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EFFECT OF ALCOHOL ON THE POSTNATAL DEVELOPMENT OF CEREBELLAR CORTEX IN RAT
II: ULTRASTRUCTURAL STUDY

(With 14 Figures)

By

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تأثير الكحول على تطور قشرة المخيخ في الفأر بعد الولادة
٢- دراسة بالميكروسكوب الألكتروني

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يحدث الكحول (١٠٪) تغيرات في تركيب خلايا بيركنج والخلايا الحبيبية بمخيخ الأر خلال مراحل العمر. تفقد خلايا بيركنج المخروط القمي ويشاهد بها عديد من الشوكات الجسدية ثم تظهر تغيرات تراجعية في عضيات هذه الخلايا . شح اتصال الألياف العصبية لخلايا الباسكت على جسم خلايا اليركنج. شوهدت تغيرات تراجعية بالزوائد الشجرية للخلايا الحبيبية والألياف العصبية.

SUMMATRY

In The present work, it was found that alcohol produced distinct changes in both Purkinje and granule cells during postnatal development. On the fifth day, the apical cones of the Purkinje cells were less developed and the cells showed an occasional somatic spines but no somatic processes. At 21 days and adult stage these cells including their dendrites showed marked degenerative changes. The number of basket cell axon synapses on Purkinje cell soma was reduced. There was also a reduction in the amount of basket fibers contributing to the pinceau around the intial segment of the Purkinje cell axons. The granule cells showed a marked change starting from five days old. These cells decreased in size and lost most of the organelles. Their axons and dendrites as well as mossy fibers were also affected. Signs of glial activation and proliferation were present in the from of formation of myelin sheaths around the degenerated fibers.

Keywarss: Effect, alchol, postnatal development, cerebellar cortex, rat, ultrastrural.

INTROUDUCTION

The cerbellum is known to be among the most vulnerable brain structures to alcohol exposure *(VOLK, 1984)*. Previous studies had shown that prolonged alcohol exposure produced alteralions in the synapses and dendritic branches as well as delayed

maturation of the neurous *(DAVIES and SMITH, 1981; McMULLEN et al., 1984)*. The effect of ethanol was examined in two major neuronal populations in cerebellar cortex the large grenatally formed Purkinje cells and the small postnatally formed granule cells. Purkinje cells were selected for study because they are the master cells of the cerebellar cortex, the recipients of all afferent information and the source of the single cortical efferent system *(ALTMAN and WINFREE, 1977)*. Granule cells were selected for investigations because they are the most numerous of all cerebellar neurons *(TAVARES and BARBOSA, 1981)*.

The study of synaptic ultrastructure is of great importance in neurobiology, since modifications of synaptic morphology has been reported to be involved in learning. memory sensitization and other C.N.S. functions *(LYNCH and BAUDRY, 1984)*.

The present study was undertaken to evaluate the effect of ethanol on the ultrastructure of Purkinje cells and granule cells as well as the synaptic contact during postnatal devolopment of cerebellum.

MATERIALS AND METHODS

Pregnant albino rats were divided into two groups: one group was given unrestricted excess of ethanol (ethyl alcohol) at 10% concentration as the only source of water at 18 days of gestation and throughout the period of lactation.. The pups were maintained on ethanol till they become adult (110 days). The animals of the second group were given tap water.

The pups of the control and treated animals were sacrificed at these ages: 0.5-21-110 (adult). five animals each. Small specimens from the cerebellum were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer PH 7.4. Then, the specimens were postfixed in 1% osmium tetraoxide in cacodylate buffer, dehydra- ted in ethanol and emedded in epon. Ultrathin sections were stained in uranyl actate and lead cirate and examined by jeol electron microscope.

RESULTS

Pinkinje Cells:

In the new born the purkinje cells of the alcohol fed group showed no changes.

Five Days:

Control: The cell was elongated and the apical part of the cytoplasm was protruded to form the future dendrite. It contained numerous ribosomas and mitochondria. Processes were originated from the soma forming both somatic spines and somatic processes which sypapse with parallel fibers and climbing fibers respectively (Fig. 1).

Treated: The Purkinje cells were decreased in size and contained less organelles. The apical cytoplasm was less developed. The nucleus contained condensed chromatin. No somatic processes could be found (Fig.2). Occasional somatic spines were observed in some cells.

Twenty One Day:

Control: The cells were pear shaped with rounded nuclei contained extended chromatin. The cytoplasm was rich in rough endoplasmic reticutum and ribosomes. Several basket cell synapses were found on the

soma (Fig.3) Typical pinceau could be observed which was formed by basket cell oxan around the intial segment of PurKinje cell oxan (Fig.4) .

Treated: The cells were small in size with illdefined boundaries, the cytoplasm lost many organelles. The nuclei were elongated and contained condensed chromatin. There was a decrease in frequency of basket oxan synapses on the soma. Many degenerated parallel fibers were found around the cells (Fig.5).

Adult:

Control: The cells became larger in size. Several basket cell synapses and typical pinceau were found (Fig.6).

