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Concanavalin a binding and neuronal differentiation

A light microscopic study on neuronal tumours*

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Summary. Concanavalin A (Con A) acceptors have been demonstrated in large differentiated neurons in a previous paper. In order to elucidate the correlation between Con A binding in normal and neoplastic neurons and lectin binding dependence upon the differentiation grade, 26 tumours of the neuronal series were examined using formalin fixed and paraffin embedded biopsy specimen. The neoplasms included 3 gangliocytomas, 7 gangliogliomas, 1 central neuroblastoma, 11 medulloblastomas, 2 retinoblastomas, and 2 sympathicoblastomas. Well differentiated neurons in gangliocytomas and gangliogliomas expressed a high intracytoplasmic Con A acceptor density comparable to the feature in large non-neoplastic neurons. Less differentiated neurons and neuroblasts showed a weak perinuclear fine granular binding or an absolute lack of binding molecules, respectively.

Our results suggest that in a variety of tumours, Concanavalin A receptor density in neurons depends upon the degree of differentiation of the cell. Well differentiated cells have a higher density than poorly differentiated neoplastic neurons.

Key words: Neuronal tumours – Neuronal differentiation – Lectin histochemistry – Concanavalin A

Con A binding structures have previously described in normal brain (Cotman and Taylor 1974; Gurd and Fu 1982; March and Thornton 1983) and neuronal tumours (Caron et al. 1981; Liwnicz 1982) both at the ultrastructural level and in tissue culture. Light microscopic characterization of lectin target cells in normal human brain and the pituitary gland (Schwechheimer et al. 1983a and b) and in brain tumours (Schwechheimer et al. 1983c) have demonstrated that lectin binding is specific and cytotypical even if formalin fixed and paraffin embedded tissue is used. The most con-

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Table 1. Concanavalin A binding to tumours of the neuronal series

Nr.	Biopsy nr.	Sex	Age (years)	Localization	Histological diagnosis[b]	Concanavalin A binding pattern[a]				
						Neurons		Neuroblasts	Astrocytes	
						Differentiated	Less differentiated		Neoplastic	Reactive
1	17094/72	F	29	Precentral, right	Gangliocytoma	+++	∅	∅	∅	∅
2	35992/75	F	53	Central, right	Gangliocytoma	+++	∅	∅	++	∅
3	8462/72	M	8	Temporal, left	Gangliocytoma	+++	∅	∅	–	∅
4	17349/72	F	29	Precentral, right	Gangliocytoma	+++	∅	∅	++	++
5	24050/77	F	46	Parieto-temporo-occipital, right	Gangliocytoma	+	∅	∅	+	∅
6	34510/78	M	16	Parietal, right	Gangliocytoma	++	–	∅	+	∅
7	22616/79	F	43	Fronto-temporal, right	Ganglioglioma	++	∅	∅	++	∅
8	6985/72	F	6	Hypothalamus	Ganglioglioma	++	+	∅	–	∅
9	33617/73	F	2	Spinal root	Ganglioglioma	+++	+	∅	++	∅
10	21118/75	F	2	Adrenal gland	Ganglioglioma	++	+	∅	+	∅
11	16842/76	F	2	Fronto-parietal, right	Neuroblastoma	∅	∅	–	∅	∅
12	2906/76	M	11	Cerebellum	Medulloblastoma	∅	∅	–	∅	∅
13	48467/76	F	8	Cerebellum	Medulloblastoma	∅	∅	–	∅	∅
14	49088/76	M	7	Fourth ventricle	Medulloblastoma	∅	∅	–	∅	∅
15	50361/76	M	9	Cerebellum	Medulloblastoma	∅	∅	–	∅	∅
16	32036/77	M	8	Cerebellum	Medulloblastoma	∅	∅	–	∅	∅
17	33015/77	M	8	Cerebellum	Medulloblastoma	∅	∅	–	∅	∅

Table 1 (continued)

