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Fine-needle aspiration biopsy of orbital tumors was first reported by Schyberg in 1975 (1), and its role in the diagnosis and management of orbital disease has been further defined (2-4). Good clinical correlation is generally required for an accurate diagnosis due to the relatively small amount of specimen and the absence of standard histologic morphology (5). In selected cases, however, the histogenesis of a tumor may be further defined using immunoperoxidase techniques combined with diagnostic cytology (6). These methods were recently used concurrently in diagnosing a case of prostatic carcinoma metastatic to the orbit (7).

Following successful application of immunocytologic methods in the aforementioned case of orbital metastasis (7), we attempted to determine if a variety of other commercially available immunoenzyme reagents were applicable to the cytologic study of selected orbital tumors. This report¶ describes four cases of orbital tumors which were studied retrospectively using immunoperoxidase techniques on previously obtained cytologic specimens. The identification of glial fibrillary acidic protein and S-100 protein in cytologic specimens and S-100 protein in extraocular orbital tissues is described. This report also introduces the combined use of the avidin-biotin-peroxidase complex staining technique (8) and orbital fine-needle aspiration biopsy.

Materials and Methods

The methods of fine orbital, fine aspiration biopsy, and cytologic preparation used in this study have been described by Kennerdell et al (2). Four cytologic specimens (four cases) were selected for study because extra slides were available from a larger series of orbital fine-needle aspiration biopsies performed by Kennerdell. These included two cases of optic nerve glioma, one case of prostatic carcinoma, and one case of malignant melanoma metastatic to the orbit (Table).

The Papanicolaou-stained specimens were soaked in xylene to remove the glass coverslips. Without destaining the

specimens, indirect immunoperoxidase stains were then performed using the avidin-biotin-peroxidase complex method (8). Three different commercially obtained primary rabbit antisera (DAKO Corporation, Santa Barbara, Calif) were used as follows. The primary antiserum was directed against glial fibrillary acidic protein (9) in the cases of optic nerve glioma, against prostate specific antigen (10) in the case of prostatic carcinoma, and against S-100 protein (11) in the case of malignant melanoma. The regents for the remaining components of the avidin-biotin-peroxidase complex were obtained from a commercially available kit (Vecastain TM ABC Kit, Vector Laboratories, Inc, Burlingame, Calif), which included biotinylated goat antirabbit IgG, avidin, and biotinylated horseradish peroxidase.

The "positive" controls used included histological slides from 1) an autopsy brain specimen stained for glial fibrillary acidic protein, 2) a biopsy specimen from a benign hypertrophic prostate stained for prostate specific antigen, and 3) a biopsy specimen from a plexiform neurofibroma stained for S-100 protein. "Negative" controls consisted of histologic specimens from unrelated tissues which were also stained for each of the three primary antigens.

Table
Summary of Cases

Case Number	Diagnosis	Antigen Studied	Result
1	Optic nerve glioma	GFAP	Positive
2	Optic nerve glioma	GFAP	Inadequate specimen
3	Prostatic carcinoma	PSA	Positive
4	Malignant melanoma	S-100 protein	Positive

GFAP = Glial fibrillary acidic protein
PSA = prostate specific antigen

Results

Each positive control demonstrated strong staining reactions for the individual primary antigens tested. The negative controls failed to demonstrate any staining reactions for the three primary antigens tested.

Of the two cases of optic nerve glioma, the specimen in Case 1 (Fig 1) was richly cellular and showed only questionable malignant characteristics. Glial fibrillary acidic protein was strongly positive. The diagnosis of optic glioma was made by computed tomographic scan, and fine-needle aspiration biopsy confirmed the diagnosis. The specimen from Case 2 was very scant, hypocellular, and showed no definite neoplastic characteristics. Follow-up information regarding histological documentation was not available; the amount of material was therefore inadequate for interpretation. Staining for glial acidic fibrillary protein in Case 2 was negative. The cytologic specimen in Case 3 (Fig 2) was consistent with prostatic adenocarcinoma metastatic to the orbit and stained strongly for prostate specific antigen. The specimen in Case 4 (Fig 3) showed a large population of malignant cells consistent with malignant melanoma, which stained strongly for S-100 protein.

The pathologic diagnosis could therefore be supported or confirmed in Cases 1, 3, and 4, which had adequate cytologic specimens.

