The fine structure of the retinal transient layer of Chievitz

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The transient layer of Chievitz appears between the inner and outer neuroblastic layers of the developing retina from six to about nine weeks of gestation in Macaca mulatta. Electron micrographs of this layer show that it is composed of intertwined neuronal and Müllerian cell processes in monkey, human, and rabbit fetuses. As the zone disappears by the scleral migration of the inner neuroblastic nuclei, portions of the layer of Chievitz probably become a part of the inner plexiform layer. A few cell junctions appear at this stage, suggestive of very early synapses.

Key words: layer of Chievitz, inner neuroblastic layer, outer neuroblastic layer, inner plexiform layer, synaptic connection, Müllerian cells, fetal retina, retinal development.

In 1887 Chievitz,1 in his study of the development of the fovea, described the distribution of the early neuroblasts in the developing human retina. Modern texts on ocular development2-4 refer to a non-nuclear layer which appears in the retina of fetuses from about six weeks to the third month of gestation. The nuclei of the primitive neuroblasts are at first distributed rather uniformly throughout the developing sensory retina. They appear to migrate sclerad so that in the early stages there is a nuclei-free zone adjacent to the vitreous and a nuclei-rich zone facing the primitive optic vesicle cavity and the pigment epithelium. The nuclei of these neuroblasts then separate into two groups by the migration of some of them vitread, leaving a nuclei-free zone between the inner and outer neuroblasts, the layer of Chievitz. Later many of these nuclei continue their migration, some from the inner neuroblastic layer migrating outwardly and some of the outer neuroblastic layer migrating vitread. In this process, the nuclei-free zone which was between them disappears, ending the transient period in which the layer of Chievitz existed. This report concerns itself with the fine structure of this layer in accurately staged primate fetuses. The material has been supplemented by the study of developing eyes of rabbits of known age.

Materials and methods

Fetal retinas from precisely timed pregnancies of M. mulatta were studied. The duration of gestation in this species is from 160 to 162 days. Speci-
mens of 43, 46, 49, 52, 54, 59, 60, 65, and 67
days were available. American Dutch rabbits were
bred in our laboratory and their fetuses removed
on the 19, 20, 21, and 22 days of gestation. The
eyes were fixed promptly in 3.5 per cent glutaralde-
hyde in 0.05 M. phosphate buffer at pH 7.3. They
were divided equatorially and an attempt was
made to remove the vitreous humor gently with
forceps. The fundus was divided into pieces with
a sharp razor blade and fixation continued for 16
hours after which they were postfixed in 1 per cent
osmic acid in the same buffer for 1 hour. Following
dehydration in a graded series of ethanol the
tissues were embedded in Epon. One micron thick
sections were stained with toluidine blue for light
microscopy and thin sections prepared from the
most developed portion of the fundus using glass
or diamond knives. The grids were examined with
a Siemens Elmiskop 1A.

**Light microscopic observations.** Sections of the
fundus of a 43-day-old Macaque retina show the
neuroblasts to be divided into an inner and outer
layer separated by the non-nucleated zone of
Chievitz. The cells of the inner neuroblastic layer
are already somewhat more differentiated than
those of the outer layer. Nuclei are larger, rounder,
and have a clear nucleoplasm containing a nucleo-
lus. Some of these nuclei are obviously of ganglion
cells which have already produced an axon run-
ing just under the internal limiting membrane
toward the optic nerve head. Therefore, the optic
erve has started to form in fetuses of this age,
but is far from complete. Not all the nuclei of the
inner neuroblastic layer are ganglion cells. Some
obviously will form amacrine and possibly some
Miillerian cells. The nuclei of the outer neuro-
blastic layer are easily distinguished from those of
the inner layer. They are smaller, more intensely
staining, and elongated, with their long axis at
right angles to the internal limiting membrane.

Fig. 1. A light micrograph of a 54-day-old monkey fetal retina. The nuclei are divided into
an inner (IN) and outer neuroblastic (ON) layers separated by the layer of Chievitz. There
are some axons in the nerve-fiber layer. The nuclei of the two layers differ in morphology,
those of the inner layer are clearer, rounder, and larger than those of the outer layer. Note
that some nuclei characteristic of the inner neuroblastic layer lie in the inner portion of the
outer neuroblasts (arrow). There is a row of differentiating cone cells at the outer retinal
margin. x768.
Fig. 2. For legend see opposite page.
Fig. 3. Electron micrograph showing two straight segments of the cytoplasm of Müllerian cells (M). They contain angular profiles of smooth endoplasmic reticulum and numerous microfilaments. x6,000.

They also possess a nucleolus. A row of cone nuclei adjacent to the external limiting membrane is obvious in the 54-day fetus (Fig. 1). The spaces occupied by the inner and outer neuroblastic layers are approximately equal. At approximately 60 to 67 days the nuclei-free zone appears to be in the process of disappearing. This is accomplished by sclerad migration of nuclei of the inner neuroblastic layer and some, less obvious, vitread movement of nuclei from the outer layer. Those which moved outward retained their characteristic morphology.

