Originals

Cong. Anom. (先天異常)
12 (1) : 1 − 9 , 1972

POSTNATAL MANIFESTATION OF HYDROCEPHALUS IN MICE CAUSED BY PRENATAL X-RADIATION

Yoshiro KAMEYAMA * and Kiyoshi HOSHINO*

The developing brain of a mammalian fetus is highly sensitive to ionizing radiation not only in the period of major organogenesis but also in the fetal period, since radiosensitive undifferentiated neural cells exist in the brain throughout the intrauterine development.

It is well known that newborn animals are involved with microcephaly when they were exposed to X-ray of over 150R during their fetal periods. The immediate and early pathological changes causing microcephaly have been studied by many investigators.[3,5,7,8] However, long-term pathological effects of X-radiation on the developing brain of mammals are less well researched.

Kaven and Ostertag[4] (1937) and Cowen and Geller[1] (1960) described a few examples of hydrocephalus among adult animals which had been exposed to X-ray in utero. The authors of this paper examined the brains of mice which had been exposed to 200R of X-ray during their fetal periods and had been permitted to survive to maturity. They found out that some of the mice had hydrocephalic involvement after birth, although these mice had been microcephalic at the time of birth.

The present experiment was designed to examine long-term or delayed pathological effects of prenatal X-radiation on the developing brain in mice. The authors' aim was to elucidate the pathogenesis of hydrocephalus manifested after birth.

METHODS

The animals employed were a closed colony of CF#1 mice, which were commercially supplied by Nippon CLEA Co. Ltd., Tokyo. Estrous virgin females were kept with potent males in cages in pairs overnight; next morning females with vaginal plugs were regarded as being in day 0 of pregnancy.

Pregnant mice were exposed to a single whole-body X-radiation at a dose of 200R on day 12, 13, 14, 15, 16 or 17 of pregnancy. X-radiation was performed with a therapeutic X-ray machine; 200KVP, 15mA, 50cm, filters of 0.5mm Cu + 0.5mm Al, and 24R/min.

Mother animals were allowed to give birth and to rear their litters. The offspring exposed to X-ray in utero were put to death just after birth, and at 1, 2, 3 and 6 weeks of age in sequence. Their brains were removed, fixed in 10% neutral formalin and examined microscopically in serial sections stained with H. E. or gallocyanine.

* Research Institute of Environmental Medicine, Nagoya University, Nagoya

RESULTS

The viability of the newborn mice exposed to 200R of X-ray in utero was generally low, and the number of mice that could survive more than 3 weeks after birth was reduced to 17% of the newborn in groups treated on day 12 and 15 of gestation ; to 33% in the group treated on day 14, and to 50% in that on day 13 (Table 1).

All live newborn in groups treated showed microcephaly with slightly enlarged lateral ventricles, a feature similar in each case. However, the postnatal sequences of X-ray induced microcephaly of the surviving animals were expressed in different ways, either depending upon which day of gestation the radiation was administered or at which postnatal age the brain was examined.

In the groups treated after day 14 of gestation, the microcephalic state remained unchanged during postnatal growth. But the lateral ventricles became narrower as the age of animals increased and hydrocephalus *ex vacuo* disappeared in the adult. In most cases in the group treated on day 12, underdevelopment and growth retardation of the brain mantle did not improve and microcephaly with enlarged lateral ventricles, namely micro-hydrocephaly, was still predominant in the adult.

Table 1 CF#1 mice survival rate following exposure to 200R of X-ray in utero

Day of gestation treated	No. of mother animals	No. of newborn mice	No. of infant mice dead within 3 weeks from birth	No. of surviving mice 3 weeks after birth (%)
12	7	70	58	12 (17.1)
13	9	94	47	47 (50.0)
14	8	90	60	30 (33.3)
15	8	86	71	15 (17.4)
16	12	130	52	78 (60.0)
17	8	78	42	37 (47.4)
Control	8	90	22	68 (75.6)

Table 2 Number of CF#1 mice with hydrocephalus manifested postnatally following exposure to 200R of X-ray in utero

Day of gestation treated	3 weeks after birth		6 weeks after birth	
	No. of autopsy	No. of hydrocephalus	No. of autopsy	No. of hydrocephalus
12	6	4	6	4
13	17	9	15	12
14	9	1	12	3

In the group treated on day 13 of gestation, the brains showed micrccephaly until about 7 days after birth, a feature similar to those in groups treated on other days (Fig. 2). Around 7 to 10 days after birth, the lateral ventricles started to enlarge rapidly and the brain mantle started to reduce in thickness. The changes became more intensive pro-

