Dept. Anatomy and Histology
Fac. Med. Assiut University.
Head of Dept. Prof. Dr. M.N. Saleh

# EFFCT OF ALCOHOL ON THE POSTNATAL DEVELOPMENT OF CEREBELLAR CORTEX IN RAT
## 1 - HISTOLOGICAL AND MORPHOMETRIC STUDIES

(With 20 Fig. & One Table)

By

M.N. SALEH; M.M. SALEH; M.A. DESOUKY;
SAADIA R. SAYED* and ENTESAR A. SABER*

(Received at 30/9/1993)

# تأثير الكحول على تطور قشرة المخيخ بعد الولاده في الفأر
## ١ - دراسة هستولوجيه ومورفومتريه

محمد صالح ، محمود صالح ، محمد دسوقى
سعديه سيد ، انتصار صابر

خلصت هذه الدراسة الى ان للكحول الاثيلى ( ١٠٪ ) تاثير ضار على تطور قشرة المخيخ فى الفأر الابيض بعد الولادة وحتى مرحلة البلوغ . ازداد سمك طبقة الحبيبات الخارجية عنه فى المجموعة الضابطة . اما الطبقة الجزيئية فقد قلت فى السُمْك وظهر بها فجوات نتيجة تحلل بعض الزوائد العصبية . كذلك وجد ان خلايا البيركنج قد وصفت فى حوالي طبقتين فى اليوم العاشر من الولادة . وحدث ان تحلل بعض هذه الخلايا وزوائدها الشجرية وازداد التحلل بتقدم العمر ، قل سمك طبقة الحبيبات الداخلية عن المجموعة الضابطة . وتحللت بعض الخلايا والزوائد العصبية وازداد التحلل بتقدم العمر .

واثبتت الدراسة المورفوميترية ان متوسط عدد خلايا البيركنج والحبيبات قد نقص عن مثيله فى المجموعة الضابطة . وظهر النقص ملحوظ فى الفصيصات ( الثانى والثالث ) فى الفص الامامى تليه الفصيصات ( التاسع والعاشر ) فى الفص الحلقى . كذلك وجد ان الكثافة العددية لخلايا البيركنج والحبيبات لكل وحدة حجم للنسيج اقل منها فى الفئران الضابطة . ووجد أيضاً ان معدل خلايا الحبيبات بالنسبة لخلايا البيركنج قد ازداد عنه فى المجموعة الضابطة .

---

*: Dept. Anatomy and Histology, Fac. Med. El-Minia University.

## SUMMARY

The effect of ethyl alcohol (10%) on the postnatal development of cerebellar cortex was studied using histological and morphometric methods. The external granular layer was much increased in thickness in alcohol fed rat than those of the control while the molecular and granular layer were reduced. The cells of the molecular layer showed neuronal degeneration and many spaces were present in this layer especially late postnatal. The Purkinje cells failed to reach their final position up the tenth day and still arranged in more than one row. The degenerative changes appeared at five days and increased with age. The extent of their dendritic tree were reduced but with long spines. The granular layer was delayed in its differentiation from the white mather. Starting from fifth day, the degenerative changes were clearly noticed in the cells and fibers. The mean number of Purkinje and granule cells in alcohol fed rats were reduced in all lobules in all ages. The most affected lobules were lobules II & III in the anteror lobe then lobules IX & X in the posterior lobe. The numerical densities of granule and Purkinje cells per unit volume of tissue were lower than the control. This density was significantly reduced at 5-21 days. The granule -to- Purkinje cell ratios were increased in alcohol fed rats than those of the control. There was a slight decrease in this ratio in the adult stage.


## INTRODUCTION

On the basis of available data, it was widely accepted that prolonged and excessive consumption of alcohol induced a large spectrum of changes in the C.N.S *(FREUND, 1973; ABRAHAM* et al., *1982 b)*. It was established that alcohol is rapidly absorpted and appeared in the blood five minutes after it has been taken by the mouth. It's concentration in the blood reaches a maximum within one hour. It is uniformly distributed throughout all tissues and fluids of the body and gains free excess to the mother's milk *(GOODMAN and GILMAN, 1990)*.

The brain has expecially rich blood supply and the concentration of alcohol in the brain quickly approachs that of the blood. So, the central effects may be obtained rapidly and

the C.N.S is more markedly affected by alcohol than any other system of the body *(KISSIN and BEGLEITER, 1974)*. Correlated physiological and morphological studies indicated that the cerebellum and hippocampus are particularly the sensitive areas to the deleterious effects of chronic ethanol treatment in rats *(ABRAHAM and HUNTER, 1982 a)*. *BAURE-MOFFET and ALTMAN (1977)* reported that the alcohol caused cerebellar growth inhibition twice as much as the rest of the brain.

Prenatal exposure to ethanol led to abnormalities of neuronal development. This was associated with abnormal growth and maturation of the different cells and impairment myelination *(HAMMER and SCHEIBEL, 1981)*. Also, postnatal alcohol exposure affected greatly the differentiation and maturations of the neurons *(SCHAPIRO et al., 1984)*.

This work was designed to study the effect of alcohol ingestion on the postnatal development of the cerebellar cortex of the offspring from birth till the adult stage.

## MATERIAL and METHODS

Albino rats of sprague dawley were used in this study. Pregnant rats were placed in individual cages on day 18 of gestation. They fed a standared diet of commercial rat chow and were divided into two groups. The pregnant animals of the first group were given unrestricted access of 10% ethanol concentration as the only available source of water. The pups of this group were housed with their treated mothers and after weaning they maintained on the same ethanol concentration till became adult. The animals of the second group were given tap water and their pups were also maintained on tap water.

