Reticulum stain for frozen-section diagnosis of pituitary adenomas

Technical note

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The authors describe a simple, quick, and reliable stain for use on frozen-section diagnosis of anterior pituitary microadenomas.

KEY WORDS • pituitary adenomas • hypophysectomy • frozen-section diagnosis • reticulum stain

Many of the clinical syndromes of hyperpituitarism, formerly thought to be due to diffuse hyperplasia of the gland, are now attributed to microadenomas 3 to 10 mm in diameter. Sensitive and specific assays for measurements of the anterior pituitary hormones and techniques of microsurgical exploration of the sella turcica permit an accurate diagnosis and effective treatment of these adenomas. Because the tumor tissue is in contiguity with normal gland and because it is sometimes difficult to differentiate them grossly, histological verification of the excised tissue is essential to insure complete and selective removal of the tumor. Continuous histopathological monitoring with frozen-tissue sections is necessary. We found that silver impregnation highlights the characteristic reticulin framework of normal and pathological tissues and facilitates their identification.

Method

The staining procedure is done on frozen sections mounted on glass slides as follows:

1. Oxidize in 10% phosphomolybdic acid solution for 30 to 40 seconds
2. Rinse in running tap water
3. Sensitize in 1.0% uranium nitrate for 30 seconds
4. Rinse in distilled water for 10 seconds
5. Place in ammoniacal silver solution for 30 seconds
6. Dip once in 95% alcohol
7. Place in reducing solution for 30 seconds (agitrate slide)
8. Rinse in distilled water
9. Tone in 0.2% gold chloride solution for 30 to 45 seconds
10. Rinse in distilled water
11. Place in 5% thiosulfate solution for 45 seconds
Reticulum stain for pituitary tumors

12. Rinse in running tap water
13. Dehydrate, clear, and mount.

Ammoniacal silver and reducing solutions should be freshly prepared. The total time required from section to mounting is less than 10 minutes.

Results

The adenohypophysis is composed of cords and clusters of cells intermingled with an extensive network of capillaries. Strands of reticulin fibers, particularly conspicuous around the blood vessels, extend among the cell nests, and by uniform branching form a delicate, regular meshwork interconnecting the capillaries. Silver impregnation of normal pituitary tissue outlines the supporting stroma of the gland and exposes it clearly (Fig. 1 left). In contrast, the adenomatous tissue exhibits a reticular framework of wider mesh and frequent fragmentation of the reticulin fibers (Fig. 1 right). The tumor cells lie in sheets or masses rather than in regular alveolar compartments. Pituitary tumors are markedly less vascularized than the normal pituitary. The method also demonstrates the nuclear membranes. The uniform, monotonous appearance of the outlined nuclei in the adenomas is an added help in diagnosis since cell nuclei in normal tissue are variable in size.

The growing tumor produces a collar-like compression of the native reticular framework like that seen in lymph nodes containing tumoral growths (Fig. 2). When included in the specimen, this feature is characteristic and renders the diagnosis almost unmistakable.

Comment

Simultaneous staining of frozen sections with hematoxylin and eosin and reticulum permits a clear-cut diagnosis of small fragments of tissue. The fibrovascular architecture is a cardinal feature in diagnosing adenomas. While it sometimes cannot be appreciated in routine hematoxylin and eosin preparations, it is markedly emphasized by silver impregnation. The greater thickness of
the frozen-tissue section reduces the clarity of
the image in hematoxylin and eosin prepara-
ations, while it increases the contrast of the
argyrophilic meshwork. The rapidity of the
staining procedure is another factor that
makes this method especially suitable for
diagnosing pituitary adenomas.

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Fig. 2. Anterior pituitary with microadenoma.
Formalin-fixed, paraffin-embedded section, reticulum
stain, × 80.