Discussion

From the few cases studied in this series, the indirect avidin-biotin-peroxidase staining technique appears well-suited as an adjunctive procedure in diagnostic cytology. Avidin is a 68,000 molecular weight glycoprotein which has an extraordinary high affinity for the small vitamin molecule biotin (12). Each avidin molecule has four binding sites for biotin, and most proteins can be conjugated with several molecules of biotin. Thus, a macromolecular complex can be formed between avidin and biotinylated enzymes. The advantages of this method over the peroxidase-antiperoxidase method of Sternberger (13) include much greater
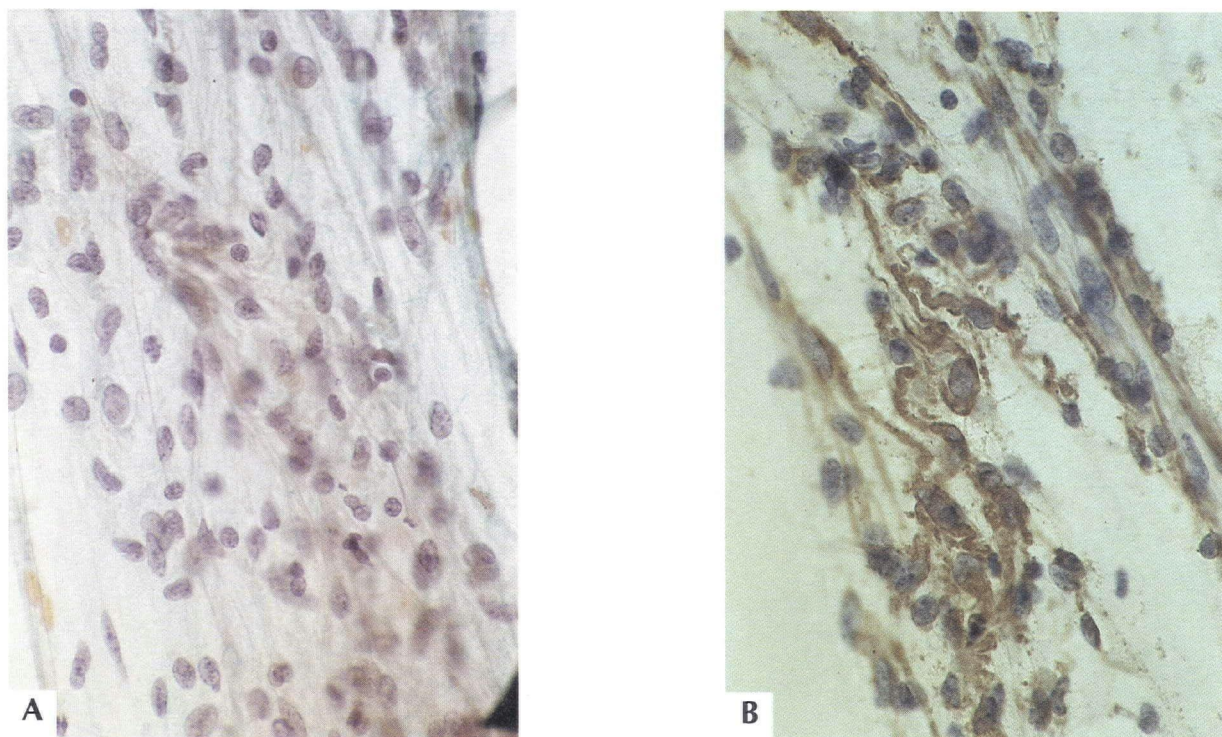
Fig 1. Case 1