The pups from the two groups were sacrificed at the following ages: 0,5,10,15,21,30 and 100 days ( adult), eight animals each. The specimens were fixed in Bouins solution and processed for paraffin sections. They were stained with Toluidine blue and Holmies silver technique according to *DRURY and WALLINGTON ( 1980)*. Other specimens were fixed in 2.5 glutareladehyde in 0.1M cecodylate buffer and processed for semithin sections ( 1 um ) then stained with Toluidine blue

For morphometric study, the paraffin midsagittal sections 6 um) stained with Toluidine blue of both control and treated animal at different ages were examined by the use of the occular micrometer at magnification x640 *( WINER, 1971)*. The mean cell number per lobule was determined for each animal from three serial sections.Granule cells were counted

Within a grid area of 10,000. um, ( 5 samples/lobule). for steriogical study, semithin sections ( 1um ) were used to

estimate the numerical density of granule and purkinje cells with the aid of an especially prepared soft ware and a digitizing set (Graphic digitizer) linked to IBM computer. Numerical densities of granule and Purkinje cells were calculated by the formula *(UNDERWOOD, 1970)* $N_v = N_a/D'$ where $N_v$= number of cells per unit volume of tissue, $N_a$= number of nuclear profiles per unit area. D'= mean nuclear diameter.

## RESULTS

**External granular layer :**

**Control group:**

It was present in newborn and young cerebellum. Thatthickness was gradually increased to reach its maximum at 10 days old, then it sharply decline to disappear completely at 30 days old (Fig. 1, 2, 3 & 4).

**Treated group :**

The external granular layer was much thicker than in the control group at different ages (Fig. 5, 6 & 7).

**Molecular layer :**

**Control group:** Thickness : It appeared as a clear zone containing few cells. It's thickness was gradually increased with advancement of age and by age 30 the molecular layer became the superficial one (Fig. 1, 2, 3 & 4).

**Cells:** At five days the molecule cells were oblong with large oval nuclei. The basket cells were clearly identifid by day ten with horizontally spread processes (Fig. 8). From 15 days onwards the molecule cells acquired the adult configuration; rounded shaped with illdefined cell membrane (Fig. 9).

**Treated group : Thickness:** Starting from five days to the adult stage the thickness of the molecular layer was thinner than the control (Fig. 1, 2 & 3).

**Cells:** The cells in the young ages (five and ten days) decreased in size and were widely distributed. The dendritic tree of some basket cells were depleted (Fig. 10). On the 15th days many cells showed neuronal chromatolysis and many vacuoles were found in this layer (Fig. 11). These degenerative changes, on the form of cytoplasmic vacuoles and depleted dendrities were continued till the adult stage.

**Purkinje cell layer :**

**Control:**

**Arrangement of the cells:** Fromnew born to five days old the Purkinje cells were arranged in several layers and cannot

demarcated from the underlying granule cells (Fig. 1). At the tenth day the Purkinje cells became arranged in one row (Fig.2).

**Cell:** The Purkinje cells at 5 days old were spindle in shape with large oval nuclei (Fig. 12). The cell at this age had many processes arose from all aspects of the body. The lateral processes were disappeared by age ten days while the apical process formed the permanent dendritic tree (Fig. 13). At 15 days old, the Purkinje cells acquired the adult appearance as the cells were flask shaped with large rounded nuclei (Fig. 14 & 15).

**Treated:** Arrangement of the cells: Unlike that of control, the Purkinje cells were arranged in more than one layer till the age of ten days (Fig. 6). Thereafter the cells formed as single row (Fig. 7).

**Cells:** On the fifth day, the Purkinje cells showed neurocyte chromatolysis (Fig. 16). At ten days the dendritic tree of some Purkinje cells were depleted but there was many presomatic processes (Fig. 10). These degenerative changes, included neurocyte chromatolysis and deplition of dendrities were continued from 15 days til adult stage leading to complete loss of some cells (Fig. 17 & 18).

**Granular layer :**

**Control :**

**Thickness:** At five days old, the granular layer was clearly defined from the underlying white matter. It was gradually increased in cell depth with advancement of age (Fig. 1, 2, 3 & 4).

**Cells:** From age five to ten days old, the granule cells increased in amount and became polyhedral in shape. Golgi cells were detected by their large sizes and large pale nuclei. From 15 days old onwards the granule cells and Golgi cells were morphologically similar to the adult (Fig. 14 & 15).

**Treated :**

**Thickness:** Unlike to the control, the granular layer was not demarcated from the subjacent white matter till the tenth day.

**Cells:** Starting from five till 15 days old, the granule cells and Golgi cells were decreased in size when compared with the control (Fig. 17). The processes of the granule cells were short, thick and carried spines (Fig. 10). Neuronal degeneration were markedly observed in late postnatal and adult stage in which complete destruction of cells were found (Fig.18).

**Morphometric results :**

The mean number of Purkinje cells and granule cells in alcohol fed rats were significantly reduced than the control in all cerebellar lobules at different ages. It was, also, found that the lobules 11 & 111 in the anterior lobe and lobules (IX & X) in the posterior lobe were the most affected lobules (Fig. 19, 20).

**Steriological results** (Table 1):

**Numerical densities of granule and Purkinje cells:** The mean numerical density of granule cells in all selected ages of alcohol fed rats tended to have lower mean densities than the control.

The mean numerical density of Purkinje cells in alcohol fed rats were significantly reduced at 5 and 21 days.

**Granule to Purkinje cell ratios:** The granule to Purkinje cell ratio was increased from five to 21 days. Then the ratio was decreased at the adult stage.

## DISCUSSION

The light microscopic and morphometric studies revealed a distinct changes in the developing cerebellum.

The external granular layer, in alcohol fed rats, was much thicker than the control group while both molecular and granular layers were reduced.

It was well established that the molecule and granule cells migrated from the extrnal granular layer *(ALTMAN, 1972 a,b)*. The increased thickness of the external granular layer may be due to failure of its cells to migrate to their final situations in the molecular and granular layers and delayed proliferation of cells derived from it *(ALTMAN, 1973)*. This in turn led to decreased thickness of the molecular and granular layers. The reduced thickness of molecular layer may be also due to decrease in cellular processes that grow thorugh this layer especially those of Purkinje cells and granule cells. This suggestion was in agreement with *BAURE-MOFFET and ALTMAN (1977)*. They mentioned that the prenatal alcohol treatment led to a reduction of the Purkinje cell number and this in turn led to decrease in the dendritic branches and spines present in the molecular layer. The empty spaces observed in the molecular layer especially in late postnatal were due to complete degeneration of cells or their processes *(MURRY et al., 1981)*.

