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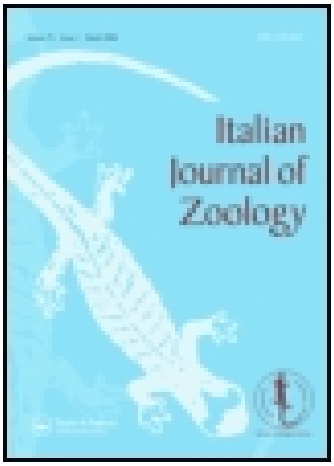


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# Regenerative capacity of the optic tectum in *Lacerta viridis*

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ABSTRACT

Regenerative capacity of the optic tectum in reptiles was studied in adults of *Lacerta viridis* kept for 24 hours at 4 °C before extraction of a dorsomedial portion of the right optic tectum. Five months after surgery brain sections were prepared with standard autoradiographic techniques.

From this investigation it emerged that five months after surgery, cell divisions in the brain were still very numerous, while in the lesioned area continuity of the ependyma had been regained. Above the ependyma in some areas, a zone of re-formed nervous tissue was present with clear signs of cell and fibre stratification.

These observations strengthen the hypothesis of a connection between loss of regenerative capacity by the C.N.S. with installation of blood-brain barrier and, conversely, of the reinstating of such capacity after alteration of this barrier.

*(Received 18 July 1980)*

## INTRODUCTION

The classic statement that the regenerative capacity of the central nervous system (C.N.S.) in Vertebrates is gradually reduced as evolution proceeds and nervous centres become more complex must be reconsidered in the light of recent investigations.

In 1961 Kirsche & Kirsche demonstrated strong regenerative capacity of the optic tectum in *Carassius carassius*, i.e. in a Vertebrate well-advanced in evolution, and in a very complex nervous area. Altman (1962) and Altman & Das (1965) reported a greatly prolonged postnatal neurogenesis in the rat but localized only in the hippocampus. Support for the hypothesis of the generative capacity of the central nervous system came from Kirsche (1967, 1968), who in a careful survey reported that in the brain of teleosts, amphibians (both anurans and urodeles), reptiles and birds, there were groups of cells with embryonic characteristics and still capable of multiplying. These areas mostly located in the neighbourhood of the ventricular ependyma of the telencephalon and mesencephalon are thought to be capable of initiating a regenerative process. Since then, investigations into the possibility of neurogenesis in adults and the regenerative capacity of cells and nerve fibres have rapidly increased (for a review, see Kiernan, 1979 and Hulsebosch & Bittner, 1980), but the results so far are inconclusive and contradictory.

When analysing the chemico-physical features of the areas and the regenerating nerve fibres, Lee & Olszewski (1959), Mellick & Cavanagh (1968), Persson *et al.* (1976) showed that these areas are always characterized by a considerable alteration in vascular permeability; Kiernan in particular (1979) and Kiernan & Contestabile (1980) demonstrated that alteration of the blood-brain barrier accompanies (or determines?) regeneration. Our research into regeneration of the C.N.S.

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in tetrapods was intended to verify this hypothesis. Since it is known that cold affects the blood-brain barrier (Rosomoff & Gilbert, 1955; Stone *et al.*, 1956; Lougheed *et al.*, 1960) we operated on animals that had been kept at 4 °C for 24 hours prior to the intervention. Using this technique we observed striking proliferative processes in the telencephalon of *Lacerta viridis* (Minelli *et al.*, 1978) after destructive surgery; the processes were mostly concentrated in the matrix areas described by Kirsche. In animals kept at room temperature (Minelli & Del Grande, 1980) neither proliferating cells nor regenerative phenomena were observed. Since the most concrete objection to this type of investigation lies in the actual nervous nature of the dividing cells, we administered Nerve Growth Factor (N.G.F.) to our operated cases and obtained an increase beyond 200% in the number of mitoses in the central horn of the second ventricle (Del Grande & Minelli, 1980). Since N.G.F. acts selectively on catecholamine neuroblasts, increasing their dividing rate (Levi-Montalcini & Hamburger, 1951; Blood, 1972; Varon, 1975, 1977), we can be more certain that the phenomena we have reported refer, at least in part, to proliferation of neuroblasts.

