HISTOLOGICAL CHANGES IN THE BRAINS OF MALFORMED FETUSES FROM THE URETHANE TREATED MOTHER MOUSE AND THEIR POSSIBLE RELATION TO OCCURRENCE OF GLIOMAS IN CHILDREN

by

Norio Yamazaki

From the 1st Surgical Division, Kyoto University Medical School
(Director: Prof. Dr. Chisato Araki)
Received for publication May 14, 1959

INTRODUCTORY NOTES

It has been known that the so-called embryonal cell rests are not infrequently found in the immature brain of a new-born infant or fetus. A number of authors (Cushing, 1930; Raff and Kernohan, 1944; Brzustowicz and Kernohan, 1952; Shimada, 1954; and Ostertag, 1956) assumed that gliomas of the brain, particularly those in children, may possibly arise from such cell rests or clusters of persistent immature cells. Seats of predilection for occurrence of the gliomas in children under 10 years of age are generally believed to be the vermis of the cerebellum, especially its posterior part, floor of the 4th ventricle, pons and quadrigeminal body, while those in persons around the adolescence the 3rd ventricle, pineal body, optic nerve, thalamus, basal ganglia and septum pellucidum. Some authors (Ostertag) believe that gliomas in adult of the olfactory brain, edge of the lateral ventricle (in the fetal period) and callosal body as well as diffuse or multiple gliomas may have something to do with developmental anomaly of the tissue.

It has recently become clear that variable exogenous factors can give rise to tissue malformation, some of which may, at the same time, play a rôle in fostering formation of tumor. Having injected methylcholanthrene to mice, for instance, Strong (1945) was able rather constantly to produce in their descendants certain malformations, such as adenomatous changes in the stomach, situs inversus visceralis, dextrocardia, apigmentatio piliaris, etc.

It seems, therefore, quite probable that the cell rests, which can be found even in the brain under the process of normal development, are liable to increase in number and show greater tendency to formation of tumor, when that brain is subjected to factors promoting tissue malformation.

In the present study, the author for the first step examined histologically the brain of normal fetus of mouse, following the developmental sequence, in the aim to see whether the same or similar cell rests as those in human fetus are present and in what part of the brain, if present, are they most likely to be found. Then, the brains of malformed fetal mice, descendants of a mother mouse having undergone intraperitoneal injection of ethylurethane solution during the pregnancy after the

method of NISHIMURA,[9] were histologically examined and compared with those of the normal fetuses.

MATERIAL AND METHOD OF EXPERIMENT

A. Experimental Material

1) 18 normal fetal mice of the hybrid strain at 12th~19th gravid day.

2) 18 malformed fetal mice, the mother mouse being treated with ethylurethane according to NISHIMURA's method: 10% ethylurethane solution, 1.2 mg per g body weight, was injected into the peritoneal cavity of the mother mouse on the 10th gravid day, On the 19th gravid day, one day prior to the delivery, the fetuses were taken out by laparotomy, because the mother animal has a habit, as SHIROTA from our laboratory already mentioned, to eat their babies malformed. Among the malformations thus produced, polydactylism on the side of the first toe of the posterior limb was most frequently observed (15 cases). Short tail was also seen in 1 case. 2 grossly normal fetuses from that same mother were also included in this group (Figs. 1 and 2).

B. Experimental Method

After decapitation, the head was fixed in toto (with the skull and scalp) in 10% neutral formalin solution. Having been embedded in gelatin, serial frozen sections, 10~15 microns in thickness, were made, the whole brain being cut either frontally or sagittaly. They were stained with PENFIELD's silver carbonate method, modification II.

RESULTS

A. Development of Nerve Cells and Glia Cells and Occurrence of Cell Rests in the Brain of Normal Fetal Mice

SHIMADA[14] from our laboratory made a detailed study histologically on the sites of persisting as well as displaced immature glia cells of the brain in 22 normal human fetuses 3 to 10 months old. He thus found that the seats of predilection for occurrence of gliomas approximately corresponded with the sites of persistence of non-pathological immature cells. A glioma is known to arise spontaneously also in the brain of mouse. In order to see whether such a correlation, as shown in the human fetus, might be true in fetal brain of the animal, the present author preliminarily investigated histologically the development of immature cells in 18 brains of normal fetal mice at their 12th~19th gravid day.

a) Mesencephalon and Rhombencephalon

1) Cerebellum and Anterior Medullary Velum

The cerebellum of 12-day-old fetal mouse was no more than a flat upheaval in appearance ("cerebellar plate"). Anterior medullary velum, on the other hand, was so thick that its boundary against the quadrigeminal body was indiscernible. In the loose tissue adjoining to the outermost layer of the matrix of the anterior medullary velum, polar spongioblasts and neuroblasts were found. In the anterior medullary velum itself and ventricular wall of the cerebellar plate was seen a rather thick

layer of argentophilic immature cells which were densely packed together. There was no distinct demarcation between the ependymal layer and subependymal zone (matrix).

In the case of 13-day-old fetus, the anterior medullary velum showed an indentation at the transitional part to the quadrigeminal body so that the distinction of the two structures became apparent. Even at this stage, the matrix of the anterior medullary velum and of the ventral portion of the cerebellar plate intermingled with the ependymal layer. In the posterior part of the cerebellar plate and near the midline, which corresponded to the cerebellar nodulus, the matrix formed a thick layer which gradually decreased its thickness coming towards the anterior medullary velum (Fig. 3).

The cells constituting the matrix were undifferentiated apolar cells (Fig. 4) which had a scanty cytoplasm and a relatively large oval nucleus with numerous fine intranuclear granules. Just external to the matrix was seen a layer of cells composed of apolar spongioblastic series possessed of an argentophilic and oval or pear-shaped nucleus and those of apolar neuroblastic series with a somewhat larger but less argentophilic oval nucleus (Fig. 5). This cell layer was found thinned around the midline of the cerebellar plate but thicker and more irregular in arrangement laterally. Near the brachium pontis, densely packed clusters of immature cells were occasionally seen (Fig. 6).

Brzustowicz and Kernohan[3] stated that they observed proliferation of the ependymal layer at the junction between the anterior medullary velum and quadrigeminal body. However, the proliferated cells seemed to us to be those of the precursor of the external granular layer but not of the ependymal layer.

Histological findings of the brain in 14-day-old fetus did not differ greatly from those in the 13-day cases, although the differentiation of the ependymal layer from the matrix was obvious in most of the former cases.

In 15-day-old fetus, the cerebellum was observed to have rapidly differentiated, and undifferentiated apolar cells were uncovered in no place on the ventral portion of the cerebellum other than the posterior medullary velum and the cerebellar nodulus. A great majority of the cells had emigrated in the medullary substance of the cerebellum where they developed into polar spongioblasts and neuroblasts. In the matrix of the anterior medullary velum, on the other hand, innumerable apolar spongioblasts and neuroblasts were still present; a fact which indicates more retarded maturity as compared with the medullary substance of the cerebellum. The external surface of the cerebellum in its entirety was covered with the external granular layer, as a thin cell layer, composed of argentophilic apolar cells which here and there formed densely packed clusters. In particular, in the posterior medullary velum where the chorioid plexus of the 4th ventricle is attached to and near the cerebellar nodulus, a considerably thickened external granular layer was seen (Fig. 7).

In the 16-day-old fetus, the internal granular layer was seen to be formed by apolar spongioblasts and more numerously by apolar neuroblasts inside the external granular layer. The internal layer appeared not uniform in thickness, but in the

cerebellar vermis it was unusually thick and in the cerebellar hemisphere rosary-like.

In the 17-day-old cases, the differentiation of the cerebellum was almost the same with that of the 16-day-group.

At the 18th fetal day, polar neuroblasts were seen to have evidently aggregated in the medullary substance of the cerebellum, forming nuclei. The glia cells surrounding these nuclei were rather mature, as contrasted with those in other portions, as they already developed into astroblasts or even astrocytes.

At the 19th fetal day, the external granular layer became uniformly thick, arranging itself parallel to the foliated outer surface of the cerebellum. Polar spongioblasts were found to have arranged, toward the molecular layer, from the external granular layer which consequently appeared thinner than that in the 18-day cases. In the vermis, near the anterior medullary velum, posterior medullary velum and around the cerebellar nodulus still persisted undifferentiated apolar cells, the density of which was most pronounced in the cerebellar nodulus; the more the nearer to the medullary substance. Accordingly, in the medullary substance were seen innumerable cell clusters which had indistinct delimitation from the surrounding tissue (Fig. 7). Similar cell conglomerates were also visible in other portions of the brain.