Purkinje cells in control group were arranged in one row at ten days old, while in alcohol fed rats the cells, at this age, failed to reach their final position and still arranged in

about two rows. The cells were greatly reduced in number and their numerical density per unit volume were reduced. Similar finding was obtained by *BAURE-MOFFET* and *ALTMAN, (1975)*. They found that ethyl alcohol, during early life, delayed the maturation of dendritic arborization of neurcns which led to vermal areal reduction. Also, many neurons were completely degene-rated and disappeared. The remaining neurons being backed into a smaller volume of tissue, thus giving this decreased numerical density of neurons.

In the present study degneration of Purkinje cells appeared at five days, complete destruction and even cell lost were found at late postnatal and adult stage. The dendritic trees of the cells were reduced but there was elongated spines. *ALTMAN (1969)* showed that the neurogenesis of Purkinje cells was formed before birth, whereas their differentiation and maturation occured during postnatal life. From the studies of *PHILLIPS and GREGG (1982)*, it was found that the Purkinje cells were susceptible to the toxic effects of alcohol at all stages of differentiation. This occured through the direct action of alcohol on the metabolism of these cells or indirectly through an alteration of their blood supply. The elongation of the dendritic spines may be a compansatory reaction to the decreased synaptic input to the Purkinje cells *(TAVARES et.al., 1983)*.

Granular layer in alcohol fed rats was ill defined from the subjacent white matter till the age of ten days while in control rats it was clearly defined at five days old. The number of granule cells was markedly reduced in all vermal lobules especially in the anterior lobe. The numerical density of the granule cells was also decreased. Neuronal degeneration started at five days old and cell lost was found in late postnatal and adult stage. The retarded growth and the degenerative changes of the granule cells were reported by *KORNGUTH* et al. *(1979)*, who found that the cerebellar histogenesis in rat, mainly granule cell maturation was, deeply affected by chronic alcohol consumption.

The reduction of granule cell number and their numerical density were in accord with *BAURE-MOFFET and ALTMAN (1977)*. They found that the postnatal alcohol exposure caused a reduction in the granule cells by 20-25%. It may be also due to the direct effect of alcohol on the maturation of granule cells *(WALKER* et al., *1981)* or indirectly by trans eural effect. Since granule cells (by their way of their axons), the parallel fibers synapse extensively with Purkinje cells *(SOTELO and TRILLER, 1979)*.

In the present work, it was found that the granule to Purkinje cell ratio, in alcohol fed rats was increased from five days to 21 days, then the ratio was decreased in the adult stage. It was indicated that alcohol during postnatal life caued its marked effect on the already formed Purkinje cells which arose mainly during prenatal lefe *(JACOBSEN, 1978)*. Therefore the decrease in granule to Purkinje cell ratio at the adult stage indicated that the long term alcohol intake caused an actual loss of granule cells and/or partial failure in the production of these cells.

Quantitative assesment of the number of Purkinje and granule cells in all cerebellar lobules revealed a marked reduction in lobules II & III in the anterior lobe, then lobules IX & X in posterior lobe. This may be explained by the fact that lobules I-IV in the anterior lobe and lobules IX & X in the posterior lobe were the earlier maturing regions of the vermis. So it was suggested that earliest maturing region was the mostly affected one *(ALTMAN, 1969)*.

From the results obtained in this work it might be concluded that alcohol led to two disinct changes, first, delayed maturation of neurons and their dendritic arborization hence causing a reduction in the molecular and granular area. Second, it caused severe damage to the neurons which led to loss of large number of them. This led to decrease in the numerical density of Purkinje and granule cells.

The effect of alcohol on cerebellum was evidented from five days old to 21 days. Then the changes were the same during the following stages till the adult stage.

## REFERENCES

*Abraham, W.C. and Hunter, B.E. (1982a):* An electrophysiological analysis of chronic ethanol neurotoxicity in the dentate gyrus: distribution of enterohinal afferents. Exp. Brain Res, 47: 61-68.

*Abraham, W.C.; Manis, P.B.; Hunter, B.E.; Zornetzer, S.F. and Walker, D.W. (1982 b):* Electrophysiological analysis of synaptic distribution in CA1 of rat hippocampus after chronic ethanol exposure. Brain Res., 237: 91-105.

*Altman, J. (1969):* Autoradiographic and histological studies of postnatal neurogenesis, III. Daling the time of production and onset of differentiation of cerebellar macroneurous in rats. J. Comp. Neur, 136: 269-294.

*Altman, J. (1972 a):* Postnatal development of the cerebellar cortex of the rat; I. The external germinal or layer ad the transitional molecular layer. J. Comp. Neurol. 145: 353-398.

*Altman, J. (1972 b):* Postnatal development of the cerebellar cortex of the rat; II. Phases in maturation of Purkinje cells and of the molecular layer. J. Comp. Neurol., 145: 399-464.

*Altman, J. (1973):* Expermintal reorganization of the cerebellar cortex. III: Regeneration of the external germinal layer and granule cell ectopia. J. Comp. Neural., 149: 153-180.

*Baure-Moffet, C. and Altman, J. (1975):* Ethanol induced reductions in cerebellar growth of infant rats. Exp. Neurol., 48: 378-382.

*Baure-Moffet, C. and Altman, J. (1977):* The effect of ethanol chronically administered to preweaning rats on cerebellar development: a morphological study. Brain Res. 119: 249-268.

*Drury, R.A.B. and Wallington, E.A. (1980):* Carlton's Histologic, technique. Fifth edition. Oxford University Press London.

*Freund, G. (1973):* Chronic central nervous system toxicity of alcohol, Ann. Rev. Pharmacol., 13: 217-227.

*Goodman, L.S. and Gilman, A.G. (1990):* The Pharmacological Basis of Therapeutic. 8th. Edition Vol. I.

*Hammer, R,P. and Scheibel, A.B. (1981):* Morphologic evidence for a delay of neuronal maturation in fetal alcohol exposure. Exp. Neural., 74: 587-596.

*Jacobsen, M. (1978):* Development Neurobiology. New York, London: Plenum press.

