Dept. of Anatomy and Histology

Fac. of Medicine, Assiut Univ.

Head of Dept. Prof. Dr. F. Zaki.

EFFECT OF ALCOHOL ON THE POSTNATAL DEVELOPMENT OF WHITE MATTER OF THE CEREBELLUM OF THE RAT

(With One Table & 15 Fig.)

By

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

(Received at 7/11/1994)

تأثير الكحول على تطور الماده البيضاء للمخيخ بعد الولاده فى الفأر

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

أوضحت هذه الدراسه الى ان الكحول الايثلى (١٠ ٪) له تأثير ضار على تطور الماده البيضاء للمخيخ فى الفأر الابيض بعد الولاده وحتى مرحلة البلوغ . فلم يحدث تميز واضح بين طبقتى الماده البيضاء والحبيبات الداخليه الا بعد اليوم العاشر من الولاده عكس المجموعه الضابطه التى وجد التميز بين الطبقتين فى اليوم الخامس من الولاده . وقد بدأ التحلل فى اليوم الخامس من الولاده حيث صغر حجم الخلايا والانويه وأيضا بدأ التحلل فى الزوائد العصبيه للخلايا وإزداد التحلل بتقدم العمر حتى ظهرت فجوات فى الماده البيضاء عند العمر الواحد والعشرين بعد الولاده وكذلك فى الطور البالغ .

ووجد أيضا ان سمك الماده البيضاء يقل عنه فى المجموعه الضابطه خاصة فى العمر الواحد والعشرين وكان الفص الامامى اكثر تأثرا عن الفص الخلفى .

*: Dept. of Anatomy, Fac. of Med., Assiut Univ.

**: Dept. of Histology, Fac. of Med., El-Minia Univ.

SUMMARY

In new born and five days old of control rats the white matter was cellular. The amount of cells started to decline by age ten days and the amount of nerve fibers gradually increased. So by the adult stage, the white matter contained mainly interlacing network of nerve fibers. Under the effect of alcohol, the white matter delayed in demarcation from the granular layer after ten days old. Neuronal degeneration appeared at five days old increased with age and at 21 days many spaces were found. In the adult stage all the dendritic network was dilated and showed localized thickenings. Morphometric study revealed that the thickness of white matter was decreased than the control in all ages especially at 21 days old. The anterior lobe was found to be more affected than the posterior lobe.

Keywords: Effect, alcohol, postnatal development, white matter, rat

INTRODUCTION

The cerebellum contains three pairs of-major projection bundles; the cerebellar peduncles. These peduncles contain both afferent and efferent fibers (*CHUSID, 1983*). Four grey masses are embedded on each side of the crebellar white core which are called intracerebellar nuclei and sometimes termed roof nuclei (*WILLIAMS and ARWICK, 1989*). Mossy fibers are afferent fibers come from the brain stem and spinal cord nuclei and pass through the white matter to terminate profusely in the granular layer on dendrites of granule cells in complex synaptic junctions called glomeruli. Climbing fibers are also afferent fibers from inferior olivary nuclei that terminate in the molecular layer near the purkinje cells (*LLINAS* et al., *1974*). Most purkinje axons end in the intracerebellar nuclei but some in the lateral vestibular nucleus (*VOOGD 1964*).

Eventually, the white matter of the cerebellum is less cellular and contains mainly nerve fibers because it repre sents the way through which afferent and efferent fibers pass to cerebellum. It is structurally different from the cerebellar cortex. There is suppose that the rate and mode of growth of these two components are different due to the difference in the elements that form them (*KORNELIUSSEN, 1986*).

The present work describes the morphological alterations of nural development of the white matter of the cerebellum at different stages of development under the effect of ethanol. Quantitative study was done because the development of the white matter of the cerebellum of the rat occurs largely during the period of the first three weeks of life *(FREUND, 1973)* [period of ethanol treatment].

MATERIALS AND METHODS

Pregnant albino rats were divided into two groups; one group was given unrestricted access of ethanol at 10% concentration as the only source of liquid at 18 days of gestation and throughout the period of lactation. Their pips were maintained on ethanol till they became adult. The animals of the second group and also, their pups were given tap water (control).

