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HISTOGENESIS OF THE CEREBELLAR CORTEX OF DOG DURING THE PRENATAL LIFE AND SUCKLING PERIOD

(With 8 Figures)

By

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تنسج قشرة المخيخ فى الكلاب أثناء الحياة الجنينية وفترة الرضاعة

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أجريت هذه الدراسة على مخيخات ستة عشر من أجنة الكلاب التى تراوحت أطوالها من ١٠-٢٠٠مم كما أشتملت علىمخيخات عشرة كلاب فى المراحل الأولى من العمر (فترة الرضاعة). فى أجنة الكلاب التى تراوحت أطوالها بين ١٠-١٥مم أنحنت الأجزاء الظهرية الوحشية للصفائح الجناحيه للدماغ التالى مكونه الشفاه المعينيه والتى أتحدت فيما بعد مكونه الصفيحه المخيخيه وذلك نتيجة الزيادة فى الثنيه الجسريه فى الأجنة التى وصلت أطوالها بين ٥٠-٦٠ مم. تميزت الصفيحة المخيخيه فى هذا العمر الى أربع طبقات هما: الطبقة الطلائيه العصبيه والطبقه الغطائيه والطبقه الجزيئيه والطبقه المحببه الخارجيه . شوهدت بشائر أنشقاق سطح المخيخ فى الأجنة التى وصلت أطوالها ١٠٠مم كما ظهرت فى هذا العمر الطبقة الحبيبية الداخليه والتى أزدادت بتقدم الحمل وما بعد الولادة. فى الاجنة التى وصلت أطوالها ١٤٠مم ظهرت طبقة خلايا بيركنجى فى أكثر من صف والتى قلت بتقدم العمر الى أن أصبحت طبقة واحدة من الخلايا المتفرقه فى نهاية هذه الدراسه . لوحظ بصفة عامه من تتابع مراحل هذه الدراسة أن الطبقة الخارجيه الحبيبية قلت تدريجيا بتقدم العمر، هذا وقد زاد سمك كل من الطبقة الجزيئيه والطبقة الداخليه الحبيبية الى أن أصبحت قشرة المخيخ مكونه من ثلاث طبقات هى الطبقة الجزيئيه وطبقة مركزيه من خلايا بيركنجى وطبقة داخليه حبيبية عند عمر ٤٥ يوم بعد الولادة مما يعكس الشكل الناضج للمخيخ فى هذا العمر.

SUMMARY

The present investigation was carried on the cerebellum of 16 dog foetuses of both sexes ranging from 10 to 200 mm CVR length. In addition, the cerebellum of 10 dogs representing the suckling period (0-45 days old) was also studied. In dog embryos ranging from 10 to 15 mm CVR length, the dorsolateral parts of the alar plates of the metencephalon bent medially and formed the rhombic lips; the primordium of cerebellum. At 50-60 mm CVR length, as a result of increasing pontine flexure, the cerebellar plate was formed by the fusion of the rhombic lips. This cerebellar plate was differentiated into 4 distinct layers; a neuroepithelial, a mantle; a molecular and an external granular layer. On reaching 100 mm CVR length, the cerebellar surface showed the beginning of fissuring as well as the internal granular layer became clearly demarcated from the mantle layer which increased with the advancement of age. The external granular layer reached its maximum thickness at 50-60 mm CVR length dog foetuses, then it decreased with the advancement of age until it disappeared in most areas of cerebellar cortex in the postnatal 45 days old dog. 0n the other hand, the molecular and internal granular layers increased in thickness on the expense of the external granular layer. The Purkinje cell layer was formed of 3-4 cells in thickness in 140 mm CVR length dog foetuses, where it was disposed into a single row of cells at 25 days old. Toward 45 days old, the Purkinje cells increased in size and attained their full mature structure. The latter age showed that the cerebellar cortex had 3 distinct layers; an outer molecular layer, a central layer of Purkinje cells and inner granular layer, the characteristic features of the adult form.

Key words: Histogenesis-cerebral cortex-Dogs-Prenatal.

INTRODUCTION

The histogenesis of the cerebellar cortex in various mammalian species was conducted by several authors *(UZMAN, 1960; NOOR EL DIN, 1966* in mice; *ADDISON, 1911; ALTMAN and WINFREE, 1977; ALTMAN and BAYER, 1978* in rats; *KENNETH, 1963; GABR, 1978* in rabbits; *GABR et al, 1991* in Camel). In man,the qualitative and quantitative histological aspects of postnatal cerebellar development have been made *(ELLIS, 1920; RAAF and KERNOHAN, 1944; ZECEVIC and RAKIC, 1976).* The early postnatal development of the canine cerebellar cortex was done by

PERKINS, (1961), as well as *PHEMISTER and YOUNG (1968)*. The previous investigators indicated that the timing of histogenetic events and the length of developmental stages in the cerebellum vary greatly from one species to another. In these differences may lie the explanation for the obvious, interspecies variations in gross cerebellar size, shape and configuration.

The present study was done to give more informations about the developmental changes in the cerebellar cortex of dog (Egyptian land race) during the prenatal and early postnatal life (suckling period).

MATERIAL and METHODS

The material employed in the present study originated from the cerebelli of 16 dog foetuses of both sexes ranging from 10 to 200 mm CVR length (Table.1),together with cerebelli of 10 dogs representing the suckling period (Table. П).