*Kissen, B. and Begleiter, H.(eds) (1974):* The biology of alcohollism Vol. 3, Clinical Pathology. Plenum press, New York.

*Kornguth, S.E.; Rutledge, J.J.; Sunderland, E.; Siegel, E.; Carlson, I.; Smollens, J.; Juhl, U. and Young, B. (1979):* Impeded cerebellar development and reduced serum thyroxine levels associated with alcohol fetal intoxication. Brain. Res., 177: 347-360.

*Murry, H.M.; Saland, L.C. and Reys, E. (1981):* Golgi and electron microscopic studies of sensorimotor cortex in rats with fetal alcohol syndrome. Anat. Rec., 199: 178-188.

*Phillips, g. and Gregg, B.G. (1982):* Changes in susceplibility of rat cerebellar Purkinje cells to damage by alcohol during fetal, neonatal and adult life. Neuropath. Appl. Neurobiol. 8: 441-454.

*Schapiro, M.; Rosman, N.P. and Kemper, T.L. (1984):* Effect of chronic exposure to alcohol on the developing brain, Neurobehev. Toxicol Teratol., G: 351-356.

*Sotelo, C. and Triller, A. (1979):* Fate of presynaptic afferents to Purkinje cells in the adult nervous mutant mouse: a model to study presynaptic stabilization. Brain Res., 175: 11-36.

*Tavares, M.A.; Paula-Barbosa, M.M. and Gary, E.G. (1983):* A morphometric Golgi analysis of the Purkinje cell dendritic tree after long term alcohol consumption in the adult rat. J. Neurocytol., 12: 939-948.

*Underwood, E.E. (1970):* Quantitative Stereology. Massachusetts: Addison-Wesley.

*Walker, D.W.; Hunter, B.E. and Abraham, W.C. (1981):* Neuro- anatomical and functional deficits subsequent to chronic ethanal administration in animal. Alc. Clin. Exp. Res. 5: 267-282.

*Winer, B.J. (1971):* Statistical Principles In Experimental Design, McGraw-Hill, New York pp: 149-160.

## LEGENDES

**Fig. 1:** Cerebellum of a five days control rat showing the external granular layer (g) and molecular layer (M). The Purkinje cell layer (P) is formed of several layers and the granular layer (G) is ill defind from the subjacent white matter (W). Toluidine blue (x200).

**Fig. 2:** Cerebellum of a ten days control rat showing the external granular layer (g) molecular layer (M), Purkinje cell layer (P) and granular layer. Notice the arrangement of Purkinje cells in a single. The granule layer is well defined from the white mather (W) Toluidine blue (x200).

**Fig. 3:** Cerebellum of a ten days control rat showing the external granular layer (g), molecular layer (M), Purkinje cell layer (p) and granular layer (G) Notice the marked decrease in thickness of external granular layer. Toluidine blue (x200).

**Fig. 4:** Cerebellum of a 30 days control rat showing the disappearance of external granular layer. Toluidine blue (x200).

**Fig. 5:** Cerebellum of a five days alcohol fed rat showing the external granular layer (g) molecular layer (M), Purkinje cell layers (P) and granular layer (G). Notice the increased thickness of external granular layer. Toluidine blue (x200).

**Fig. 6:** Cerebellum of a ten days alcohol fed rat showing the marked increase of external granular layer (g) and the arrangement of Purkinje cells in several rows (P). Note that the granular layer (G) is not demarcated from the white matter (W). Mollecular layer (M). Toluidine blue (x200).

**Fig. 7:** Cerebellum of a 15 days alcohol fed rat showing that the external granular layer is thicker than control. Notice the arrangement of Purkinje cells in one row (P). Toluidine blue (x200).

**Fig. 8:** Cerebellum of a ten days control rat showing the basket cells with therir spread processes. Holmes silver technique (x1250 oil immersion)

**Fig. 9:** Cerebellum of a 15 days control rat showing the molecule cells. Semithin section stained with toluidine blue (x1250 oil immersion).

**Fig. 10:** Cerebellum of a ten days alcohol fed rat showing a basket cell with depleted dendritic arborization (b). Notice a Purkinje cell (P) has no dendritic tree but with many perisomatic processes (arrows). Notice also the thickened and short processes of granule cells. Holmes silver technique (x1250 oil immersion).

**Fig. 11:** Cerebellum of a 15 days alcohol fed rat showing the degeneration of some molecular cells and the presence of many spaces. semithin section stained with toluidine blue (x1250 oil immersion).

**Fig. 12:** Cerebellum of a five days control rat showing the spindle shaped Purkinje cells. Semithin section stained with toluidine blue (x1250 oil immersion).

**Fig. 13:** Cerebellum of a ten days control rat showing a Purkinje cell with branching dendritic tree (P). Holmes silver technique (x1250 oil immersion).

**Fig. 14:** Cerebellum of a 15 days control rat showing the flask shaped Purkinje cells (P) granule cells (G) and Golgi cells (arrow) semithin section stained with toluidine blue (x1250 oil immersion).

**Fig. 15:** Cerebellum of an adult contral rat showing Purkinje cells (P)., granule cells (G) and Golgi cells (arrow). semithin section stained with toluidine blue (x1250 oil immersion).

**Fig.16:** Cerebellum of a 15 days alcohol fed rat showing degenerated Purkinje cells (P) Notice the complete destruction of a cell leaving empty space (double arrows). The granule cells (G) and Golgi cells (arrow) are decreased in size (arrow). Semithin section stained with toluidne blue (x1250 oil immersion).

**Fig.17:** Cerebellum of a 15 days alcohol fed rat showing degenerated Purkinje cells (P). Notice the complete destruction of a cell leaving empty space (double arrows). The granule cells (G) and Golgi cells (arrow) are decrease in size. Semithin, section stained with toluidine blue (x1250, oil immersion).

**Fig.18:** Cerebellum of an adult alcohol fed showing the degenerated Purkinje cells (P), granule cells (G) and Golgi cells (arrows). Semithin section stained with toluidine blue (x1250 oil immersion).