The pups of both control and ethanol fed rats were sacrificed at these ages: 0-5-10-15-21-30 and 110 days (adult), eight animals each. The cerebellum of each animal was dissected carfully and fixed in Bouin's solution and processed as usual for paraffin sections. The sections were stained with toluidine blue and holmes silver technique according to *DRUY and WALLINGTON (1980)*. Other small specimens were fixed in 2.5% glutaraldehyde in o.1.M cacodylate buffer ph 7.4 and then processed for semithin sections (1 U) that stained with toluidine blue.

The thickness of the white matter was measured using eye piece gracticule with a Linear scale caliberated against a stage micrometer. At a magnificantion x 640 the total areal thickness of the white matter in the midsagittal region of the ten lobules in both the anterior and posterior of the cerebellum were measured. This was repeated in each age in both control and treated groups.

RESULTS

Control:

In new born, the white matter cannot demarcated from the granular layer (Fig.1). It started to become distinct: at age five days (Fig.2). At this stage, the white matter was highly cellular, their cells were oval or fusiform with large oval nuclei (Fig.3). Each cell had two processes, one was directed towards the cortex while the other process was directed towards the roof of the fourth ventricle (Fig.4).

At ten days, the demarcation between the white matter and the granular layer well defined. The cells had the same characters of the previous stage with long branched processes.

The amount of the cells in the white matter began to decrease in amount at this age and age 21 days old, the white matter contained few amount of cells (Fig.5). Their dendrities became much more elongated and extensively branched so by the adult stage, the white matter contained an interlacing network of fibers (Figs. 6&7). Axons were not demonstrated in older stages because they were so delicate.

Treated:

In new born and till the tenth day the white matter was still ill defined from the granular layer (Fig.8). The demarcation between the two layers were clearly distinct in the subsequent ages.

Signs of degeneration appeared at five days old, where the cells were decreased in size with little Nissl substance, and some cells contained pyknotic nuclei (Fig.9). Their processes were wavy, dilated and less impregnated with silver (Fig.10).

As age advanced the process of degeneration became more, the cells were much smaller and by age 21 days old many cavities were found (Fig.11). The spaces between the fibers (Fig.12).

In the adult stage, the dendritic network was dilated and less impregnated with silver. The fibers showed localized thickening at an interrupeted areas (Fig.13). Climbing fibers during their course through the white matter were found to have a beaded appearance or segmented into small pieces (Fig.14).

Morphometric Study:

In control groups, the white matter area was increased with age. In alcohol fed groups the white matter area waas below controls at all ages. This decrease in the thickness of the white matter layer was marked at 21 days old. The differences in thicknes of the white matter between the control and alcohol fed groups was marked in the anterior lobe than in the posterior lobe (Table 1).

DISCUSSION

The light and morphometric studies showed that the white matter, similar to cerebellar cortex (*SALEH*, et al., *1993*) was greatly affected by alcohol during postnatal development. It was found that the demarcation between the white matter and the granular layer was still indistinct till the age of ten days, then became evident in the ages after.

In countrol, the demarcation between the two layers started to appear at age five days old and became more evident with advancement of age. This was due to the diminution in the number of cells present in the white matter. Thus, the transition became abrupt between the densely populated granular layer and the sparsely populated white matter *(NOOR-EL-DIN, 1966)*. The delayed demarcation between the white matter and the granular layer was due to delayed diminusion of the amount of cells in the white matter and also due to growth retardation of alcohol intoxication *(KORNGUTH* et al *1979)*. Degenerative changes were evident in the fibers passing through the medullary layer including dendrites of the cells of the medullary layer, Mossy and climbing fibers. These changes appeared at five days old and increased till the stage. This was in agreement with *ALTMAN and BAURE-MOFFETT (1977)* who found that ethanol affected, prenatally as well as postnatally the cerebellar components including purkinje cell axons, climbing and Mossy fibers. *SINCLAIR* et al *(1983)* observed that ethanol acts also upon inferior olive neurons (The origin of cerebellar climbing fibers). *WEST* et al *(1981)* found altered organization of hippocampal Mossy fibers in rats exposed prenatally to ethanol.

A significant inhibition in brain protein synthesis was reported by *TEWARI and NOBLE (1979)* in animals maintained on ethanol for prolonged periods. These induced metabolic changes which could explain the deteriorative alterations found in cerebellar cells and fibers.

