Virchows Arch. A Path. Anat. and Histol. 372, 175–182 (1976)

Virchows Archiv A

Proliferation of Blood Vessels and Stroma in Brain Tumours*

An Enzyme-Histochemical Study

G.Th.A.M. Bots

Department of Pathology (Prof. Dr. Th.G. van Rijssel, Prof. Dr. A. Schaberg), University of Leiden, Wassenaarseweg 62, Leiden, The Netherlands

Summary. Proliferation of the vascular endothelium occurring in brain tumours is accompanied by a proliferation of histiocytes in the peripheral part of the vessel wall. These histiocytes infiltrate the tumour tissue in a very regular pattern. Enzyme-histochemically, there are marked differences between the activities of alkaline phosphatase, 5-nucleotidase, and ATPase in the normal and proliferating blood vessels. The whole process encompasses reactive changes evoked by the destroyed perivascular sheath of astroglial foot processes and the subsequent oedema in the tumour and the surrounding parenchyma. There are often tumour areas where diminished vascular permeability is established by proliferation of perivascular connective tissue. Here the oedema has completely disappeared. A clearcut influx of monocytes from the blood into the vessel wall is seen only in the vicinity of necrotic foci; the number of histiocytes is increased and their turnover is observed in swollen macrophages. In the rest of the tumour influx of monocytes and activity of macrophages are inconspicious.

Key words: Endothelial proliferations – Enzyme-histochemistry – Influx of monocytes – Oedema of brain tumours.

Introduction

The proliferation of the walls of the blood vessels and stroma (PBS) in malignant brain tumours is well known, but its significance poorly understood (Calvo, 1971; Henschen, 1955; Russel and Rubinstein, 1971; Rubinstein, 1972). These changes are most marked in capillaries and veins, and are less prominent in arteries. Light-microscopical and particularly electron-microscopical studies (Henschen, 1955; Long, 1970) have shown that the process is very complex and that the proliferation of the endothelium is only one part of it. The work of Kitamura (1972) underlined the importance of the influx of monocytes into

* This study was supported by the Koningin Wilhelmina Fonds under grant N 75.30

traumatic brain lesions. Because monocytes like their tissue counterparts the histiocytes are characterized by the activity of α-naphthylesterase, we thought it worthwhile to try to find out whether this influx could also be dected enzyme-histochemically in brain tumours and, if so, whether this process is part of the PBS.

Material and Methods

Samples of 200 gliomas were embedded in paraffin for conventional histological investigation and frozen in liquid nitrogen for enzyme-histochemical procedures. Five enzymes were studied with the indicated methods: alkaline phosphatase (Gomori, 1952; Burstone, 1962), acid phosphatase (Gomori, 1952; Barka and Anderson, 1963), 5-nucleotidase (Wachstein and Meisel, 1957) adenosine triphosphatase (Wachstein and Meisel), and α-naphthylesterase (Barka and Anderson, 1963).

Results

Figure 1 gives a typical example of PBS, showing proliferating endothelial cells, connective tissue fibres, and deposits of a hyaline material in the vessel wall.

In PBS many enzymes show different activity patterns from those seen in normal blood vessels. Alkaline phosphatase activity, which occurs in the endothelium of some of the blood vessels in brain tissue, is diminished in PBS, and in oedematous regions occurs not only in the endothelium but also in an ill-defined perivascular zone and between the adjacent tumour cells (Fig. 2a and 2b). This peripheral zone of activity is even present in the blood vessels without endothelial alkaline phosphatase activity.

The 5-nucleotidase activity of the endothelium disappears completely in PBS, whereas the neuropil and the tumour usually show strong activity in this enzyme (Fig. 3a and 3c). In the oedematous (outer) parts of the tumour the activity is lost in the neuropil and tumour cells (Fig. 3a), but positive cells still are present in the innermost layer of the proliferated endothelium and stroma around most of the vessels still is positive (Fig. 3b).

The endothelium of the proliferated vessels, like that of the normal blood vessels, does not show α-naphthylesterase activity. But in PBS a positive monocyte is sporadically seen adhering to the endothelium, and in some vessels a number of the innermost lining cells show the same α-naphthylesterase activity as monocytes (Fig. 5c). In the wall and especially at the periphery of most blood vessels, strongly positive histiocytes with slender protrusions occur as well as less markedly positive swollen macrophages (Fig. 5b). The tumour tissue invariably shows many positive histiocytes (Fig. 5a) whose distribution is remarkably even, but is denser around necrotic areas. They are also present in the absence of PBS.