TABLE 1 Data on cerebellar granule and Purkinje cells in control and experimental rats

| | 5 days | | 21 days | | 110 days | |
|---|---|---|---|---|---|---|
| | Control | Alcohol | Control | Alcohol | Control | Alcohol |
| $N_{vg}$ | 1320.456 | 1152.792 | 3200.376 | 31112.626 | 3537.983 | 3340.616 |
| $N_{vp}$ | 2115 | 1830 | 4402 | 4127 | 4690 | 4455 |
| G : P | 624.32 | 629.94 | 727.02 | 754.21 | 754.36 | 749.85 |

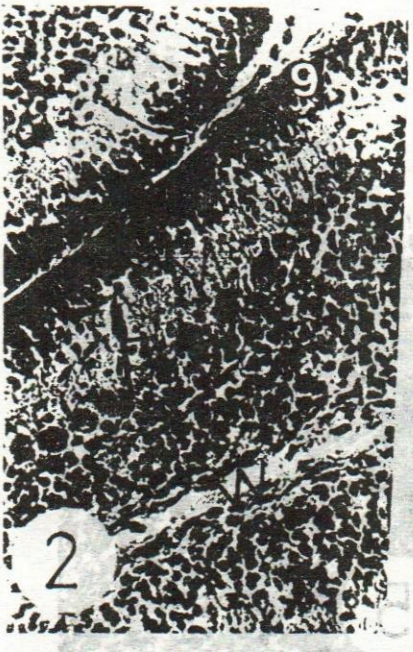
$N_{vg}$ = Numerical density of granule cells / 0.001 $mm^3$

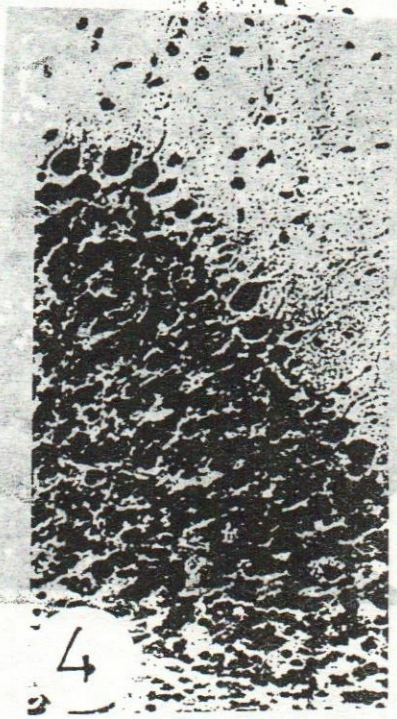
$N_{vp}$ = Numerical density of Purkinje cells / $mm^3$

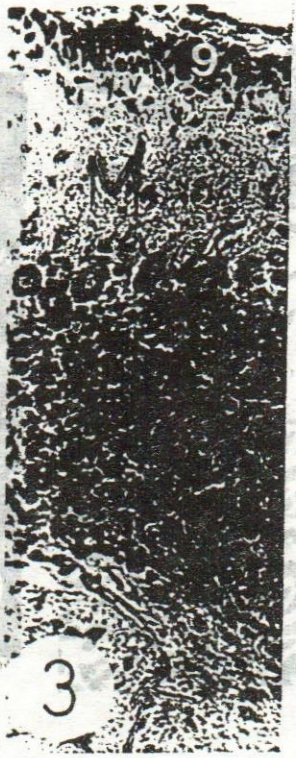
G : P = Granule - to - Purkinje cell ratios

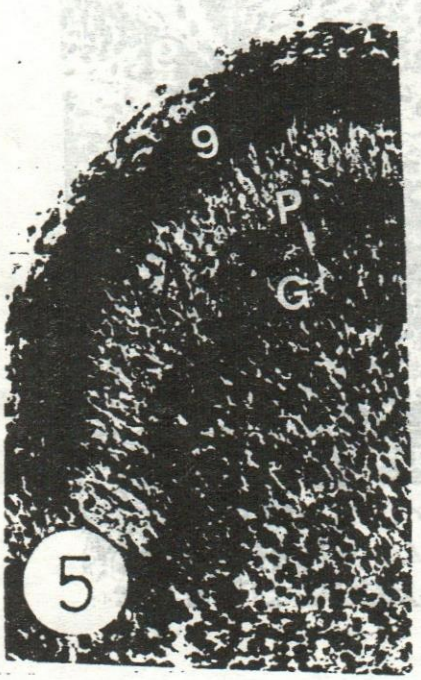
Each value represents the mean of six sections of 3 animals of each age.

