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OBSERVATIONS ON THE GLIA CELLS OF THE TOAD *BUFO ARENARUM* HENSEL. EVIDENCE OF MORPHOLOGIC PLASTICITY.

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Les cellules gliales du système nerveux du crapaud *Bufo arenarum* Hensel sont étudiées par l'intermédiaire de la réaction Golgi. Elles sont classifiées selon les critères classiques morphologiques, en astrogliales, oligodendrogliales, microgliales et en cellules épendymales. On accorde une attention spéciale à l'existence possible de changements morphologiques saisonniers dans les cellules gliales.

INTRODUCTION

The analysis of structural relationship between neurons and their surrounding glia cells has enjoyed considerable attention in recent years. Although the important contributions of electron microscopy changed many of the structural concepts about the interrelationship between neurons and glia cells, some of the ideas of classic authors concerning the functions of glia cells remain still unmodified (Cajal, 1911; Achúcarro, 1915; Del Rio Hortega, 1928, 1942; De Castro, 1946; Palay, 1958; Brightman, 1961, Polak, 1965).

In most parts of vertebrates the identification of glia cells is made without difficulty. In addition a subdivision into three main classes can be made in mammalian, and to some extent in lower vertebrate brain, on the basis of silver staining procedures and electron microscopy. These are the astrocytes, the oligodendrocytes and the microgliocytes.

In lower vertebrates there is also a great number of glial cells which in many aspects are comparable to those of mammals. However there is usually less agreement concerning a classification into distinct types.

In the present work attention was especially focused upon the astroglial cells and their morphological changes in different periods of the year cycle of the animals. Three main problems concerning the structure and architectonics of glial cells in the CNS of the toad *Bufo arenarum* Hensel were studied: 1) the general basic structure and architectonics of glial cells: 2) the appearance of relevant structural changes in the astrocytic population during the period of activity of the animals (Spring-Summer) and 3) the occurrence of a special type of angioblastic elements.

MATERIAL AND METHODS

26 adult specimens of both sexes of *Bufo arenarum* were studied in the present work. 15 were captured during the months of December and January (Summer), and the remaining 11 in June and July (Winter). The last were in natural hibernating conditions. In all cases the whole central nervous system was dissected and prepared according to the original Golgi-Del Río Hortega technique (Del Río-Hortega, 1956) and to the Golgi-Cox method slightly modified (Pessacq, 1970). Frozen sections were cut 150 to 300 μ mounted on slides and covered with a layer of Canada balsam without coverslip. In some cases the sections were counterstained with 1% Toluidine blue.

DESCRIPTION

The different types of glial cells were classified according to the morphological criteria of normal cells given for ependymal cells and astroglia by Ramón y Cajal (op. cit.) and for oligodendroglia by Del Río-Hortega (1956). Some luminous stellate angioblastic-like elements found in the forebrain were described without including them in any of the three classes although some of the observed forms exhibit some similarities with the microglial cells described by Stensaas and Stensaas (1968) in the spinal cord of the toad.

I) Ependymal cells

The ventricular cavities are lined by a layer of cuboid cells possessing a long caudal process addressed to the superficial layers and almost constantly ending by means of enlarged extremities beneath the pia. As it was pointed out in classic descriptions they show fine excrescences of the protoplasmic tail. These are especially noticeable by their richness in the optic lobe. In some areas of the anterior brain the expansions of the ependimocytes are tended to subventricular blood vessels constituting a sort of vascular feet which entirely cover the vascular wall (Fig. 1 A).

II) Astroglial cells

a) Spongioblastic-like cells.

According to classic and recent studies this is the unique type of astroglia present in the amphibian brain (Stensaas and Stensaas, op. cit.). The spongioblastic-like astrocytes are fusiform elongate cells with processes extended toward the pial surface. The processes end by means of enlarged extremities on the pial surface of the CNS. From the principal process originate a great number of fine short collaterals ended by acute sometimes lamellar extremities. This is the unique type of glial astrocytic cells we have been able to observe in animals captured during the hibernating period.