Optic nerve glioma. (A) Note elongated cells with oblong-shaped nuclei and fibrillar cytoplasm. Panaicalaou-stained smear, 630X. (B) Brown intracytoplasmic granules from same specimen confirm presence of glial fibrillary acidic protein. Indirect avidin-biotin-peroxidase complex staining technique, 630X.
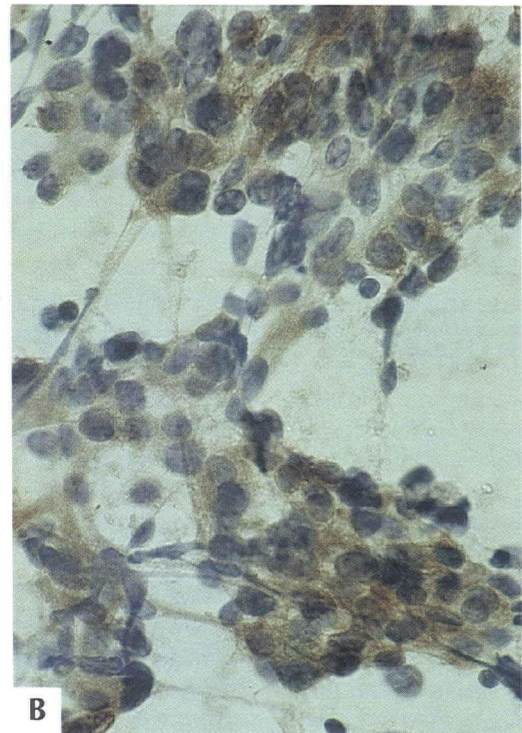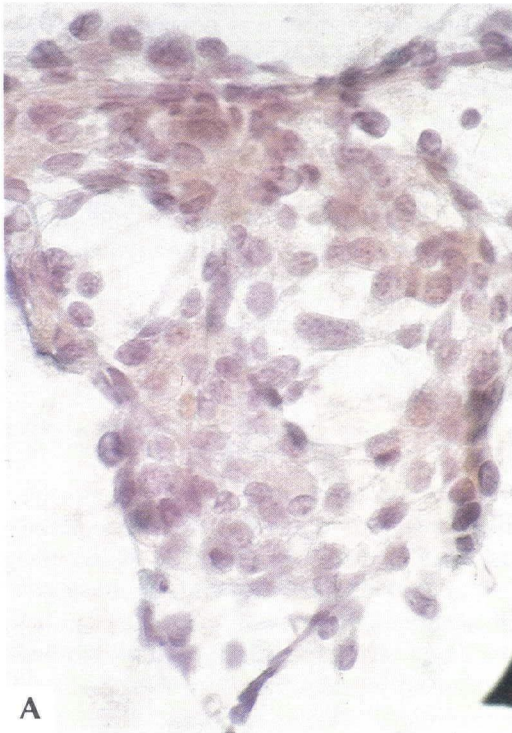
Fig 2. Case 3
Orbital metastasis from prostatic carcinoma. (A) Tissue fragment showing incomplete acinar arrangement of small cuboidal cells with vesicular nuclei having coarse chromatin micronucleoli. Papanicalaou-stained smear, 630X. (B) Brown intracytoplasmic granules from same specimen confirm presence of prostate specific antigen. Indirect avidin-biotin-peroxidase complex staining technique, 630X.
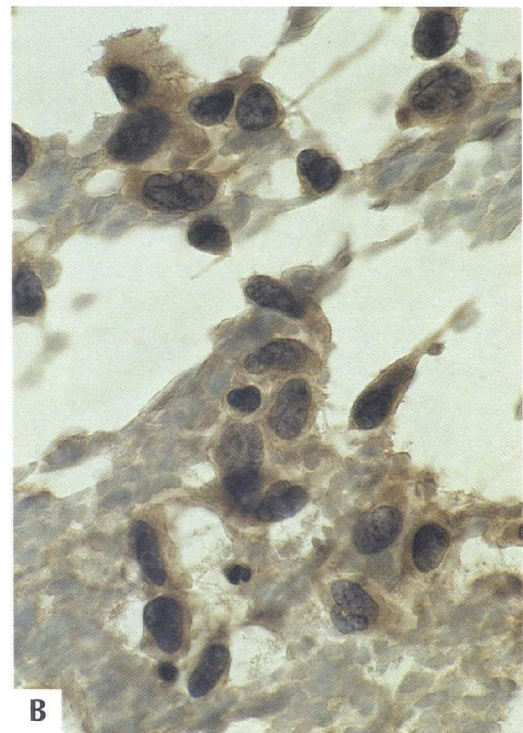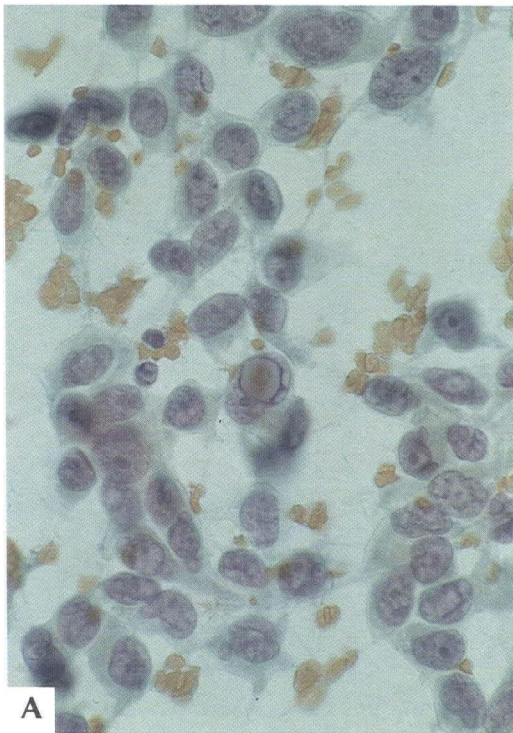

