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Detection of Acid Mucopolysaccharides in Human Brain Tumors by Histochemical Methods

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Summary. Acid mucopolysaccharides (glycosaminoglycans) are identified by histochemical methods in biopsies of 107 human brain tumors. Isomorphous oligodendrogliomas and astrocytomas stained with alcian blue show marked, weblike, or diffuse distribution and concentration of acid mucopolysaccharides. Histochemically, the characteristics of hyaluronic acid and chondroitin sulphate are found. They seem to be closely associated with the cytoplasmic membrane of the tumor cells. Increased dedifferentiation and malignancy lead to a progressive loss of alcianophilia. In tumors such as ependymomas, meningiomas, sarcomas, and medulloblastomas, concentration of alcian blue is found only in the blood vessel walls and connective tissue. In neurinomas a greater amount of acid mucopolysaccharides can be shown not only in the collagen fibers but also in tumor areas of the Antoni-B-type. One case of cerebral neuroblastoma revealed marked alcianophilia of the parenchyma and stroma. As with findings in experimental brain tumors, an altered regulation of the carbohydrate metabolism of the glia cells during neoplastic transformation is discussed as a reason for the alcianophilia of gliomas.

Key words: Brain tumors – Acid mucopolysaccharides (glycosaminoglycans) – Histochemistry

Acid mucopolysaccharides which are clearly detectable by histochemical methods in the CNS of human adults appear to be bound to certain structures, such as basal laminae, connective tissue fibers, and the so-called "corpora amylacea". To demonstrate positive reactions in neuroepithelial tissue therefore signifies a striking finding. After the detection of neutral mucopolysaccharides in brain tumors by Earle (1959) and Arseni (1967) by the PAS method an increase of acid mucopolysaccharides in human oligodendrogliomas and neurinomas was shown by Smith and Butler (1973) using alcian blue. Schiffer et al. (1974, 1975, 1978) repeatedly described a positive reaction in experimental oligodendrogliomas with alcian blue. Studies by Engelhardt and Bannasch (1976, 1978) revealed alcianophilic areas to represent the earliest change in incipient rat gliomas and correlated the alcianophilia with various stages of glioma histiogenesis.

The concept of this study is to demonstrate whether there is a relationship in experimental and human gliomas as to the occurrence of acid mucopolysaccharides. In addition, the reaction of several types of human brain tumors to alcian blue is examined.

Material and Methods

Biopsies of 107 tumors of the human CNS were fixed in 4% neutral formalin and embedded in paraffin.

The histochemical reactions carried out after sectioning include: 1% solution of alcian blue 8 GS in 3% acetic acid according to Steedman (1950), modified by Mowry (1963), at pH 2.5, counterstained with Kernechtrot; 1% solution of alcian blue in 0.1 N HCl at pH 1.0 (Lev and Spicer, 1964) for detection of sulphated acid mucopolysaccharides, counterstained with Kernechtrot.

"Critical electrolyte concentration (CEC)" for differential staining of acid mucopolysaccharides according to Scott and Dorling (1965): 0.05% solution of alcian blue in 0.025 M sodium-acetate-buffer at pH 5.8 with varying $MgCl_2$ concentrations (0.3 M and 0.8 M $MgCl_2$) for 24 h. Control reaction in buffer.

Enzymatic reaction with hyaluronidase (Merck, 250 USP-E/mg) 0.05% in 0.1 M phosphate buffer, pH 6.0, at 37°C for 6 h (Leppi and Stoward 1965). Control reaction in buffer without enzyme. Subsequent staining with alcian blue at pH 2.5. Counterstain with Kernechtrot.

For histological diagnosis sections stained by H.E. were used complemented by special staining methods. Table 1 shows the distribution of the types of brain tumors.

Results

Isomorphous oligodendrogliomas show distinct staining features with alcian blue; characteristic honeycomb- and weblike appearances predominate (Fig. 1a). Occasionally, peripheral and poorly cellular

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areas are diffusely stained. Marked staining occurs also in the vicinity of calcified tissue. In a few tumor types, areas of decreased reaction may be found. A characteristic feature of malignant oligodendrogliomas is the coexistence of clearly alcianophilic, weakly positive and completely negative areas, in which the detection of residues of acid mucopolysaccharides between the cells may be taken as an indication of a progressive loss of alcianophilia (Fig. 1b).