2) Quadrigeminal Body

In the 12-day-old fetus, the quadrigeminal body was in general thin, its caudal portion being protruded posteriorly and then continuing to the anterior medullary velum. The mesencephalic aqueduct, therefore, had an enormous cavity, so-called mesencephalic cavum. All the cells in the quadrigeminal body at this stage were undifferentiated apolar ones which constituted remarkably cellular matrix. Outside this matrix was found a less cellular part in which apolar spongioblasts and neuroblasts were observed.

In the 13～14-day-old fetuses, the quadrigeminal body increased its thickness and the mesencephalic cavity narrowed. As the caudal portion of this cavity is thinner than the rostral, the cellular density of the former appeared larger than that of the latter (Fig. 8).

The same findings were confirmed in all the 15～19-day-old cases, as well.

In the 15-day-old cases, the thickness of the quadrigeminal body further increased, particularly in its rostral portion. At the middle of that structure a bending and folding of the floor of the aqueduct was occasionally found (Fig. 9).

Brzustowicz and Kernohan[3] pointed out that folding and bending of the ependymal layer were responsible for occurrence of ependymal cell rests which seemed to be most liable to appear around the aqueduct, as far as the brain of the fetal mouse was concerned. Complicated ramified form as observed by Shimada in the brain (aqueduct) of 5-month-old human fetus was, however, not present (From this fact it can be conjectured that the brain of the mouse has a more simple architecture and differentiation than that of the man has.). In the roof of the mesencephalic aqueduct, the matrix could be distinguished from the ependymal layer which was multi-stratified. The cells constituting the matrix were largely apolar spongioblasts and neuroblasts excepting its caudal part. In the outermost layer were seen polar

spongioblasts, while apolar spongioblasts and neuroblasts reappeared in and near the subpial region.

In the 16-day cases, the ependymal layer became so thin that it was composed of only one layer of rather mature cells of the ventricular wall, although multistratified parts could be seen in some places. Such an irregularity in the thickness of the ependymal layer may be accounted for the frequent occurrence of ependymal cell rests around the mesencephalic aqueduct.

In the 17- and 18-day cases, the cells of the quadrigeminal body were generally matured. In the lateral portion, in particular, the cells developed into astroblasts or even astrocytes. Near the midline and in the subpial zone as well as in the caudal portion, immature cells were seen still persisting.

In the 19-day old fetus, the state remained almost unchanged. That is to say, the quadrigeminal body showed, among the brain structures distal to the midbrain, a more retarded development. Such a tendency was in much resemblance with what was found by SHIMADA in the human fetal brain.

3) Cerebral Peduncle, Pons and Medulla Oblongata

In the floor of the mesencephalic aqueduct and 4th ventricle of the 12-day-old fetus, the demarcation inbetween the ependymal layer and matrix was yet indistinct where a densely clustered layer of undifferentiated apolar cells was seen. Polar cells were already visible in the subpial zone of the ventral side of the cerebral peduncle, pons and medulla oblongata.

In the 13-day-old cases, the matrix of the floor of the aqueduct was the thinnest and that of the taenia rhombencephali (ponticulus) the thickest (Fig. 10). At the midline of the floor of the 4th ventricle, the ependymal cells outgrew ventrally to form a long processus, striae medullaris. In the subpial zone of the ventral surface and at the midline, unipolar spongioblasts and embryonic rod cells appeared. But on the ventral part of the pons and in the subpial zone, apart from the midline, apolar neuroblasts tended to conglomerate. In the floor of the 4th ventricle at this stage, a deep transverse groove began to appear, around which were found densely cellular ependymal layer and matrix (Fig. 11). Immature cells were confirmed to be persisting even at the very end of the fetal period.

In the 14-day-old fetus, polar cells were about to migrate from the floor of the aqueduct and 4th ventricle ventralward and polar spongioblasts and neuroblasts increased in number in the cerebral peduncle, pons and medulla oblongata. On the ventral surface of the medulla oblongata, the nucleus originis olivaris was found to be formed, the glia cells therearound being remarkably matured.

In the 15-day cases, all the nuclei from the cerebral peduncle to the medulla oblongata, inclusive, were apparently constituted. And the area densely packed together with undifferentiated apolar cells around the taenia rhombencephali markedly decreased its extension. These apolar cells developed into apolar spongioblasts and neuroblasts. On the ventral side of the pons (nucleus pontis), apolar neuroblasts gathered together so that they made a semilunar cluster of cells. On the dorsal side, networks composed of astroblasts, astrocytes and multipolar neuroblasts were

seen.

In the 18- and 19-day cases, all the cells inbetween the cerebral peduncle and medulla oblongata further differentiated, and astroblasts and astrocytes with fine processes were uncovered especially in the lateral parts and adjacent to the pia mater. The nerve cells constituting various nuclei also progressively differentiated, although apolar spongioblasts and neuroblasts were observed to have persisted in some part of the taenia rhombencephali. Ventral to the pontine nucleus and subpially were also seen apolar neuroblasts and spongioblasts (Fig. 12).

b) Prosencephalon

1) Diencephalon

The 3rd ventricle of the 12-day-old fetus was very large and the constituent cells of the massa intermedia, thalamus and hypothalamus were nothing but undifferentiated apolar cells.

In the 13-day cases, the thalamus and hypothalamus thickened medially and, consequently, the 3rd ventricle became flattened, enfolded, so that sagittal sections revealed many grooves. In the periventricular matrix were found areas of dense cellularity and numerous argentophilic apolar round cells were seen in the parts from the infundibulum to the mammillary body, while polar spongioblasts were present in the part lateral to the thalamus and hypothalamus and near the internal capsule.

In the 14- and 15-day cases, the ependymal layer of the 3rd ventricle appeared comparatively thick, but was evidently delimited from the matrix. In the hypothalamus, irregularly shaped clusters of ependymal cells were frequently seen in the olfactory fossa and infundibulum. In the massa intermedia, the ependymal layer was occasionally found to have ditched in the interior of the parenchyma (Fig. 13).

In the 16- and 17-day-old fetuses, the findings were similar to those of the 15-day. Immature cells were abundantly found in the periventricular areas.

In the 18-day case, such immature cells were about to differentiate into polar spongioblasts and neuroblasts with short and thick processes. The cellularity was dense and immature cells persisted in the lateral wall of the 3rd ventricle, tuber cinereum and mammillary body, in particular. Spielmeych, Schob and Schwarz's[13] observation of cell clusters of variable forms in the perivascular areas in the hypothalamus of human fetus seems to be in accord with our finding.

Even in the 19-day-old fetus, persistence of immature cells in the periventricular areas was confirmed. Clusters of cells of irregular shapes were not infrequently seen in the hypothalamic region.

2) Telencephalon

The telencephalic mantle of the 12-day-old fetal brain appeared thin and its cellular density was as great as that of the quadrigeminal body. Innumerable columns of undifferentiated apolar cells were arranged in perpendicular direction against the wall of the lateral ventricle. The so-called "Ganglienhügel" at this stage occupied the floor of the lateral ventricle, protruding medially in semi-globoid form, where the matrix had a considerable thickness (Fig. 14).

In the 13-day case, the mantle increased in thickness and apolar spongioblasts and neuroblasts were seen emigrating from the matrix of the lateral ventricle toward the intermediate zone which was yet quite narrow. In the cortex, apolar neuroblasts were found to have formed a thick layer and apolar spongioblasts were arranged in the marginal zone in parallel direction with the pia mater.

In the 14-day case, the lateral ventricle extended to the rhinencephalon and the telencephalon was almost completed in configuration. The matrix of the lateral ventricle was as a whole rather thick, particularly so in the frontal portion as compared with the occipital. Around the junction between the anterior and olfactory horn and also in the temporal horn, the thickness of the matrix of the lateral ventricle was uniform and clusters of immature cells were found occasionally.

In the 15-day case, polar spongioblasts emigrating from the outermost layer of the caudate nucleus into the internal capsule increased in number and polar elements were observed to be emigrating from the matrix of the roof of the lateral ventricle toward the intermediate zone and callosal body.

In the 16-day-old fetus, the hippocampus began to develop. In its cortex were found apolar neuroblasts arranged regularly and in the subcortex a number of apolar spongioblasts conglomerated, the cellularity being quite dense.