On these grounds we thought it opportune to investigate whether repair process were observable in lacertilians, even in a nervous region where the cytoarchitecture was more complex, such as the optic tectum.

## MATERIALS AND METHODS

*Lacerta viridis* adults (26-30 cm long) were kept for 24 hours at 4 °C before extracting a dorsomedial strip of the optic tectum with a fine pipette connected to a vacuum pump. During surgery the animals were kept in hypothermia with a refrigerated plate, they were subsequently brought to room temperature gradually and kept in terraria at constant temperature (20-22 °C).

Five months after surgery 6-H3 thymidine (Radiochemical Centre, Amersham) was injected intraperitoneally at a dose of 1 μCi per gram body weight. 24 hours later the brains were removed and fixed in 10% neutral formalin. After being embedded in paraffin, the brains were sectioned transversely (10 μ thick) and after deparaffination were covered with emulsion (Kodak NTB 2) and exposed for three weeks at 4 °C. The slides were then developed with Kodak D 19 and fixed with Kodak Unifix. The sections were weakly stained with 5% Giemsa's stain at a low temperature for 10 minutes.

## RESULTS AND DISCUSSION

In our previous investigations we had observed, using the technique described above, that cell proliferation and repair in the area of nervous tissue was present during a long time. In the present experiments we examined animals five months after surgery, on the assumption that regenerative processes, when present, would be clearer and more evident. We also found that extraction of an area of nerve tissue by suction with a glass capillary does not make for identical lesions in the different brains; in some cases almost the whole optic tectum on one side was extracted; whereas in others about one third was left *in situ* (Fig. 1); this difference produced a different response in the repair process. Examination of the histological preparations revealed that there had been a partial re-formation in the optic tectum in the gap left by the area extracted. Above all, continuity of the ependymal layer was always re-established, a fundamental pre-requisite for regeneration as we had seen in all our previous researches. Above this layer different conditions could be observed depending on the extent of extraction. The characteristics of the regenerated area appeared in fact linked to the integrity, or lack of it, of the contiguous layers of the tectum. If at operation, the optic tectum of one side was extracted, on the regenerated ependymal layer of that side only a few cells and fibres could be observed (Figs. 1C, 2A). If surgery left part of the optic

tectum *in situ*, above the regenerated ependymal layer a number of layers of cells and fibres could be found which reached about one-third of the original thickness (Figs. 1D, 2B). Moreover in some cases there was a definite stratification of the cells in this area (at times in some areas we noted even two layers of cells) which appeared continuous with internal plexiform layer, and, above this, the piriform and fusiform neuron layer of the periventricular zone can be located, although it is not always visible or may be extremely thin. Above this layer no ordered structure was seen.

The hypothesis that these layers are newly formed and not a normal untouched part of the optic tectum that had been

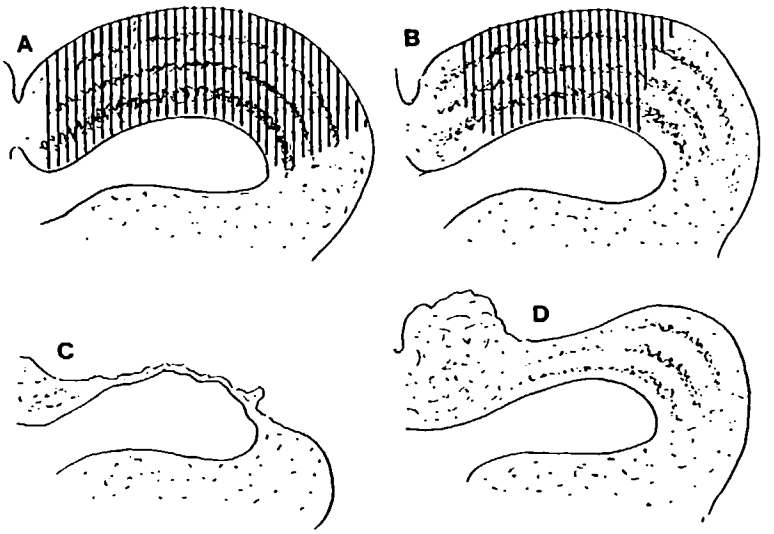


Fig. 1. — Diagram of the extent of operation on the optic tectum of *Lacerta viridis* and the relative consequences. A: extraction affects the whole of the optic tectum on one side. B: extraction is limited to a strip of the optic tectum on one side. C: results of extraction, type A, where only one ependymal layer is restored with few cells and fibres. D: results of a more limited extraction, type B, where above the ependymal layer a layer of cells and fibres may be reconstructed tending to stratification, and, in the median plane, a considerable disorganized mass of cells and fibres. (Frontal sections).

the deeper layers of the remaining part of the optic tectum (Figs. 2B, C).