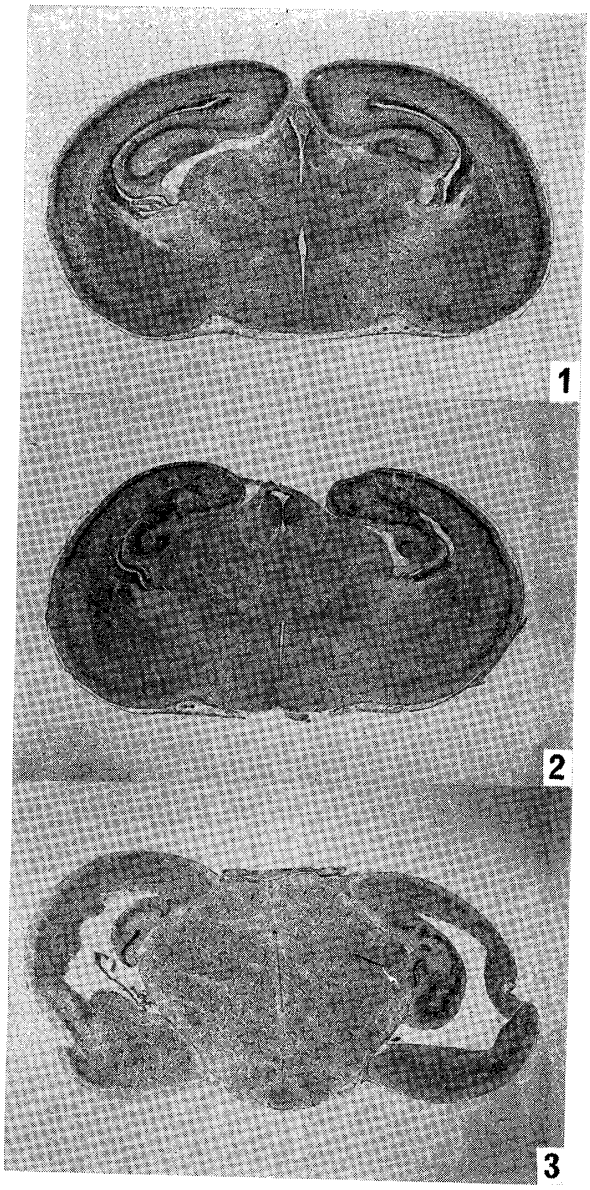

Fig. 1-3 Coronal sections of the brains of CF #1 mice (gallocyanine stain).

1. Normal brain of newborn mouse in the untreated control group.

2. Microcephaly of newborn mouse in the group irradiated with 200R of X-ray on day 13 of gestation. The development of the dorsal neocortex and hippocampus are severely disturbed.

3. Hydrocephalus of 14 day-old mouse in the same group as figure 2. The brain mantle is reduced in thickness and the lateral ventricles are markedly enlarged.

gressively with the advance of age (Fig. 3). At 4 weeks, the brains showed a typical internal hydrocephalus, which could be detected externally by a vaulted cranium. The parietal and temporal bones became very thin like semitransparent paraffin-paper, indicating an increase of pressure in cerebrospinal fluid. The third venticle was also enlarged, but the fourth ventricle was not so much dilated. Neither obstruction nor stenosis in the ventricular system could be found before or during the ventricular enlargement.

This type of hydrocephalus was seen in the group irradiated on day 12 of gestation, but was less extensive in degree compared with that in the group treated on day 13. Hydrocephalus was also produced in the group treated on day 14 as a rare finding, and was never found in those treated after day 15 of gestation (Table 2). It was noticed that almost all cases of hydrocephalus were produced in the group irradiated on day 13 of gestation, and most of them manifested it during the lst and 2nd week after birth.

Prior to manifestation of hydrocephalus, venous congestion and hemorrhages in the subependymal white matter of the brain mantle were observed in infant mice which had been irradiated on day 13 of gestation. These circulatory changes were predominant in the radiatio corpus callosi near the lateral edges of the lateral ventricles in the coronal sections cutting through between the optic chiasma and the infundibulum (Fig. 4). At the same time or a little later, ablation of the ependymal layer and periventricular hemorrhages were sen in places along the

lateral ventricles (Fig. 5). The choroid plexus in the lateral ventricles showed also strong blood congestion (Fig. 8).

