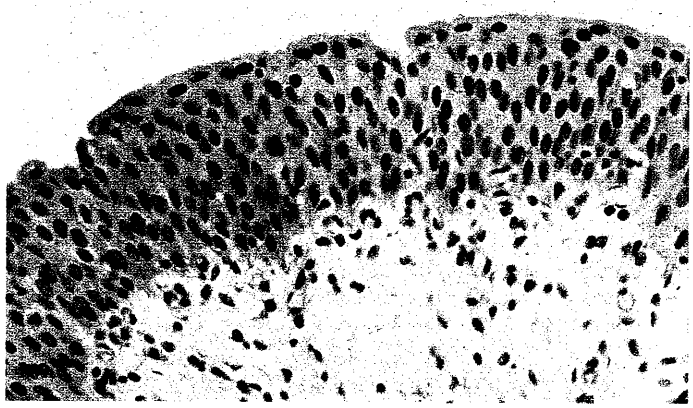
CHAPTER

ONE

SPECIFICITY
ONLY

PRINCIPLES OF IMMUNOHISTOCHEMISTRY

Lawrence D. True



to varying levels of illumination should be known. In contrast to the eye (and to photographic emulsions), which responds logarithmically to illumination, the response of digital cameras is closer to linearity (Inoue, *1986*).

Adequacy of the detection system for identifying a sought antigen is checked with a positive control. This is a section previously shown to contain the antigen, under identical conditions of immunohistochemistry. Selecting an adequate positive control can be difficult for antibodies to a new antigen; a negative immunostain of a tissue reported to contain the antigen cannot be fully interpreted and the distinction cannot be made between inactive antibody and inactive or absent antigen. In such a case, there is little choice but to immunostain other tissues reported to contain the antigen (at a range of antibody dilutions) or to return the antibody to the supplier.

Enhancement Methods

Diaminobenzidine reactivity can be enhanced by counterstaining with heavy metals such as osmium (Graham, *1966*), colloidal gold followed by silver, and nickel or cobalt (Figs. 1.29 and 1.30). Repeat bridges, ie, with subsequent peroxidase–antiperoxidase, also enhance detectability (Vacca, *1975*) (Fig. 1.31).

SPECIFICITY

Nonspecific immunoreactivity represents deposition of reaction product at a site other than the location of the desired antigen. Such false positivity can arise either from antibody binding or from nonantibody-specific binding.

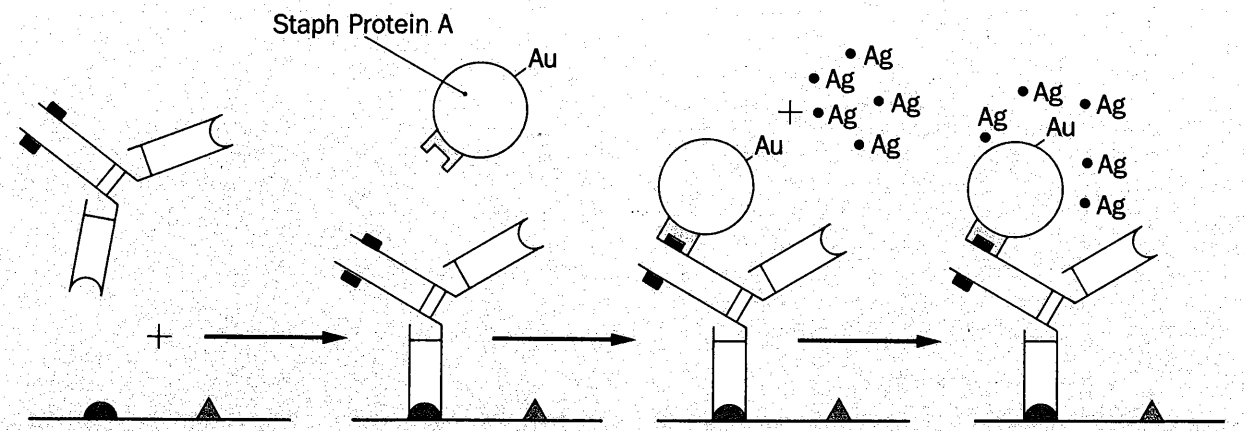


Figure 1.29. *Silver-enhanced, gold-labeled Staph Protein A immunohistochemistry. The primary antibody must contain a Staph Protein A-binding site, to which the Protein A–gold complex binds. Subsequent development of silver (Ag) deposits black reduced silver grains at the site of sought antigen A.*