The empty spaces observed in 21 days and adult stage were due to the complete degeneration and loss of nerve fibers *(MURRAY* et al., *1981)*.

In the present work the white matter layer in alcohol fed rats was reduced in all ages of postnatal life and reach its maximum reduction at 21 days. This was in accord with *BAURE-MOFFETT and ALTMAN (1975)*. They found that ethanol caused medullary areal reduction 34% than control. They referred this reduction to the dificit of Purkinje cell axons, the effect of ethanol on glial cells and myelinated afferent fibers. They found also that the myelinogenesis of medullary fibers was relatively of late onset and this contributed to the areal increase of the vermis between 21 and 90 days postnatally. This finding confirms the result of this work in which the medullary layer was severly affected by ethanol at 21 days old.

In the present work, the medullary reduction in the anterior lobe was markedly affected than in the posterior lobe, it was suggested that the earliest maturing region was the mostly affected one *(ALTMAN, 1969)*.

It is concluded that the white matter of the cerebellum was greatly affected by ethanol during postnatal life. Degeneration of cells and fibers started from the fifth day and gets worser later on till the adult stage. The maximum effect as regard cell degeneration and decrease in the thickness of the white matter layer appeared at the day 21. The anterior lobe was more affected than the posterior lobe.

REFERENCES

Altman, J. (1969): Autoradiographic and histological studies of postnatal neurogenesis, III. Dating the time of production and onset of differentiation of cerebellar macroneurons in rats

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

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

Chusid, J.G. (1983): Correlative neuroanatomy and functional neurology. 18 th ed. Lang Medical puplication Los. Altos Calif. 37.

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

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

Korneliussen, A.K. (1968): On the ontogenetic development of cerebellum (Nuclei, fissures and cortex) of the rat, with special reference to regional variations in corticogenesis. J. Hernforsch. 10: 379-412.

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

Liinas, R.; Hillman, D.E. and Precht, W. (1973): Neuronal circuit reorganization in mammalian agranular cerebellar cortex. J. Neurobiol., 4: 69-94.

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

Noor-EL-Din. (1966): Qualitative and Quantitative studies of the postnatal development of the cerebellum of mouse. M.d. Thesis. Assiut Univ., Assiut, Egypt.

Saleh, M.N.; Saleh, M.A.; Desouky, M.A.; Sayed, S.R. and Saber, E.A. (1993): Effect of alcohol on the postnatal development of cerebellar cortex in rat 1-Histological and morphometric studies. Assiut. Vet. Med. J. 30 (59) Oct.: 13-31.

Sinclair, J.G.; Lo, G.F. and Harris, D.P.(1983): Ethanol inhibits bits inferior olive neurons. Proc. West phatmacal. Soc. 26: 155-165.

Tewari, S. and Nobel, E.P. (1979): effects of ethanol on cerbral protein and ribonucleic acid synthesis. Pages 541-548 in e.Majchrowicz and E,P. Nobel, Eds., Biochemistry and pharmacology of Ethanal, Vol. 1, Plenum, new York/London.

Voogd, J. (1964): The cerebellum of the cat structure and fibers connexions. Proefschr. Van Gorcum; Assen.

West, J.R.; Hodges, C.A. and Black, A.B. (1981): Prenatal exposure of ethanol alters the organization of hippocampal mossy fibers in rats. Science, 211:957-959.

Williams, P.L. and Arwick, R.W. (1989): Gray's Anatomy 37 the ed. Edinburgh London Melbourno and New York.

Winer, B.J. (1971): Statistical principles in Experimental Design. Mc graw-Hill. New York. PP.149-160.

Legends of Figures

Fig. 1: Photomicrograph of the cerebellum of a new born control rat showing the illdefined white matter(W) from the granular layer(G). External granular layer(g). (Toluidine blue x 125).

Fig. 2: Photomicrograph of the cerebellum of a five days old control rat showing the well defined white matter (W) from the granular layer (G). (Toluidine blue x 125).

Fig. 3: Photomicrograph of the cerebellum of a five days old control rat showing the white matter cells. They are oval with large nuclei. Blood vessel(B). Semithin section stained with toluidine blue (x 1250).

Fig. 4: Photomicrograph of the cerebelum of a five days old control rat showing that each cell has two processes, one is long (P) and the other is short (arrow) Holmes silver technique (x 1250 oil immersiorn).