In the brains of 7-day old infant mice, small tissue defects with hemorrhages were found in the radiatio corpus callosi and the tapetum (Fig. 6). In the brains which lateral ventricles began to enlarge, some tissue defects of the periventricular white matter chan-

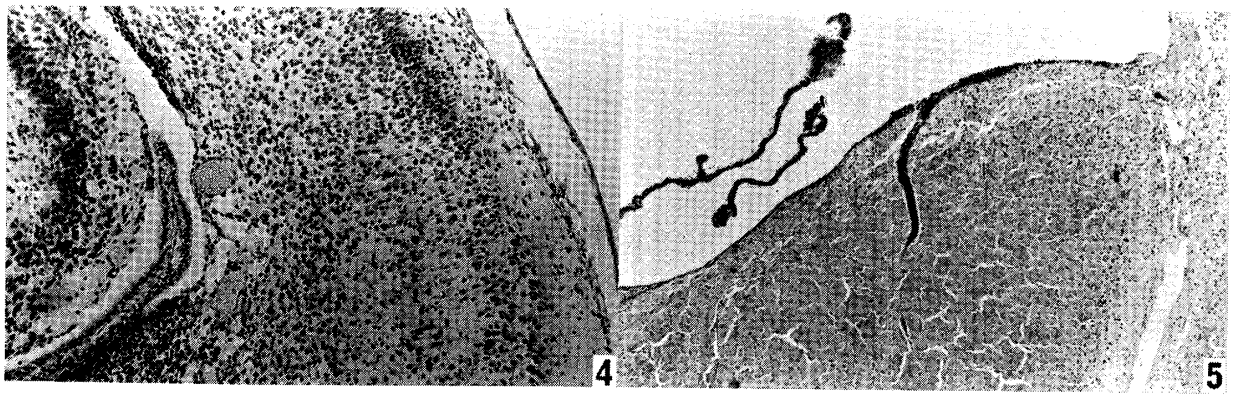

Fig. 4-5 Coronal sections of the brains of 7 day-old mice in the group irradiated with 200R of X-ray on day 13 of gestation.
4. Blood congestion and hemorrhage are seen in the subependymal white matter at the lateral edge of the lateral ventricle (gallocyanine stain).
5. Blood congestion and periventricular hemorrhage are predominant along the lateral ventricle (H. E. stain).