Even in the 17- and 18-day cases, undifferentiated cells were to be found in the matrix of the lateral ventricle, especially in the so-called "Ganglienhügel". However, the cells emigrating therefrom in the intermediate zone, increased in number, broadening the zone where polar spongioblasts and neuroblasts were arranged in networks (Fig. 15). Occasionally, apolar spongioblasts were seen emigrating from the matrix of the ventricle toward the intermediate zone and attached to the blood vessels. In the cortex, polar neuroblasts with short processes were differentiated and in the marginal zone polar spongioblasts were present in large numbers. In the cortex of the olfactory brain, polar neuroblasts were arranged quite regularly but the matrix still had a considerable thickness. In the anterior commissura, corpus callosum and fornix were found many polar spongioblasts and piloid astrocytes. Around the interventricular foramina and sulcus limitans, on the contrary, persisting immature cells were to be found. The finding may correspond with that confirmed by Globus and Kuhlenbeck.[5]

In the 19-day-old fetus, differentiation of individual cells, generally speaking, further proceeded, although the immature cells above-mentioned still persisted. In other words, the telencephalon showed a more retarded development than the brain distal to the midbrain. It is presumed that the matrix still contains a thick layer of persisting immature cells when the fetus comes to birth (Fig. 16). Allen[1] reported that the mantle layer of the lateral wall and roof of the lateral ventricle persisted in residual form even two years after the birth. The same tendency was also observed in the brains of the 19-day-old fetal mouse. Shimada found that clusters of apolar cells were found in the transitional part between the rhinencephalon and frontal lobe in the human fetal brain, while the present author confirmed that the matrix of the rhinencephalon was very thick and not uniform in fetal brain of mouse.

B. Development of Cells in the Brain of Fetal Mouse with Malformed Posterior Limb or Tail Resulting from Injection of Urethane Solution and in That with Grossly Normal Body Appearance from the Same Mother Mouse.

The malformations obtained by treatment of urethane were, as already mentioned, polydactylism of the posterior limb and short tail. In all the cases were there found no gross malformations in the brain, but slight dilatation of the lateral and 3rd ventricle in a few cases. However, in such brain tissues presumed to be in normal ranges, some changes probably due to injection of urethane solution were present. The findings in the 18 cases will be described hereunder.

a) Mesencephalon and Rhombencephalon

1) Cerebellum

Brzustowicz and Kernohan classified the cell rests found in the periventricular tissues of the 4th ventricle of man in the following four groups: (1) mixed cell rests, (2) ependymal cell rests, (3) external granular cell rests, and (4) neural cell rests. In the case of malformations in fetal mouse as a result of urethane treatment of the mother mouse, clusters resembling the mixed cell rests and composed of irregular immature cells were occasionally found in the medullary substance of cerebellar hemisphere. Such clusters were, however, readily confused with the rosary-like cell clusters resulting from incomplete differentiation of the internal granular layer of the cerebellum of the normal fetus 16~17 days old. Such states may be considered as retarded development of the cerebellum. In Fig. 17 are shown the mixed cell rests found in the vermis, near the anterior medullary velum. Internal to the internal granular layer are found densely cellular clusters of large spindle-shaped cells, apolar neuroblasts and spongioblasts. In other cases, too, were also found rests of immature cells arranged irregularly in the same region. Fig. 18 shows the mixed cell rests, well delimited from the surrounding tissue, in the medullary substance of the rostral part of the cerebellar hemisphere and near the wall of the 4th ventricle. These are thought to be the persisting immature cells, normally recognizable in the brain of 14-day-old fetal mouse in the transitional part between the quadrigeminal body and cerebellum. Fig. 19 shows the mixed cell rests found internal to the internal granular layer of the lateral part of the cerebellar hemisphere. In the cerebellar nodulus, which was pointed out by Brzustowicz and Kernohan as the most frequent seat of mixed cell rests, undifferentiated apolar cells were found, but not in a larger number than in normal fetus (Fig. 20). Ependymal cell rests were most frequently observed around the brachium pontis, but they were not the continuation from the ependymal layer of the lateral wall of the 4th ventricle. In the Fig. 21 are shown argentophilic apolar cells which have a small nucleus, and large cells resembling those of the ependyma which have a fusiform nucleus, both kinds of cells being arranged parallel to the wall of the 4th ventricle. In variable parts of the cerebellum were observed presumable external granular cell rests. In some parts, it appeared that the external granular layer increased its width (Fig. 22), or just caved in. Such findings were frequently seen in the vermis and posterior medullary velum. As was confirmed by Brzustowicz et al., neural cell rests were

found quite rarely. In Fig. 23 were seen cell clusters rich in apolar spongioblasts, but apolar neuroblasts were also seen intermingled. Beside the variable cell rests above-written, the pial membrane of the cerebellum was occasionally seen to have caved in the cortex. Proliferation of cells took place also in some part of the chorioid plexus of the 4th ventricle, forming nodules (Fig. 24).

2) Mesencephalon

The cell rests in the midbrain appeared largely around the mesencephalic aqueduct. This can be reasoned from the fact that the wall of the aqueduct, which was initially quite a large cavity, was gradually enfolded as the quadrigeminal body and cerebral peduncles became thicker and that the thickness of the ependymal layer was not uniform in some parts (at the 14th and 15th gravid day). In the brain of the malformed mouse (malformation either of the posterior limb or tail), such a tendency was much pronounced and clusters of cells simulating ependymal cell rests were uncovered around the aqueduct. The Fig. 25 shows densely packed cell conglomerates arranged parallel to the aqueduct in the caudal portion of the quadrigeminal body, some of which were in close contact with the ependymal layer. In the transitional part between the aqueduct and 3rd ventricle, the ependymal layer was seen proliferating (Fig. 26). In the cases with urethane treatment, proliferative abnormality was confirmed not only around the aqueduct but also in other parts of the brain. Changes also of the pia mater were noticed in the midbrain. As shown in Figs. 27 and 28, cells of the pial membrane which had indistinct outline and a large fusiform nucleus were seen proliferated, forming large clusters in the dorsolateral surface of the quadrigeminal body, where this was in close contact with the occipital lobe. These proliferative changes of the pia mater were in resemblance with the outgrowth of the chorioid plexus of the cerebellum of the mouse treated with urethane (Fig. 24).

3) Pons and Medulla Oblongata

Cell rests were scarcely noticed in the pons and medulla oblongata. However, around the transverse sulcus of the 4th ventricle resulting from bending and folding of the pons at the 13th gravid day, persisting immature cells could be observed even in the normal cases. In the brain of the mouse treated with urethane, such a tendency was much conspicuous and more marked and characteristic proliferative changes could be seen. Fig. 29 is one of the most representative illustrations, in which are shown multiple protrusions in irregular forms of the ependymal layer toward the ventricular cavity. The cells which constituted these protrusions were immature cells which had scanty cytoplasm and an argentophilic oval nucleus. The ependymal layer in other parts was thin and the ependymal cells were quite matured. Brzustowicz et al. and Shimada reported that ependymal cell rests were frequently observed in the taenia rhombencephali of the human fetal brain. In the brain of mouse treated with urethane, no such cell rests were present and protrusions of immature ependymal cells similar to those described above were predominantly found.

b) Prosencephalon

1) Diencephalon

The diencephalon, in general, had a dense cellularity. Particularly, around the 3rd ventricle, thalamus and hypothalamus were found clusters of apolar neuroblasts and spongioblasts, undifferentiated apolar cells and irregularly shaped ependymal cells. Such cell clusters were, however, also present in the normal cases, though different in cell constituents and not always so large in number as in the mouse treated with urethane. But, there were a few cases in which rosette-like clusters of argentophilic immature cells were disclosed in the part distant from the wall of the 3rd ventricle (Fig. 30) or proliferation of subependymal cells in the floor of the same ventricle, protruding into the ventricular cavity, was noted (Fig. 32).

2) Telencephalon

Cell rests were more frequently observed in the telencephalon than in other parts of the brain. Between the intermediate zone and the outermost layer of the subependymal matrix, which persisted in the roof and lateral wall of the lateral ventricle, argentophilic immature cells with a round nucleus were seen to have conglomerated alongside the blood vessels (Fig. 33). The similar finding was already confirmed in the human fetal brain by SHIMADA who termed this as "perivascular cuff of apolar spongioblast (ARAKI-SHIMADA)". Around the transitional part between the caudate nucleus and intermediate zone, ring-form clusters of so densely packed cells were also seen that the individual cells could not be identified from each other (Fig. 34). Such clusters may likewise be considered as the perivascular cuff. Here and there were seen cell clusters which had no connection with the blood vessels (Figs. 35 and 36). Fig. 37 shows the immature cell clusters seen in the transitional part from the rhinencephalon to the frontal lobe. In Fig. 38 are shown cell clusters of peculiar form resembling eye-glasses. In one case was there found in the parietal lobe round shaped cluster of densely packed immature cells at the tip of the pial protrusion which ditched deeply in the cortex together with the blood vessels (Fig. 39).

COMMENTS

I. Differentiation of the immature cells and occurrence of cell rests in different parts of the brain were studied in normal fetal mice at their 12th~19th gravid day.