Nr.	Biopsy nr.	Sex	Age (years)	Localization	Histological diagnosis[b]	Concanavalin A binding pattern[a]				
						Neurons		Neuroblasts	Astrocytes	
						Differentiated	Less differentiated		Neoplastic	Reactive
18	34106/77	F	5	Fourth ventricle	Medulloblastoma	∅	∅	–	∅	∅
19	48663/78	F	4	Fourth ventricle	Medulloblastoma	∅	∅	–	∅	∅
20	23462/79	M	43	Cerebellum	Medulloblastoma	∅	∅	–	∅	∅
21	40748/79	F	9	Cerebellum	Medulloblastoma	∅	∅	–	∅	∅
22	49043/79	M	6	Cerebellum	Medulloblastoma	∅	∅	–	∅	∅
23	10814 B[c]	F	3	Orbita	Retinoblastoma	∅	∅	–	∅	∅
24	10682 B[c]	F	1/4	Orbita	Retinoblastoma	∅	∅	–	∅	∅
25	16107/72	F	7	Thoracal node	Sympathicoblastoma	+ +	+	–	∅	∅
26	16132/72	M	1	Paravertebral, left	Sympathicoblastoma	∅	+	∅	∅	∅

[a] Reactivity: very strong (+ + +), strong (+ +), weak (+), negative (–), cell type not present ∅

[b] Histological diagnosis according to: Histological typing of tumours of the central nervous system, World Health Organization, Geneva, 1979

[c] The two retinoblastomas were kindly provided by Prof. Dr. Kraus-Mackiw, Universitäts-Augenklinik Heidelberg (Director: Prof. Dr. W. Jaeger)

spicious feature of Con A in normal human brain was its strong intracytoplasmic granular reactivity in large neurons.

The present study was undertaken to further evaluate Con A binding in tumours of the neuronal series including highly differentiated and undifferentiated neoplasms. Peculiar emphasis was laid on the possible change of Con A acceptor density in normal and neoplastic cells and in differentiated and undifferentiated neuronal cells, i.e. mature neurons or immature neuroblasts.

Material and methods

3–5 μm paraffin sections of formalin fixed biopsy specimen from the files of the Pathological Institute Heidelberg were used. These included 3 gangliocytomas, 7 gangliogliomas, 1 central