Treated: The cells contained fragmented mitochondria dilated and short sement of rough endoplasmic reticlum ribosomes were decreased in amount. The amount of the basket cell oxon synapses on the purkinje cell soma and the typical pinceau were decreased. The intial segment of the axon and the dendrities of the cells showed signs of degeneration and many spaces were present (Fig.7 and 8).

Granule Cells:

In new born the granule cells of alcohol fed rats showed no changes.

Five days:

Control: The nucleus was large and filled most of the cell body. The cells had recognizable dendrites with packed ribosomes. (Fig. 10).

Treated: The granule cells were decreased in size. They had dense nuclei. There were wide spaces between the cells and the degenerated axons and dendrites. Glial proliferation around the granule cells was also found (Fig. 11).

21 days:

Control: The cells contained large nuclei and the cytoplasm was rich in Nissle bodies and mitochondria. The amount of mossy rossettes were also increased but with less synaptic vesicles. The majority of them became complex with a lrge number of dendritic profiles and dendritic spines (Fig. 12).

Treated: The granule cell became smaller in siz and elongated.

The nuclei were pressing against the cell membrane with a thin rim of Nissl substance around the perimeters of the cells. Mossy fibers were swollen with complete loss of synaptic vesicles and fragmented mitochandria. Glial proliferation was also observed around the degenerated mossy fibers (Fig. 13).

Adult:

Control: The granule cell had angular appearance. The cytoplasm fromed a thin rim around the large nucleus. It contained a large amount of ribosomes and mitochondria. The mossy rossettes were increased and the accumlation of synaptic vesicles were also increased. The glomeruli which were formed of synapses between the granule cell dendrites and mossy rossettes became more complex in organization (Fig. 14).

Treated: The cytoplasm of the granule cells lost most of the organelles. There was an increase in the amount of glial processes synapsing on the cell soma. Mossy rossettes

showed marked varicosity. They lost their vesicles and contained fragmented mitochondria. Glial proliferation was found around the mossy rossettes (Fig. 15).

DISCUSSION

At newly born stage the Purkinje cells were slighty differentiated and had no synapses on their soma. At five days the apical cones started to form the future dendritic tree and some processes originating from the soma formed both somatic spines and somatic processes. In alcohol fed rats, the cells showed an occsional somatic spines but no somatic processes. At 21 days the Purkinje cells of the control group were highly developed simulating the adult stage. There was occasionl bsket cell axons synapsing with the smooth surface of the Purkinje cell soma and occasionally on the smooth dendrites.

In alcohol fed rats, the Purkinje cells showed degenerative changes Additianally there was a reduction in the number of basket cell axon synapses on the Purkinje bodies and there was also a reduction in the amount of basket fibers forming the pinceau around the intial segment of the Purkinje cell axon. The dendrites and axons of the Purkinje cells showed also degenerative changes. Similar observations were reported by *BARNES and WALKER (1980)* who found that the pre-and postnatal alcohol administration caused changes in all stages of development of Purkinje cell, dendritic branches, axons and synapses.

The Purkinje cells in this work showed a marked decrease in free ridosomes and rough endoplasmic reticulum. Such reduction in Nissle substance may reflect impaired Purkinje cell metablolism. Altered protein boisyntnesis by alcohol had been reported by *RAWAT (1975)* in the fetal and neonatal brain, he found a marked decrease of ribosomal protein synthesis Also, *MOHAMED et al (1987)* showed that prolonged ethanol administration interfered with the protein synthesizing system. Possibly at the ribosomal level in the cell (both the transcription and translation levels).

In the present work, it was found that the dendritic spine areas were increased. This may be a compansatory process due to the degeneration of the dendrities.

The decrease of basket cell synapses on the Purkinje cell som in alcohol fed rats was found by *LARRAMENDI (1969)* who reported that, basket cell synapses began to increase on Purkinje bodies as the frequency of climbing fibers synapses on the soma decreased. Therefore, the reduced basket cell synapses found in alcohol exposed pups, were perhaps a reflection of impaired climibing fibers maturation.

The granule cells of alcohol fed rats showed marked change. The cytoplasmic organelles were affected, spacing between cells increased and degenderated axons and dendrites were present. Mossy fibers showed also manifestation of degeneration including swelling complete loss of synaptic vesicles and fragmented mitochondria. A sign of glial activation and proliferation appeared in the form of formation a myelin ensheathment around the degenerated mossy

terminal. These results were in agrement with *KORNGUTH et al (1979)* who found a delayed maturation and cell damage in the granule cells of cerebellar cortex of rats prenataly exposed to alcohol. On the other hand, *BAURE-MOFFETT AND ALTMAN (1977)* reported that the early postnatal exposure to alcohol led to damage of granule cells and permenent reduction in their number. They added that these alterations persisted throughout adulthood even after postweaning rehabitation period.

The degenerative changes of the granule cells were explained in the results of *WALKER et al (1981)* by the neurotoxic effect of alcohol on the neurons of the cerebellum or it may be also followed degeneration of purkinje cells by a process of transynaptic degeneration which would follow the deterioration of parallel fibers *(SOTELO and TRILLER, 1979)*.