Nearly the same process of differentiation as that found in human fetal brain by IDE,[6] SHIMADA[14] or GLOBUS and KUHLENBECK[5] was thus obtained. And the parts of the brain where undifferentiated apolar cells persisted were mostly the same as in the case of human fetal brain. The cell rests were thought to appear, at the 13th~15th gravid day when every part of the brain promptly developed, owing to, as BRZNSTOWICZ and KERNOHAN mentioned, (1) erroneous folding or bending of a cell layer on itself, (2) incomplete fusion of several cell layers, (3) arrest in the migration of immature elements from the germinal epithelium, and (4) alteration or dérangement of the normal process of differentiation of germinal cells. As the brain of mouse is much simpler than that of man, the cell rests found were less in number in mouse than in man.

Ⅱ. The brains of malformed fetuses and those of normal by appearing ones, both from one and the same mother mouse treated with intraperitoneal injection of urethane solution, revealed somewhat more retarded cell differentiation if compared with the brains of normal fetuses of the same gravid days. For example,

1. Dérangement of the internal granular layer of the cerebellum,
2. Persistence of the external granular layer in the vermis, particularly of the spindle-shaped undifferentiated apolar cells,
3. Clusters of immature cells with a small round nucleus in the cerebellar hemisphere and near the lateral wall of the 4th ventricle,
4. Clusters of undifferentiated apolar cells (though not unusually numerous) around the cerebellar nodulus,
5. Neoplastic outgrowth of the chorioid plexus of the 4th ventricle,
6. Proliferative protrusions of the matrix of the 4th ventricle,
7. Ependymal cell rests around the mesencephalic aqueduct,
8. Proliferation of ependymal cells in the transitional part between the aqueduct and 3rd ventricle,
9. Thickening of the pial membrane on the dorso-lateral surface of the midbrain,
10. Rosette-like cell conglomerates around the 3rd ventricle and proliferation of the matrix in the floor of the same ventricle,
11. Perivascular cuff of apolar spongioblast in the neighborhood of the lateral ventricle and in the transitional part between the caudate nucleus and intermediate zone,
12. Clusters of immature cells in the adjacent part to the matrix of the rhinencephalic cavity, and
13. The pial protrusion which caved in the cerebral cortex of the temporal lobe was found in 1～2 cases.

It seems, however, impossible to draw a definite conclusion, on the base of the mere fact that the 19-day-old fetal mouse treated with urethane show somewhat more retarded development than the normal one, that the clusters of immature cells written above are really the so-called embryonal cell rests.

Spielmeyer, Schob and Schwarz were of the opinion that the variable cell clusters found in the hypothalamus, alongside the blood vessels, were not identical with the cell rests, but cell conglomerates normally appearing during the process of differentiation. Murakami expressed the view that production and absorption of the cerebrospinal fluid before the stadium when plica chorioidea was formed took place by way of the ependymal layer, proliferation of which would result in overproduction of the fluid. In some of the urethane-animals were confirmed proliferative changes of the ependyma or outgrowths of cells in the floor of the 4th ventricle and the wall of the aqueduct. However, it could not be clarified whether such changes had a parallel relationship with occurrence of the hydrocephalus.

In summarizing, the changes which the brain tissue might suffer by urethane were that (1) the number of the cell rests was larger than usual and (2) unusual proliferation of the cells was in some parts observed, though not remarkable.

Urethane had rather slight influence on the central nervous system. This can readily be presumed, as gross malformations rarely occur in the brain by urethane treatment. It should, however, be noted that some histological abnormality may be detected in the brain, when some malformation is actually or potentially present in some part of the body other than the brain. Since gross malformations are not always uncovered in patients harboring a glioma, we must mean histological abnormalities, when we say that congenital developmental anomalies may have something to do with occurrence of gliomas. Therefore, cell rests and or persisting immature cells were called in question, which, however, were not particularly rich in number, as far as the present study was concerned, in the cerebellum, the most frequent seat of predilection for occurrence of gliomas in children. On the contrary, such cells were more frequently found in the cerebrum, a fact indicating the complicacy of the problem under discussion.

CONCLUSIONS

(1) The process of development of nerve cells and glia cells in the brain and occurrence of cell rests were studied in the 18 brains of normal fetal mice at their 12～19th gravid day. The results obtained were that the process of differentiation of the brain of fetal mouse roughly corresponded with that of man.

(2) In the brains of 18 malformed fetuses (either with polydactylism or with a short tail) from one and the same mother mouse treated with urethane, somewhat more retarded development tended to take place if compared with the normal contrasts.

(3) Both the malformed and seemingly normal fetuses, derived from the same mother mouse treated with urethane, revealed more frequent occurrence of cell rests than contrast fetuses, though the difference was not quite conspicuous. There were found a few cases in which the ependymal layer, subependyma, pial membrane and chorioid plexus showed proliferative changes.

(4) When some gross malformation was present in some part of the body or when such a defective constitution was inherited in spite of no gross abnormality, more or less abnormal changes histologically might be found in the brain. Should congenital developmental anomaly have something to do with occurrence of a glioma, such histological anomalies must be called in question. Cell rests and persisting immature cells may be given as such, which, however, were not always rich in number, as far as the present study was concerned, in the cerebellum but they were rather more in the cerebrum. This may indicate the complicacy of the problem under discussion, i. e. congenital histological anomalies and occurrence of gliomas.

The present study was supported by a grant in aid from the Ministry of Education.

REFERENCES

1) Allen, E.: The cessation of mitosis in the central nervous system of the albino rat. J. Comp. Neurol., **22**, 547, 1912.
2) Bailey, P., and Cushing, H.: A classification of tumor of the glioma group etc. Philadelphia, Lippincott, 1926.
3) Brzustowicz, R. J., and Kernohan, J. W.: Cell rest in the region of the fourth ventricle,

(a) Their site and incidence according to age and sex. Arch. Neurol. and Psychiat., **67**, 585, 1952.
(b) Histologic and embryologic condition. ibid, **67**, 592, 1952.
(c) Their relation to the development of gliomas. ibid, **67**, 602, 1952.
4) Cushing, H.: Experiences with cerebellar astrocytomas. Critical review of 76 cases. Surg. Gynec. and Obst., **52**, 129, 1931.
5) Globus, J. H., and Kuhlenbeck, H.: Tumor of the striatothalamic and related region. Their probable source of origin and more common forms. Arch. Path., **34**, 674, 1942.
6) Ide, Z.: Embryologic studies on the cytogenesis and structure of glial cell. Japan Path. S., **38**, 163, 1949.
7) Murakami, U.: Manifestation of some abnormalities in abnormal environments. Studies on phenocopies in mice (First report). Jap. J. Genet., **27**, 176, 1953.
8) Nakahara, W.: Problem in cancer research. Sogoigaku, **10**, 10, 517, 1953,
9) Nishimura, H.: How do environmental factors put influence on the congenital constitution, as viewed from the experimental study of constitution in the human fetus. Saishinigaku, **11**, 12, 1956.
10) Ostertag, B.: Missbildungen, in Lubarsch et al.: Handbuch d. spez. path. Arat. u. Histol., Bd. **13**, Ⅳ. Teil, Kap. Ⅹ, 577, 1956.
11) Pfleger, L.: Beobachtungen über heterotopic grauer Substanz im Mark des Kleinhirns. Zentralbl. f. med. Wissensch., **18**, 468, 1880.
12) Raff, J. and Kernohan, J. W.: Relation of abnormal collection of cells in posterior medullary velum of cerebellum to origin of medulloblastoma. Arch. Neurol. & Psychiat., **52**, 163, 1944.
13) Schwarz, H., Goolker, P. & Globus, J. H.: The normal histology of infant's brains. Am. J. Dig. Chile, **43**, 889, 1932.
14) Shimada, M.: Persistence and misplacement of immature glial cells in various parts of the human fetal brain and their possible relation to the development of gliomas. Folia Psychiat. et Neurol. Japon., **8**, 238, 1954.
15) Shirota, G.: On the possible relationship of the predilection site for the glial abnormalities in the malformed fetal mouse brain to that for the human infant's gliomas. Arch. f. Jap. Chir., **37**, 579, 1958.