The cell types with α-naphthylesterase activity also show slight to moderate acid phosphatase activity (Fig. 6), but the level of acid phosphatase activity is higher in the swollen macrophages than in histiocytes. The proliferated endothelial cells show a stronger ATPase activity than is seen in normal blood vessels. ATPase activity is also present in histiocytes (Fig. 4).

The most important findings are shown in Table 1.

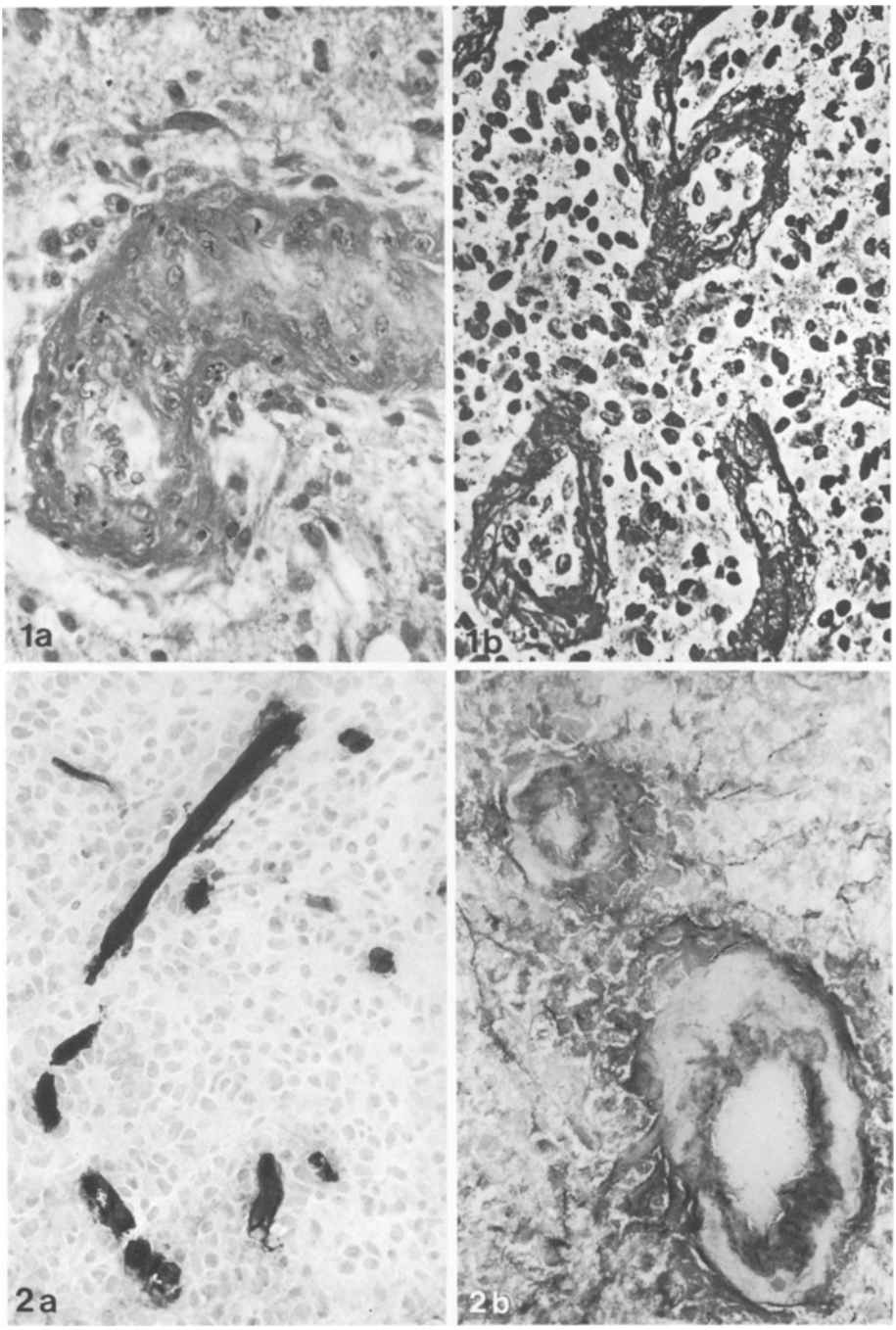

Fig. 1 a and b. Proliferation of the blood vessels in a glioma. **a** Proliferation of endothelial cells and peripheral cells. Several mitotic figures can be seen at the periphery. Between the nuclei a partially hyaline, partially more fibrous material is present. HE ×225. **b** Proliferation of reticulin fibres. Gomori's reticulin stain. ×225