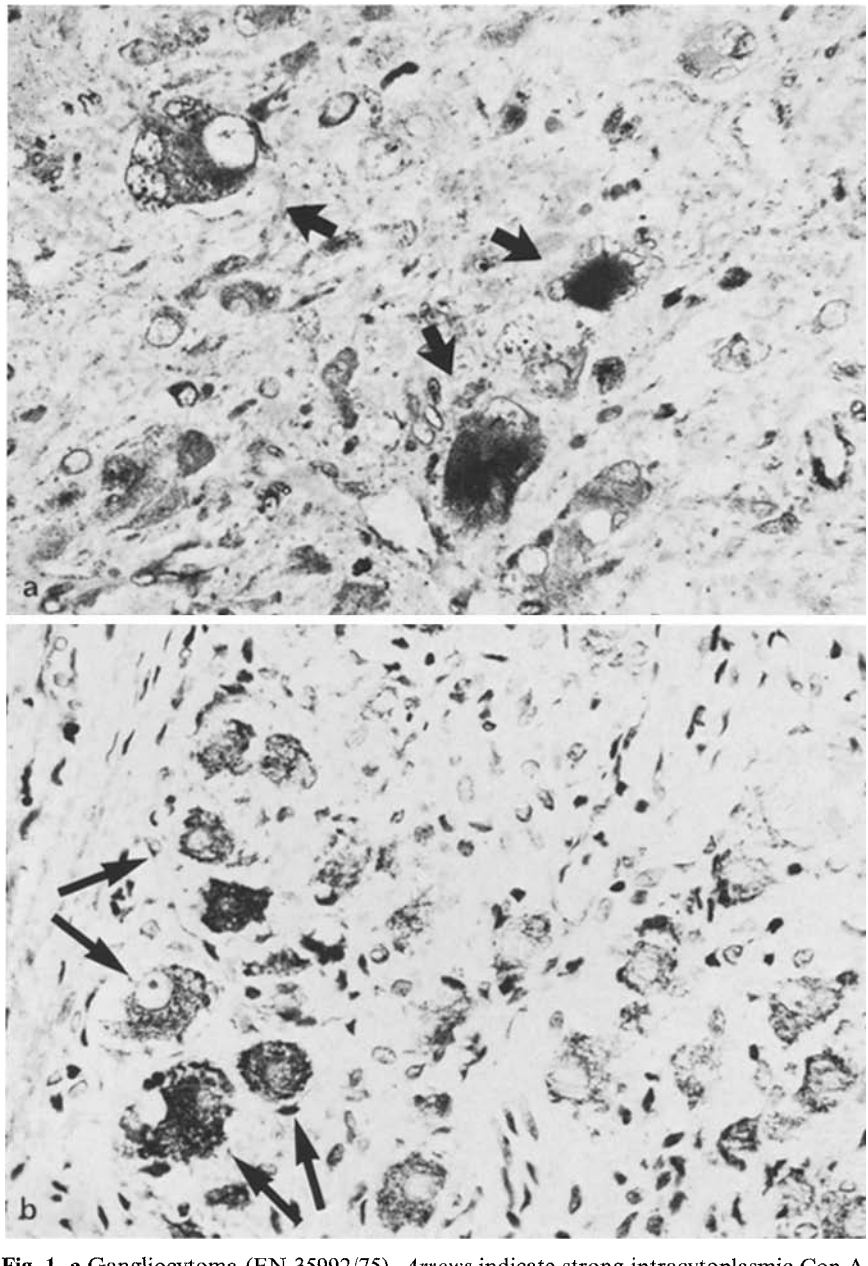

Fig. 1. a Gangliocytoma (EN 35992/75). *Arrows* indicate strong intracytoplasmic Con A binding in giant and multinucleated neurons. Con A, peroxidase. Haematoxylin counterstain. × 110. **b** Sympathicoblastoma (EN 16132/72). Well differentiated non-neoplastic neurons (arrows) of the ganglion in the environment of the tumour with coarse granular cytoplasmic staining. Con A, peroxidase. Haematoxylin counterstain. × 110