A more detailed analysis of this regenerated area makes it possible to evaluate the cytoarchitecture and to state that the reconstruction affects, at this stage, the deeper layers. The periventricular zone can be clearly recognized (Leghissa, 1962) (Fig. 3), including the ependyma and the deep plexiform layer with above it a layer of cells that can be interpreted as the deep fusiform neuron layer. Next lies the partially extracted, is supported by the observation that the thickness of these layers is significantly different from the normal. The periventricular zone was reduced by 30%, while the deep fusiform neuron layer which normally consists of 2-3 layers of nerve cells shows a single layer of cells sometimes with very wide spaces between them in the regenerated area (Fig. 2C). Finally the internal plexiform layer and the piriform and fusiform neuron layer, when clearly present, are

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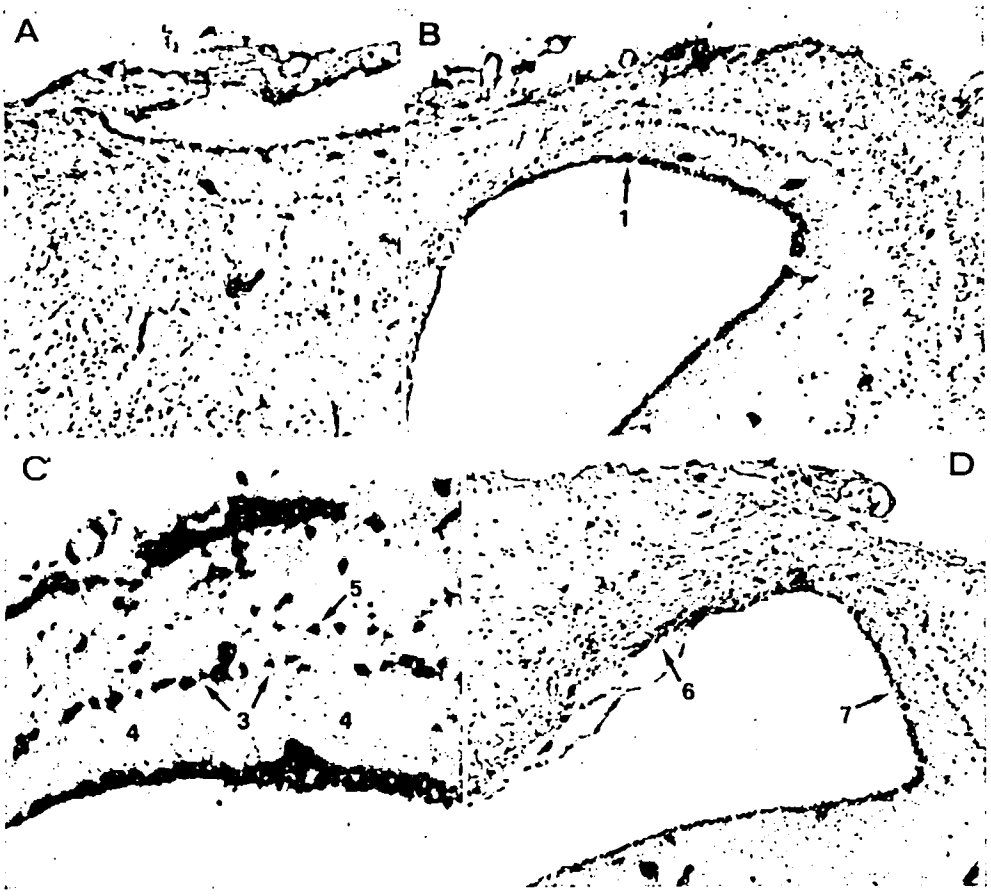