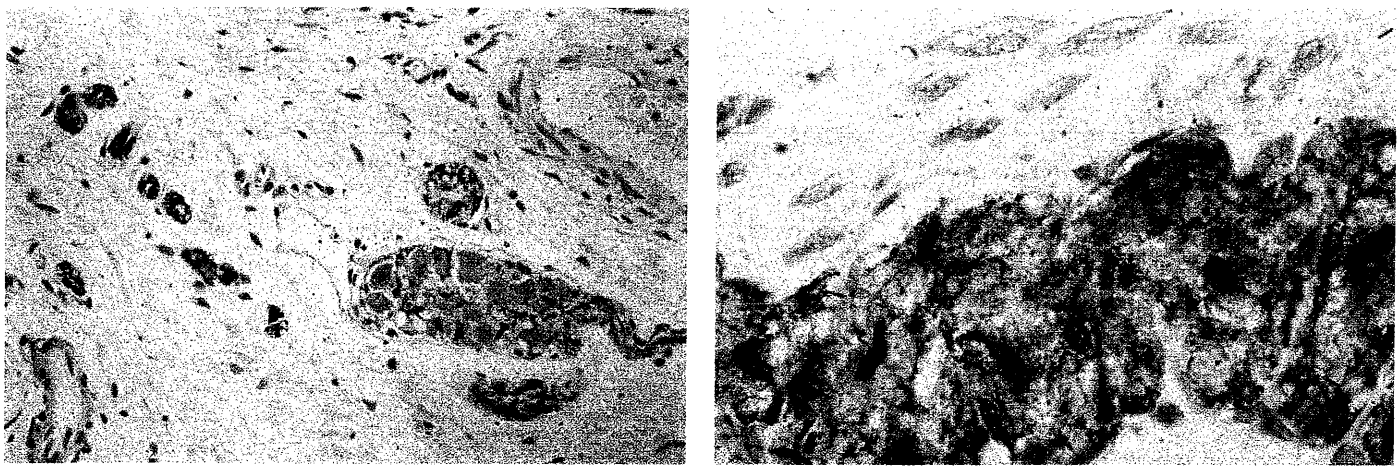


Figure 1.30. (Left) *Section of colon reacted with anti-S-100 protein, followed by Staph Protein A complex and silver. Granules of reduced silver stain nerve sheath cells in Auerbach's plexus black.* (Right) *Amplification of PAP staining. At higher magnification, the granular nature of the silver precipitate is seen. There is virtually no nonspecific binding.*

Immunologic Nonspecificity

Here, immunologic nonspecific reaction refers to the successful immunohistochemical localization of an antigen which provides a false result. Sources of such false positivity include the following.

Sequence Homology

Many molecules share partial amino acid identity, ie, gastrin/cholecystokinin, the intermediate filaments, α_1-AT/α_1-ACT, and S-100/calmodulin. Antibodies to the homologous sequence may localize both molecules. Unless the investigator is aware of both the partial potential antigenic identity of these two molecules and the specificity of the antibody to the common antigen determinant, she/he may falsely conclude that one molecule has been specifically localized. For example, localization of antineurofilament activity to nuclei could represent cross-reactivity with nuclear lamin, which is partially homologous with the intermediate cytoplasmic filaments (see Chapter 4) (Figs. 1.32 and 1.33).

Similar Antigenicity

There need not be amino acid identity for there to be antigenic similarity. For example, Leu-7 monoclonal antibody binds any proteins containing a certain carbohydrate group of restricted configuration, and some lupus antibodies bind to a phosphodiester epitope regardless of whether the epitope is on DNA or cardiolipin (Lafer, *1981*).

Contaminating Antibodies

The primary antibody preparation may contain various antibodies in addition to the one expected. If directed towards endogenous molecules, these are termed "autoantibodies." They may be present congenitally. Six percent of hybridomas from plasma cells of newborn mice produce autoantibodies, most frequently directed to such cytoskeletal proteins as tubulin and actin (Dighiero, *1985*). Virtually all adult humans contain antibodies to the 200,000 kd neurofilament protein; these immunohistochemically localize to neurons at 1:50 dilutions (Stefansson, *1985*). Apparently normal people have a 4% to 8% incidence of antivimentin and/or antikeratin antibodies.

Rabbits are known to have endogenous antikeratin antibody activity. That a polyclonal antiserum may be localizing an antigen other than the desired antigen was probably the explanation for reported immunostaining of epidermal cells and of proven squamous-cell carcinomas using a polyclonal anti-factor VIII-related antibody (Wilson, *1984*) (Fig. 1.34).