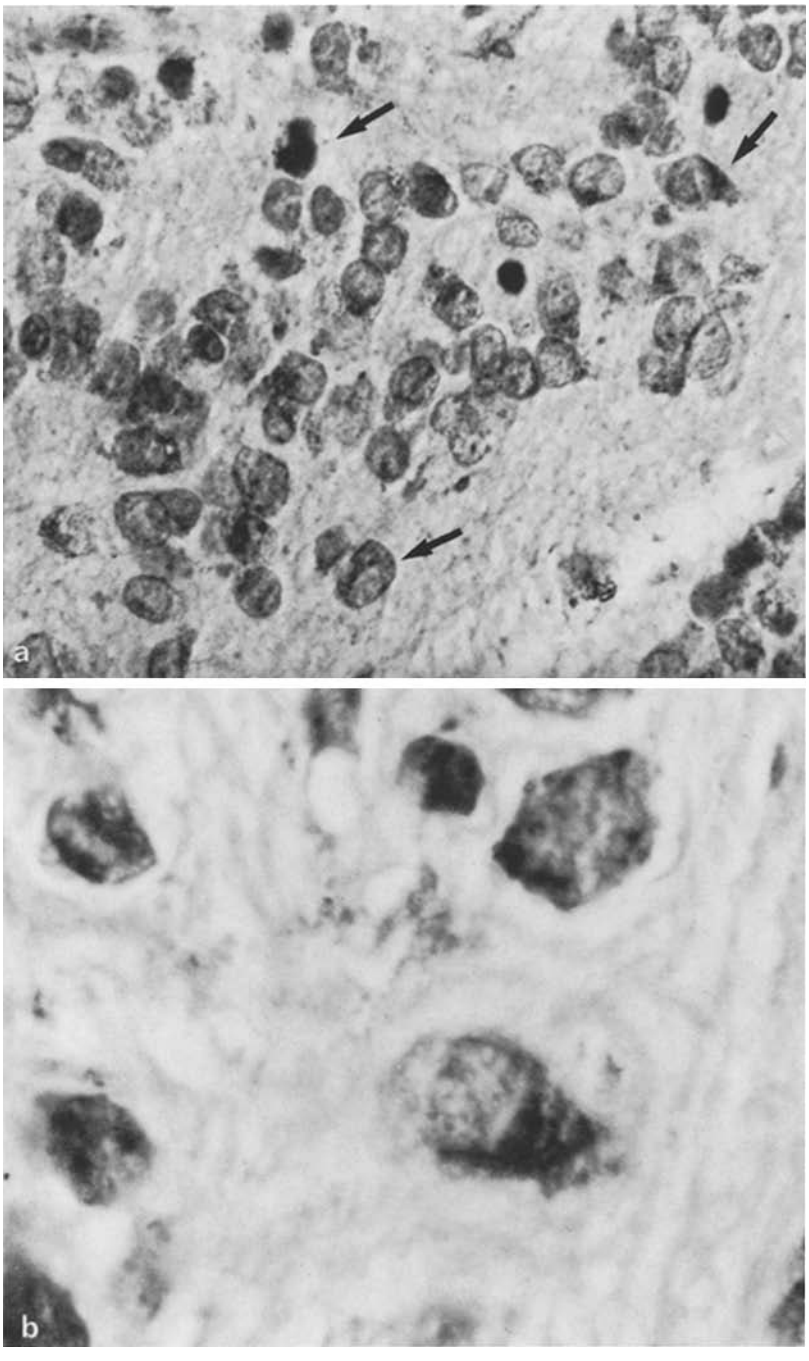

Fig. 2a, b. Sympathicoblastoma (16107/72). **a** Less differentiated neurons with fine granular perinuclear staining (*arrows*). × 110. **b** High power view indicating a perinuclear staining beside the Haematoxylin stain of the nuclei. × 440