Fig. 5: Photomicrograph of the cerebellum of a 21 days control rat showing the less cellular white matter(wm) Granular layer(G). Semithin section stained with toluidine blue (1250 oil immersion).

Fig. 6: Photomicrograph of the cerebellum of a 21 days old control rat showing the highly silver impregnated nerve fibers pass in the white matter (arrows) Granular layer(G). Holmes silver technique (x 1250 oil immersion).

Fig. 7: Photomicrograph of the cerebellum of an adult control rat showing the interlacing dendritic fibers in the white matter. Neuroglia cells(N). Holmes silver technique (x 1250 oil immersion).

Fig. 8: Photomicrograph of the cerebellum of a ten days old alcohol fed rat showing the still ill-defined white matter(W) from granular Layer(G) External granular layer(g). (Toluidine blue x200).

Fig. 9: Photomicrograph of the cerebellum of a five days old alcohol fed rat showing the cells in the white matter. Notice their small and condensed nuclei (arrows). Semithin section stained with toluidine blue (x1250 oil immersion).

Fig.10: Photomicrograph of the cerebellum of a five days old alcohol fed rat showing the dilatation of the processes of the white matter cells. Notice that the fibers are less impregnated with silver. Holmes silver technique (x 1250 oil immersion).

Fig.11: Photomicrograph of the cerebellum of a 21 days old alcohol fed rat showing the presence of multiple cavities in the white matter (arrows). Granular layer(G). Semithin section stained with toluidine blue (x 1250 oil immersion).

Fig.12: Photomicrograph of the cerebellum of a 21 days old alcohol fed rat showing the marked dilatation of the dendritic branches (arrow) and the presence of wide spaces(S) between these fibers. Holmes silver technique (x 1250 oil immersion).

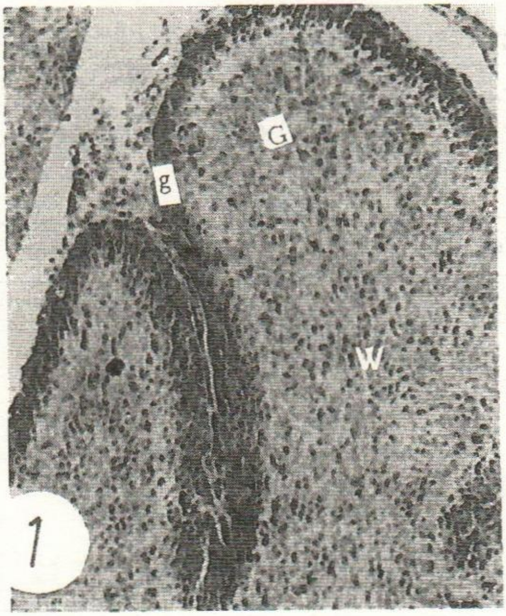
Fig.13: Photomicrograph of the cerebellum of an adult alcohol fed rat showing the dilated fibers of the dendritic netowork and the presence of localized thickenings (arrow). Holmes silver technique (x 1250 oil immersion).

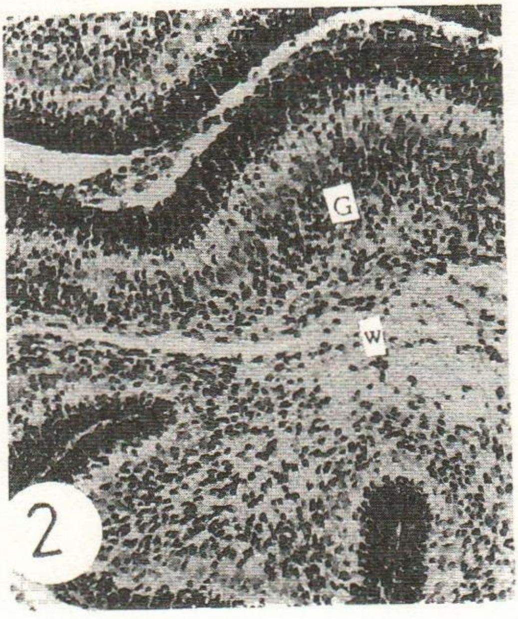
Fig.14: Photomicrograph of the cerebellum of an adult alcohol fed rat showing the beaded and segmented appearance of a climbing fiber(cf). Holmes silver technique (x 125).