Fig. 2. — Histoautoradiographic sections of the mesencephalon of *Lacerta viridis* 150 days after intervention. A: extraction was too widespread, thus only the ependymal layer is reconstructed with a few cells and fibres, 100 ×. B: presence of an untouched area of the optic tectum (2) allows reconstruction of part of the tectum (1), 100 ×. C: newly formed area under high magnification allows reconstruction to be visualized of the deep plexiform layer (4), of a layer of cells that can be interpreted as a layer of deep fusiform neurons (3), while some cells (5) hint at a cellular stratification which can be interpreted as a layer of piriform and fusiform neurons, 100 ×. D: in the central sections of the optic tectum, in addition to the untouched area, one can observe a disordered mass of cells and fibres that tend mostly to concentrate on the median plane, 200 ×.

reduced to less than 50% of their normal thickness.

In the regenerated area there is another detail almost constant in every individual: above the ependyma and in the centre of the regenerated tectum, there is a mass of fibres and cells which is almost equal in thickness to that of the original tectum, but it is extremely disorganized and has a large number of labelled cells (Fig. 2D).

We investigated the location and number of the labelled cells in our preparations to find out how many cells began dividing in the 24 hours before the brain was fixed. From this study it emerged that many labelled cells appeared along

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the entire brain axis from the telencephalon to the medulla oblongata, though the values differed considerably between one area and another, as we had already found in previous investigations, they fluctuated around 3 000 units. Thus we can affirm that the rate of cell division in the brain of animals five months after surgery may be considered as very high, since in control animals values reached a few tens of units.

Analysis of the location of labelled cells (Table 1) indicates that most (72%) occur in the telencephalon, whereas only 21% appear in the mesencephalic vesicle, and the remaining 6% in the medulla oblongata.

We carefully examined also the diencephalon because Kirsche (1967, 1968) had described two areas situated near the diencephalic ventricle rich in cells with embryonic characteristics, but in our diencephalic preparations we noticed labelled cells only occasionally.

On the whole, surgery affected only one side of the optic tectum, but in a few cases it spread over a little into the opposite side. In both types of lesion, we observed little difference between the two sides in the concentration of labelled cells, whether in the telencephalon, mesencephalon or medulla oblongata (Tab. 1). This finding is not in harmony with what was observed in the repair process in the telencephalon of *Lacerta viridis* (Minelli *et al.*, 1978), where there was a sharp