The fetuses were obtained from pregnant bitches (Egyptian-land race) sacrificed at various periods of gestation. The fetuses were removed shortly after evisceration and the crown to rump (CVR) length was measured to the nearest millimeter. The entire embryos (10-60 mm CVR length) and the cerebelli of the remaining stages were removed and fixed in 10% neutral buffered formaline and Bouin's fluid. After proper fixation, the material was dehydrated, cleared and embedded in paraffin wax. Sections at 5-7 μm thickness were stained with Haematoxylin and Eosin, Einarson's gallocyanine and Holm's silver stain *(DRURY and WALLINGTON, 1980)*.

The thickness of different cortical layers of the cerebellum was measured using the measurment system of Visualis (1.2).

Material available for study

Table (I)

Age (CVRmm)	10	15	50	60	100	130	140	200
No. .of foetuses	2	1	2	2	3	2	2	2

Table (II)

Age (day)	0	3	25	30	40
No. of dogs	2	2	2	2	2

RESULTS

10-15mm CVR length dog embryos:

The dorsolateral parts of the alar plates of the metencephalon bent medially and formed the rhombic lips; the primiordium of cerebellum (Fig.1).

The rhombic lip consisted of a neuroepithelial layer, a mantle layer and a marginal layer (Fig. Ia).

The neuroepithelial layer lining the fourth ventricle showed several mitotic figures. The mantle layer formed the major part of rhombic lip and is formed of differentiating neuroblasts. The marginal layer was composed of the fibers of both the neuroepithelial and mantle layers.

The roof plate of the metencephalon was formed of one layer of columnar ependymal cells overlyed by vascular mesenchymal tissue, which formed together the tela choroidea. The vascular mesenchyme formed a sac like invagination which projected into the fourth ventricle (Fig. 1).

50-60 mm CVR length:

At this stage of intrauterine life, the cerebellar plate which was formed by the fusion of the rhombic lips as a result of increasing pontine flexure , projected into the fourth venticle .The tela choroidea of the fourth ventricle was formed of multiple folds which projected completely in the cavity of the fourth ventricle (Fig. 2).

Histologically, the cerebellar plate was differentiated into 4 distinct layers; neuroepithelial, mantle, molecular and external granular layer (Fig. 2a). The latter was covered by discontinuous layer of undifferentiated mesenchymal cells forming the primitive cells of pia matter (pia cells). These cells had elongated oval nuclei arranged with their longitudinal axes parallel to the pial surface.

The external granular layer (99.69 μm in thickness) consisted of 6-10 cell layers. The cells of this layer were arranged into two strata; a superficial dense one, with more densely packed cells and an inner loose one. The cells showed large dense oval or rounded nuclei occupying most of the cells. Several mitotic figures could be demonstrated within the cells of this layer (Fig. 2b) .

The molecular layer was obviously very thin, occupied by a few cellular elements, similar to the cells of the external granular layer.

The mantle layer represented most of the bulk of the cerebellar plate and was traversed by primitive blood capillaries. Other few differentiating blood capillaries were observed ramifying within the molecular and external granular layer perpendicular to the pial surface.

The neuroepithelial layer measuring about 80.93 μm in thickness and was formed of closely packed 6-8 cell layers. The cells had large oval nuclei arranged perpendicular to the ventricular surface. Several mitotic figures were demonstrated within the cells of this layer.

100 mm CVR length:

The cerebellar surface showed the beginning of fissuring (Fig.3, 3a).

The external granular layer decreased in thinckness than those observed at the previous ages with an average thickness of 55.37 μm.

The molecular layer was very thin measuring about 45.84 μm in thickness with a few cellular elements. Some of these cells were oval in shape with their longitudinal axes perpendicular to the surface. Other cells were rounded with darkly stained, large nuclei.

The internal granular layer (115.0 μm in thickness) was clearly demarcated from the mantle layer rostrally but gradually faded out caudally and ranged from 7 to 9 cell layers. Some cells had darkly stained rounded nuclei, while others had large oval vesicular nuclei.

The mantle layer showed somewhat loosely arranged cells than that observed at the previous age, with a few fibrillar elements.

The neuroepithelium layer was obviously decreased in thickness than that of the previous age with an average thickness of 30.58 μm.

130-140 mm CVR length:

The cerebellar surface showed more fissuring than that observed at the previous age (Fig. 4). The external granular layer was decreased in thickness (32.0 μm) while both the molecular and internal granular layers were increased in width, measuring about 63.35 and 129 μm respectively .

In this period of intrauterine life, the internal granular layer covered large area of cerebellum which increased with advancement of age. This layer was more or less paler than those of the external granular layer and relatively thicker at the top of the folia than at the bottom and side of the fissures.

The Purkinje cell layer was intermingled with the cells of the internal granular layer and appeared in more than one level (3-4 cell thick) layers.The cells appeared larger than those of the external and internal granular layers with large elongated oval nuclei and fine apical processes.(Fig. 4a).

Toward 200 mm CVR length, the different layers of the developing cerebellum had no qualitative other than quantitative changes . In addition, the fissures increased in depth and number which increased toward the end of gestation period.

Newly born pups (0-3days old):

The cerebellum was differentiated into 4 distinct layers:external granular layer, molecular layer, purkinje cell layer and internal granular layer (Fig. 5 and 5a).

The external granular layer (78.78 µm in thickness) ranged between 6-9 cell layers. The cells of this layer appeared into two strata; a superficial dense one and an inner loose one. The cells had large rounded or oval deeply stained nuclei occupying most of the cell bodies, and illdefined cell borders.