Figure 1: Posterothalamic region of the toad brain showing the ependymary cells with their caudal processes, covering a subependymary vein (arrows). The elements appearing in the deep cerebral substance are fibroblastoid astrocytes. B and C show the astrocytic cells with great magnification. The arrows point out capillaries. A: × 300; B: × 1000; C: × 3550 a prov. Golgi method.

a) Protoplasmic astrocitic cells.

In the specimens captured during the period of activity (Summer) the CNS seems to change its astroglial pattern. The nervous parenchyma is invaded by roundish densely ramificate astroglial cells which seem to constitute at this period the predominant glial type. In many aspects they are comparable to protoplasmic astrocytic cells of higher vertebrates. They are present at all levels of the CNS of the toad although they seem to be more numerous in the white substance and the spinal cord. It is evident that these cells are in close relationship with the blood vessels (Fig. 3). Although they do not possess vascular end feet identical to those of mammalian astrocytes, it is possible to find the existence of clear cut thin vascular processes (Fig. 3 B). Very frequently the astrocytic bodies are closely applied to the vascular wall constituting a veritable glial sheet (Fig. 1 C). In the white matter of the spinal cord their processes are longer running parallel to the myelinated fibers. In transverse sections it is observable that they give rise to a veritable plexus of laminated varicose fibers, perforated by the running nerve fibres (Fig. 2).

III) Oligodendroglial cells

Oligodendrocytes possess in the studied species morphological and architectural characteristics similar to those shown in higher vertebrates. Their morphological details have been analysed in the important contribution

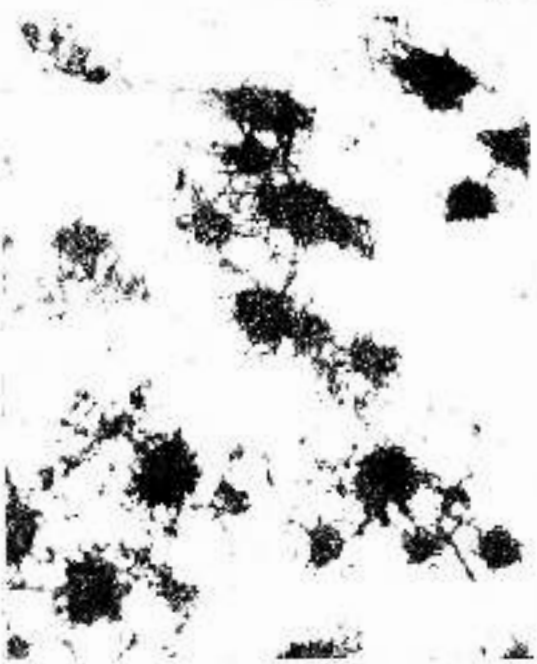


Figure 3: Protoplasmic astrocytes in the spinal cord of a toad captured in Summer. Golgi method. 1000.

of Stensaas and Stensaas (op. cit.). Figures 4 and 5 illustrate different types of oligodendroglial cells in the white matter of the spinal cord.

IV) Angioblastic reticular cells

These elements are particularly numerous in the toad hemispheres during the periods of activity. They are laminous stellate anastomozing cells which are in connection with newly formed blood vessels (Fig. 1 C).

It is difficult to range them in any defined cellular class. In view of their angiogenic properties they should be ascribed to mesenchymal (microglial) cells although they present some structural characteristics transitional to the precedingly described astrocytic cells.