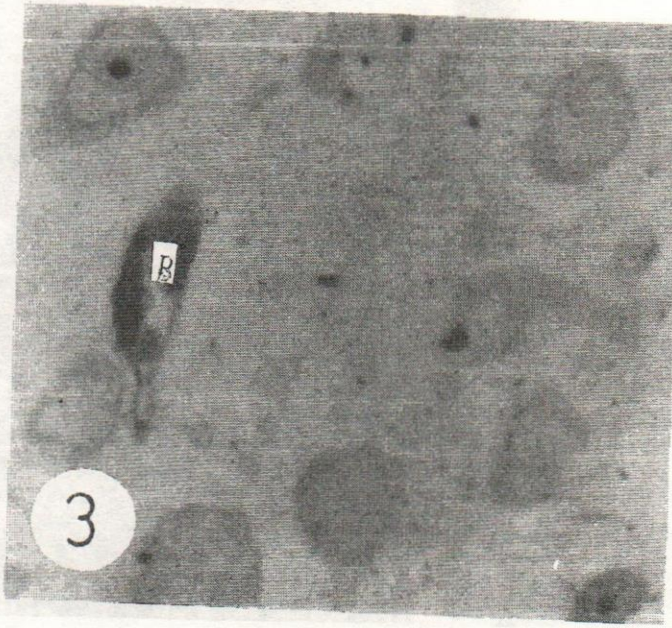
Fig.15.A,B: The white matter area are less thickned in all ages of alcohol fed rat than the control group. Also, the anterior lobe is more affected than the posterior lobe. The black bars represent the control, while the hatched bars represent the alcohol fed groups.

Table 1: Comparison between midsagittal white matter areas of the cerebellum in (Um) at control and treated rats at different ages of postnatal life. Note that: The anterior lobe is always more affected by ethanal than the posterior lobe.

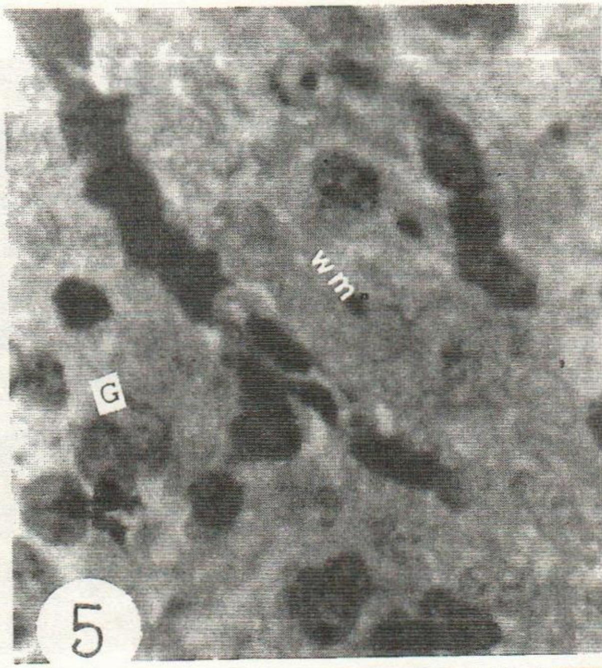
Age	Anteriior lobe		Posterior lobe	
	Control	Treated	Control	Treated
5	70	60	100	85
7	130	120	150	130
10	150	130	175	160
15	150	130	175	162
21	175	62	180	70
30	176	60	182	65
50	185	75	190	75
110	200	110	220	115