The degeneration of the dendrites and axons of the cells of the granular layer observed in this study may be due to the metabolic changes of these fibers following the same changes that occurred in their cells *(TAVARES et al, 1985)*. It was also reported that the degenerative changes observed in neuronal dendritic domains after longterm alcohol treatment might result from the abonormalities of the smooth endoplasmic reticulum which had failed in its role to transport proteins into the dendrite, leding to metabolic changes and eventual degeneration of the dendrite *(RILEY 1977)*.

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LENGDS OF FIGURES:

Fig. 1: Electronmicrograph of the Purkinje cell of a five days control rat, showing the protrusion of the apical cone to form the future dendrite(d). It contains ribosome and mitochondria(M). Notice the presence of somatic spine (double arrows) which synapses with parallel fiber(L) and two somatic processes(P) that synapse with climbing fibers(C). (X 14.000)

Fig. 2: Electronmicrograph of the Purkinje cell of a five days alcohol fed rat, showing the less developed apical cytoplasm. Notice the absence of somatic processe. (X 14.000)

Fig. 3: Electronmicrograph of the Purkinje cell of a 21 days control rat, showing the rounded pale nucleus(N) and the numerous rough endoplasmic reticulum (RER). Notice the basket cell synapses on the soma (arrows) (X 10.000)

Fig. 4: Electronmicrograph of the basal portion and axon(A) of the Purkinje cell of a 21 days control rat showing the typical pinceau(S). The arrows point to the outer limit of the axon. (X 27.000)

Fig. 5: Electronmicrograph of the Purkinje cell and its dendrite of a 21 days alcohol fed rat. Showing the illdefined cell boundary and the elongated condensed nucleus. The cytoplasm contains less organelles. Notice the absence of basket cell axon synapses on the soma. The arrows point to the outer limit of the cell. (X 20.000)

Fig. 6: Electeronmicrograph of the Purkinje cell of an adult control rat showing the several basket cell synapses (arrow) and the pinceau(S) A: axon (10.000).

Fig.7: Electronmicrograph of the portion of purkinje cell and its axon of an alcohol fed rat showing the fagmented miochondria (M) and dilated rough endoplasmic reticulum. (R). Notice the Gegeneration of the axon (A) and the presence of vacuoles (V). The arrows point to the outer limit of the cell and its axon. (X 20.000)

Fig. 8: Electtonmicrograph of the dendrite of purkinje cell of an qadult alcohol bed rat showing the degenerative changes in the denorite (d). Notice the large vacuole (V) in the dendrite. F= degeneratedd parallel fiber. P = apex of purkinje cell. (X 20.000)

Fig. 9: Electronmicrograph of the granule cell of a five days control rat The nucleus is large. The cytoplasm contains free ribosomes. The arrow points to the dendrite (X 14.000)

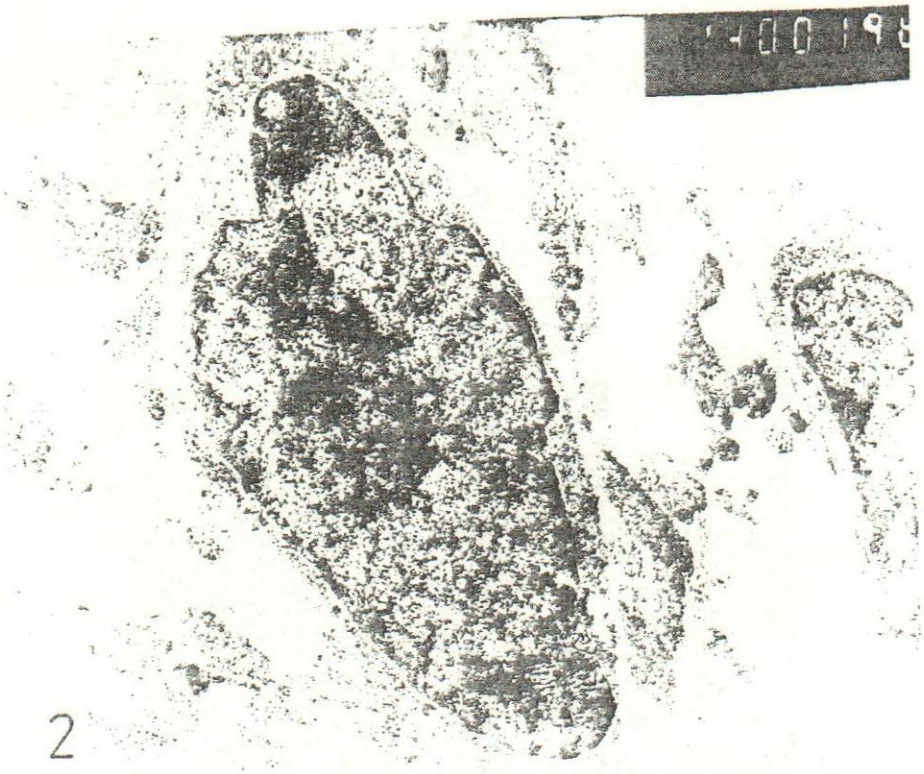
Fig.10: Electro0nmicrograph of the granule cell of a five days alcohol fed rat, showing the degenerative changes in the cell and the surrouding dendrities (d). Notic the wide spaces between the cells and the glial proliferation engulfing the cell (arrows). (X 10.000)