Fig. 2. a Alkaline phosphatase activity in normal capillaries. ×225. **b** Alkaline phosphatase activity in PBS. Activity is lowered in the swollen endothelial cells and now also occurs in the oedematous perivascular zone and extracellular space. Positive areas and fibres in and around the vessel wall indicate formation of fine collagen fibres. ×225

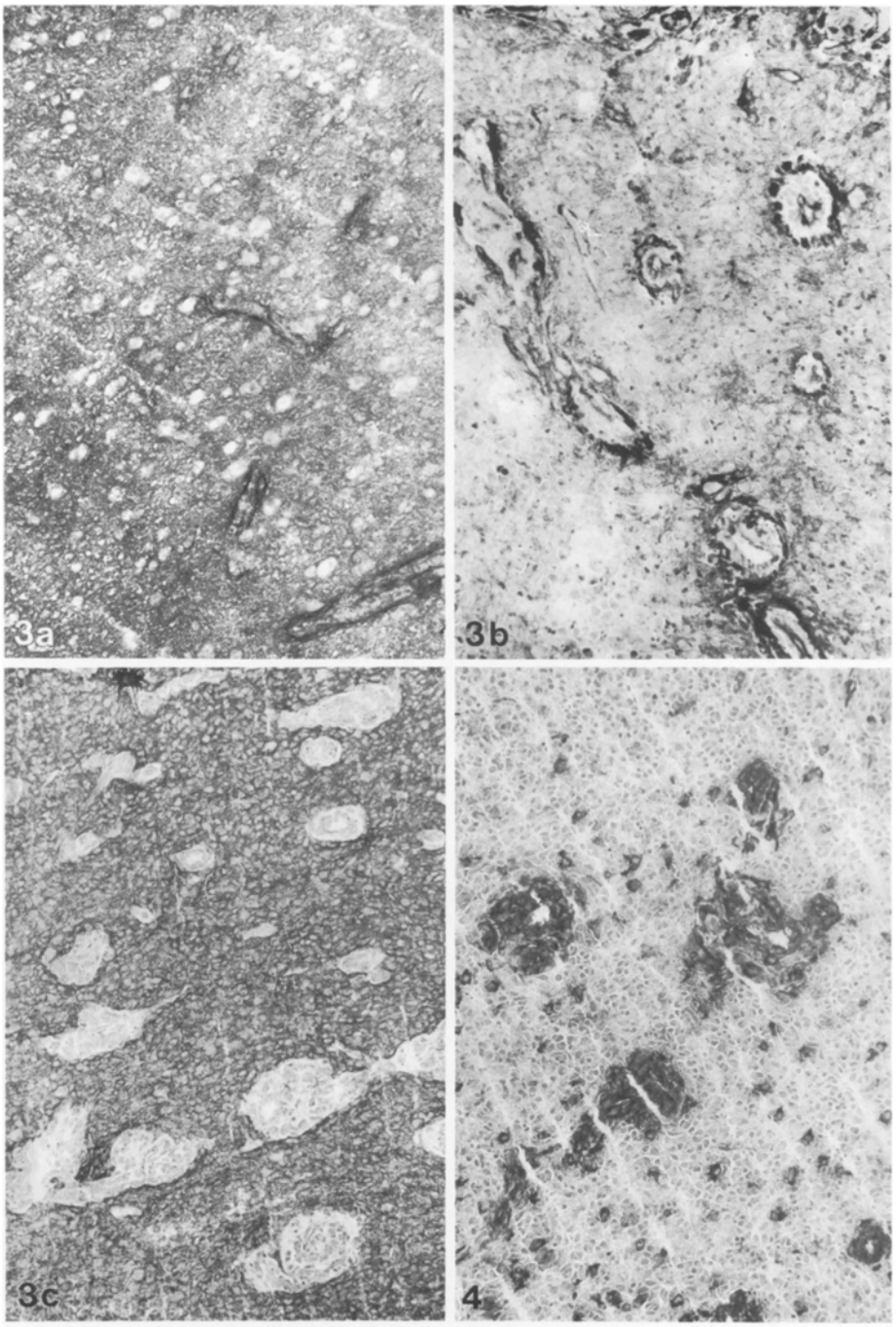

Fig. 3. a 5-nucleotidase activity of the cerebral parenchyma, the endothelial cells, and (even more pronounced) of the vascular stroma. ×90. **b** 5-nucleotidase activity in the oedematous outer part of the tumour: a number of proliferated blood vessels show only some activity in the innermost layer of the proliferated endothelium. There is no activity in tumour tissue. The activity of the stroma is again pronounced. ×90. **c** 5-nucleotidase activity in the tumour tissue. No activity in the endothelium or the vascular stroma. ×90