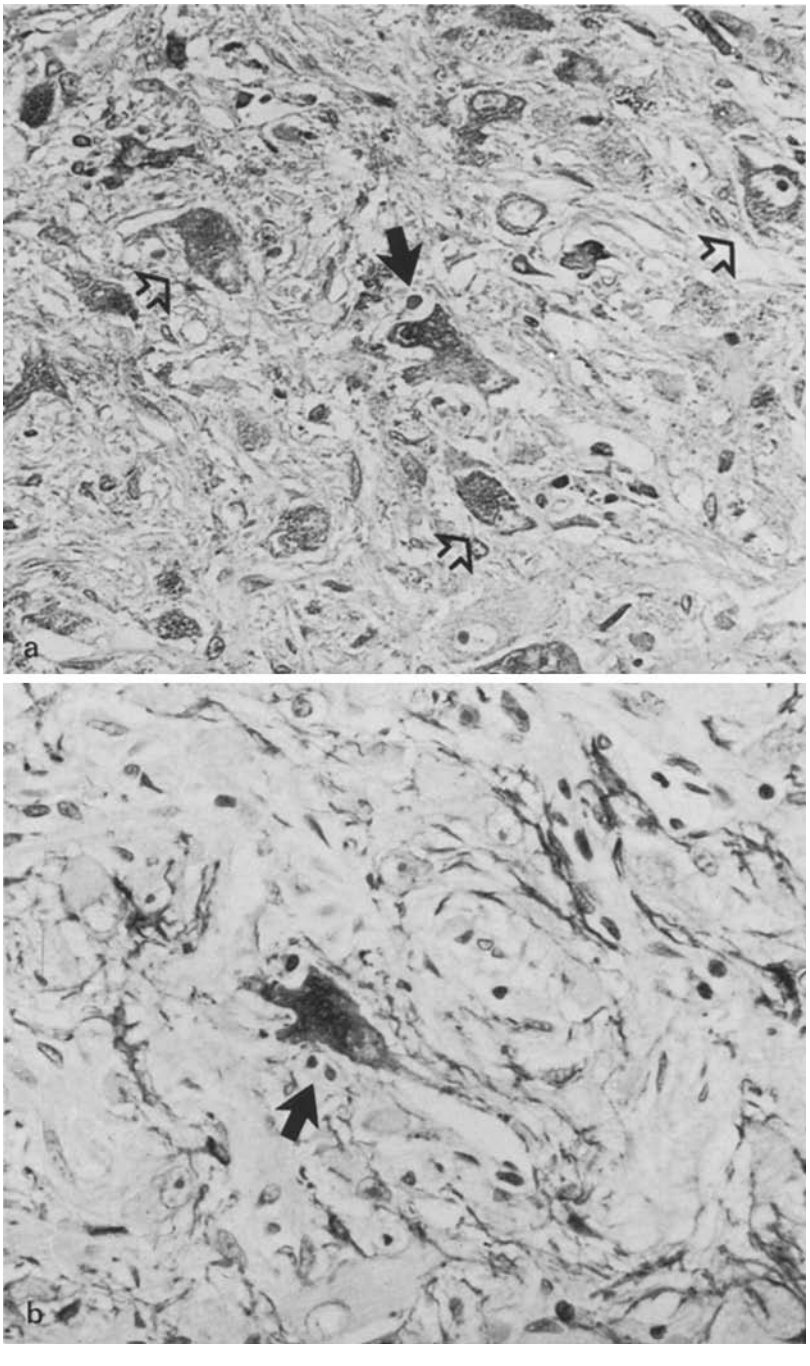

Fig. 3a, b

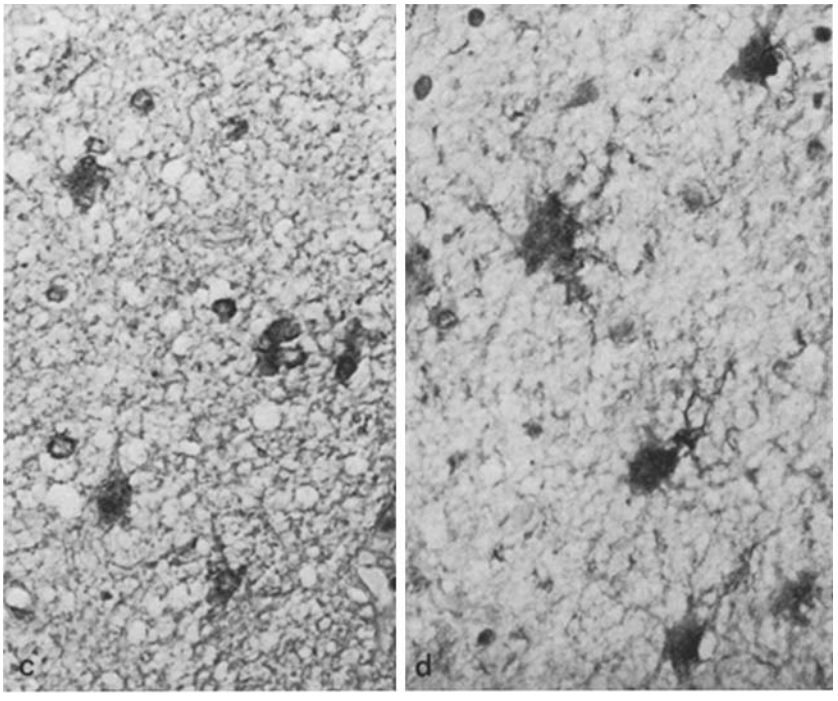

Fig. 3a–d. Ganglioglioma (EN 8462/72). **a** Pittoresque cellularity. Strong granular cytoplasmic Con A binding in neoplastic astrocytes (*filled arrows*) as well as in neoplastic neurons (*open arrows*). Con A, peroxidase, Haematoxylin counterstain. ×110. **b** Demonstration of GFAP in neoplastic astrocytes. anti-GFAP, PaP technique. Haematoxylin counterstain. ×110. **c** Reactive irregularly shaped astrocytes with strong intracytoplasmic Con A reaction in the environment of a ganglioglioma (EN 17349/72). Con A, peroxidase. Haematoxylin counterstain. ×110. **d** Same tumour. Demonstration of anti-GFAP indicating the glial nature of Con A positive cells in **c**. anti-GFAP, PaP technique. Haematoxylin counterstain. ×110

neuroblastoma, 11 medulloblastomas, 2 retinoblastomas, and 2 sympathicoblastomas. The details are quoted in Table 1. In order to demonstrate Con A binding the method as described by Avrameas et al. (1976) was employed. Con A (Lot Nr. 1104) was available from Medac Hamburg (FRG), and used at a final concentration of 0.1 mg/ml; the peroxidase (Lot Nr. 39) was purchased from Serva Heidelberg (FRG) and assayed at a final concentration of 10 μg/ml. 3,3′-diamino-benzidine (DAB, 50 μg/ml; Firma Merck, Darmstadt, FRG) was used as chromogen. Control experiments were done by omitting the lectin or by addition of the complementary sugar (α-methyl-manno-pyranoside, final concentration: 0.1 M). In gangliogliomas, the expression of glial fibrillary acidic protein was additionally examined by means of the unlabeled peroxidase-antiperoxidase technique of Sternberger (1970). Anti-GFAP raised in rabbits was a kind gift of D. Dahl (Department of Neuropathology, Harvard Medical School, Boston, USA). Anti-GFAP and PaP complex (DAKO, Denmark) were both used in a dilution of 1:100. Reaction was visualized by DAB (see above). – Tumour diagnosis was done on routinely processed and conventionally stained sections.