Table 1. Types and numbers of brain tumors. Reaction with alcian blue pH 2.5

		Parenchyma	Stroma
Isomorphous oligodendrogliomas	(n = 10)	+++	(+)
Malignant oligodendrogliomas	(n = 8)	+	+
Astrocytomas grade I/II	(n = 19)	+++	(+)
Astrocytomas grade III/IV	(n = 24)	+	++
Neurinomas	(n = 14)	(+)	+
Ependymomas	(n = 7)	(+)	(+)
Cerebral neuroblastoma	(n = 1)	+	++
Medulloblastomas	(n = 2)	−	+
Meningiomas	(n = 16)	−	++
Sarcomas	(n = 6)	−	+++

Astrocytomas of a more benign type exhibit a high content of acid mucopolysaccharides. Small-cystic areas of fibrillar astrocytomas (Fig. 1c) and cerebellar astrocytomas (Fig. 1d) present the strongest reaction. An increased degree of anaplasia is accompanied by a loss of alcianophilia. Large unstained areas are found in astrocytomas with features similar to those of glioblastomas. On the other hand, the same tumors and a few polymorphous oligodendrogliomas show marked staining of the proliferated vascular connective tissue. In these gliomas showing marked dedifferentiation the positive staining of the stroma contrasts clearly with the alcian blue negative areas of the parenchyma (Fig. 3a).

Ependymomas show only occasionally very thin alcianophilic fibers in their stroma. A positive margin within the vessel walls is found especially in "high-malignancy ependymomas" (Fig. 3b). As an additional point, it should be mentioned that we studied a case of myxopapillary ependymoma of the filum terminale. Compared with other ependymomas, it presented