Fig. 4. ATPase activity in proliferated blood vessels, in the stroma, and in the dispersed histiocytes. ×90

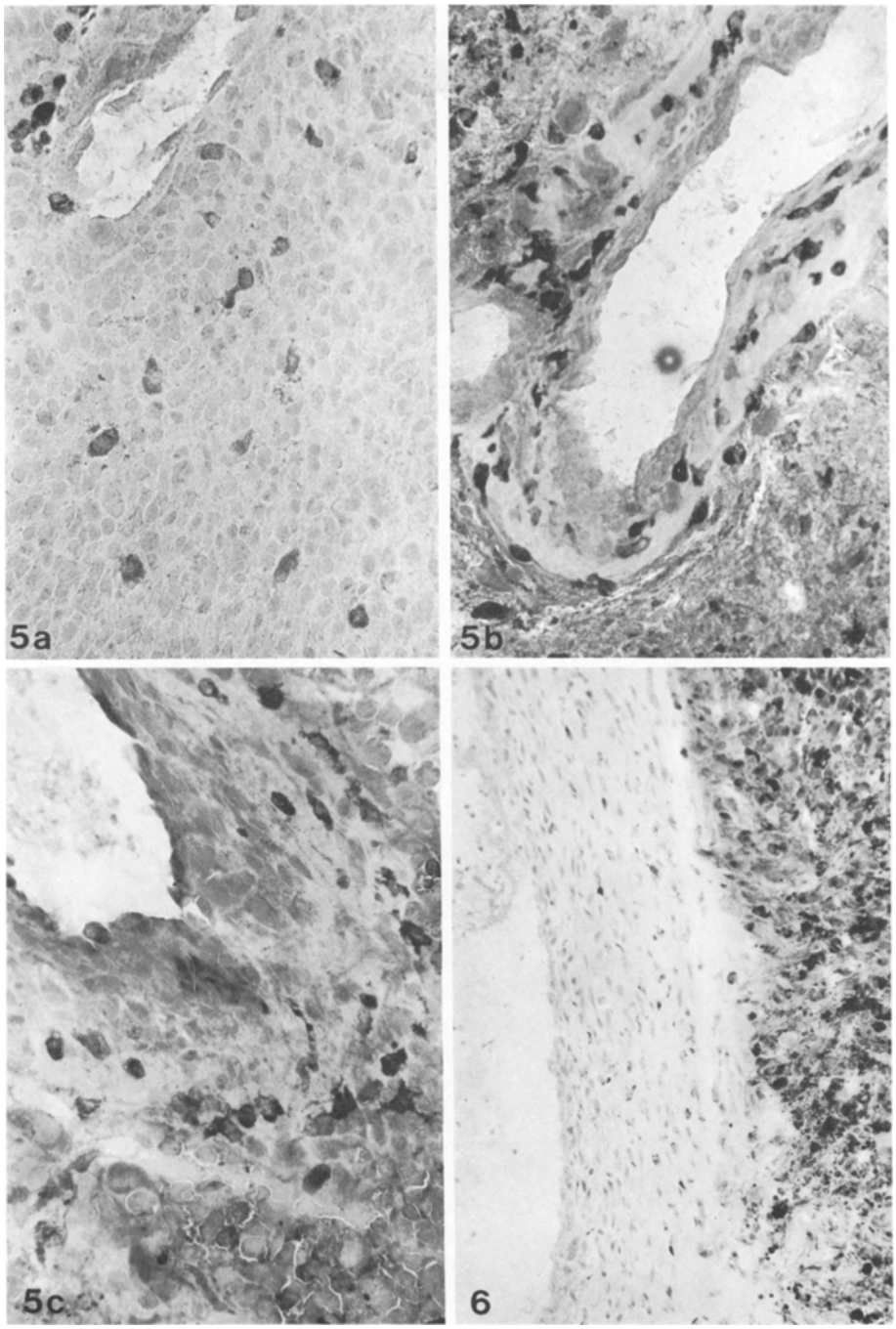

Fig. 5. a α-naphthylesterase-positive histiocytes are evently distributed in the tumour tissue but aggregated around the vessel wall. ×225. **b** α-naphthylesterase-positive histiocytes in the vessel wall, with accumulation at the periphery. ×225. **c** The same kind of tissue as in b, but with some positive cells adherent to the innermost layer of the vessel wall. ×225