G
g
W
1

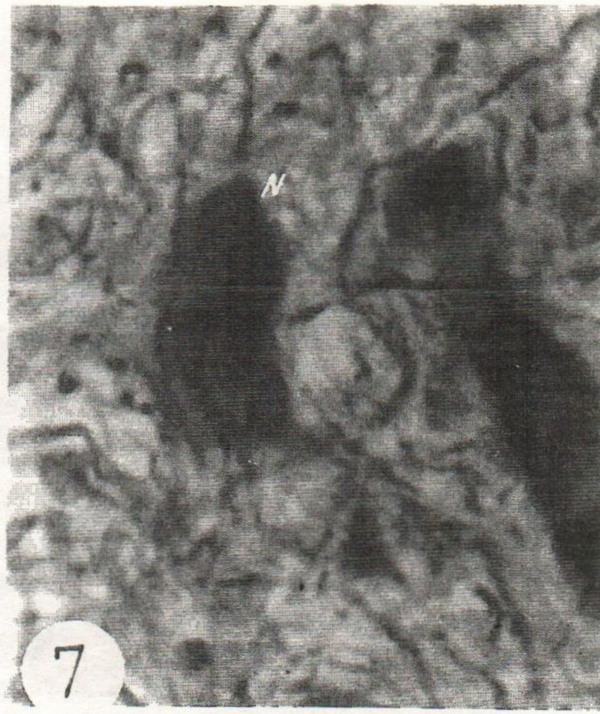

G
W
2


3
P

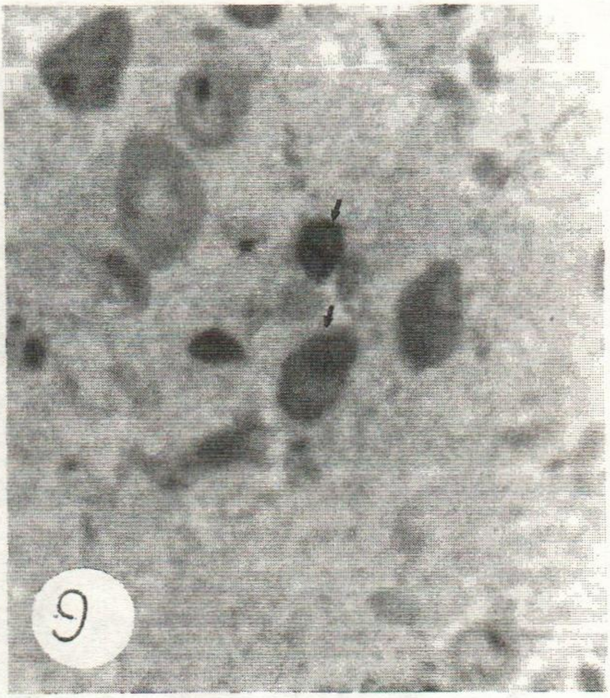

4
P


wm
G
5

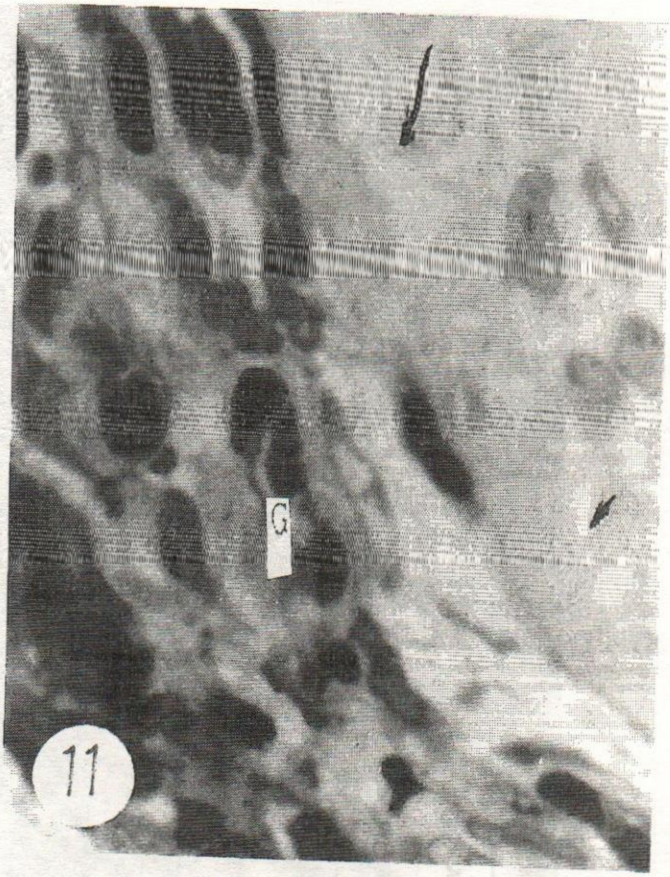

6