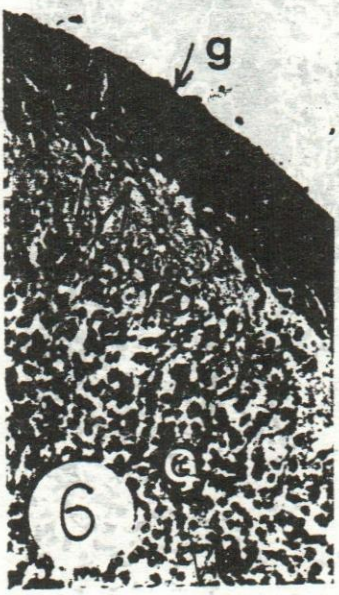

9
W
1


9
2

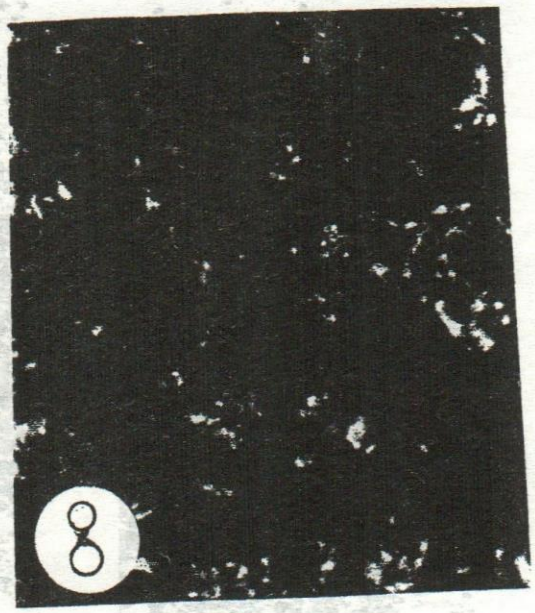

4


9
3

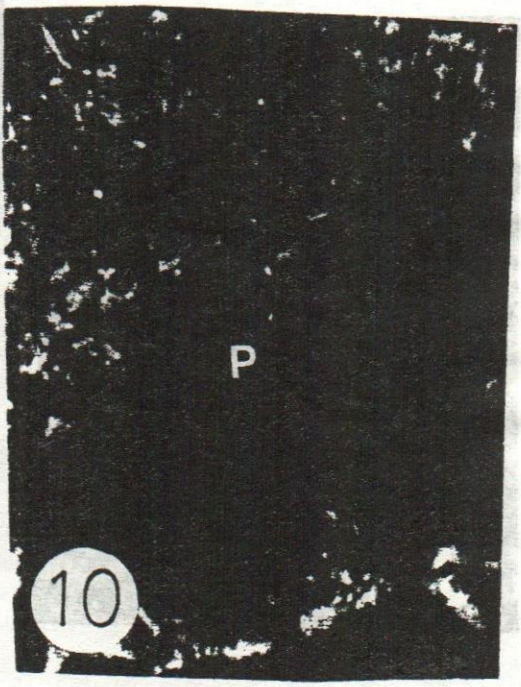

9
P
G
5

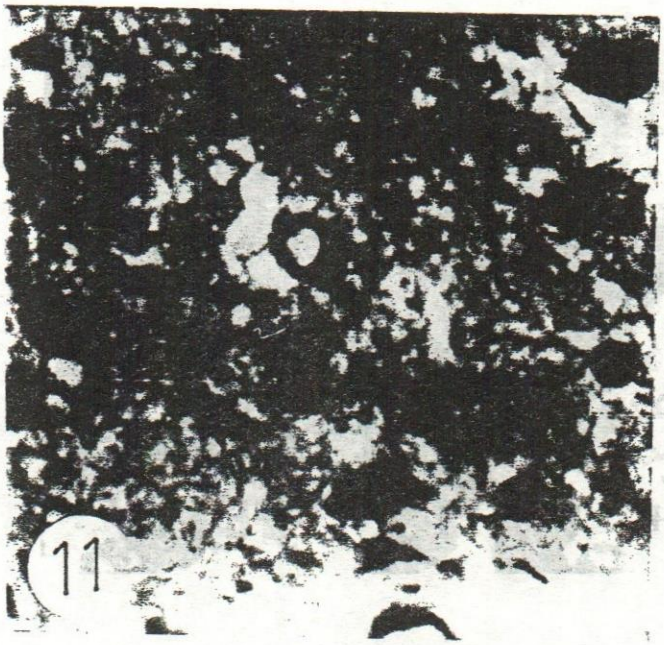

g
6

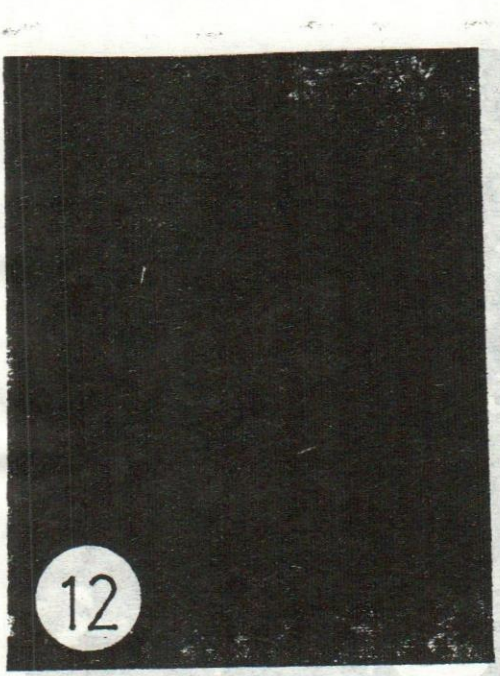

7

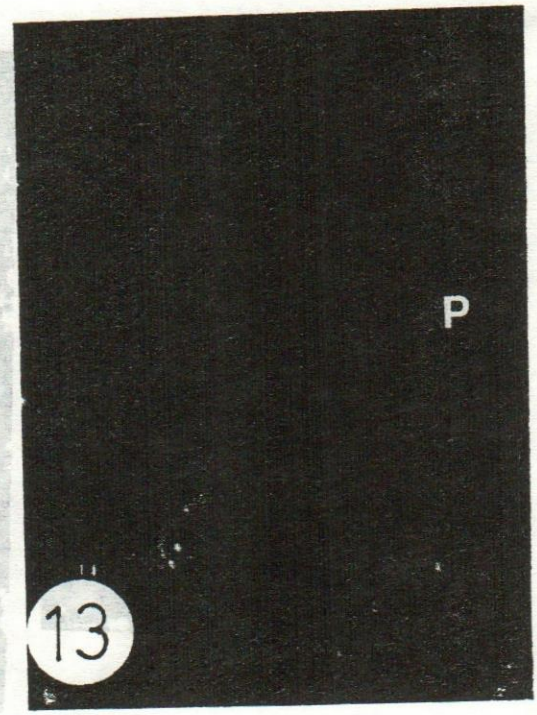

8

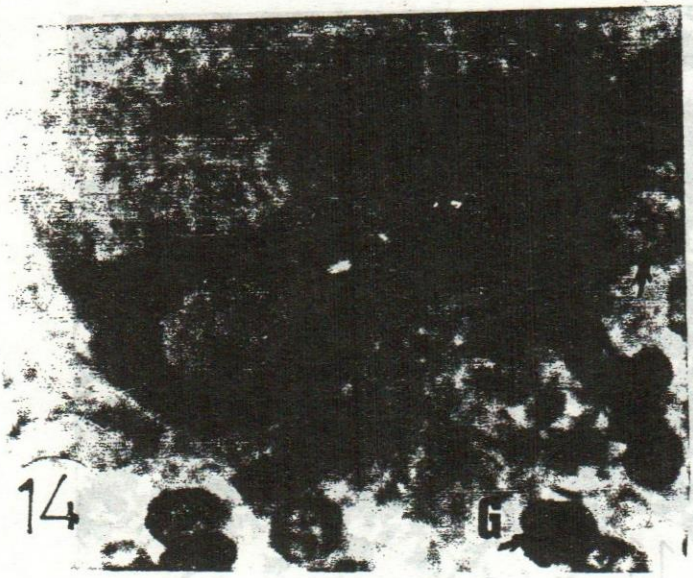

9

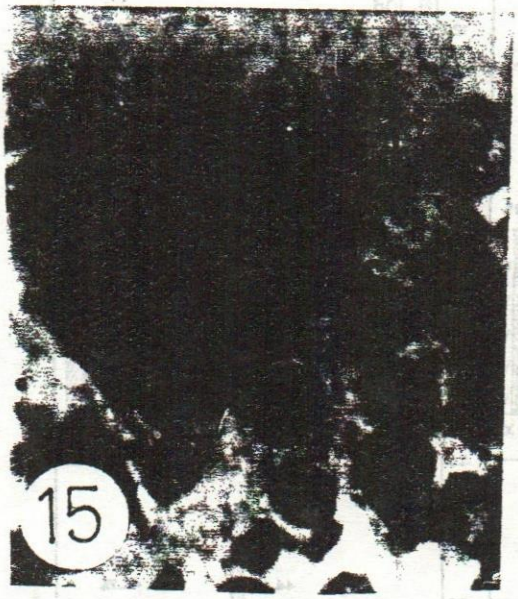