Fig. 11: Electronmicrograph of the granule cells of a 21 days control rat, showing that the cell contains ribosomes and mitochondria (M). Notice the mossy terminals (m) which are complex with a large number of dendrites ((d) and dendritic spines (s). (x 14.000)

Fig.12: Electronmicrograph of the granule cells of a 21-days alcohol fed rat The nuclie (N). press against the cell membrane with a thin rim of Nissl substance around the perneters of the cells. Notice the degenerated mossy fibers (m) with thich myelin sheath around them. (X 10.000)

Fig.13: Electronmicrograph of the granule cell of an adult control rat showing thin rim of cytoplasm around the large nucleus (N). The cytoplasm contains ribosomes and mitochondria (M). Notice the cynap- ses on the cell body (arrow) and the synaptic gloeruli between granule cell dendrites (d) and mossy rossettes (m) (X 10.000)

Fig.14: Electronmicrograph of the granule cell of an adult alcohol fed rat, showing the disappearance the Nissl bodies and the fragmented mitochondria (M0. Notice the increase of glial processes synapsing on the cell body (arrows) and degenerated mossy rossette (m) with glaial proliferation. (X 14.000)



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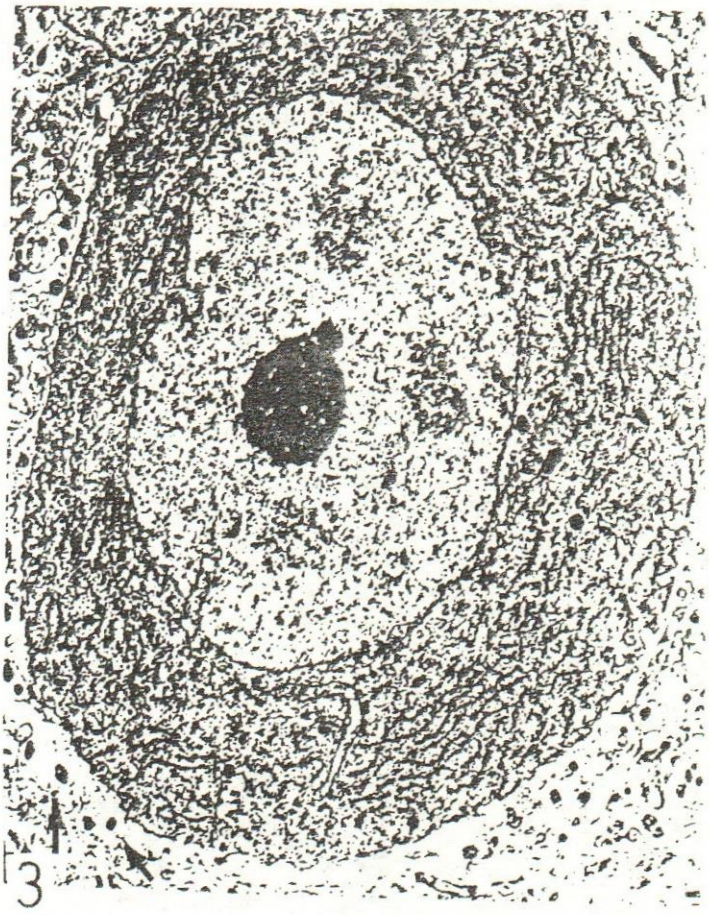