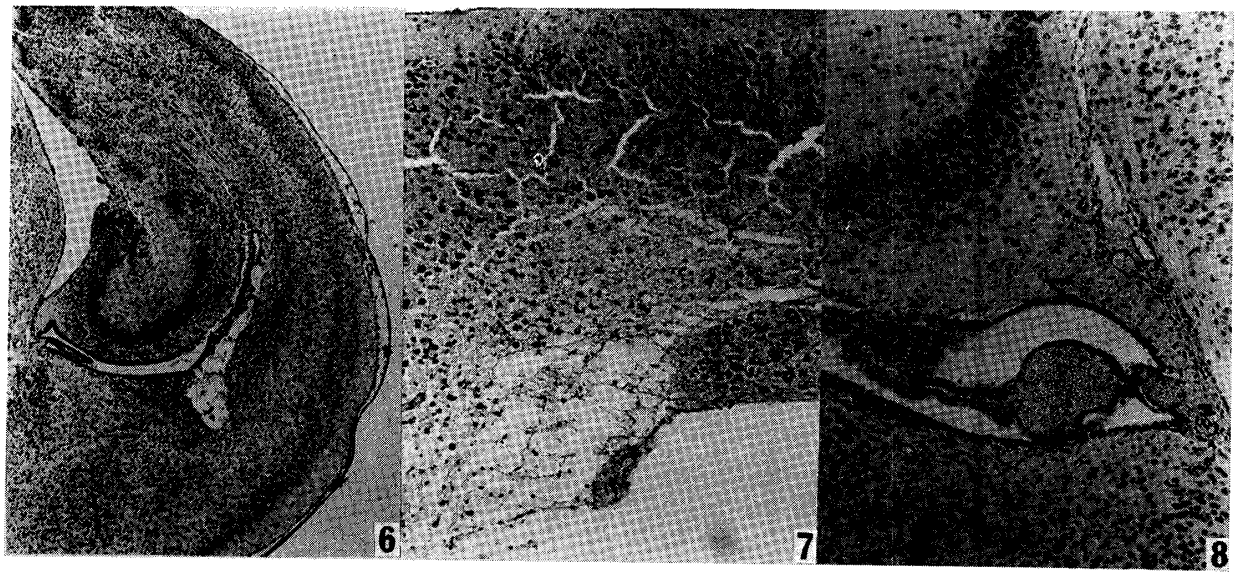

Fig. 6-8 Coronal sections of the brains of 7 and 14 day-old mice in the group irradiated with 200R of X-ray on day 13 of gestation.
6. Tissue defect following hemorrhage is seen in the periventricular white matter. 7 day-old mouse (gallocyanine stain).
7. Spongy degeneration of the periventricular white matter are spreading over the deep layers of the neocortex. 14 day-old mouse (H. E. stain).
8. The choroid plexus in the lateral ventricles shows strong blood congestion. 7 day-old mouse (gallocyanine stain).

nelled to the ventricular cavity and became ventricular diverticla. Simultaneously, spongy degeneration started in the periventricular white matter and spread over the deeper layer of the neocortex (Fig. 7). Neither hemorrhage nor tissue defect could be detected in the outer layer of the cortex.

The above pathological process was prevalent in the brains involved with severe histogenetic abnormalities of neopallium such as aberrant and insufficient cortical structure, heterotopic gray and white matter (Fig. 10). The brains with mild histogenetic abnormalities of neopallium showed mild circulatory changes and no marked tissue degeneration.

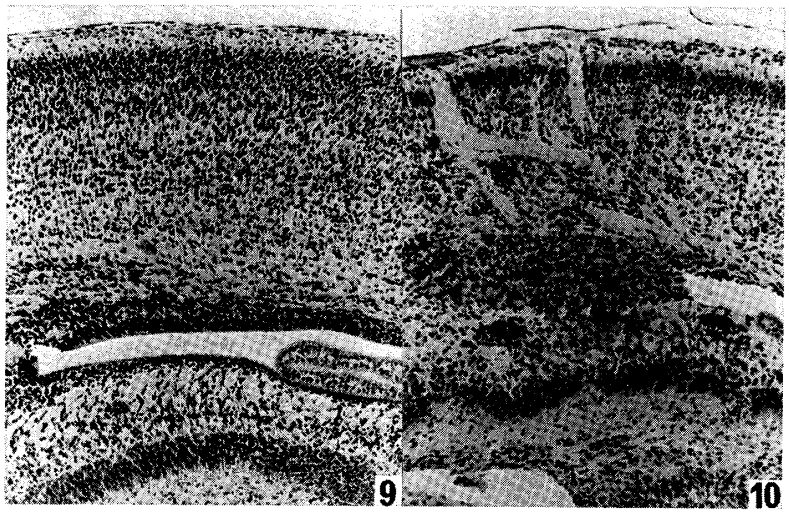

Fig. 9-10 Dorsal neopallia of newborn mice in the coronal sections cutting through the optic chiasma (stained with H. E.).
9. Normal neopallium in the untreated control group.
10. Malformed neopallium in the group irradiated with 200R of X-ray on day 13 of gestation. Aberration of cortical structure and heterotopia are evident.

After the lateral ventricles had enlarged, the congestion and hemorrhage diminished as the brain mantle reduced in thickness. In the advanced state of hydrocephalus, extensive degeneration of the mantle tissue obscured original histogenetic abnormalities which were predominant in the fetal and neonatal periods.

COMMENTS

X-ray induced hydrocephalus of the fetus and newborn animals has been studied by many investigators and its morphogenesis is regarded as a type of hydrocephalus *ex vacuo*, which manifests itself as a consequence of developmental arrest of the brain mantle due to direct radiation-injuries of the undifferentiated neural cells. This type of hydrocephalus should be called "dysgenetic" hydrocephalus or microhydrocephaly, and therefore distinguished from true hydrocephalus, in which brain enlargement of the ventricles and reduction in thickness of the cerebral hemispheres are progressive after birth.

Kaven and Ostertag[4] (1937) first described communicating hydrocephalus of adult mice that had been exposed to X-ray in utero, which feature was similar to that in this experiment. They considered it to be a hypersecretory hydrocephalus, based on the fact of no obstruction in the ventricular system. Cowen and Geller[1] (1960) also reported as a rare finding a similar hydrocephalus of adult rats irraidated in utero, but they did not mention its morphogenesis.