Results

The details are quoted in Table 1. The findings clearly demonstrate that Con A binding is cytotypical and not histotypical. For this reason it might

be appropriate to subdivide the neuronal tumours according to their cell types. Thus, the principal cell types are the well-differentiated and the less differentiated neurons, the neuroblasts, and the neoplastic and reactive astrocytes.

In the large neurons of the well differentiated neoplastic neurons a strong, granular Con A binding was observed. A granular or diffuse lectin staining was also seen in the double-nucleated neurons (Fig. 1a). Non-neoplastic, well-differentiated neurons in the environment of a tumour (Fig. 1b) expressed the features of Concanavalin A binding characteristic for normal neurons. Lectin staining was only weak and finely granular in less differentiated neurons and was restricted to the perinuclear cytoplasm (Fig. 2a, b). Such less differentiated neurons were observed in gangliocytomas and -gliomas as well as in the sympaticoblastomas to a variable extent. In undifferentiated precursor cells, which are the main cell type in neuro-, retino- and medulloblastomas, no intracytoplasmic Con A binding was noted (data not shown).

Intracytoplasmic lectin acceptor density was very high both in reactive astrocytes of the tumour surrounding tissue and in neoplastic gemistocytic and giant, sometimes multinucleated astrocytes. Pilocytic astrocytes as the glial component of the gangliogliomas, showed a very weak granular perinuclear reaction or none at all (Fig. 3a, c). GFAP was expressed in some of the tumour cells as well as in the reactive astrocytes (Fig. 3b, d).