Fig. 6. The histiocytes in and around the vessel wall show little acid phosphatase activity, but many positive macrophages and degenerated tumour cells are seen around the vessel wall. ×90

Table 1. Enzyme patterns in normal blood vessels and in the proliferated blood vessels of brain tumours

	Normal blood vessels		Proliferated blood vessels and stroma (PBS)	
	endothelium	stroma	endothelium	stroma
Alkaline phosphatase	++	−	+	+
5-nucleotidase	+	+	−	−
ATPase	+	−	++	−

Discussion

The autoradiographic studies performed by Kitamura and co-workers (1972) showed that in experimental brain lesions there is an influx of blood-borne monocytes. As we reported earlier (Bots, 1970), an important feature of brain tumours is that they are always diffusely infiltrated by large number of histiocytes. These cells are distributed in more or less the same way as the microglia cells and pericytes in the unaffected brain tissue, but differ from the latter two cell types by their astrocyte-like, rather bulky cell body, their high α-naphthylesterase activity, and their lack of dehydrogenase activity. This suggested that these tumour histiocytes, too, might derive from a detectable influx of blood monocytes. The presence of a few monocytes (characterized by α-napthylesterase activity) adhering to or present in the innermost endothelium cell-layer in some of the PBS material might indicate that a similar monocyte influx occurs in brain tumours. However, large numbers of strongly positive histiocytes were found in the outer layers of most of the proliferated vessel walls and the surrounding tumour tissue. This suggests (together with the mitotic figures occurring in these areas) that the proliferation of histiocytes in the peripheral part of the vessel wall and in the extracellular spaces is also an important source of these histiocytes in the tumour. The large number of proliferating cells in the outer vascular layers probably represent the ultimate offspring of a relatively small number of influx monocytes. This conclusion is consistent with the work of Spector et al. (1967) on subcutaneous chronic inflammatory granulomata and some recent reports on the monocyte influx in brain lesions (Oehmichen et al., 1972). Spector et al. (1967) concluded that fresh emigration of monocytes made only a minor contribution to the chronicity of inflammation compared with mitotic division of histiocytes. Regions surrounding necrotic foci showed increased numbers of monocytes in the inner vessel wall, of histiocytes in the outer vessel wall and in the tumour, and of swollen macrophages, as demonstrated by the α-naphthylesterase and acid phosphatase activity. This suggests that in these vessels the influx of monocytes is considerably increased, and that this influx is strongly correlated with the increased proliferation of histiocytes and the differentiation of histiocytes into macrophages.

It is accepted (Reese and Karnovsky, 1967; Karnovsky, 1967; Long, 1970) that the blood-brain barrier in the CNS is formed by the combination of the

pentalaminar tight junctions between the endothelium cells of the blood vessels and the sheath around those vessels formed by the pericapillary foot processes of the astrocytes. This sheath is interrupted by the narrow entrances to the extracellular space between the parenchymal cells. In the fast-growing gliomas this smoothly fitting sheath of astroglia foot processes is disrupted and disappears completely (Long, 1970) and many pentalaminar tight junctions become abnormal (Long, 1970). In this way the blood-brain barrier is destroyed. This gives rise to oedema in the tumour and surrounding parenchyme. Possibly as a consequence of the increased permeability, the endothelium reacts by cell proliferation and the production of more basal membrane material (Long, 1970). Later on collagenous material is deposited around the vessels.