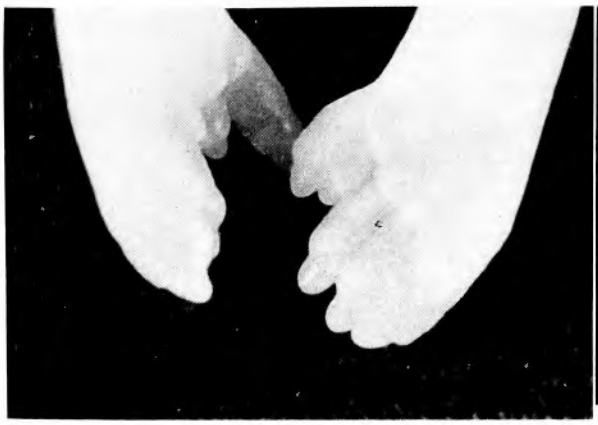

Fig. 1 Malformed fetal mouse (polydactylism) derived from the mother mouse treated with urethane at the 19th gravid day

Fig. 2 Short tail in the same descendant at the same fetal day

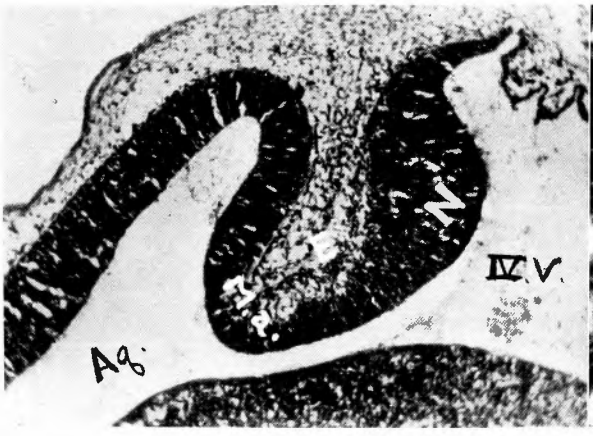

Fig. 3 Cerebellum and quadrigeminal body of 13-day-old fetal mouse (Sagittal section × 400)
N : cerebellar nodulus
Aq : mesencephalic aqueduct
E : external granular layer of the cerebellum
Ma : anterior medullary velum

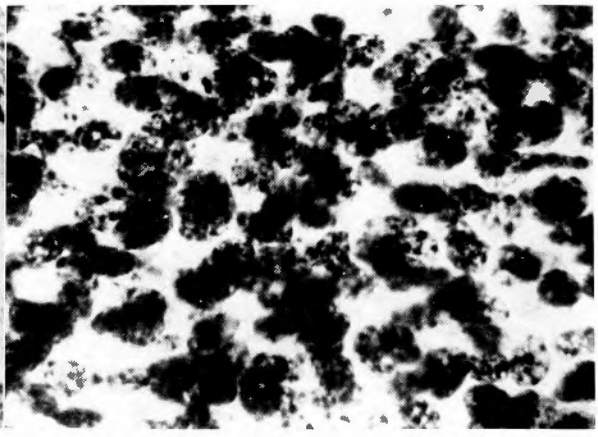

Fig. 4 Showing undifferentiated apolar cells (Silver diamino-carbonate method ×1000)

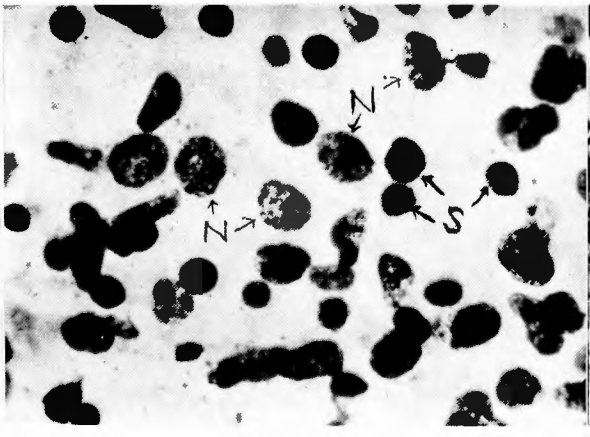

Fig. 5 Showing apolar cells of neuroblastic and spongioblastic series (Silver diamino-carbonate method × 1000)
N : apolar neuroblast
S : apolar spongioblast

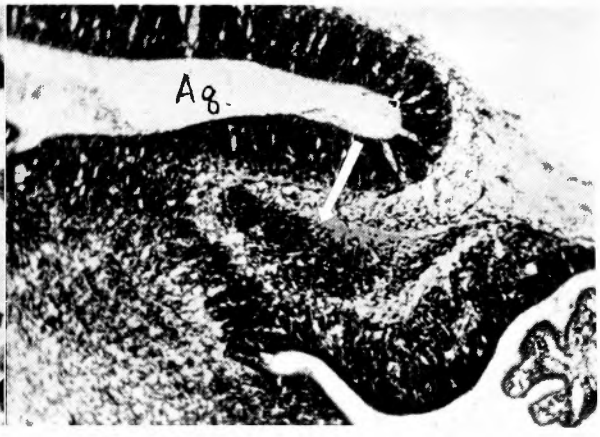

Fig. 6 The arrow indicates percursor of the external granular layer of the cerebellum of 15-day-old normal fetal mouse
(Sagittal section × 400)

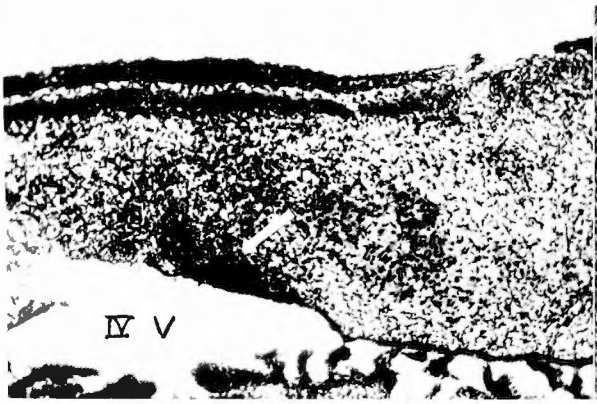

Fig. 7 Persisting immature cells in the cerebellar nodulus and cell clusters therearound of 19-day-old normal fetus (Sagittal section × 100)

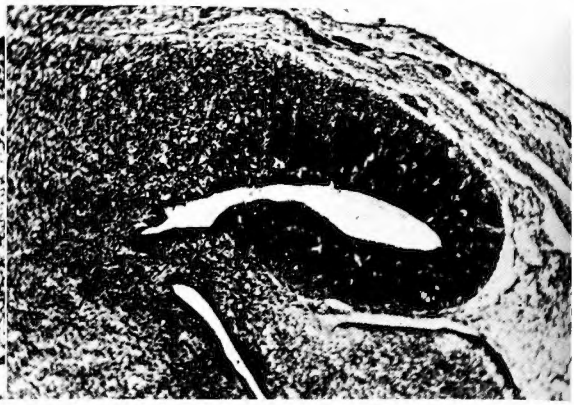

Fig. 8 Clusters of immature cells seen in the caudal part of the quadrigeminal body of 14-day-old normal fetus (Sagittal section × 100)

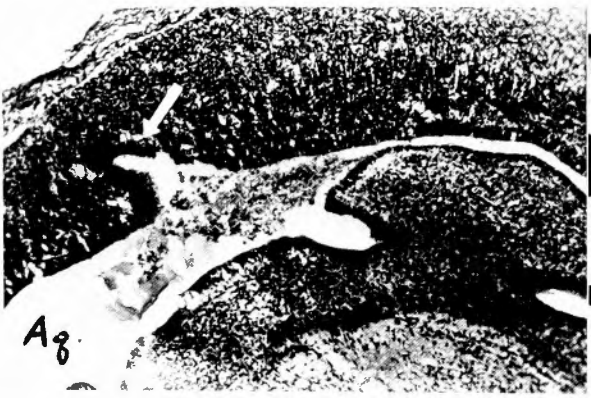

Fig. 9 Enfolding at the quadrigeminal body of 14-day-old normal fetus (Sagittal section × 100)

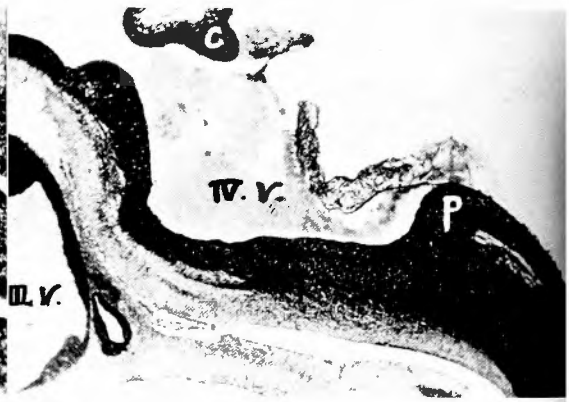

Fig. 10 Matrix of the floor of the aqueduct and 4th ventricle of 12-day-old normal fetus
(Sagittal section × 40)
P : ponticulus(taenia rhombencephali)
C : cerebellum