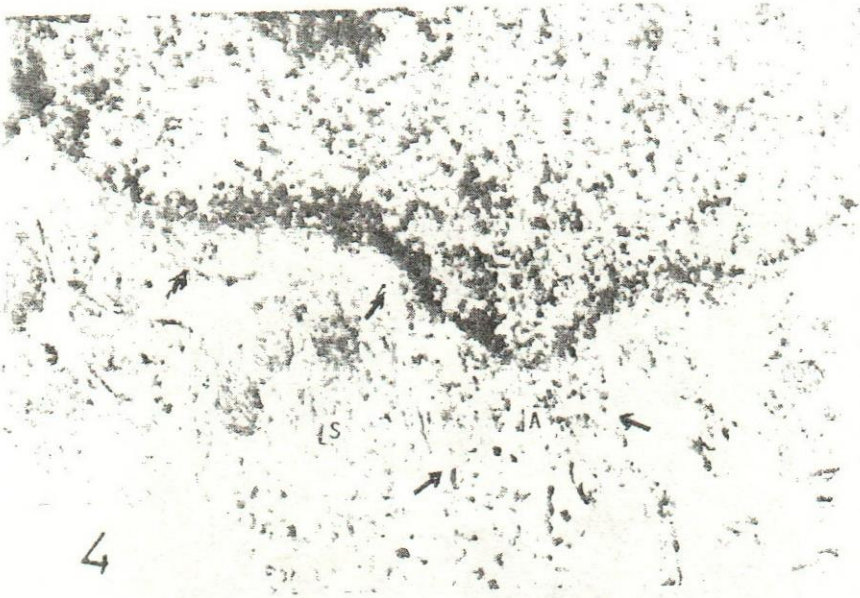
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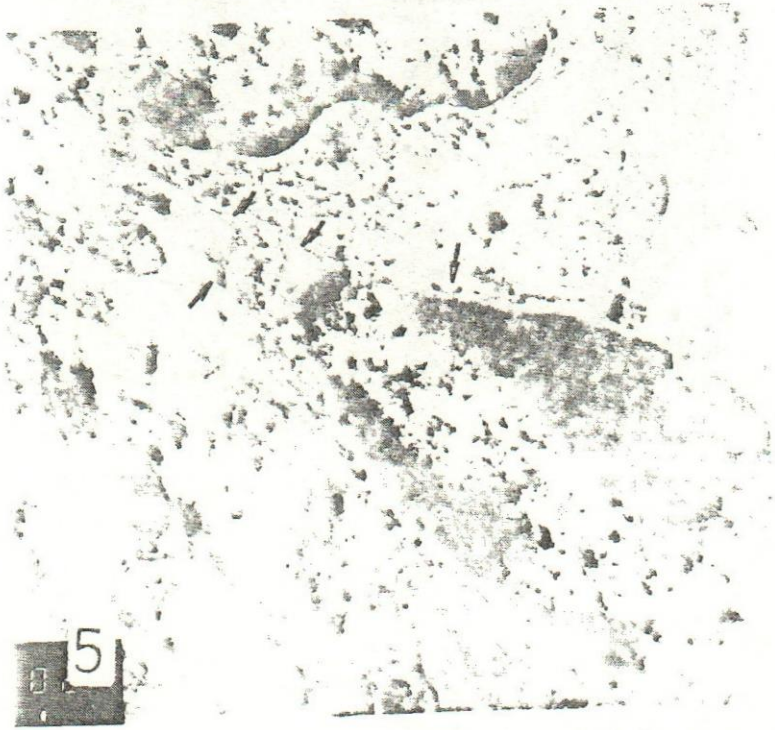
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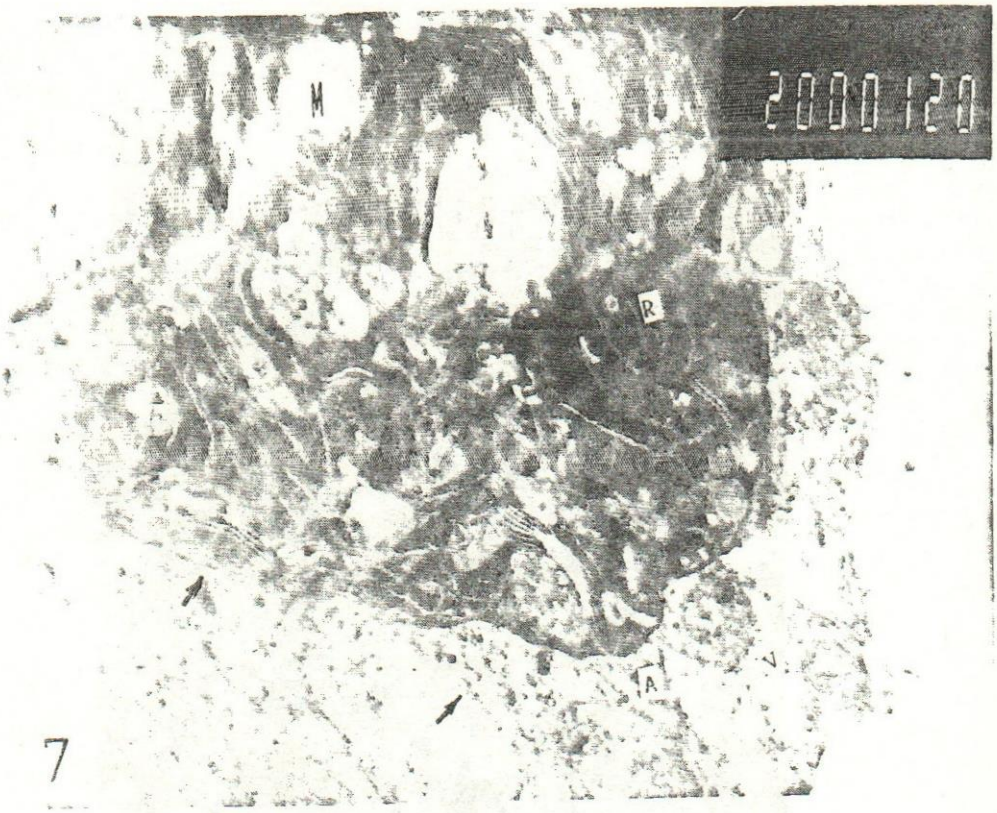