Study of the 19-day rabbit fetuses revealed that the retina consisted of neuroblasts which were divisible into an inner and outer zone separated by a series of small spaces, apparently the beginning of the layer of Chievitz. Like the monkey fetuses the internal layer of the retina was occupied by axons, though the inner neuroblasts appeared cytologically still quite undifferentiated. By the twentieth day of gestation, two layers of neuroblasts were present but not precisely defined. The shape of the nuclei in these layers was very similar to that described in the monkey fetuses. However, the inner neuroblasts occupied a zone approximately ¼ as wide as the outer neuroblasts. By the twenty-first and twenty-second day of gestation there were two distinct neuroblastic layers, separated by a nuclei-free zone, the transient layer of Chievitz.

Electron microscopic observations. Electron micrographs of the youngest monkey studied (43-days of gestation) revealed small discontinuous nuclei-free areas in the region in which we believe the.....
Fig. 4. For legend see opposite page.
layer of Chievitz was destined to lie. A few days later (46-days of gestation) it was easy to find this nuclei-free zone. It was occupied by relatively blunt neuronal cell processes which were twisted and entangled with one another. Already at this early age the cytologic characteristics of the Müllerian cells were established (Fig. 2). These were easily seen as quite straight, long segments of cytoplasm containing irregularly-shaped profiles of smooth endoplasmic reticulum. In addition, these cells contained microfilaments and relatively few ribosomes (Fig. 3). The cross-sections of some neuronal processes showed, in a few cases, mitochondria, some vacuoles, and sections of neurotubules. The processes with large diameters presumably were nearer the perikaryon and were the ones with mitochondria. There were also a few dead cells scattered in this area (Fig. 2). The tangled mass of processes occasionally joined by punctate desmosomes were somewhat loosely fitted together and there were spaces between them which we do not interpret as artifactitious. The slightly older, 49 day fetus, revealed a rather discrete nuclei-free zone. It, like that of the earlier fetus, was composed of relatively thick processes entangled with one another and interspersed with a few spaces. The processes contained microfilaments, some neurotubules and a few vesicles, including coated ones. Some of the thicker cytoplasmic extentions, presumably nearer their cell body, also had some ribosomes. Rough-surfaced endoplasmic reticulum and Golgi vesicles were occasionally seen. The processes were at times attached to one another by small junctions. The impression is gained that the layer consists of a felt-like mass of intertwined cytoplasmic extentions. Some of these were relatively straight and the sections passed through a considerable extent of the cells. When the micrograph included cells of the outer and inner neuroblastic layer, their cytoplasm was seen to contain the masses of polysomes characteristic of immature cells. The nuclei of the inner neuroblastic layer were often deeply indented. Their outer nuclear membrane was frequently lifted from the nuclear mass, giving the appearance of blebs. Some of these membranes were studded with ribosomes and were actively contributing to the development of cisternae of rough-surfaced endoplasmic reticulum.

In the 54-day stage the layer of Chievitz consisted of somewhat more slender processes loosely fitted together as before (Fig. 4). The cells adjacent to the layer of Chievitz had much rough-surfaced endoplasmic reticulum, mitochondria, and occasionally, a Golgi apparatus. Many of the processes contained neurotubules which were cut either in longitudinal or cross-section. There were coated vesicles in their cytoplasm and numerous polysomes and glycogen particles were observed particularly in larger diameter processes presumably proximal to their cell body. They were not found in the more slender neurotubule-containing processes. Until this stage, excepting some punctate desmosomes, there was no indication of firmly fixed junctions between processes, however, in this specimen structures were observed which had the appearance of either a very early synapse or a more mature desmosomal linkage (Fig. 4, inset). The light micrograph showed that several nuclei with the character of those in the neuroblastic layer had migrated outwardly and taken up position in the internal portion of the outer neuroblastic layer (Fig. 1). The layer of Chievitz is at its greatest development at this stage, this migration indicates that it is beginning to regress and that portions of it may be the layer which lies between amacrine and ganglion cells, the future internal plexiform layer. It is possible that the junctional complexes we observed are the very earliest stages in differentiation of the synapse in the internal plexiform layer. There were many more such structures in the 60-day specimen in which we are sure that the layer of Chievitz is disappearing by the outward migration of inner neuroblast nuclei to join those of the outer layer to form the inner nuclear zone. The disappearing layer of Chievitz (67-day fetus) is shown in Fig. 5. In this specimen we may confuse remnants of the layer of Chievitz with areas which are destined to remain and will contribute to the beginning of the internal plexiform layer. A membrane densification with filaments projecting into the cytoplasm and associated with a structure which may be a synaptic vesicle suggests that this region is in a very early stage of synaptic development (Fig. 5, inset). Such structures are still quite rare. The outer plexiform layer has not yet made its appearance at this time.

**Rabbits.** Electron micrographs of developing rabbit retinas were essentially the same as those

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Fig. 4. Electron micrograph of the layer of Chievitz of a 54-day-old monkey fetus. The nuclei of the inner neuroblast layer are characteristically rounder and often indented. The layer of Chievitz which lies between the inner and outer neuroblasts is a "felt-like" mass of intertwined processes which at this age are more slender than formerly. ×6,000.