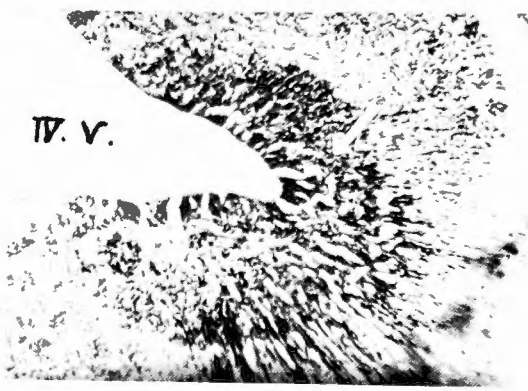

Fig. 11 Rests of immature cells in the transverse sulcus of the floor of the 4th ventricle of 13-day-old normal fetus (Sagittal section × 100)

Fig. 12 Conglomerate of immature cells ventral to the nucleus pontis of 19-day-old normal fetus (Sagittal section × 100)
P : nucleus pontis

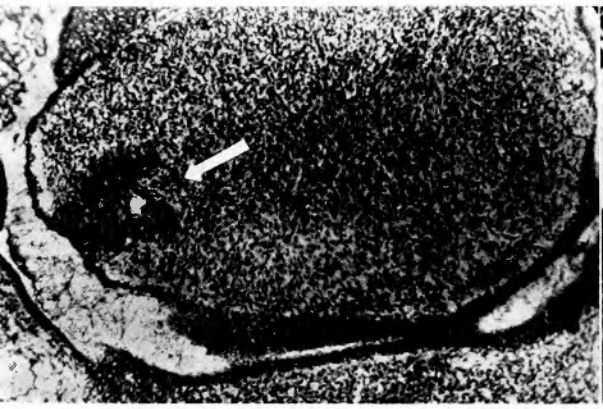

Fig. 13 Clusters resembling ependymal cell rests found in the massa intermedia of 15-day-old fetus (Sagittal section × 100)

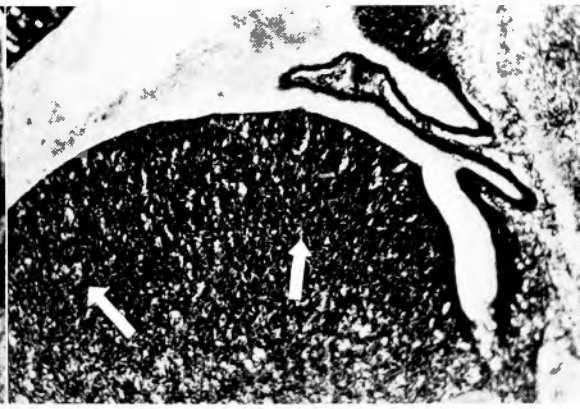

Fig. 14 Matrix of the "Ganglienhügel" at the 13th gravid day (Sagittal section × 100)

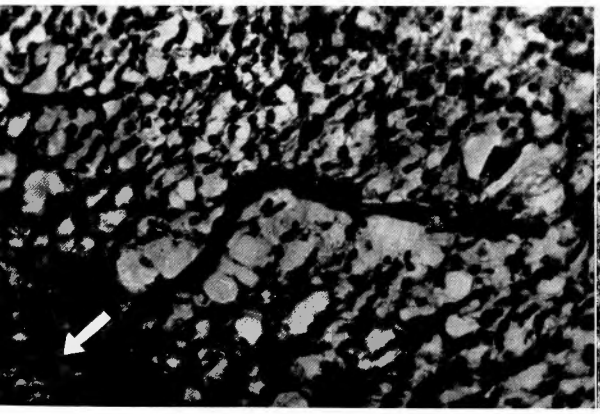

Fig. 15 Intermediate zone and the matrix (as indicated by an arrow left left down) in the 18-day-old normal fetus (Sagittal section × 400)

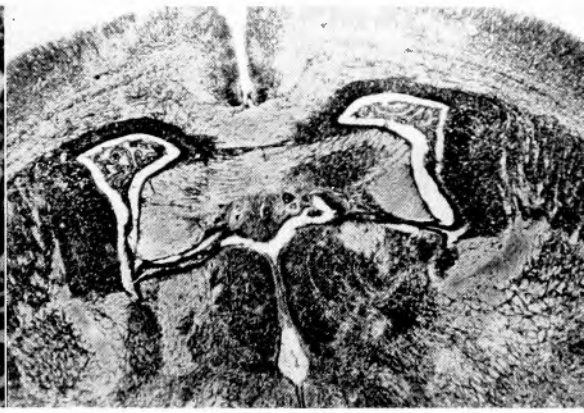

Fig. 16 Matrix of the lateral ventricle at the 19th gravid day (Frontal section × 40)

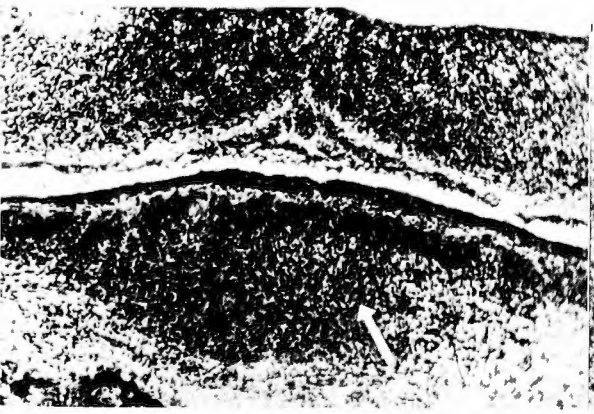

Fig. 17 Mixed cell rest found in the vermis of malformed fetal mouse derived from the mother treated with urethane (Frontal section × 100)

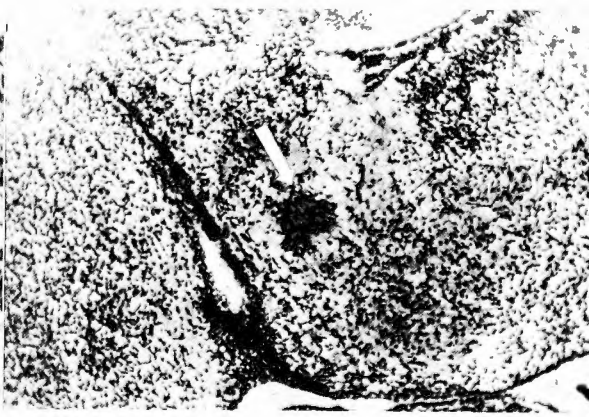

Fig. 18 Mixed cell rest found in the rostral part of the lateral wall of the 4th ventricle of the malformed fetus from the same mother (Frontal section × 100)

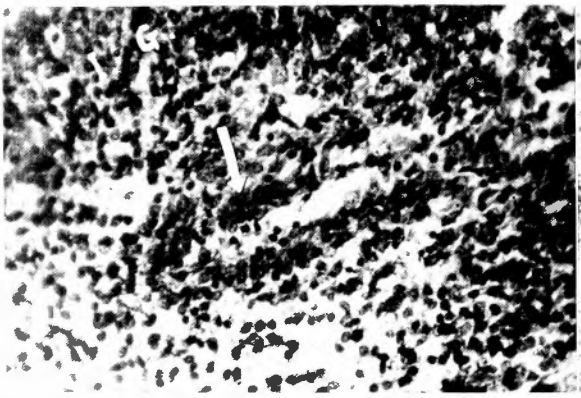

Fig. 19 Mixed cell rest found internal to the internal granular layer of the malformed fetal mouse(Frontal section × 400)
I.G. : internal granular layer

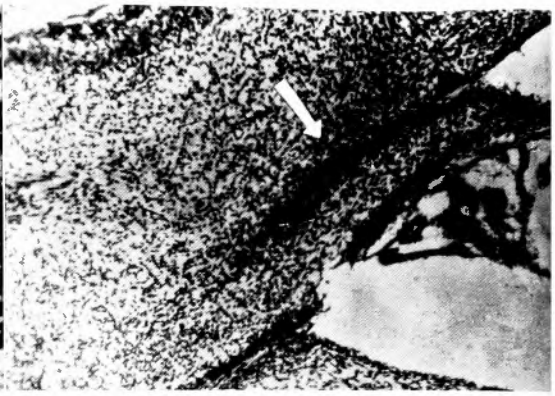

Fig. 20 Immature cell rest found in the cerebellar nodulus of the malformed fetal mouse (Frontal section × 100)

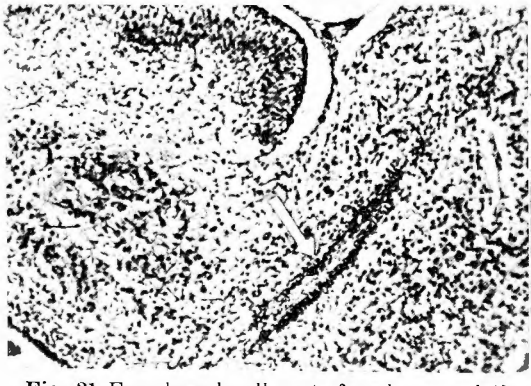

Fig. 21 Ependymal cell rest found around the brachium pontis of the malformed fetal mouse (Sagittal section × 100)