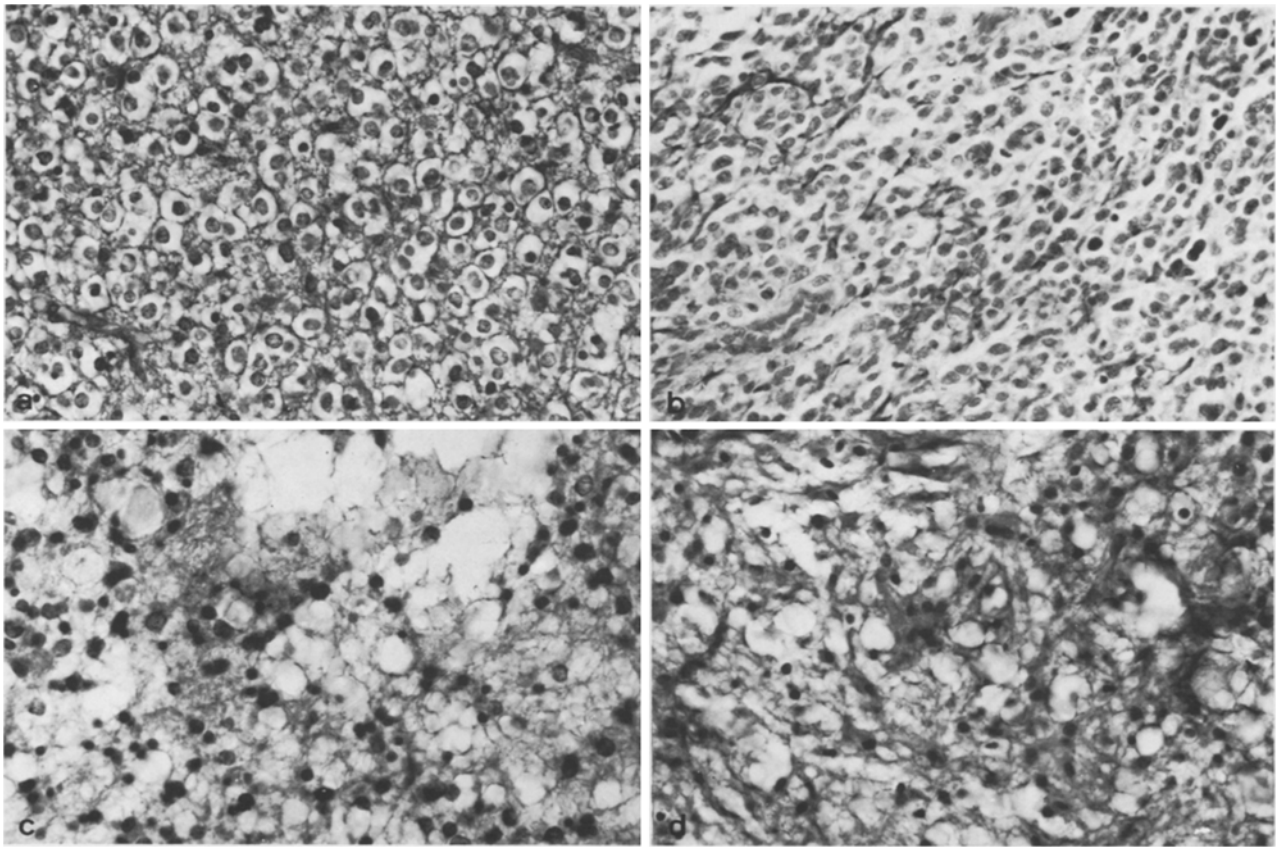

Fig. 1. a Weblike alcianophilic isomorphous oligodendroglioma. Alcian blue pH 2.5. ×250. **b** Malignant oligodendroglioma. At the left residues of acid mucopolysaccharides. Alcian blue pH 2.5. ×250. **c** Alcianophilic fibrillar astrocytoma. Alcian blue pH 2.5. ×250. **d** Alcianophilic cerebellar astrocytoma. Alcian blue pH 2.5. ×250

extremely positive acid mucopolysaccharides staining in the cysts and connective tissue fibers.

Neurinomas exhibit a varying concentration of acid mucopolysaccharides. Typical fibrillar parts of the Antoni-A-type do not usually stain with alcian blue whereas parts of the Antoni-B-type may show reticular positive areas, sometimes together with lipid storage (Fig. 2). Parts with a very positive reaction almost invariably represent collagen fibers or poorly cellular connective tissue.

One case of cerebral neuroblastoma revealed marked alcianophilia both of the parenchyma and the stroma.

The parenchyma of endotheliomatous meningiomas does not show any positive staining with exception of the trabeculae of connective tissue traversing the tumor. Psammoma bodies also are surrounded by a marginal capsule containing acid mucopolysaccharides. The fibroplastic type of meningiomas shows slightly alcian positive collagen fibers between the cells. Amorphous alcianophilic material is found rather often in the midst of whirls especially in the so-called transitional type (Fig. 3c).

Sarcomas show notable staining only if there is abundant fiber production (Fig. 3d).

Medulloblastomas similarly show negative staining of the parenchyma and a more or less positive staining of the vascular connective tissue.

The differential staining methods for acid mucopolysaccharides we carried out yield similar reactions at pH 2.5 and pH 1.0, being a bit weaker in the latter. Using alcian blue in 0.3 M $MgCl_2$ both the stroma and parenchyma are clearly positive (Fig. 4a), whereas there is no staining of the parenchyma in solutions of 0.8 M $MgCl_2$ (Fig. 4b). The collagen shows a positive reaction even at this concentration. Treatment with hyaluronidase reduces considerably the reactions in all parts of the tumor (Fig. 4c and d). Areas with a high concentration of mucopolysaccharides on the other hand may even then exhibit residues of alcian blue positive material.