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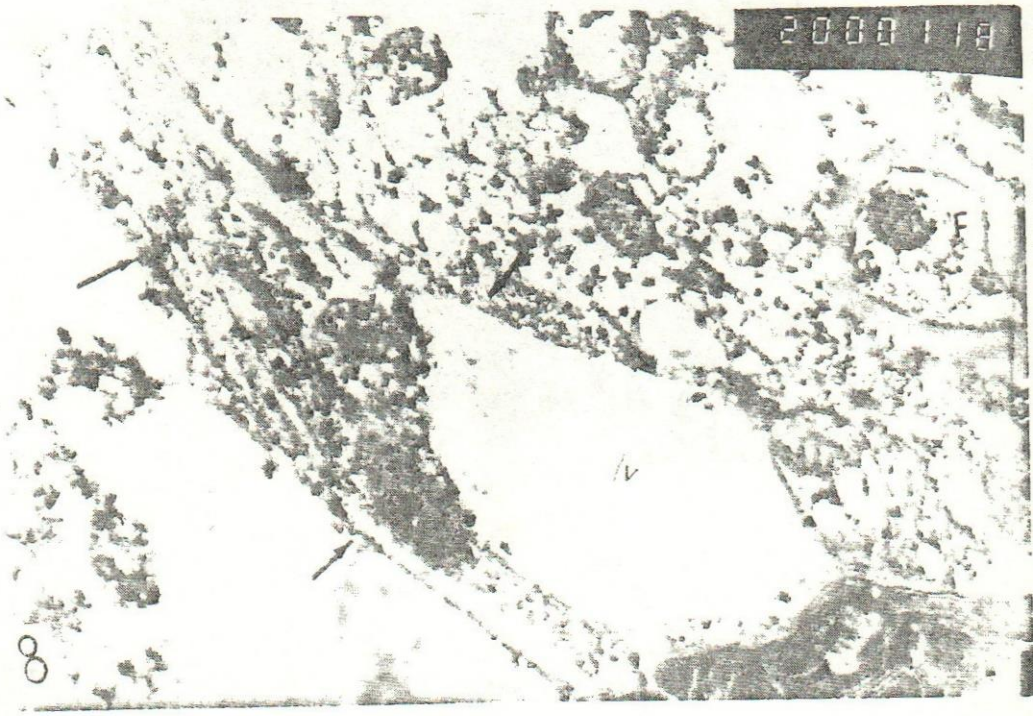
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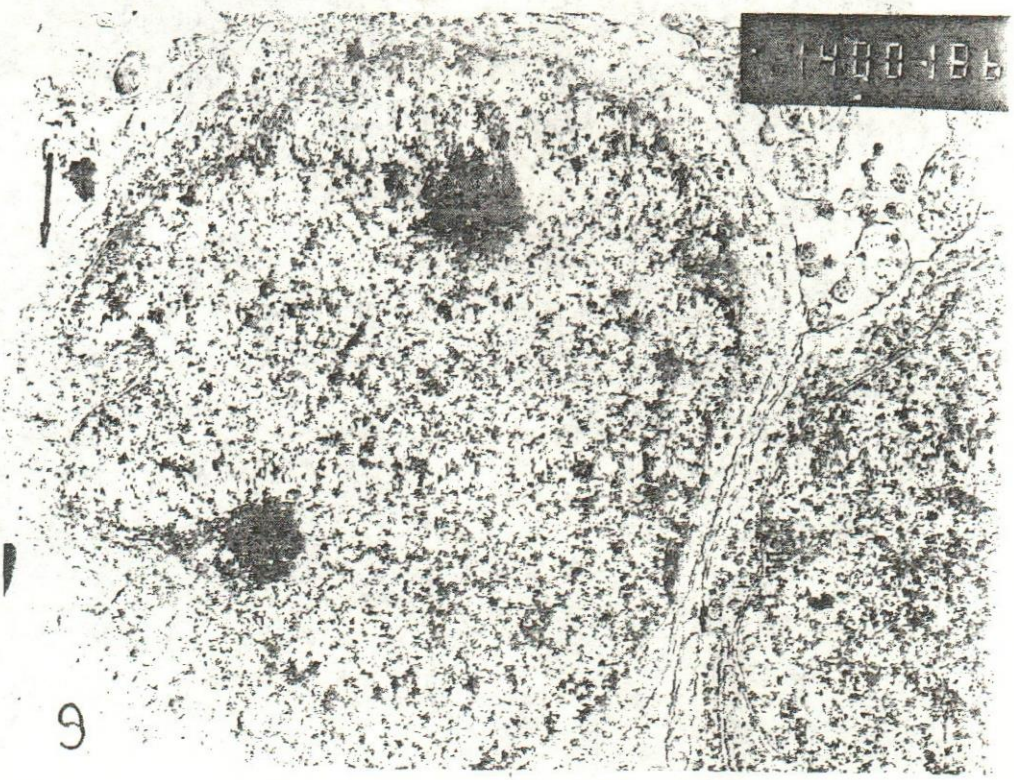
ALCOHOL, POSTNATAL DEVELOPMENT OF CEREBELLAR & CORTEX IN RAT