P
10


11

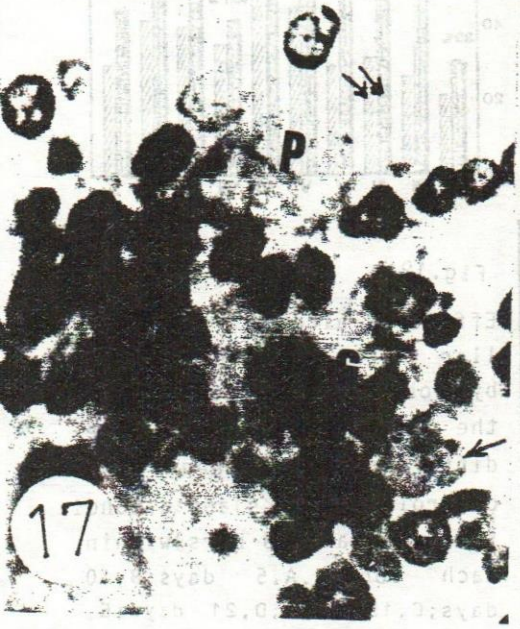

12

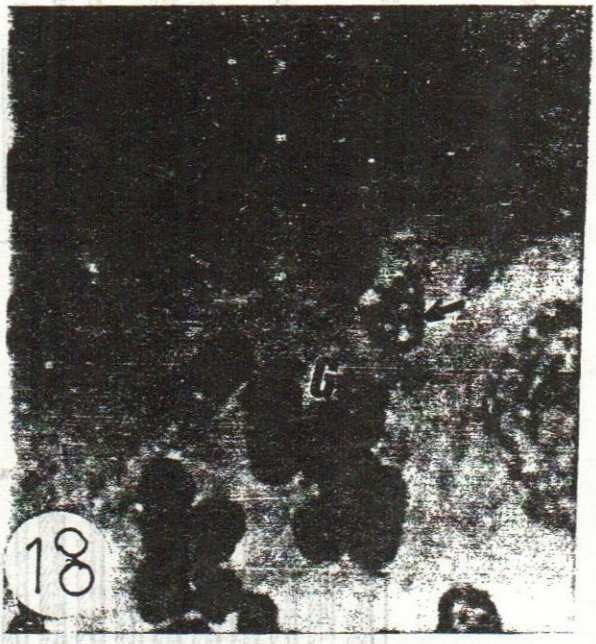

P
13


14
G


15


16


P
17


18

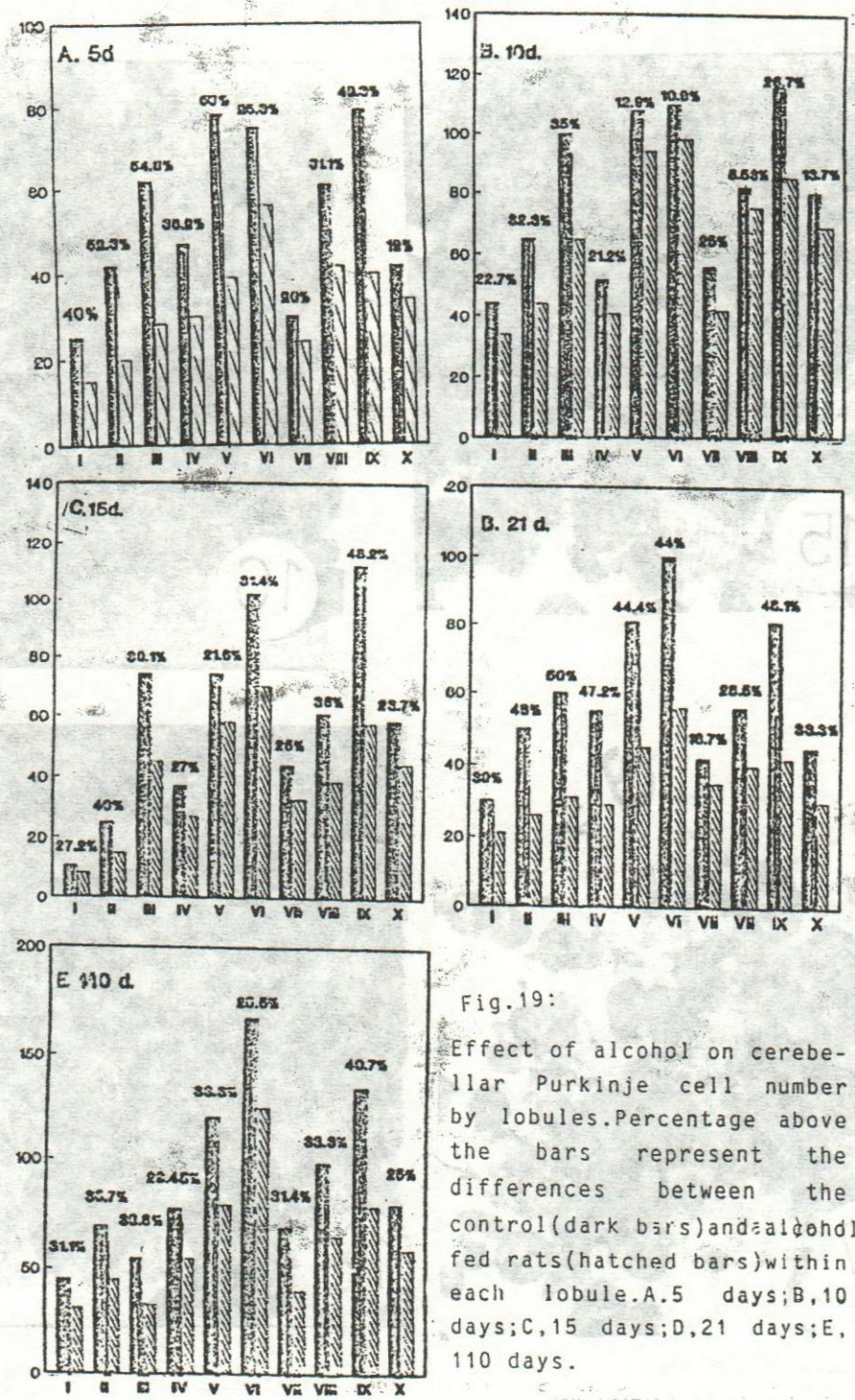



Fig.19:

Effect of alcohol on cerebellar Purkinje cell number by lobules. Percentage above the bars represent the differences between the control (dark bars) and alcohol fed rats (hatched bars) within each