The molecular layer (68.57 µm) was mainly composed of fibrillar elements running perpendicular to the cell surface and a few cellular elements.

The Purkinje cells had large nuclei and arranged themselves in one irregular row parallel to the pial surface. Fine processes arised from the tapering ends of these cells, coursing within the molecular layer.

The internal granular layer (131.0 µm) became a distinct layer at this period of life. Their nuclei were rounded or oval paler in staining than those of the external granular layer.

The white matter was obviously demarcated from the internal granular layer. It was formed mainly of horizontal fibers which ran parallel to the pial surface,as well as few cellular elements intermingled with these fibers.

25 days old:

The external granular layer decreased clearly in thickness (13.4 µm) and was formed of 3-4 rows of cells, while the molecular layer increased in width (117.6 µm) with numerous cellular elements than that observed at the previous age (Fig.6 and 6a). In addition, the molecular layer presented vertical and horizontal fibers which were the axons of the internal granular layer, as well as the dendrities of Purkinje cells (Fig. 6b) .

The purkinje cells obviously increased in size than at the previous age and became flasked shaped. The cells were arranged in one row and were widely spaced from each other. Each Purkinje cell body (Fig.6b) was observed to be enveloped by the processes of basket cells which lied in the deep portion of the molecular layer.

The internal granular layer increased in thickness (135.5 µm) than at the previous age. The cells of this layer appeared in small clusters (Fig.6a) with small rounded nuclei and fine granular chromatin.This layer presented large number of processes (Fig. 6b) representing the dendrites of the internal granular cells as well as the axons of Purkinje cells. Golgi cells could hardely be localized among granular cells at this stage.

There was a sharp line of demarcation between the internal granular layer and white matter.

30 days old:

The changes at this stage were more quantitive than qualitative. The external granular layer decreased in thickness with an average 9.0 μm and was formed of 1-3 rows of cells. On the other hand, both the molecular and internal granular layers increased in thickness (185.5 and 187.2 μm respectively, Fig.7). In addition, the cells of the internal granular layer became more organized in clusters than those observed in the previous age.

45 days old:

The histological changes occured at this age of development could be summarised as follows.

The external granular layer almost disappeared in most of the cerebellar cortex except for a few areas represented by discontinuous one layer of few cells (Fig. 8 and 8d).

The molecular and the internal granular layers were relatively increased in width (192 and 193 μm respectively) than those at the beforementioned age (Fig. 8a).

The Golgi cells of the internal granular layer were obviously increased in number than those observed in the previous ages.

They had large vesicular, rounded or oval nuclei occupying most of the cell bodies (Fig. 8c).

The Purkinje cells relatively attained their full mature structures and had a flask shaped somata with centrally located, large rounded vesicular nuclei, and distinct nucleoli. Each cell had one or two thick primary dendrites arising from the narrow end of the body, traversing the molecular layer where arborization took place (Fig. 8d). The axons of the Purkinje cells arised from the broad base within the granular layer (Fig. 8b).

So, at this period of life, the cerebellar cortex, had 3 distinct layers; an outer molecular layer, a central layer of Purkinje cells and an inner granular layer a characteristic picture of the adult form.

DISCUSSION

In the early stages of development, the external granular layer was rapidly developed and occupied completely the marginal zone of the cerebellar plate. This layer was formed from the migrating neuroblasts of the mantle layer *(HAMILTON and MOSSMAN, 1975)* or from the migrated cells of the neuroepithelium *(SADLER, 1988)*.

Mitotic figures were frequently observed within the cells of both neuroepithelium and external granular layer in the early stages of development. This phenomenon reflected the active cellular proliferation and differentiation of other types of cells within the developing cerebellar cortex. *JACOBSON (1978)* stated that the cells of the cerebellar cortex originated from two separate germinal zones. A zone in the roof of the fourth ventricle gave origin to the Purkinje and Golgi 11 cells as well as some glial cells, all of which migrated outward toward the pia mater to form the mantle layer of the cerebellar plate. Somewhat later another germinal zone called the external granular layer was formed immediately beneath the pia mater covering the cerebellar plate and this gave origin to granule, stellate, basket cells and some glial cells all of which migrated deeper into the cerebellar cortex.

The latter view explained the continuous decrease in the thickness of both the external granular and neuroepithelium layers with advancement of age in the present study.

The present work revealed that, the external granular layer decreased in thickness with the advancement of age until disappeared in most areas of cerebellar cortex around 45 days old postnatally. On the other hand the molecular and internal granular layers increased in thickness on the expense of the external granular layer. The general growth patterns of the different cerebellar cortical layers of the dog were found to be similar to those reported for rats *(ADDISON, 1911; IBRAHIM, 1976)*, mice *(UZMAN, 1960; NOOR EL DIN, 1966)*, rabbit *(GABR, 1978)* and man *(ELLIS, 1920; RAAF and KERNOHAN, 1944)*, however certain species differences could be demonastrated .The time of disappearance of the external granular layer varied from one animal to another. *PHEMISTER and YOUNG, (1968)* in dog found that, this layer completely disappeared at 72 days old postnatally, in rabbit *(GABR, 1978)* at about 3 months old, in mice *(NOOR EL DIN, 1966)* at an age beyond 15 days old and in man *(RAAF and KERNOHAN, 1944)* at 18-20 month of age.