The altered metabolism of the endothelial cells in PBS can be demonstrated very clearly with enzyme-histochemical methods: compared with normal vessels the alkaline phosphatase activity is decreased, the ATPase activity is increased, and the 5-nucleotidase activity disappears completely. This last observation is important, because 5-nucleotidase has been assumed to have a function in the maintenance of the integrity of the blood-brain barrier (Torack and Barnet, 1964; Varkonyi and Jóo, 1968). Thus the disappearance of the 5-nucleotidase activity of the endothelium and the stroma of the blood vessels of the tumour might demonstrate the disappearance of the barrier function of the PBS, whereas the high activity in the central parts of the tumour tissue itself might suggest a partly re-established vascular permeability. This suggestion is supported by the absence of oedema. The disappearance of the 5-nucleotidase activity in the oedematous outer parts of the tumour and surrounding brain probably also reflects a disturbances of the blood-brain barrier in those regions. The oedematous parts are also characterized by extracellular alkaline phosphatase activity. This probably indicates new collagen formation in the increased mucopolysaccharide ground substance deposited around the vessels (Danielli, 1945; Willighagen, 1960). Decreased activity of alkaline phosphatase in the endothelium is probably related to swelling of these cells. Increased activity of ATPase is difficult to interpret, because this enzyme occurs in so many tissue components, but it is certainly related to the increase in basement membrane material produced by the endothelium.

In conclusion, endothelial proliferation and the replacement of the missing perivascular astroglial foot processes by perivascular connective tissue, are an adaptation of the increased vascular field to the tumour growth. Thus nonoedematous fields of tumour tissue are often established. This does not result in the re-establishment of a true blood-brain barrier.

The author wishes to thank Miss N.J. Dijkshoorn, Miss M.J.M. Louwe, and Mrs. B. Ras-Muller for their valuable technical assistance.

References

Barka, T., Anderson, P.J.: Histochemistry. New York: Harper and Row 1963

Bots, G.Th.A.M.: Enzymhistopathology of primary tumours of the human nervous system in relation to the accompanying tissue reaction. Proceedings of the VIIth International Congress of Neuropathology, pp. 497–511. Paris: Masson et Cie 1970

Burstone, M.S.: Enzyme histochemistry. New York: Acad. Press 1962
Calvo, W.M.: Growth patterns of intracranial neoplasm. In: Pathology of the nervous system. Vol. 2 edit. by J. Minckler. New York: McGraw-Hill 1971
Danielli, J.F.: The enzymes in woundhealing. Brit. J. exper. Path. **26**, 367–376 (1945)
Gomori, G.: Microscopic histochemistry; principles and practice. Chicago: University of Chicago Press 1952
Henschen, F.: Tumoren des Zentralnervensystems und seiner Hüllen. In: Handbuch der speziellen pathologischen Anatomie und Histologie. XIII-3. Berlin-Göttingen-Heidelberg: Springer 1955
Karnovsky, M.J.: The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J. Cell Biol. **35**, 213–236 (1967)
Kitamura, T., Hattori, H., Fujita, S.: Autoradiographic studies on histogenesis of brain macrophages in the mouse. J. Neuropath. exp. Neurol. **31**, 502–518 (1972)
Long, D.M.: Capillary ultrastructure and the blood-brain barrier in human malignant brain tumours. J. Neurosurg. **32**, 420–427 (1973)
Oehmichen, M., Grüninger, H., Saebisch, R., Narita, Y.: Mikroglia und Pericyten als Transformationsformen der Blutmonocyten mit erhaltener Proliferationsfähigkeit. Acta neuropath. (Berl.) **23**, 200–218 (1972)
Reese, T.S., Karnovsky, M.J.: Fine structural localization of a blood-brain barrier to exogenous peroxidase. J. Cell Biol. **34**, 207–217 (1967)
Rubinstein, L.J.: Tumours of the central nervous system. Washington D.C.: Armed Forces Institute of Pathology 1972
Russell, D.S., Rubinstein, L.J.: Pathology of tumours of the nervous system. London: Arnold 1971
Spector, W.G., Lykke, A.W.J., Willoughby, D.A.: A quantitative study of leucocyte emigration in chronic inflammatory granulomata. J. Path. Bact. **93** (1), 101–107 (1967)
Torack, R.M., Barnett, R.J.: The fine structural localization of nucleoside phosphatase activity in the blood-brain barrier. J. Neurpath. exp. Neurol. **23**, 45–59 (1964)
Varkonyi, T., Jóo, F.: The effect of nickel chloride on the permeability of the blood-brain barrier. Experientia (Basel) **24**, 452–453 (1968)
Wachstein, M., Meisel, E.: Histochemistry of hepatic phosphatases at a physiologic pH. Amer. J. clin. Path. **27**, 13–23 (1957)
Willighagen, R.G.J.: Histochemisch onderzoek van de activiteit van alkalische en zure fosfatase in normaal en in pathologisch veranderd menselijk weefsel. Leiden: Thesis 1960

Received August 26, 1976