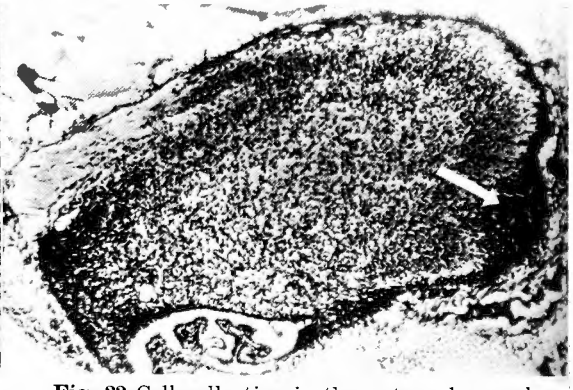

Fig. 22 Cell collection in the external granular layer of the malformed fetal mouse (Sagittal section × 100)

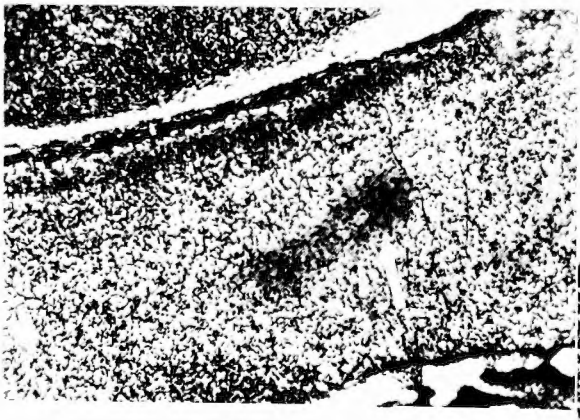

Fig. 23 Cluster of cells resembling neural rest around the cerebellar nodulus of the malformed fetal mouse
(Frontal section × 100)

Fig. 24 Nodular outgrowth of the chorioid plexus seen in the cerebellum of the malformed fetus
(Sagittal section × 100)

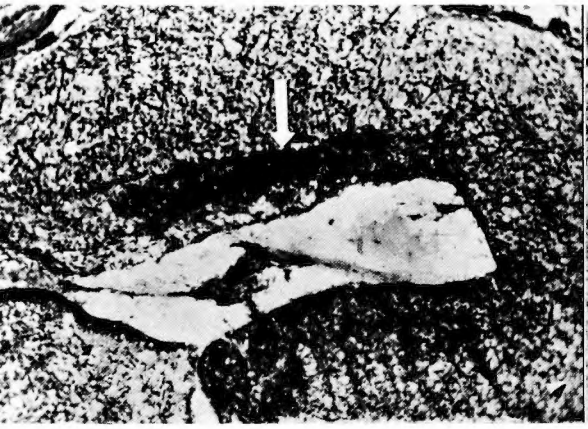

Fig. 25 Immature cell rest found in the quadrigeminal body of the malformed fetal mouse (Sagittal section × 100)

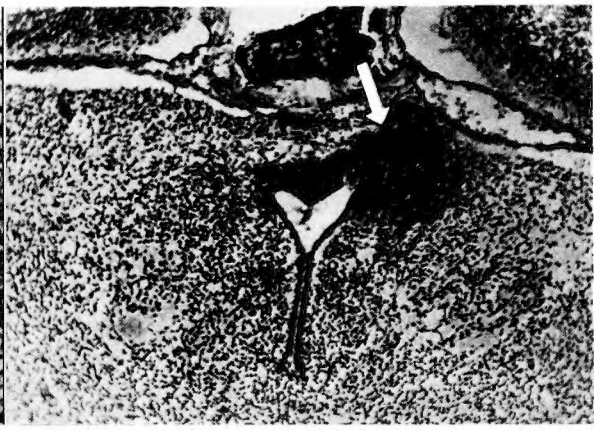

Fig. 26 Mesencephalic aqueduct and proliferative changes of the ependymal layer therearound seen in the malformed fetal mouse (Frontal section × 100)

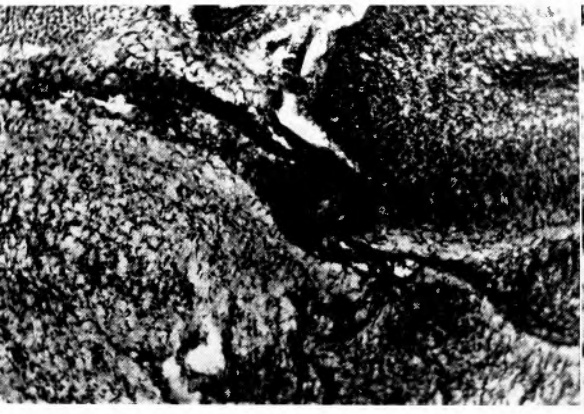

Fig. 27 Proliferation and thickening of the pia mater at the dorsolateral part of the midbrain (Sagittal section × 100)

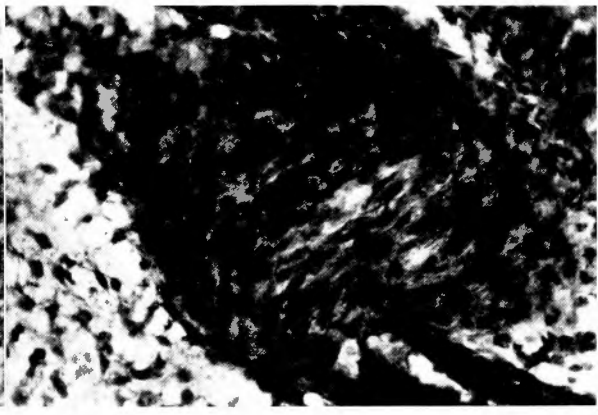

Fig. 28 Higher magnification of the part indicated by arrow in the fore-going figure (× 400)

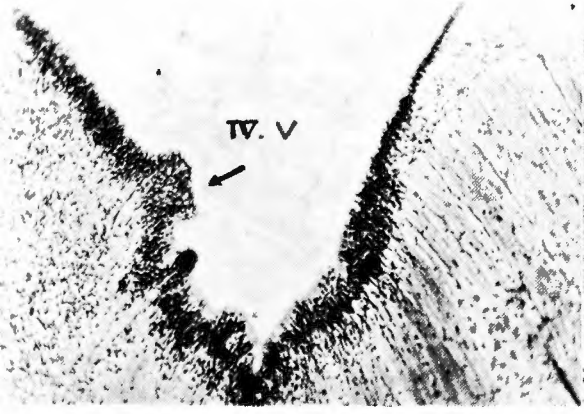

Fig. 29 Proliferative changes of the ependymal layer in the floor of the 4th ventricle of the malformed fetus (Sagittal section × 100)

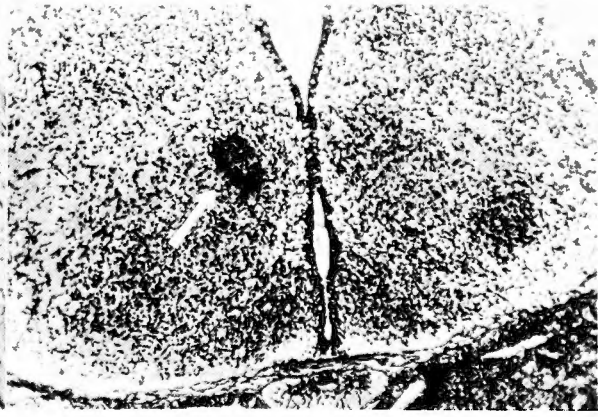

Fig. 30 Rosette-like cell aggregate around the 3rd ventricle of the malformed fetal mouse (Frontal section × 100)

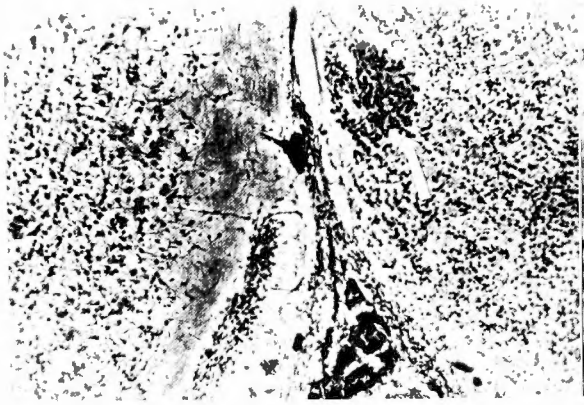

Fig. 31 Immature cell clusters around the mammillary body of the malformed fetus (Sagittal section × 100)