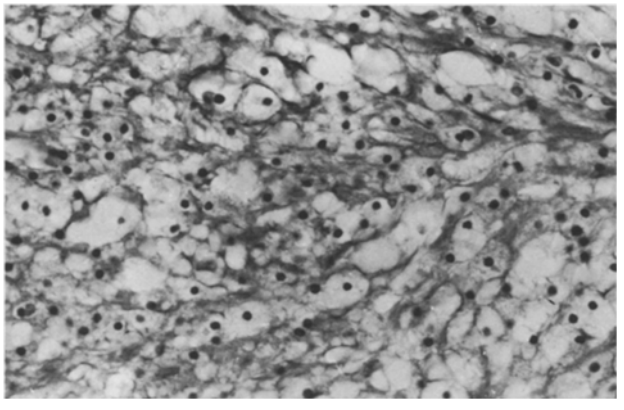

Fig. 2. Neurinoma. Reticular alcianophilic Antoni-B-type. Alcian blue pH 2.5. ×230

Discussion

Studies with rat brain tumors which were experimentally induced by methyl-nitroso-urea demonstrated that the rise in concentration of histochemically detectable acid mucopolysaccharides ("alcianophilic areas") represents the earliest light-microscopical change during glioma histiogenesis (Engelhardt and Bannasch 1978). This histochemical equivalent to the early stages of neoplastic transformation could not be demonstrated yet in human brain material. But findings in obvious

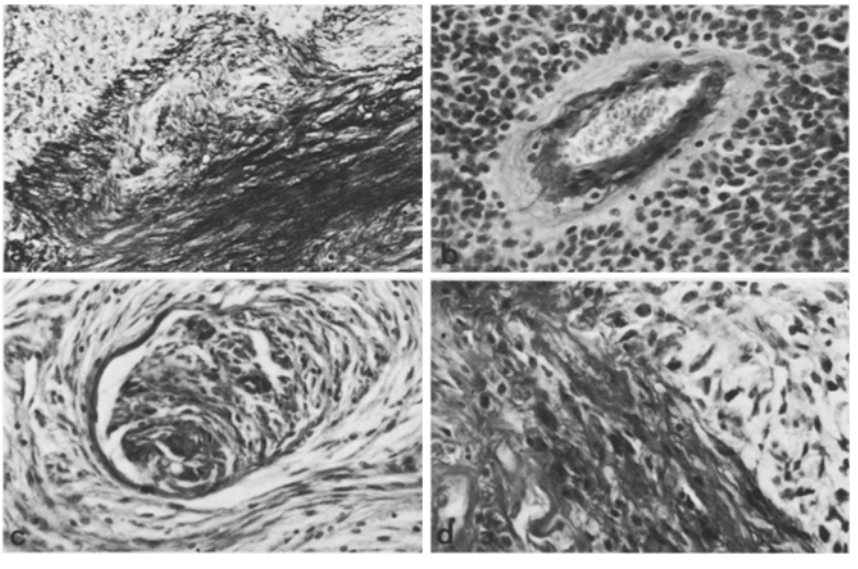

Fig. 3a–d. Alcian blue positive reaction of the stroma. **a** Polymorphous glioma. Alcian blue pH 2.5. ×110. **b** Ependymoma. Alcian blue positive blood vessel wall. Alcian blue 0.3 M $MgCl_2$. ×190. **c** Meningioma. Amorphous alcian blue positive substance in the center of the whirl. Alcian blue pH 2.5. ×190. **d** Sarcoma. Alcian blue positive fibers. Alcian blue pH 2.5. ×190