The molecular layer was progressively increased in thickness to reach its adult form at 45 days postnatally.The growth of the molecular layer was largely a reflection of the development of Purkinje cell dendrites as well as the axons of the internal granular layer. The same explanations were obtained by the results of *PHEMISTER and YOUNG (1968)* in dog, and *GABR (1978)* in rabbit.The latter author mentioned that the increased thickness of the molecular layer could be attributed to the increase in cellular processes and neuropil by the advancement of age.

The present study also revealed that the Purkinije cell layer was formed of 3-4 cell rows in 140 mm CVR length dog foetuses, whereas it was disposed into a single row of cells at 25 days old. Toward 45 days, the Purkinje cells increased in size and attained their full mature structure. The differentiation of Purkinje cells in Egyptian land race dog followed the same sequence of other mammals *(NOOR EL DIN, 1966 in mice; IBRAHIM, 1976* in rat ; *GABR, 1978* in rabbit). However, the camel Purkinje cells followed a relatively rapid rate of differentiation and growth. This could explained the earlier cerebellar maturity and independency of camel at brith *(GABR et al, 1991).*

In all vertebrates, the Purkinje cells were derived from the ventricular germinal zone and migrated out into the superficial region of the mantle layer of the cerebellar plate. They were initially small and arranged in an irregular row up to 12 cell thick. They were thinned out to a single row during the subsequent growth of cerebellum *(JACOPSON, 1978).*

The present work revealed that the internal granular layer was small and intermingled with the mantle layer at 100 mm CVR length dog foetuses. This layer showed a steady increase with advancement of age until the 45 days at which time there was an indication of stability in its size. This layer was formed by inward migrating granule cells of the external granular layer *(JACOBSON, 1978).* So, the development of the granule cells in dog corresponded to the general pattern reported in other species such as mice, rat, rabbit by *NOOR EL DIN, 1966, ALTMAN; 1969 and GABR, 1978* respectively.

In conclusion, the different elements of cerebellar cortex of the Egyptian land dog were developed and changed in a way nearly or closely similiar to those described for other vertebrates including mammals along the period of pre- and postnatal life. Also the stage of development of cerebellum can be correlated with newborn animals powers of locomotion and motor coordiratian. In animals that are able to walk soon after birth such as the chick, guinea pig and camel, the cerebellum is well developed at birth, where as in animals such as mouse, rat, rabbit, man and dog that are helpless at birth, the cerebellum is in corresponding state of its maturity, and its histogenesis and morphogenesis mainly occure after birth.

REFERENCES

Addison, W.H.F. (1911): The development of the Purkinje cells and of the cortical layers in the cerebellum of the albino rat. J. Comp. Neur.21: 459-485.

Altman, J. (1969): Autoradiographic and histological studies of postnatal neurogenesis 111. Dating the time of production and onest of differentiation of cerebellar microneurons in rat. J. Comp. Neur.136: 269-294.

Altman, J. and Bayer, S.A. (1978) : Prenatal development of cerebellar system in the rat 1- Cytogenesis and histogenesis of the deep nuclei and the cortex of the cerebellum. J. Comp. Neur. 179 : 23- 48.

Altman, J. and Winfree, A.T. (1977) : Postnatal development of the cerebellar cortex in the rat. V. spatial organization of Purkinje cell Perikarya. J. Comp. Neur. 171 : 1-16.

Drury, R.A. and Wallington, E.A. (1980): Carletons Histological Technique 5th ed. Oxford University Press.

Ellis, R.S. (1920): Norms for some structural changes in the human cerebellum from birth to old age. J. Comp. Neur., 32: 1- 33.

Gabr, M.A. (1978): Histogenesis of the cerebellar cortex in rabbit Master Degree (Anatomy), Assiut Univ.

Gabr,M.A.; Alam El-Din M.A.; Abdel-Moneim, M.E.; and Hassan, S.A.S. (1991): Histogenesis of the cerebellar cortex in the camel Assiut Vet. Med. J. Vol. 25 No. 50.

Hamilton, W.J. and Mossman, H.W. (1972): Human embryology, prenatal development of form and function. 4th ed. Cambridge: W. Herrer& Sons ZTD. Baltimore: Williams & Wilkins Company.

Ibrahim, M.W. (1976): Qualilative and Quantitative studies on the postnatal development of cerebellum of white rat. M.D. Thesis, Fac. of Medicine, Al Azhar Univ..

Jacobson, M. (1978): Developmental Neurobiology 2nd, Plenum Press-New York and London.

Kenneth, R.S. (1963) : The cerebellar cortex of the rabbit. J. Comp. Neur.: 459-484.

Noor El-Din, M.A. (1966) : Qualitative & Quantitative studies on the postnatal development of the cerebellum of mouse and an experimental on the site of termination of spinocerebellar tract M.D. Thesis, Assiut Univ.

Perkins, L.C. (1961): The early postnatal development of the cerebellar cortex in the dog and the effect of early spinocerebellar tractomy, Ph.D. Thesis. Duke Univ. 58 P. Univ. Microfilms, Ann Arbor, Mich. (Diss.Abstr., 22-706).

Phemister, R.D. and Young, S. (1968): The postnatal development of the canine cerebellar cortex . J.Comp. Neur., 134: 243- 254.

Raaf, J. and Kernohan, J.W. (1944) : A study of the external granular layer in the cerebellum . Am. J. Anat. , 75: 151- 172.

Sadler, T.W. (1985) : Langmans medical embryology 5th ed. William& Wilkins, Baltimore, Hong Kong, London, Sydney.

Uzman, L.L. (1960): The histogenesis of the mouse cerebellum as studied by its tritiated thymidine uptake. J. Comp. Neur. , 114: 137- 159.