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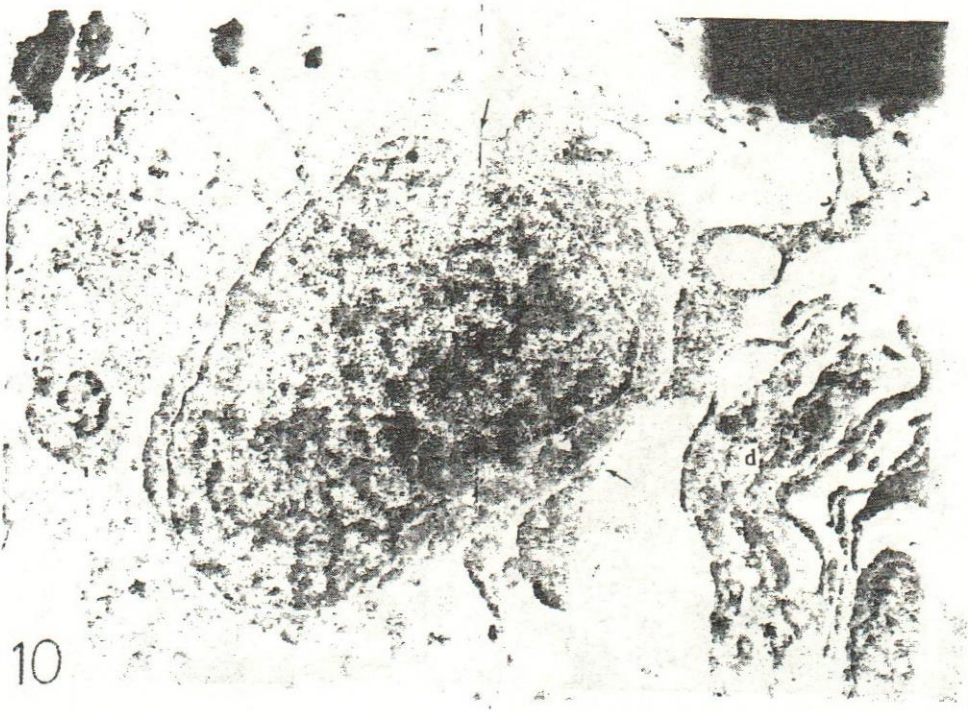