Discussion

As far as the classification of the tumours of the neuronal series is concerned, we follow the proposal of Russell and Rubinstein (1971): the neuroblasts develop from the primitive germinal cells. They undergo progressive differentiation and become mature ganglion cells. Neuroblast-derived tumours are the neuroblastomas of the central nervous system, the cerebellar medulloblastomas, the retinoblastomas as well as their counterparts in the peripheral ganglia. As far as the medulloblastoma is concerned, various publications (e.g. Rorke 1983) indicate that some 50% of these tumours cells contain GFAP. Twenty-five percent of medulloblastomas appear to contain neurofilament protein, the neuron-typical intermediate-sized filament, 10% neither neurofilament protein no or GFAP and 13% both of these proteins. These results suggest both a neuronal and glial differentiation potency of these tumours. In gangliocytomas, the mature neurons represent the principal neoplastic elements. Both the neuronal and the glial cells in various stages of maturity form part of the mixed ganglioglioma.

Our results indicate a high Con A acceptor density in the well-differentiated neurons of the neoplastic tissue and the normal neurons in the areas surrounding the tumours. Furthermore, it is obvious that the number of Con A binding intracytoplasmic molecules depends upon the neuronal differentiation. In primitive neuroectodermal tumours, such as neuroblastoma, retinoblastoma and medulloblastoma, lectin binding was completely lacking. Less differentiated neurons with broader cytoplasm occurring in sympathi-

coblastomas and in cells in the environment of pseudorosettes showed a perinuclear fine granular cytoplasmic staining. Well-differentiated neoplastic, sometimes double-nucleated neurons were strongly marked.

Even if the light microscopic appearence of the well differentiated neurons was characteristic and allowed their identification, the less differentiated cell elements are more difficult to classify. The most reliable method to prove the neuronal origin of a cell is the demonstration of the neuron-specific enolase and/or of the neurofilament protein(s). Unfortunately, the required antisera are not available to us.

From the present study it is not possible to decide if a differentiation-dependent cytoplasmic binding capacity is relevant for the astrocytes, too. Reactive astrocytes (case 4) demonstrated a strong reactivity of the cytoplasm and the processes while the binding of the neoplastic astrocytes was ambiguous. The most intensive staining was observed in neoplastic multinucleated astrocytes with abundant cytoplasm. In pilocytic astrocytes which sometimes formed the glial component of the gangliogliomas, only a weak fine granular perinuclear or a negative reaction was observed.

Cotman and Taylor (1974) demonstrated Con A acceptors on the surface of postsynaptic membranes by electron microscopy while March and Thornton (1983) showed Con A binding glycoproteins in synaptic vesicles. These observations were confined by Gurd and Fu (1982) using biochemical methods. Feldman et al. (1982) reported Con A binding to neurites of goldfish retinal explants and Liwnicz (1982) studied the effects of mitogenic lectins on the growth rate of cells derived from glial, neural crest and meningiomas in tissue culture. Our method is not as sensitive as tissue culture, biochemical analysis or ultrastructural immunocytochemistry. By our method, that is light microscopic lectin histochemistry, it is not possible, of course, to visualize and to demonstrate membrane structures such as the postsynaptic membrane exactly. In our present context the paper of Caron et al. (1981) is of interest. They found peanut lectin surface acceptors on neuroblastoma cells using rhodamine isothiocyanate coupled lectin. Peanut lectin, however, is not necessarily comparable with Con A because the two lectins differ with respect to their sugar affinity. In addition, it is very difficult to demonstrate surface binding sites after formalin fixation of the tissue. These two reasons may explain why we were not able to demonstrate Con A surface acceptor molecules in our material.

The influence of the fixation procedure on lectin binding has recently been documented by Rittmann and Mackenzie (1983).

By our method it is not possible to determine the nature of the Con A acceptor molecule and/or the related cellular structures. In order to solve this problem, biochemical and ultrastructural work is recommended. From theoretical grounds one may speculate that there are multiple lectin acceptor molecules which are glycoproteins and/or -lipids.

The present results are summarized as follows:

Con A binding is specific, is characteristic for both differentiated normal and neoplastic neurons, is therefore cytotypical and not histotypical, and the acceptor density in neuronal cells decreases with descending differentiation.

The later finding might be best explained by regarding it as a functional alteration in carbohydrate metabolism accompanying neuron maturation and differentiation, which can be visualized by means of lectin histochemistry.

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