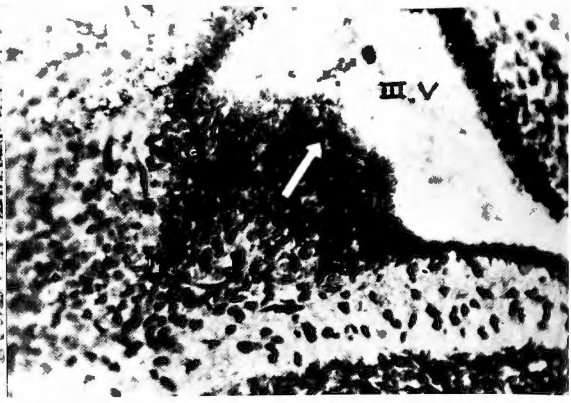

Fig. 32 Proliferative changes of the matrix of the floor of the 3rd ventricle of the malformed fetus
(Frontal section × 400)

Fig. 33 Perivascular cuff of apolar spongioblast seen around the intermediate zone of the malformed fetus
(Sagittal section × 100)

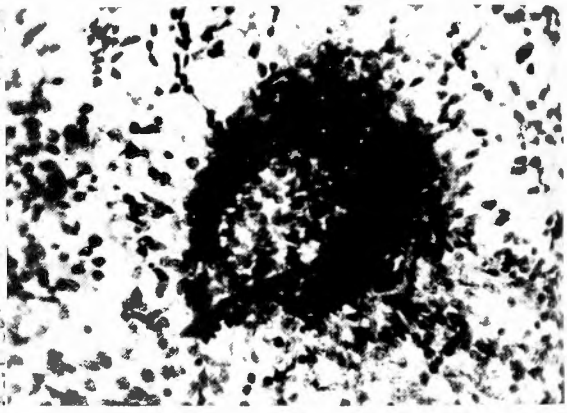

Fig. 34 Collection of cells found in the boundary between the intermediate zone and striate body of the malformed mouse
(Sagittal section × 400)

Fig. 35 Immature cell rest seen in the subcortex of the malformed fetal mouse
(Sagittal section × 100)

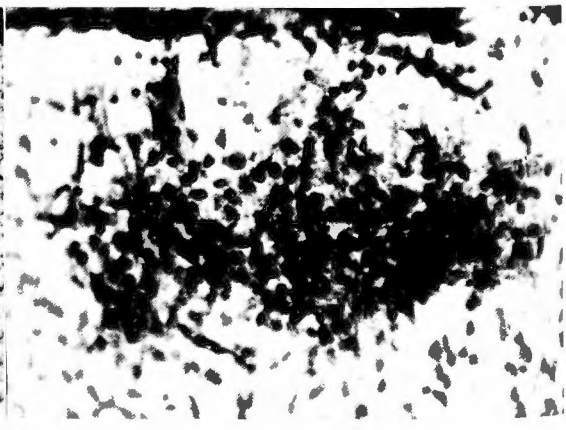

Fig. 36 Higher magnification of Fig. 35 (× 400)

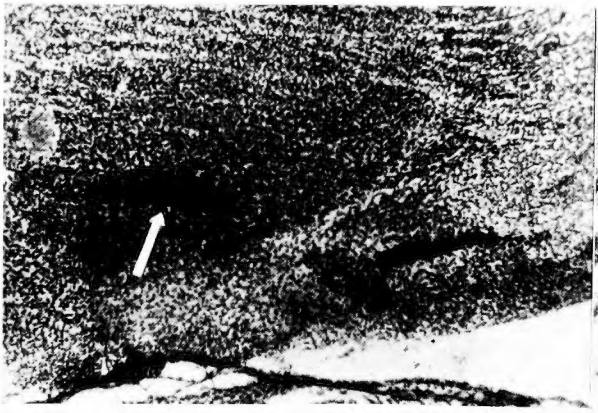

Fig. 37 Immature cell rests (↑) found in the transitional part between the olfactory brain and frontal lobe of the malformed fetus (Saittal section × 100)

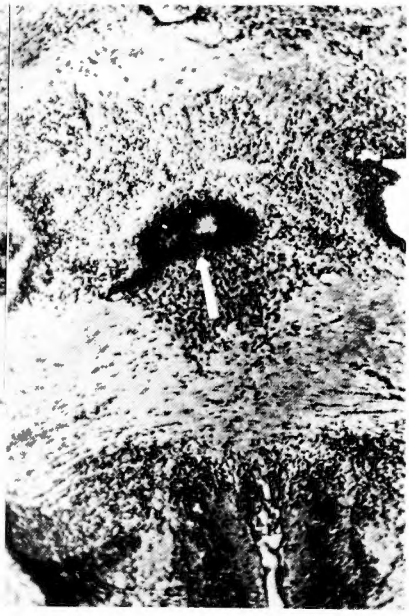

Fig. 38 Immature cell rest found around the septum pellucidum of the malformed fetus (Frontal section × 100)

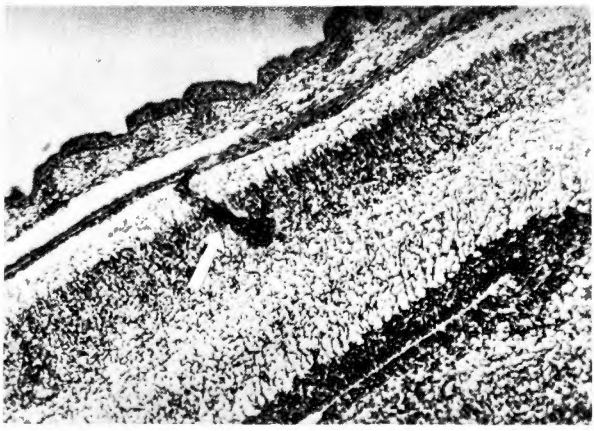

Fig. 39 Caving in of the pia mater in the parietal region of the malformed fetal mouse (Sagittal section × 100)

和文抄録

ウレタン処置畸形マウス脳の組織学的変化

——人間小児脳グリオームの発生より見たる——

京都大学医学部外科学教室第1講座 (指導：荒木千里教授)

山崎徳雄

最近実験的に，種々な外因で畸形を発生せしめ得ることが分つたが，畸形を発生させる外因の中のあるものは腫瘍をも惹起せしめ得るのでは無いかと考えられている (Strong 1945)．それで脳組織の様に，その正常な発育過程中に於ても，一種の組織畸形と見做すべきCell restsを生ずる臓器に，催畸形因子を作用させれば，更に多くのCell restsを発生するのではないか，そしてそれはより大なる腫瘍発生素因を意味するものではないかということが考えられる．それで，私は正常マウス胎仔脳の発育過程を組織学的に検索し，マウス胎仔脳に於けるCell restsの存在及び未成熟細胞残存好発部位を追求した後，西村氏法に従つて妊娠10日目の母マウス腹腔内にエチールウレタンを注入す第ることによつて生じた畸形胎仔の脳（妊娠第19日目に開腹して取出した）を同様組織学的に検索し，次の結果を得た．

A. 正常マウス胎仔脳に於ける神経細胞及びグリア細胞の分化過程及びCell restsの発生について．

正常マウス胎仔脳に於ける神経細胞並びにグリア細胞の成熟過程は，先に島田，井出，Globus及びKuhlenbeckが人間胎児に就いて検索したと略々同様であり，未成熟細胞残存部も人間胎児のものに似ており，小脳外顆粒層，小脳結節，菱脳紐，四丘体特に下丘，間脳腹側，側脳室周辺 Ganglienhügel 及び嗅脳室周辺であつた．Cell restsの発現に関しては，胎生13日目頃から15日目頃にかけて，脳各部が急速に発達する時期に生ずると考えられ，Brzustowicz及びKernohanの見解に一致する様である．併しマウス脳は人間の脳と比べると単純である為Cell restsの発現数は人間胎仔脳に比べて遙かに少数である．

B. ウレタン処置を行つた畸形胎仔及び同腹の外見上正常胎仔脳に就いて．

この場合同日数の正常胎仔脳に比べ，多少成熟が遅延する傾向が認められ，Cell restsの発現も若干対照例よりも多い．併し小児グリオームの最大好発部位である小脳よりもむしろ大脳に多く認められた事は，Cell restsの発現と脳腫瘍の関連性という問題の複雑さを示唆するものと思われる．又，第四脳室底部上衣下層，中脳水道周辺Matrix，第三脳室底部Matrixに於ける増殖性変化，第四脳室脈絡叢の増生，中脳背外側軟脳膜肥厚等の変化をも認めたが，ウレタンの中枢神経系に与えた作用は一般にいつて軽微であつた．