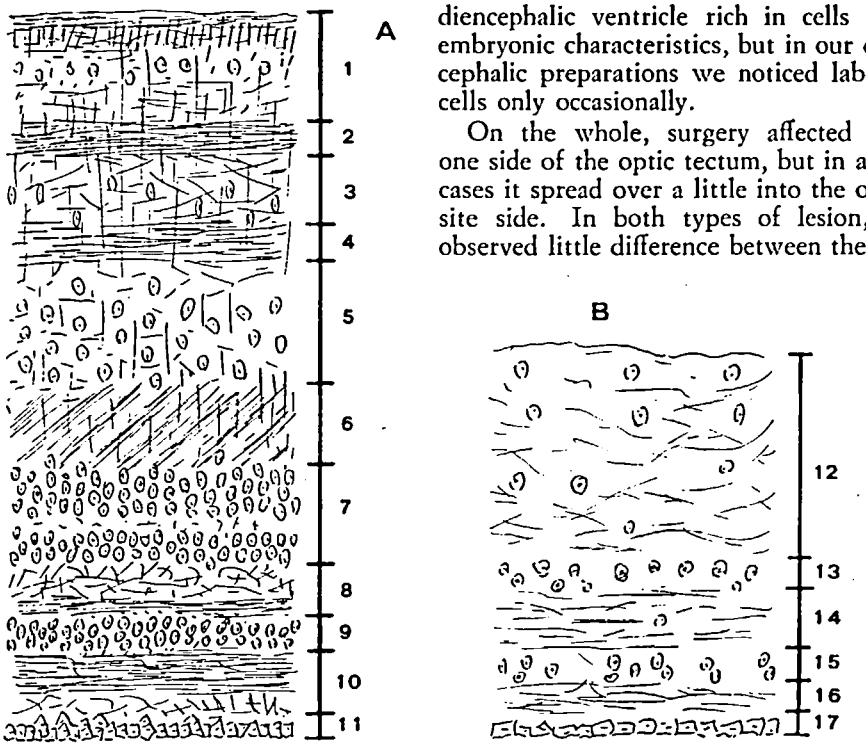


Fig. 3. — A: diagram of the architecture showing the different layers in the optic tectum in *Lacerta viridis*. (After Leghissa, 1962). 1: white outer layer; 2: outer plexiform layer; 3: layer of fusiform retinal neurons; 4: central plexiform layer; 5: multipolar neuron layer; 6: inner white layer; 7: piriform and fusiform neuron layer; 8: inner plexiform layer; 9: deep fusiform neuron layer; 10: deep plexiform layer; 11: ependymal layer. B: diagram of the reconstructed tectum at the level where the stratification of cells and fibres begins. 12: layer with no stratification; 13: piriform and fusiform neuron layer; 14: inner plexiform layer; 15: deep fusiform neuron layer; 16: deep plexiform layer; 17: ependymal layer.

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TABLE I — *Percentage distribution of labelled cells in the different areas of the brain in* Lacerta viridis.

| Area | % of total | % operated side | % healthy side | (*) |
|---|---|---|---|---|
| Telencephalon | 72 | 41 | 31 | |
| Mesencephalon | 21 | 4 | 5 | 12 (**) |
| Medulla oblongata | 6 | 4 | 2 | |

(*) In some animals operation extended, if only a little, into the controlateral tectum.

(**) These labelled cells appear in the mass of cells and fibres evident in the regenerated area towards the centre of the optic tectum.

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difference between the number of labelled cells in the operated and healthy sides.

Finally 12% of the labelled cells observed in every case appeared in the regeneration area about the middle of the mesencephalon. The area lacked a definite cytological structure but was certainly of recent formation, yet because of its position, it is arbitrary to assign it to one side or the other.

In the telencephalon we also studied the localization of the labelled cells and we noted that 37% were found in the neighbourhood of the ependyma related to the palaeocortex, the dorsal hippocampus and the more dorsal part of the medial hippocampus; whereas 63% of the labelled cells of the telencephalon were found near the ependyma in the more ventral zone of the medial hippocampus. From these investigations therefore it emerged that the great majority of labelled cells in the telencephalon appeared near the ependyma either scattered or concentrated in well-defined areas which Kirsche had already indicated as rich in cells with embryonic characteristics (1967, 1968). In the mesencephalon labelled cells were not observed in particular areas or in the region of the ependyma, but rather were scattered here and there in both the intact and regenerated areas. Even the few labelled cells in the medulla oblongata had no preferential site.

The original hypothesis that if with the technique employed five months after surgery there were regeneration processes of the optic tectum these would provide unequivocal pictures and data is confirmed by the observations reported. In fact, five months after surgery, the rate of cell division was still very high, since we observed up to 3 000 labelled cells in the telencephalon and mesencephalon of each animal: all this points to a very high mitotic rate and a process of repair still active.

Attempts to detect the areas in which these cells concentrated showed that in regeneration of the optic tectum the telencephalon is also involved. More than 70% of the labelled cells appeared in the telencephalic hemispheres, with slight predominance of the hemisphere ipsilateral to the optic tectum operated on.

Areas of the telencephalon where these labelled cells appear, correspond to the germinative areas already detected by Kirsche (1967, 1968); the ventral germinative area appears most active since 63% of labelled cells are found there. The remaining 37% can be seen on the dorsal ependyma, corresponding mostly to the dorsal germinative area.

From these data, and from what has been observed above on the regenerative capacity of the telencephalon, one may suppose that the embryonic characteristics of the germinative zones make them available for regenerative processes of both the telencephalon and the mesenchephalon. Particularly interesting is our observation in some cases of reconstruction of a number of layers of cells and fibres in the optic tectum. The hypothesis that this area is newly formed is supported by the regular stratification and by the fact that the characters of the observed layers are significantly different from those of the controls, so that this area cannot be considered as an original (i.e. un-

touched) part remaining of the optic tectum after experimental surgery. This point is quite important because for the first time we noticed the tendency in a regenerated area to reinstate the characteristic architecture; this formation occurred only when on that side a part of the optic tectum still remained *in situ*. This observation more than anything else supports our belief that the phenomena recorded in our research refer to regenerative processes of the C.N.S. and confirms the hypothesis of a link between cold, blood-brain barrier and regenerative capacity.