Fig 3. Case 4
Orbital metastasis from cutaneous malignant melanoma. (A) Population of isolated malignant cells with prominent nucleoli. Note absence of identifiable intracytoplasmic pigment. Papanicalaou-stained smear, 630X. (B) Same specimen shows coarse brown granules present in every malignant cell. Indirect avidin-biotin-peroxidase complex staining techniques, 630X.

sensitivity, reduced background staining, and stability of the essentially irreversible biotin-avidin complex (8).

Fluorescent histochemistry has also been applied to the diagnosis of orbital tumors. For example, the demonstration of steroid-hormone receptors in orbital metastasis from breast carcinoma may be of great value in directing further palliative therapy with hormonal therapy (14). Histochemical documentation of steroid-hormone receptors in orbital metastases has been achieved using cryostat sections and specialized fluorescence microscopic techniques. Obviously, many difficulties exist in maintaining cytologic specimens in a frozen state for fluorescent cytologic examination. The stability of immunoperoxidase reagents offers decided advantages for applications in cytologic diagnosis.

Glial fibrillary acidic protein is a subunit of intermediate-sized cytoplasmic filaments within astrocytes (8). Tissue specifically for normal, reactive, or neoplastic cells showing astrocytic differentiation has been demonstrated by immunohistochemical methods (15,16). The presence of glial fibrillary acidic protein has been detected in normal retina (17), retinoblastoma (18), and hemangioblastoma (19), but has not been extensively studied in extraocular orbital tissues.

Prostate specific antigen has been detected in normal, benign hypertrophic, and malignant prostatic tissues, but not in other human tissues (10). Using immunoperoxidase techniques, identification of prostate specific antigen was found to be 100% sensitive and specific in the diagnosis of prostatic carcinoma (20). The concurrent application of orbital fine-needle aspiration biopsy and immunoperoxidase staining for prostate specific antigen was recently used to confirm a diagnosis of carcinoma metastatic to the orbit (7). Another prostatic specific antigen, acid phosphatase isoenzyme 2, is not as sensitive or specific for prostate tissue as prostate specific antigen (21).

S-100 protein is present in a variety of tissues but is particularly associated with cells of glial and neural crest origin, including peripheral nerve sheath tumors, malignant melanoma, pigmented nevus, and carcinoid tumor (22).

Several types of tumors commonly found in the orbit fail to stain for S-100 protein, making this characteristic potentially useful in pathologic diagnosis.

A negative fine-needle aspiration biopsy is a characteristic of orbital tumors with a predominantly fibrous matrix where intercellular cohesion is strong and cellularity is diminished (2). A metastatic scirrhous carcinoma to the orbit is a classic example of an orbital tumor that may not be amenable to diagnosis by fine-needle aspiration biopsy (23). In debilitated patients or those refusing major surgery, a large bore-needle biopsy may be considered to obtain a cylinder of tissue that can be processed by standard histologic and immunoenzyme techniques.

Although fine-needle aspiration biopsy is less invasive and is associated with less morbidity than open orbital biopsy, cytologic confirmation is often necessary for planning treatment. Immunoenzyme methods may enhance our ability to provide histogenetic origins of an orbital mass. Interesting biochemical characteristics of neoplasms may also be revealed with these techniques (6,7).

This study combined orbital fine-needle aspiration biopsy and immunoperoxidase staining techniques and suggests the potential for applying immunocytologic methods to a wide variety of tumors. Due to the specialized techniques involved, useful clinical application of these methods requires the coordinated effort of the cytopathologist and ophthalmic pathologist in consultation with the ophthalmic surgeon, preferably prior to biopsy (2,4,5,7). Such a multidisciplinary approach combined with further advances in the field of immunohistochemistry may improve our ability to diagnose and manage malignant diseases affecting the orbit.

Acknowledgments

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