Zecevic, N. and Rakic, P. (1976): Differentiation of Purkinje cells and their relationship to other components of developing cerebellar cortex in man J. Comp. Neur. 167: 27- 48.

LEGENDS

Fig. 1: Transverse section of metencephalon of 10 mm CVR length dog embryo showing: rhombic lip (head arrow), roof plate (F), tela choroidea (T) and fourth ventricle (V).
(Haematoxylin and Eosin, X 40).

Fig. 1a: Transverse section of 10 mm CVR length dog embryo showing rhombic lip with a neuroepithelial layer (n), a mantle layer (ma) and a marginal layer (I). mitotic divisions (arrow).
(Haematoxylin and Eosin, X 250).

Fig. 2: Sagittal section in dog embryo (50 mm CVR length) showing; cerebellar plate (r), fourth ventricle (V), tela choroidea (T), pontine flexure (arrow), lateral ventricle (L), mesencephalon (m), metencephalon (P) and myelencephalon (M) .
(Haematoxylin and Eosin, X 50).

Fig. 2a and b : Cerebellar plate of 50 mm CVR length dog embryo showing: external granular layer (e), molecular layer (o), mantle layer (ma), neuroepithelial layer (n), pia cells (p), mitotic divisions (↓) and blood capillaries (c).
(Haematoxylin and Eosin, 2a: X 63 and 2b: X250)

Fig. 3 and 3a : Cerebellum of dog foetus (100 mm CVR length) showing external granular layer (e), molecular layer (o), internal granular (g), mantle layer (ma) and neuroepithlial layer and fissure (arrow).
(Haematoxylin and Eosin, 3: X 100)
(Einarson's Gallocyanine, 3a: X250)

Fig. 4: Cerebellum of 130 mm CVR length dog foetus showing that the cerebellar surface had more fissures than those observed at the previous age.
(Haematoxylin and Eosin, X63)

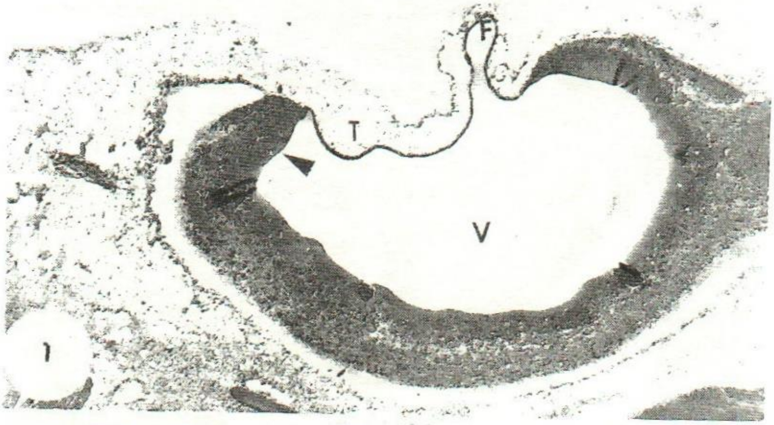
Fig. 4 a: Cerebellum of 140 mm CVR length dog foetus showing that the Purkinji cell layer (k) intermingled with the internal granular layer.
(Haematoxylin and Eosin, X1000).

Fig. 5 and 5a : Cerebellum of newly born dog showing : external granular layer (e), molecular layer (o), layer of Purkinje cells (k), internal granular layer (g) and white matter (w).
(Haematoxylin and Eosin, 5 : X63)
(Holm's silver, 5a : X250)

Fig. 6, 6a and 6b : Cerebellum of 25 days old dog showing external granular layer (e), molecular layer (o), layer of Purkinje cells (k), internal granular layer (g) and white matter (w). Notice.: Basket cell axons traversing molecular layer and descending to form terminal baskets over somata of Purkinje cells (→), also the internal granular layer presented large number of fibers.
(Haematoxylin and Eosin, 6:X63, 6a : 250)
(Holm's silver, 6b : X250).

Fig. 7: Cerebellar cortex of 30 days old dog showing : external granular layer (e), molecular layer (o), layer of Purkinje cells (k) and internal granular layer (g).
(Haematoxylin and Eosin , x 250).