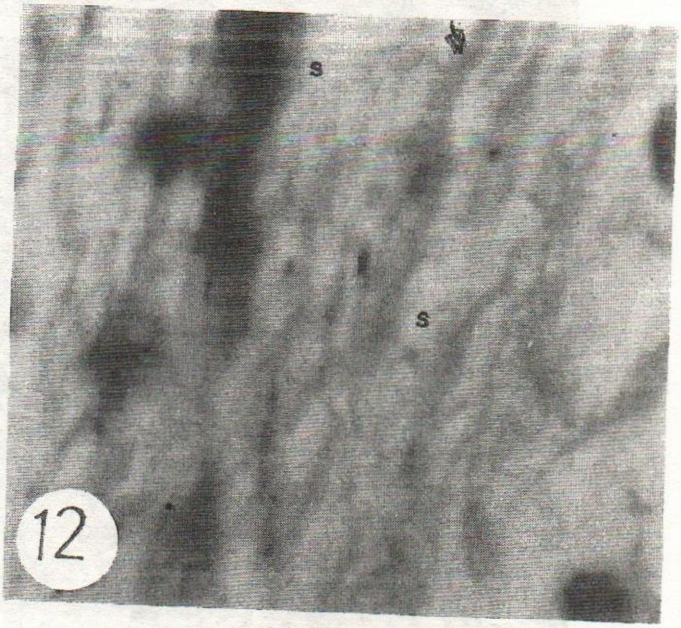

N
7

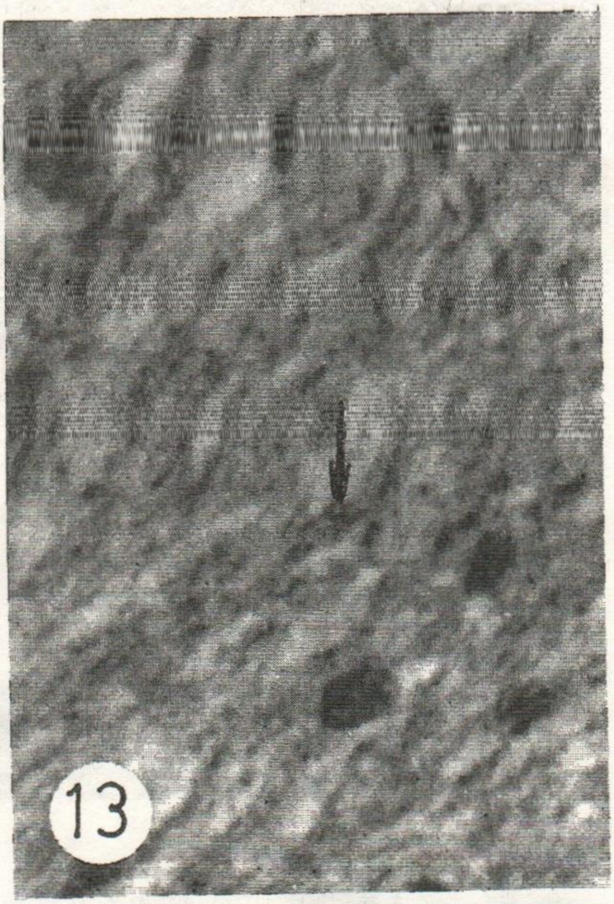

W
G
8

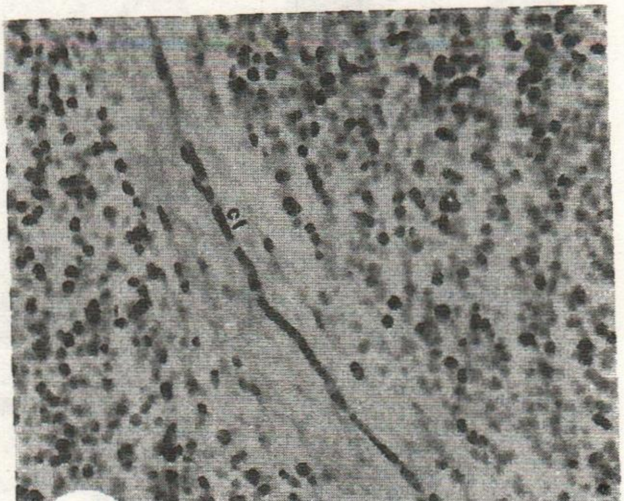

9


10


11
G


12
S
S


13


cl
14