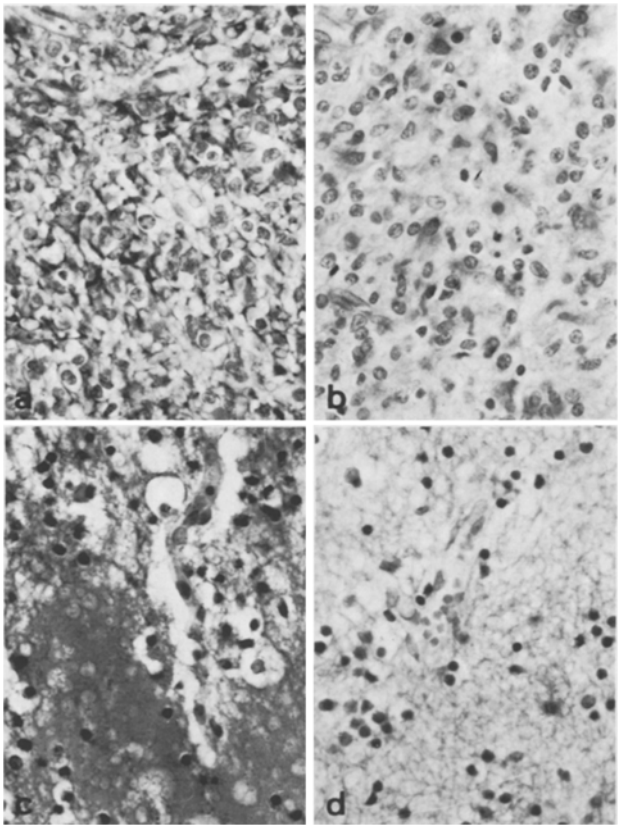

Fig. 4a–d. Differential staining of acid mucopolysaccharides in gliomas. **a** Alcian blue 0.3 M $MgCl_2$. **b** Alcian blue 0.8 M $MgCl_2$. **c** Control reaction in buffer without enzyme. AB pH 2.5. **d** After treatment with hyaluronidase. AB pH 2.5

tumors which are naturally occurring in man correspond with these found in experimental tumors: both oligodendrogliomas and astrocytomas exhibit a raised concentration of acid mucopolysaccharides with the histochemical characteristics of hyaluronic acid and chondroitin sulphate. Increasing dedifferentiation leads to a progressive decrease in concentration, and even complete loss of alcianophilic material.

There is no doubt about the alcianophilia of oligodendrogliomas, but concerning astrocytomas there is controversy in the literature. Arseni (1967) and Smith and Butler (1973) consider astrocytomas to be alcian blue negative. Schiffer and Giordana (1976) use the term "astrocytic focus" to describe alcian blue negative areas in gliomas. According to our findings there is no difference between oligodendrogliomas and astrocytomas as to the increase in concentration of acid mucopolysaccharides. Recently, Böck and Jellinger (1979) reported an increase of sulphated glycosaminoglycans in isomorphous human oligodendrogliomas and astrocytomas. Already in 1970, Dorfman and Ho succeeded in demonstrating acid mucopolysaccharides in tissue cultures of astrocytoma cells.

The alcianophilia of the parenchyma of gliomas must be separated strictly from the positive reaction of vascular connective tissue; the latter could be demonstrated in polymorphous gliomas, many neurinomas, ependymomas, and medulloblastomas. In addition, the connective tissue fibers produced by the tumor cells in meningiomas and sarcomas usually exhibit positive staining. This might be due to the reaction of native collagen with alcian blue (Scott and Dorling 1965). A quantitative increase of connective tissue fibers in many tumors must lead, therefore, to an increased alcian blue positive reaction. Additionally, it seems that in several cases there is also a qualitative change with masses of amorphous alcianophilic material within the stroma.

The alcian blue positive material in the parenchyma of gliomas has no connection with collagen fibers because benign tumors with poor connective tissue exhibit an increase of acid mucopolysacchrides. The alcian blue positive material seems to be associated predominantly with the cytoplasmic membranes of the tumor cells where it can be found preferably in normal glia cells (Margolis and Margolis 1977). Occasionally, storage of alcian blue positive material can be observed within the cytoplasm, especially in astrocytomas.

The reason for the alcianophilia of gliomas so far remains unclear. Schiffer and Giordana (1975) suppose that there is a connection between the development of oligodendrogliomas and the stage of myelinogenesis during which an increase of acid mucopolysaccharides takes place, too. Smith and Butler (1973) also emphasize the alcianophilia particularly of tumors of myelin producing cells, oligodendrogliomas, and neurinomas. In our opinion a pathological mechanism shared by the astroglia is very likely because of the alcianophilia of astrocytomas. Polysaccharide storage phenomena which appear during the development of extraneural tumors are known to occur to some extent at the preneoplastic stage (Bannasch 1974). Analogous to that, we consider a faulty regulation of the carbohydrate metabolism during neoplastic transformation of the glia cell to be responsible for the alcianophilia of gliomas. At the moment an enzyme deficiency in gliomas leading to inhibition of decomposition or increased synthesis of acid mucopolysaccharides is not definitely known.

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