Fig. 8, 8a, 8b, 8c and 8d: Cerebellum of 45 days old dog showing : molecular layer (o), layer of Purkinje cells (k), internal granular layer (g),white mater (W), pia matter (p), dendrites of Purkinje cell (→). axons of Purkinje cells (short arrow), axons of granular cells (head arrow), Notice The external granular layer disappeared except in some areas is represented by few cells (e) Golgi cells of the granular layer (G).
(Haematoxylin and Eosin, 8 : X63)
(Holm's silver, 8a : X250, 8b : X400, 8c: X1000, 8d: X250).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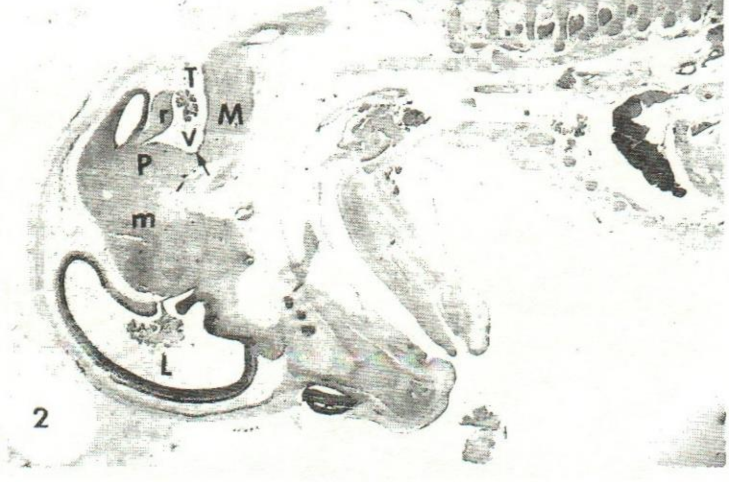
