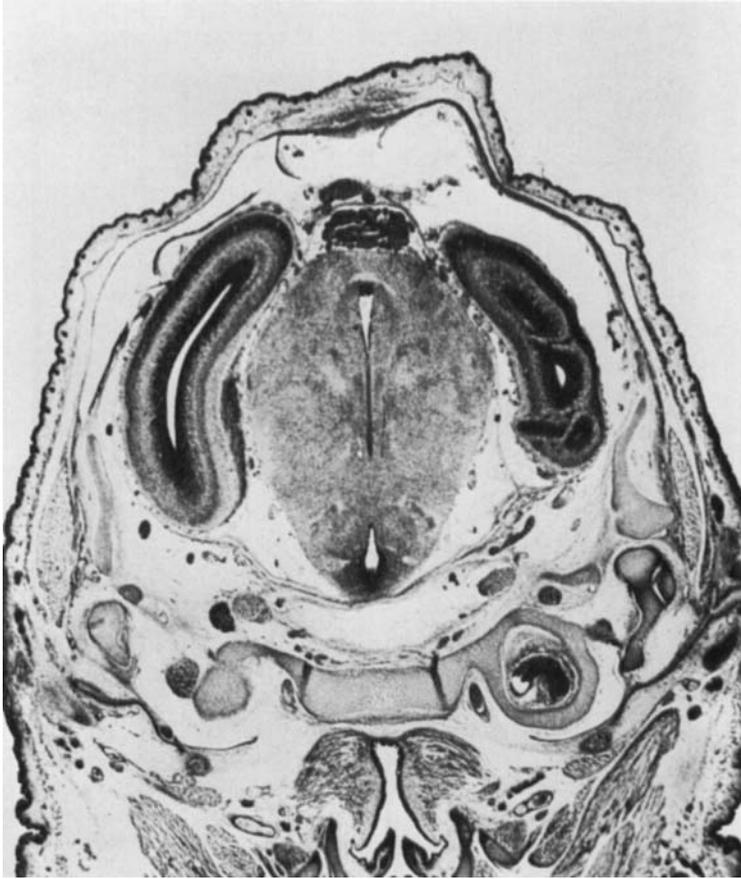
Fig. 14 Section through the midbrain of the same fetus (figs. 12, 13) showing open aqueduct. Note folding of the cortex of the left hemisphere. × 15.

B_{12}, pantothenic acid, and niacin were found to be teratogenic if combined with metabolic antagonists. Whereas pure deficiencies become effective only after prolonged deprivation of the mother, deficiencies combined with antimetabolites rapidly induce teratologic results (See reviews by Kalter and Warkany, '59, and Giroud, '70.) In fact some of the antimetabolites can produce congenital malformations in humans without maternal dietary deficiencies if given during sensitive periods of embryonic development (Thiersch, '52; Warkany et al., '60; Diamond et al., '60; Emerson, '62; Shaw and Steinbach, '68; Milunsky et al., '68). There is little proof that the convincing teratogenic animal experiments using chronic nutritional deficiencies are simulated by human situations and that dietary vitamin deficiencies cause malformations in man. On the other hand, it is well established that chronic iodine deficiency of women resulting in goiter can have adverse effects on the fetus and can cause endemic cretinism, a disorder of physical and mental impairment (Quervain and Wegelin, '36). In this context it seems of great importance that short-term zinc deficiency can bring about congenital malformations in rats without noticeable effects on the mothers which cannot mobilize zinc stored in their bodies for the benefit of their embryos and fetuses. Although applications of these experiments to human conditions are merely speculative at this time it is worth pointing out that zinc deficiency can occur in man (Prasad, '66) and that certain foods

Figure 16

Figure 15

Figs. 15 and 16 Transverse sections through spinal cords at pelvic levels. Both cords have abnormal arrangements of cell groups and of tracts. The central canals are in atypical positions (myelodysplasias). × 80.
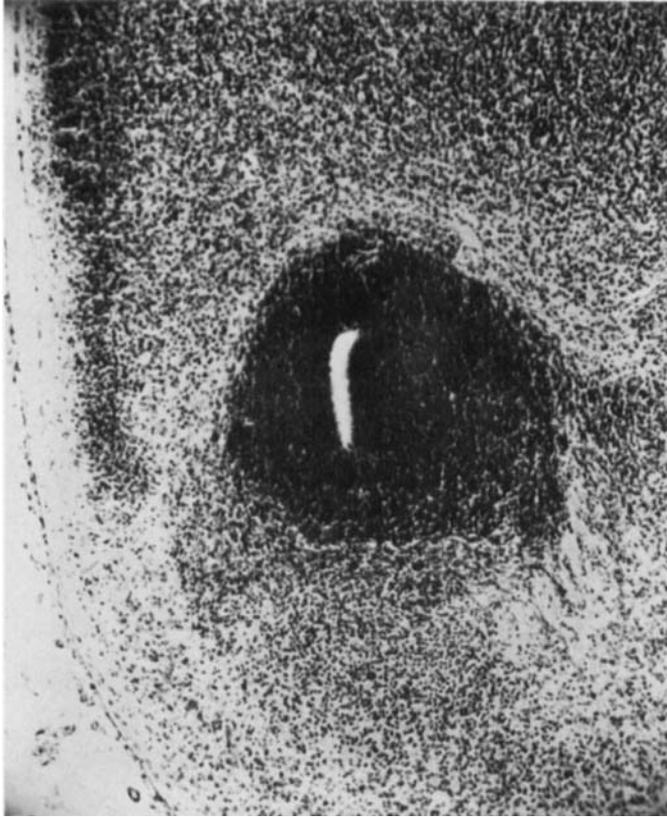
Fig. 17 Frontal section through the forebrain of a control rat fetus (21st day) at the level of the rostral end of the left lateral ventricle showing periventricular walls made up of dark-staining germinal cells. × 80.

used by man are either poor in zinc or contain nutritional elements that make zinc unavailable for absorption (Oberleas et al., '66; Halsted, '66). There are no reports of congenital malformations in children attributable to zinc deficiency. But it has been pointed out that the existence of zinc deficiency in humans suggests that it could occur in pregnant women and be aggravated if other factors interfere with zinc absorption or utilization (Hurley, '68). It is most doubtful that such brief deficiencies during early pregnancy could be recognized after a deformed child is born. The experimental approach should alert observers of human malformations to the possibility of subtle and complex environmental teratogenic constellations in early pregnancy.

The gross malformations produced by zinc deficiency are very impressive but minor changes observed and described in this communication are equally important because they raise further questions concerning recognition of prenatal causes of congenital anomalies in postnatal life. The tissue anomalies described may not elicit signs and symptoms until functional disabilities can be assessed. One can only speculate about the postnatal fate of heterotopias, rosettes, tract anomalies, and myelodysplasias. Such minor congenital anomalies deserve further study by postnatal observations of animals that survive birth and can be raised.

Fig. 18 Frontal section through the head of a control rat fetus at the level of the anterior commissure. The periventricular walls about the lateral ventricles are clearly defined by dark-staining cell layers. × 14.

O'Toole in the nutritional and experimental work reported.

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