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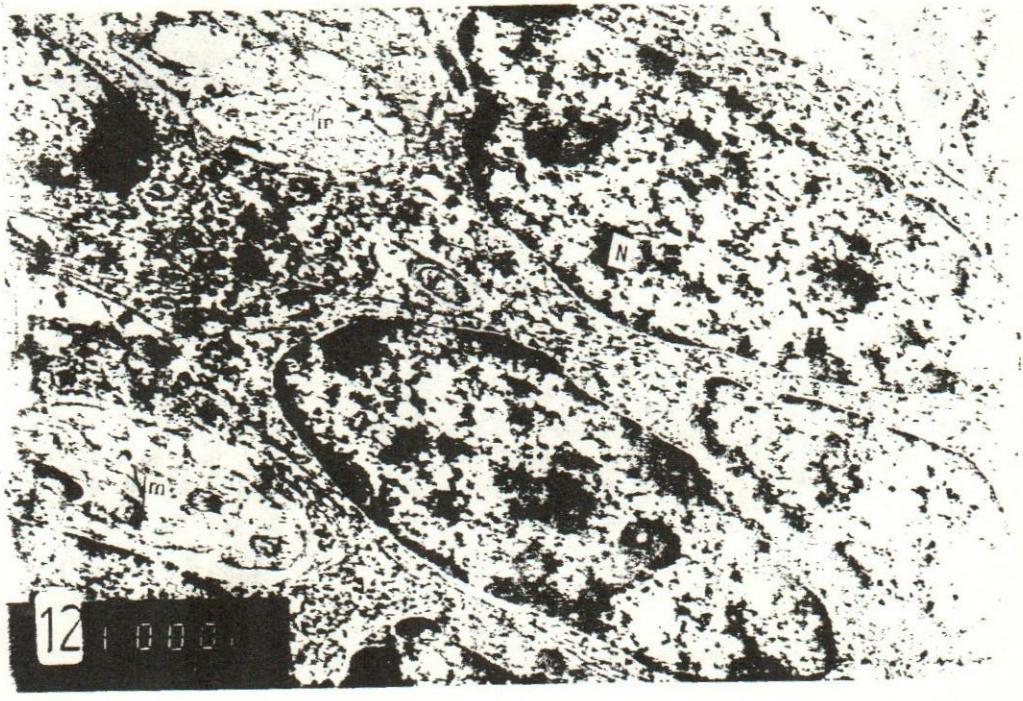
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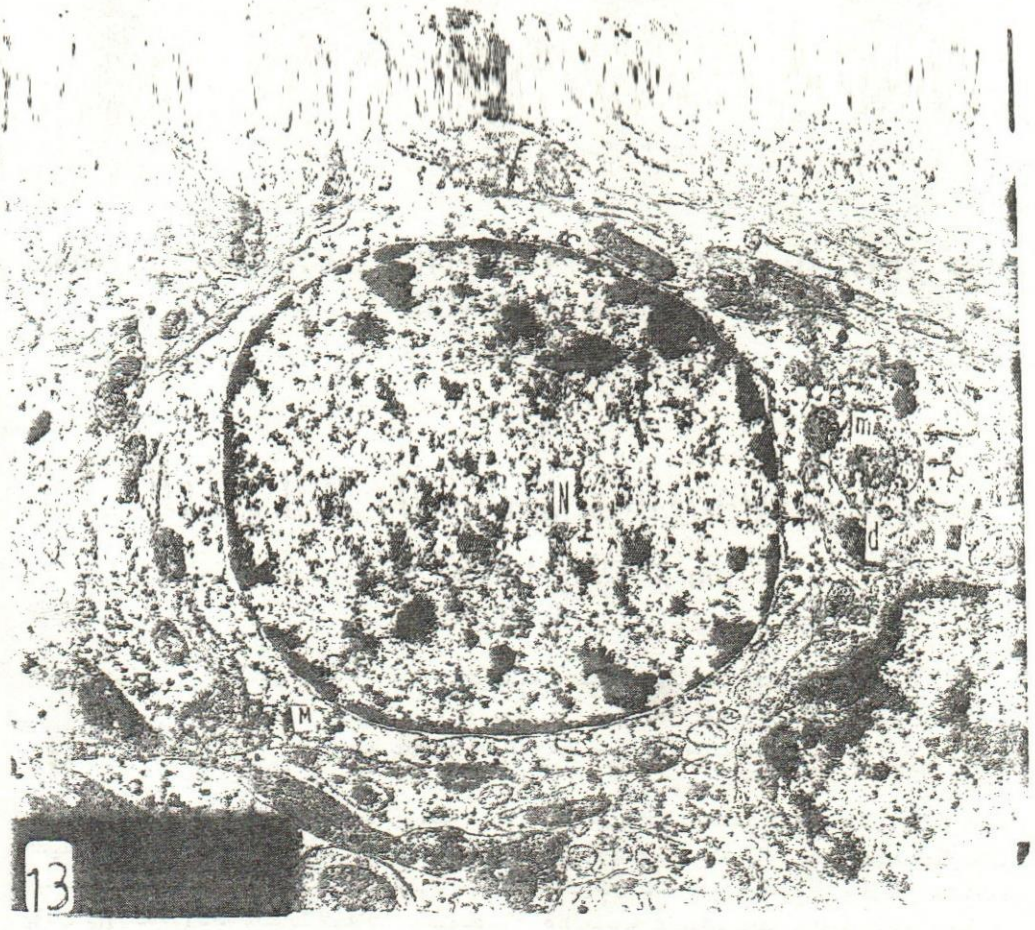
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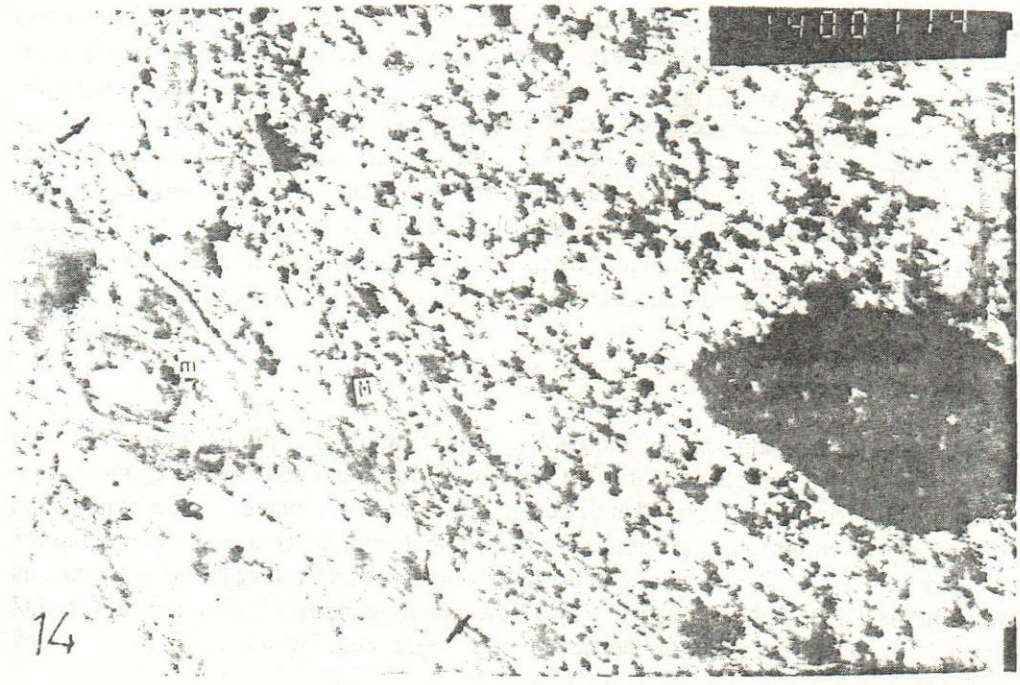
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