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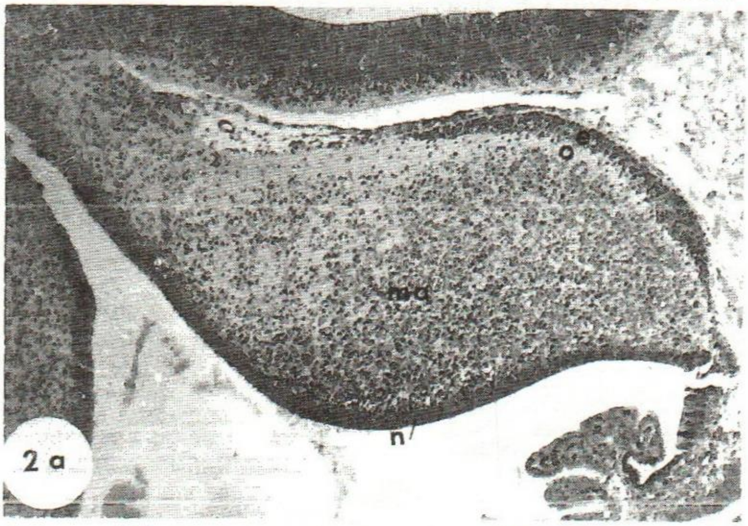
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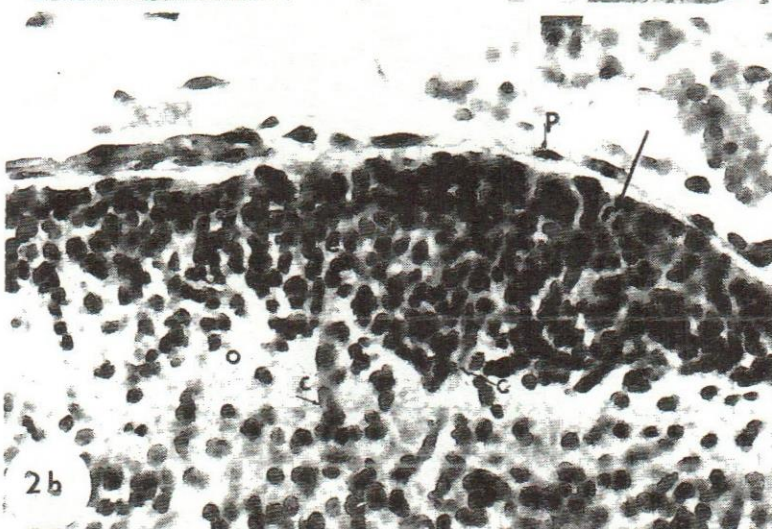
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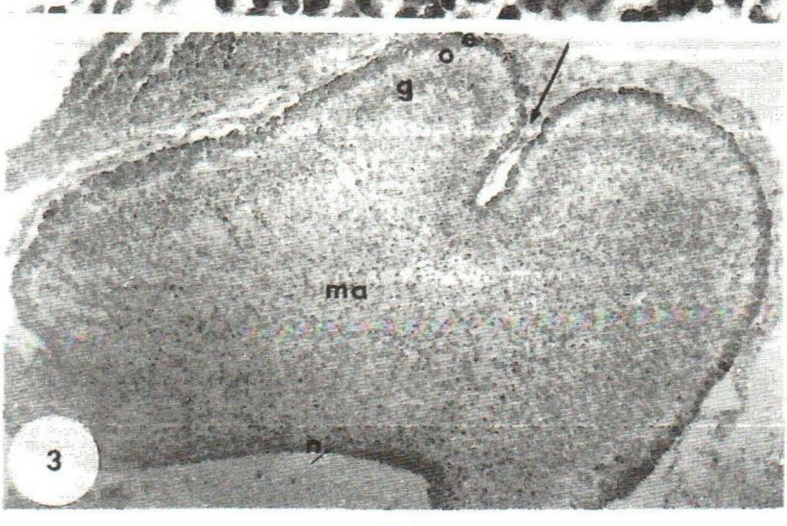
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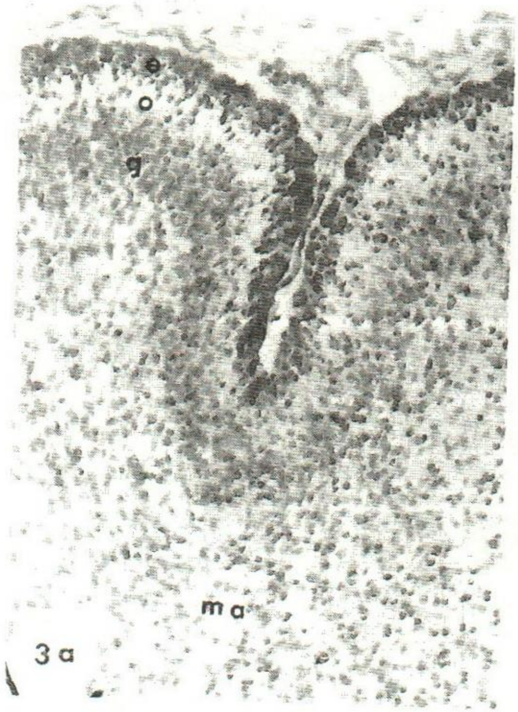
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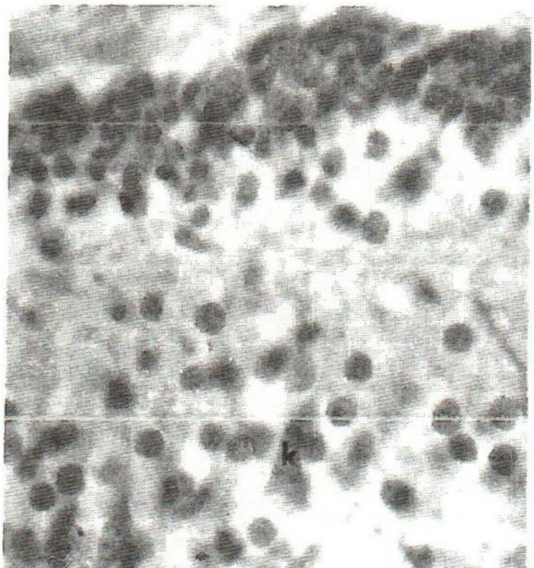
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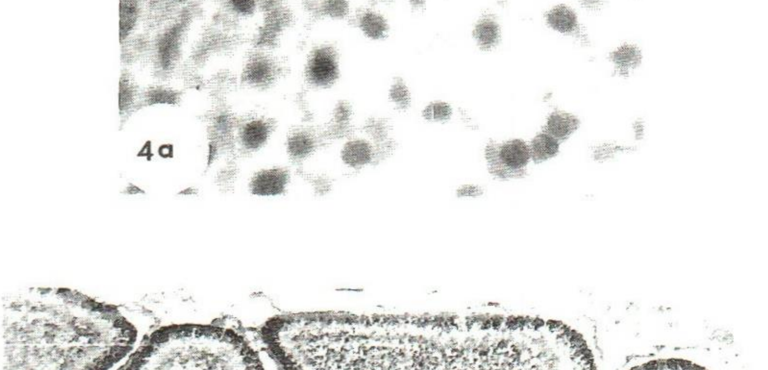
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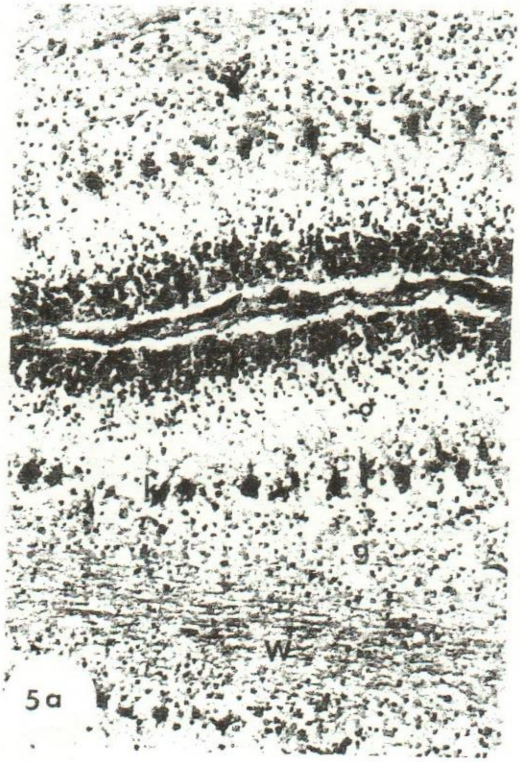