lobule. A. 5 days; B, 10 days; C, 15 days; D, 21 days; E, 110 days.

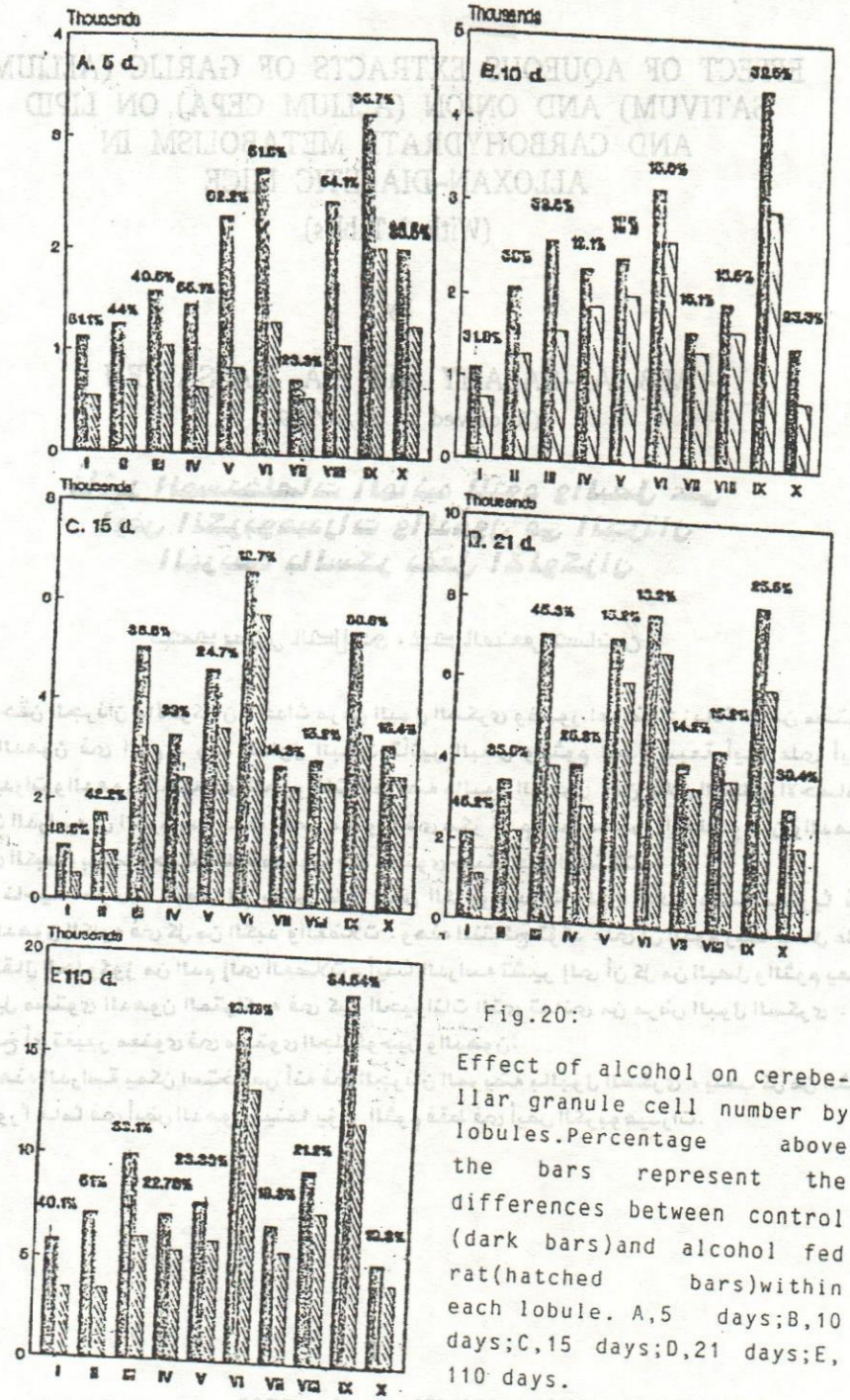



Fig.20:

Effect of alcohol on cerebellar granule cell number by lobules. Percentage above the bars represent the differences between control (dark bars) and alcohol fed rat (hatched bars) within each lobule. A, 5 days; B, 10 days; C, 15 days; D, 21 days; E, 110 days.