In hydrocephalus in this experiment, enlargement of the lateral ventricles started after birth and became progressively intensive with advance of age. Prior to manifestation of hydrocephalus, venous congestion and hemorrhages occurred in the neopallial white matter. Neither stenosis nor obstruction in the ventricular system could be detected before or during the ventricular enlargement. These results suggested long-term pathological effects of X-radiation upon the vascular system in the developing brain, and a possible causal relationship between the disorders of blood circulation in the brain mantle and manifestation of hydrocephalus. The finding that postnatal hydrocephalus occurred mostly in the brain involved with severe aberration of the cortical configurations seems to be significant for the pathogenesis of circulatory disorders in the brain.

The morphogenetic process of hydrocephalus in this experiment can be explained as follows: Neural injuries as an initial damage following radiation and subsequent structural aberration of the brain mantle would account for a disturbance of vascular formation and a deranged vascularity. This abnormal vascularity would later bring about a circulatory disorder in the tributaries of the cerebral venous system at the time when blood flow in the brain mantle increased rapidly. These circulatory changes would cause a secondary destruction of the mantle tissue and also would be a basis for accumulation of the cerebrospinal fluid.

The reason why postnatal hydrocephalus was manifested mostly in the group irradiated on day 13 of gestation may be answered by the following facts: The mice exposed to 200R of X-ray on day 13 showed marked abnormalities of the cortical structure at birth and many of them could survive. The mice irradiated with the same dose on day 12 or earlier were involved with severe brain abnormalities as well, but most of them could not survive more than 3 weeks after birth. Those treated after day 14 or later were not involved with marked cortical maldevelopment.

The exact role of vascular injury in the pathogenesis of effects of X-radiation upon the fetal brain has not yet been conclusively established. Roizin, Rugh and Kaufman[6] (1962) reported numerous hemorrhages and pathological changes of the blood vessels in the brain of rat fetuses irradiated with 150R of X-ray on day 8 or 9 of gestation. On the other hand, Hicks[2] (1953), Cowen and Geller[1] (1960) found no evidence of vascular changes in the brains of adult rats exposed to X-ray in utero.

Although no positive evidence of direct injury to cerebral blood vessels by X-radiation could be found in this experiment, it might be possible, even probable that secondarily abnormal vascularity could be produced by structural aberration of neural tissue in the brain. The cerebral vascularity of mice exposed to X-ray in utero will be reported in a further study.

SUMMARY

1. The postnatal changes of X-ray induced microcephaly were observed in sequence in CF#1 mice which had been exposed to a single dose of 200R of X-ray from day 12 to 17 of gestation.

2. In the groups irradiated on day 12 and after day 14 of gestation, X-ray induced microcephaly remained unchanged or became less predominant as the animals grew older. In the group treated on day 13, the lateral ventricles of microcephalic brains started to enlarge one to two weeks after birth, and hydrocephalus became intensive progressively with the advance of age. Postnatal hydrocephalus was manifested in brains involved with marked aberration of cortical structure.

3. Prior to manifestation of hydrocephalus, venous congestion, hemorrhages and consequent tissue degeneration were evident in the subependymal white matter of neopallium. Neither stenosis nor obstruction in the ventricular system could be detected.

4. The results suggested possible causal relationships among maldevelopment of the cortical structure, circulatory disorders in the brain mantle and manifestation of hydrocephalus.

The authors wish to thank Dr. Shinji Takahashi, Professor of Radiology, Nagoya University School of Medicine, for his aid in X-ray administration. The authors also acknowledge the technical assistance of Mrs. Yoneko Ito anb Miss Shizu Kokubo, Research Institute of Environmental Medicine, Nagoya University.

(Received on March 5, 1971)

REFERENCES

1) Cowen, D. and Geller, L. M. Long-term pathological effects of prenatal X-irradiation on the central nervous system of the rat. J. Neuropath. Exp. Neurol., 19 : 488-527, 1960.

2) Hicks, S.P. Effects of ionizing radiation on the adult and embryonic nervous system. Res. Pub. Ass. Ner. Ment. Dis., 32 : 439-462, 1953.

3) Hicks, S.P. and D'Amato, C. J. Effects of ionizing radiation on mammalian development. Advances in Teratology, Vol. 1, (Woollam, D.H.M. ed.), 195-250, Academic Press, New York, London, 1966.

4) Kaven, A. and Ostertag, B. (1937) : cited from Ostertag, B. Behavior, mortality and anatomical findings in mice surviving prenatal irradiation. Radiation Biology of the fetal and Juvenile Mammal. (Sikov, M. R. and Mahlam. D. D. ed.), 289-300, AEC 17 Symposium Series, U.S. AEC, 1969.