DISCUSSION

Recently, Stensaas and Stensaas (op. cit.) have analysed by means of the Golgi reaction and other technical procedures, the morphology of the glial cells of the CNS of the toad *Bufo arenarum*. They describe the precise morphology of astrocytes, oligodendrocytes and microgliocytes. Concerning the former they found in agreement with classic statements of Cajal (op. cit.) that adult toad astrocytic neuroglial cells possess comparable morphology and architectonics as mammalian spongioblasts during embryonic development. They did not find true stellate forms similar to adult astroglial cells of higher vertebrates. In our material corresponding to specimens captured in Summer we have been able to recognize true protoplasmic astrocytes possessing vascular expansions (Fig. 3). As we have employed basically the same impregnating procedure we believe that these differences in the results could perhaps be attributed to differences in the periods of the year in which the animals were collected or to other functional circumstances. Nevertheless we can not discard that they correspond to slight differences in the employed technique. The intimate relationships shown between astrocytes, capillaries, and nerve cells since the classic works of Golgi (1903, 1907) served as the basic for Golgi's concept of glia being a channel for the distribution of nutritive materials between blood and neurons. The findings of electron microscopy have provided additional support for this point of view (De Robertis and Gerschenfeld, 1961). On the other hand, Sjöstrand (1966) has reported relevant hypertrophic changes in the hypoglossal nucleus of the rabbit during experimental nerve regeneration. The author points out that "the reactive astrocytes return towards normal cytology after the seventh week and by the 60th day they do not differ from those of controls". If astrocytic cytoplasm really serves as a pathway for metabolites between blood and nerve cells and vice versa, we may expect to find an increase in the cytoplasmic volume, and eventually a change in the histochemical properties of neuroglia during increased metabolic requirements of nerve tissue. The last condition certainly occurs during the period of motor activity of the animals and structural adaptative modifications of astroglial cells for increased transport could account for the appearance of numerous protoplasmic astrocytic forms, which are inapparent under other functional situations of the animal.

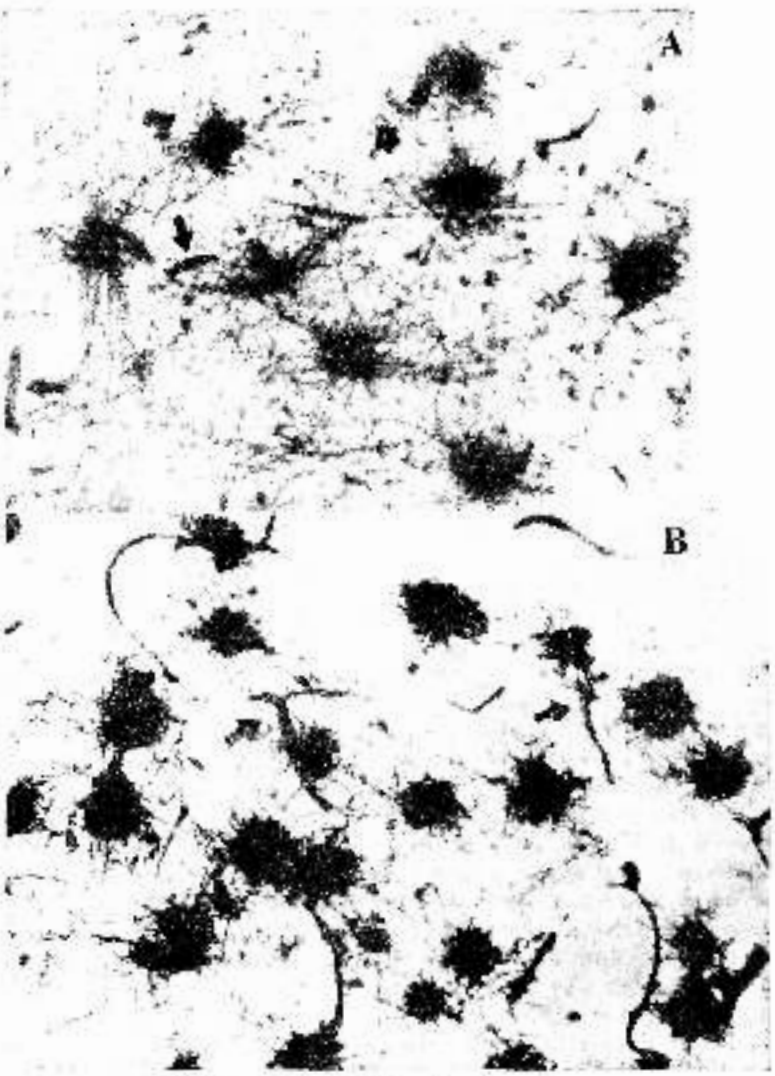


Figure 3: Protoplasmic astrocytes in the middle brain of a toad captured in Summer. To note the thin network of glial expansions enveloping the nerve fascicles. The arrows point out capillary branches. Golgi method. ×1000.