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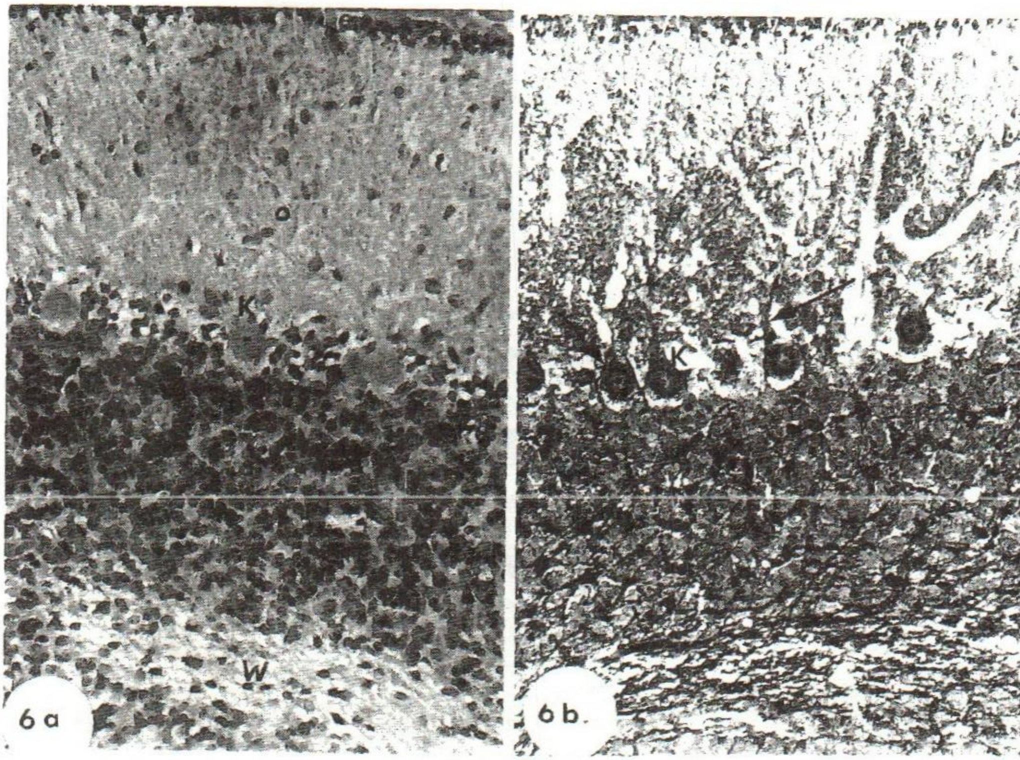
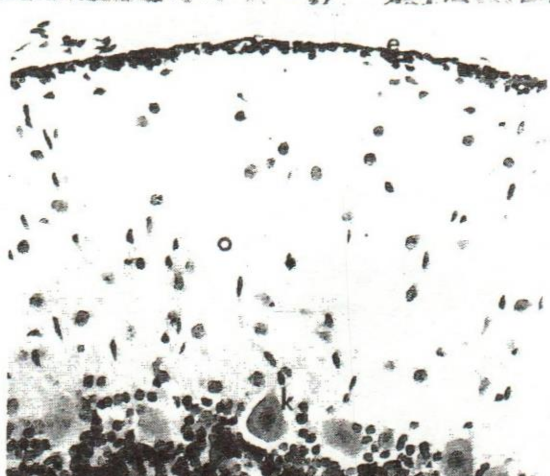
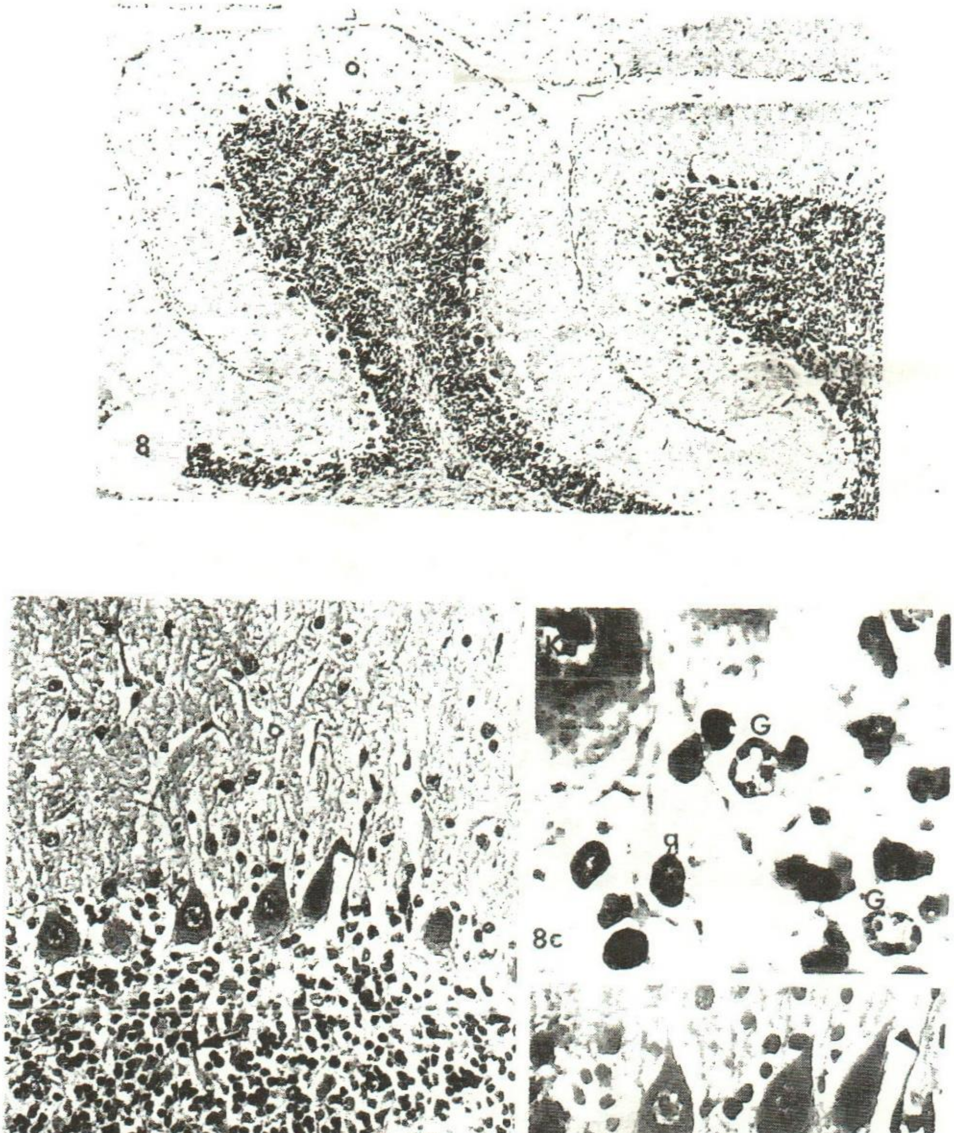

e
o
K
W
6 a
K
6 b.


e
o
k
7


8
8a
8b
8c
G
G

8d