5) Murakami, U., Kameyama, Y. and Majima, A. A dynamic observation on the experimental formation of developmental abnormalities of the fetus caused by X-radiation. Ann. Rep. Environ. Med. Nagoya Univ., 8 : 101-115, 1960.

6) Roizin, L., Rugh, R. and Kaufman, M.A. Neuropathlogic investigation of the X-irradiated embryo rat brain. J. Neuropath. Exp. Neurol., 21 : 219-243, 1962.

7) Rugh, R. Major radiobiological concepts and effects of ionizing radiation on the embryo and fetus. Response of the Nervous

System to Ionizing Radiation. (Haley, T. J. and Snider, R. S. ed.), 3-26, Academic Press, New York, London, 1962.

8) Wilson, J. G., Jordan, H. C. and Brent R. L. Effects of irradiation on embryonic development. II. X-rays on the 9th day of gestation in rat. Am. J. Anat., 92 : 153-188, 1953.

胎生期X線被曝によるマウスの晩発性水頭症の成立について

亀山 義郎*・星野 清*

抄 録

マウス，ラットの胎仔の大脳が器官形成期のみでなく胎児期より新生児期にかけて高い放射感受性をもち，150R以上の胎児期のX線被曝によって小頭症が成立することはすでに知られている．かような小頭症をもたらす胎仔神経組織の初期変化は多くの研究者によって明かにされているが，成立した小頭症の生後の経過と晩発性脳発達障害についてはあまり検討されていない．

マウスの胎生12～17日にX線200Rを照射すると，大脳実質の広範な損傷により被曝した胎令にかかわらず，胎生末期の胎仔には小頭症あるいは小水頭症が成立した．これらの被曝仔の50～80%は出生直後に死亡したが，生き残ったものの生後の経過は被曝胎令によって差異が認められた．すなわち胎生12日に被曝した仔では脳外套の発達障害がつよく小水頭症のまま成体に達するものが大半を占めた．胎生14日およびそれ以降の胎児期に被曝した仔では出生時広くなっていた側脳室は次第にせまくなり補空性水頭の状態は消失するが，小頭症自体は成体に至るまで変化はなかった．ところが胎生13日に被曝した仔では出生時は小頭症を示したが，生後7～10日に側脳室の急速な拡大と大脳外套の菲薄化が始まり，日令とともに進行性に増強し生後3週以降には大半の例が典型的な内水頭症に発展した．なおこれらの例には脳室系の閉鎖，狭窄はなく，第3脳室は側脳室と同様に強い拡大がみられたが，第4脳室の拡大は軽度であった．

かような出生後に発現する水頭症は胎生12日被曝にもみられたが程度は軽く，また胎生14日被曝仔にも少数みられたが，胎生15日以後の処理には全く成立せず，胎生12～17日処理群中では胎生13日処理群に集中する傾向が明瞭であった．

水頭症の多発する胎生13日被曝仔の大脳の組織学的所見のうち，脳室の拡大に先だってあらわれる注目すべき変化は大脳外套の上衣下白質の静脈系の血行障害とそれに引きつづく脳実質の二次的退行変性の像であった．まづ脳梁放線の側脳室外側角に近い部位に白質の鬱血と拡大した静脈の破綻による出血があらわれ，ついで同部位を中心に側脳室上衣下の線状出血，上衣層の脱落がみられた．生後1週前後になると白質内に出血を伴う組織欠損があらわれ，脳室の拡大開始とともに欠損部は脳室に連絡し憩室状をなす例が多かった．さらに上衣下白質より皮質深層におよぶ海綿状変性をみる例もあった．側脳室脈絡叢には脳外套と同様に側脳室の拡大前につよい鬱血がみられた．なお大脳外套の表在性の血管および髄膜血管には鬱血は著明でなく，出血はみとめられなかった．これらの血行障害と脳実質の退行変性は皮質構築の攪乱や灰白質異所形成のような大脳外套の組織発生障害のつよい被曝仔につよく，組織構築異常の軽度の個体では血行障害も軽度であった．

以上の所見はX線小頭症に出生後発現する大脳外套の血行障害が水頭症の成立に主要な役割をもつことを示唆する．この血行障害は胎生期のX線被曝による脳実質の損傷と外套の組織構築の攪乱にともなっておこる血管の走行，分布の異常を基盤として発現したものと解釈される．

* 名古屋大学環境医学研究所　病理・胎生部門