In recent works much attention has been addressed to ependymal lining cells and to other glial types (spider cells, hipependymal cells) in hypothalamic regions attributing to them specific transportive functions between the cerebrospinal fluid and hypothalamic neurons or vice versa (Bleier, 1971; Leonhardt and Eberhardt, 1971; Kendall, Jarobs and Kramer, 1971).

Although the presented morphological and experimental evidence are quite strong for supporting this view, it must be pointed out that this type of glial architectonics does not seem specific for this region. We can observe similar types of glial cells and glial vascular relations in the periventricular layers of the optic lobes of the amphibians, and in the fourth and lateral ventricles of most vertebrates.

OBSERVAȚII ASUPRA CELULELOR GLIALE ALE BROAȘTEI *BUFO ARENARUM* HENSEL. DOVADA PLASTICITĂȚII MORFOLOGICE.

REZUMAT

Celulele gliale din sistemul nervos central al broaștei *Bufo arenarum* Hensel sînt studiate prin intermediul reacției Golgi. Ele sînt clasificate con-

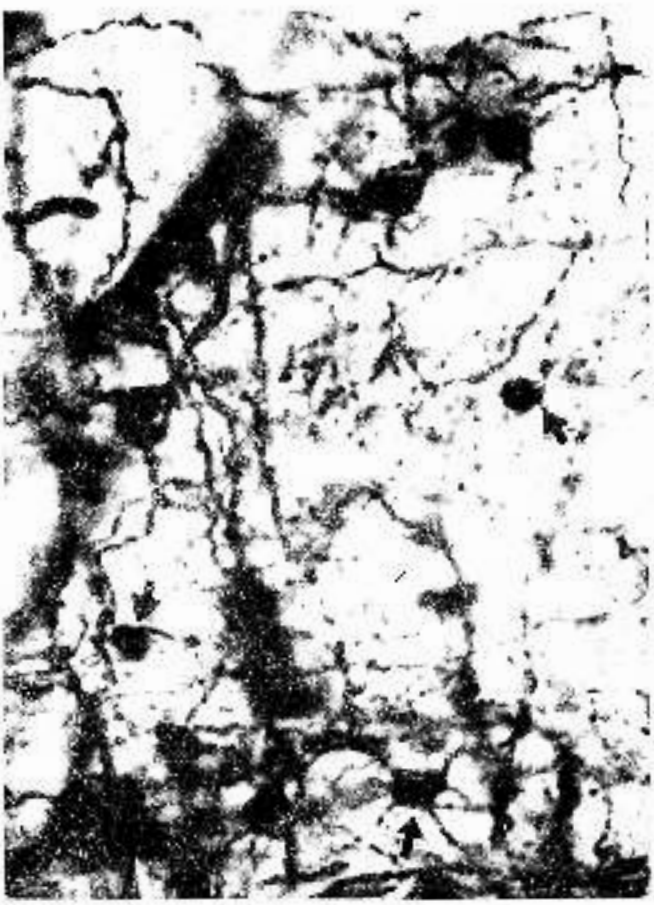
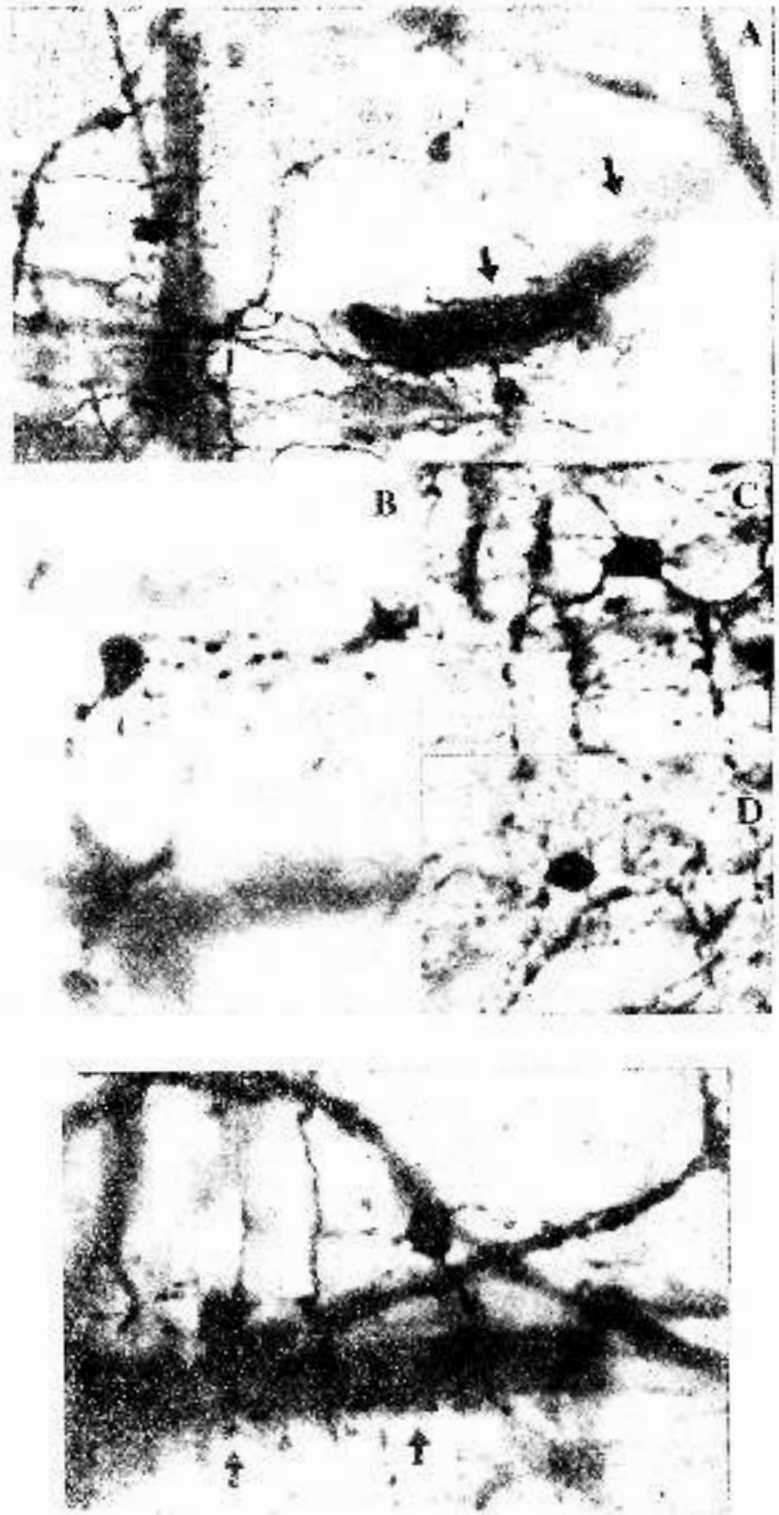


Figure 4: Oligodendrocytes in the peripheral plexus of the spinal cord of the toad. To note the tenuous varicose expansions of the oligodendrocytes (arrows). Golgi method. × 2000.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form criteriilor clasice morfologice în astrogliale, oligodendrogliale, microgliale şi celule ependimale. Se acordă o atenţie specială existenţei posibile a unor schimbări morfologice survenite în celelule gliale.

BIBLIOGRAPHY

ACHUCARRO (N.), 1915 — De l'évolution de la névroglie et spécialement de ses relations avec l'appareil vasculaire. *Trav. Lab. Invest. biol. Univ.*, **13**: 169—212.