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## REFERENCES


Altman J., 1962 - Are new neurons formed in the brains of adult mammals? Science, *135*: 1127-1128.

Altman J. & Das G. D., 1965 - Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J. Comp. Neurol., *124*: 319-336.

Blood L. A., 1972 - Some quantitative effects of nerve growth factor on dorsal root ganglia of chick embryos in culture. J. Anat., *112*: 315-328.

Del Grande P. & Minelli G., 1980 - Response of the regenerating telencephalon of *Lacerta viridis* to nerve growth factor. Z. mikrosk.-anat. Forsch., *94* (5): 785-793.

Hulsebosch C. E. & Bittner G. D., 1980 - Evolution of abilities to regenerate neurons in central nervous systems. Am. Anat., *115*: 276-284.

Kiernan J. A., 1979 - Hypotheses concerned with axonal regeneration in the mammalian nervous system. Biol. Rev., *54*: 155-197.

Kiernan J. A. & Contestabile A., 1980 - Vascular permeability associated with axonal regeneration in the optic system of the Goldfish. Acta Neuropathol., *51*: 39-45.

Kirsche W. & Kirsche K., 1961 - Experimentalle Untersuchungen zur Frage der Regeneration und Funktion des Tectum opticum von *Carassius carassius*. L. Z. mikrosk.-anat. Forsch., *67*: 140-182.

Kirsche W., 1967 - Über postembryonale Matrixzonen im Gehirn verschiedener Vertebraten und deren Beziehung zum Hirnbauplan. Z. mikrosk.-anat. Forsch., *77*: 315-406.

Kirsche W., 1968 - Über die Bedeutung postembryonale Matrixzonen verschiedener Vertebraten fur die Hirnregeneration. Tag. Ber. der Ges. Anat. Histol. Embryol., Akad Wiss., "Reaktive und regenerative Prozesse im Nervensystem", Tbilissi: 25-26.

Lee J. & Olszewski J., 1959 - Permeability of cerebral blood vessels in healing of brain wounds. Neurology, *9*: 7-14.

Leghissa S., 1962 - L'evoluzione del tetto ottico nei bassi Vertebrati. Arch. Ital. Anat. Embriol., *47*: 343-413.

Lougheed W. M., Sweet W. M., White J. C. & Brewster W. R., 1960 - Use of hypothermia in surgical treatment of cerebral vascular lesions, preliminary report. J. Neurosurg., *12*: 240-248.

Levi-Montalcini R. & Hamburger V., 1951 - Selective growth stimulation effects of mouse sarcoma on the sensory and sympathetic nervous system of the chick embryo. J. exp. Zool., *16*: 321-362.

Mellick R. S. & Cavanagh J. B., 1968 - Changes in blood vessel permeability during degeneration and regeneration in peripheral nerves. Brain, *91*: 141-160.

Minelli G., Del Grande P. & Mambelli M. C., 1978 - Preliminary study of the regenerative processes of the dorsal cortex of the telencephalon in *Lacerta viridis*. Z. mikrosk.-anat. Forsch., *91*: 241-256.

Minelli G. & Del Grande P., 1980 - An hypothesis on the influence of the temperature on the telencephalic reparative processes in *Lacerta viridis*. Z. mikrosk.-anat. Forsch., *94* (2): 209-216.

Persson L., Hansson H. A. & Sourander P., 1976 - Extravasation, spread and cellular uptake of Evans blue-labelled albumin around a reproducible small stab wound in the rat brain. Acta Neuropathol., *34*: 125-136.

Rosomoff H. L. & Gilbert R., 1955 - Brain volume and cerebrospinal fluid pressure during hypothermia. Am. J. Physiol., *183*: 19-28.

Stone H. H., Donnelly C. & Frobese A. J., 1956 - Effect of lowered body temperature on cerebral hemodynamics and metabolism of man. Surg. Gynec. & Obst., *103*: 313-327.

Varon S., 1975 - Nerve growth factor and its mode of action. Exp. Neurol., *48*: 75-92.

Varon S., 1977 - Neural growth and regeneration: a cellular perspective. Exp. Neurol., *54*: 1-6.