*Inset:* A junctional complex uniting two processes in the layer of Chievitz. The membranes forming this structure are asymmetrical suggesting that it may be a predecessor of a synaptic union. ×42,000.
Fig. 5. For legend see opposite page.
of the fetal monkey. At 19 days of gestation, the layer of Chievitz was just becoming obvious. This area was characterized by a tangled mass of relatively thick cytoplasmic extentions fitting together loosely. Intercellular spaces were again visible. These were not filled with debris and the cell membrane of the processes running through the spaces were intact. Therefore, we believe that this area is characterized by loosely fitting cells without attachment devices between them. The larger processes contained some coated vesicles and a few polysomes. The slightly older, 20-day specimen was very similar to that of the nineteenth day gestation. At 21 days gestation the layer of Chievitz appeared as a plexus of variable-sized neural extensions. A good many of these contained polysomes, others had numerous neurotubules. Some of the processes were cut in relatively long profiles of cytoplasm which was electron dense. These had the same staining characteristic as Mullerian cells in this species and with this fixative. The neuroblasts adjacent to the zone of Chievitz contained many polysomes, some profiles of rough-surfaced endoplasmic reticulum and a few small mitochondria characteristic of immature growing neurons. Coated vesicles were seen, but we did not observe the Golgi apparatus, indicating that it was not particularly extensive.

One human fetus was examined. It had an estimated age of 11 to 12 weeks. A narrow nuclei-free zone lay between two masses of neuroblasts, the transient layer of Chievitz. This fetus had very few axons, so we may believe it to represent a fairly early stage in development of the zone of Chievitz. Electron micrographs were quite indistinguishable from those just described of the rabbit of 21 days gestation.

Discussion

In his now famous and often quoted papers, Chievitz was not interested particularly in the nuclei-free zone which he observed in developing human retinae. His purpose was to describe the differentiation of the area of the macula and fovea. His illustration shows the retina divided into two masses of nuclei separated by a very narrow nuclei-free zone. This transient layer came to bear his name. It contributes nothing to the adult retina excepting in the area of the fovea. It is not established what necessitates the inward and later outward migration of the nuclei in the retina. We believe that it should not be thought of as a migration of the entire cell body, but rather the relative movement of the nucleus in the cytoplasm. It would be difficult to imagine entire cells wandering through the essentially solid retinal mass. Rather, we are of the opinion that the retinal cells are simply changing their shape, withdrawing some processes and pushing others into new areas where they will eventually make contact and synapse with specific neurons. Originally, the neuroblasts of the retina consisted of columnar cells which rested on the basement membrane facing the vitreous with their free apical tips joined to one another at the external limiting membrane. The apices of the Mullerian cells face the vitreous cavity into which they insert their villi which form later the "baskets of Schultze." They are attached at the external limiting membrane to the rod and cone cells. All of the other neuroblasts lose contact with both surfaces and it is they who apparently put forth and withdraw their processes, which form the plexiform layer to which Chievitz's name has been attached. It is traversed by trunks of branching Mullerian cell cytoplasm and portions of it may eventually become part of the internal plexiform layer. The migration of the processes through it is aided by its loose texture and the paucity of attachment devices, at least in the earlier stages. The looseness of the retinal elements at this stage contrasts sharply with the compactness of the adult. Therefore, we suspect that the retinal

Fig. 5. An electron micrograph of the layer of Chievitz in a 67-day-old monkey fetus. It consists of intermingled processes with some spaces between them. The neuroblasts (outer layer) have their cytoplasm filled with mitochondria, Golgi (G) apparatus, polysomes, and rough-surfaced endoplasmic reticulum. It can be seen in one cell originating from the nuclear envelope (arrow). Large trunks of Mullerian cells (M). ×6,000.

Inset: A junction which may be an early synapse showing a vesicle associated with it and one or two other vesicles in the adjacent cytoplasm. ×48,000.
intercellular space is larger in the fetus than in the adult and that the mechanism for keeping it to a minimum has not yet developed. A large intercellular retinal space has been observed in other studies. This may provide space in this area in which the neurons may rearrange themselves more readily than in the compact mass of neuroblasts.

This paper has dealt with the transient layer of Chievitz as it is described in modern texts. Chievitz's original work had a different emphasis. He described a "transitory radial fiber layer" which he showed in his figures Nos. 8 and 13, as part of the internal nuclear layer in the region of the developing fovea of the 7 1/2 to 8-months-old human fetus. We have been describing structures which occur earlier than those discussed by Chievitz. Our observations have been confined to the fundus of the retina, probably in the region where the fovea would have developed much later.

In his paper Chievitz says: "An internuclear space arises in the internal nuclear layer between the spongioblasts (amacrines) and the other elements which (the space) is diagonally traversed by the elongated and diverging radial fibers. This 'transitory radial fiber layer' whose traces are lacking in the adult is still present in the 9-months-old fetus."

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REFERENCES