BLEIER (R.), 1971 — Structural relationship of ependymal cells and their processes within the hypothalamus. *In:* KNIGGE (K. M.), SCOTT (D. E.), WEINDL (A.), Brain-Endocrine Interaction, Median Eminence Structure and Function (a Symposium) 300—318. *Basel.*

BRIGHTMAN (M. W.), 1961 — The fine structure of ciliated ependyma. *Anat. Rec.* **139**: 210

BRIGHTMAN (M. W.), 1965 — The distribution within the brain of ferritin injected into cerebrospinal fluid compartments. I. Ependymal distribution. *J. Cell. Biol.*, **26**: 99—123.

CAJAL (S. R. Y.), 1911 — Histologie du système nerveux de l'Homme et des Vertébrés. *Paris.*

DE CASTRO (F.), 1946 — Sobre el comportamiento y significación de la oligodendroglia en la sustancia gris central y de los ganglios nerviosos periféricos. *Arch. Hist. Norm. patol. (Bs. As)*, **3**: 317—348.

DEL RIO HORTEGA (P.), 1942 — La neuroglia normal. Concepto de angioglioma y neuroglioma. *Arch. Hist. norm. patol. (Bs. As)*, **1**: 6—71.

DEL RIO HORTEGA (P.), 1928, 1956 — Tercera aportación al conocimiento morfológico e interpretación funcional de la oligodendroglia. *Mem. Soc. esp. Hist. nat.* **14**: 5 — 54. Republished in: *Arch. Histol. normal patol. (Bs. As.*, **6**: 132—183.

DE ROBERTIS (E.), GERSCHENFELD (H. M.), 1961 — Submicroscopic morphology and function of glial cells. *Int. Rev. Neurobiol.*, **3**: 1—61.

GOLGI (C.), 1883, 1903 — Sulla fina anatomia degli organi centrali del sistema nervoso. *Rio. spez. Freniat.* Republished in: Opera Omnia. Ulrico Hoepli, 397—356, *Milan.*

GOLGI (C.), 1907 — La dottrina del neurone. *Arch. Patog. Cienc. af.* (Univ. Nac. de La Plata), **5**: 31—46.

KENDALL (J. W.), JACOBS (J. J.), KRAMER (R. M.), 1971 — Studies on the transport of hormones from the cerebrospinal fluid to hypothalamus and pituitary. *In:* KNIGE (K. M.), SCOTT (D. E.), WEIN WEINDL (A.), Brain-Endocrine Interaction, Median Eminence Structure and Function (a Symposium), 342—349. *Basel.*

LEONHARDT (H.), ENERHARDT (H. G.), 1971 — Dye transport from the median eminence to the hypothalamic wall. *In:* KNIGGE (K. M.), SCOTT (D. E.), WEINDL (A.), Brain-Endocrine Interaction, Meddian Eminence Structure and Function (a Symposium), 331—341, *Basel.*

PALAY (S. L.), 1958 — An electron microscopical study of neuroglia. *In:* THOMAS (C. C.) (Ed.), Biology of Neuroglia, Chap. 2, W. F. Windel: 24—38. *Springfield, Illinois.*

PESSACQ (C. P.), 1970 — A chloral hydrate modification of the Golgi-Cox method for impregnation of glia cells. *Acta anat.*, **76**: 131—135.

POLAK (M.), 1963 — Neuroglia central y periférica. Su estudio con el microscopio óptico. *Arch. Histol. norm. patol. (Bs As)*, **9**: 3—39.

SJÖSTRAND (J.), 1966 — Glial cells in the hypoglossal nucleus of the rabbit during nerve regeneration. *Acta Physiol. scand.*, **67**: 1—17.

STENSAAS (L. J.), and STENSAAS (S. S.), 1968 — Astrocytic neuroglial cells oligodendrocytes and microglioeytes in the spinal cord of the toad. I. Light microscopy. *Z. Zellforsch.*, **84**: 473—489.

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Figure 5: A, B, C and D. Different types of oligodendrocytes in the spinal cord of the toad. The arrows signal a blood vessel. Golgi method. A: ×1000; B, C and D: ×3000

Figure 6: Two oligodendrocytes related to a blood vessel of the spinal cord (arrows). Golgi method. ×3000.