False positivity owing to contaminating or autoantibodies can be corrected by purifying antibody preparations. When pure antigen is available, affinity chromatography can remove spe-

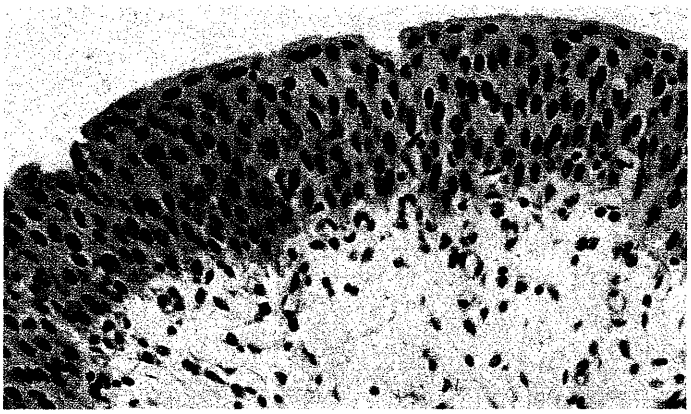


Figure 1.32. *Nuclei of urothelium localizing antineurofilament antibody. One explanation is that the nuclear lamin proteins of these urothelial nuclei share a specific epitope with neurofilament protein.* (DAB-PO; H-counterstained.)

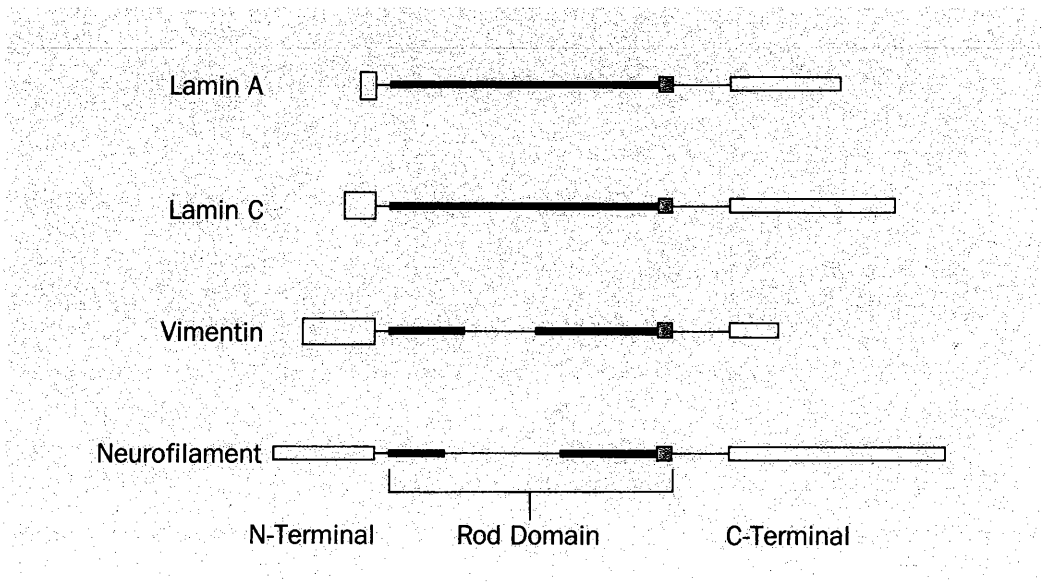


Figure 1.33. *Schematic of areas of extensive amino acid homology between the nuclear-membrane-associated proteins lamin A and lamin C. The cytoskeletal filaments vimentin and neurofilament protein are indicated in red. These four proteins differ at the amino and carboxy terminals. (McKeon, 1986).*

cific antibodies from a solution of multiple antibodies. When the specific antigen is unknown, the specific antibody cannot be isolated, but it can be concentrated using methods that isolate immunoglobulins, such as ammonium precipitation of immunoglobulins or separation with a Protein A column.

False positivity due to epitope identity cannot be corrected because successful antibody–antigen binding in these instances represents immunologic activity. Solutions of polyclonal antibodies containing multiple antigen-specific antibodies can be purified of antibodies that cross-react by affinity chromatography using the cross-reacting molecule as the immunoreactant. Only antibodies directed to unique epitopes will remain.

Nonimmunologic Nonspecificity

Many sources of non-antibody-binding false positivity exist. Fc portions of whole immunoglobulins may bind receptors of Fc phagocytes and mast cells (True, *1981*). Fc receptors are labile and readily inactivated by fixation. Complement-binding IgG molecules may localize to complement already present in tissue. (Buffa, *1979*). Complement is also labile. Certain antibodies and gut endocrine cells have an electrostatically mediated affinity that can mimic antibody–antigen reactivity (Grube, *1980*). Changes in the solution pH and salt concentration of reagents abolish this activity. Free aldehyde groups from incompletely reacted fixatives may nonspecifically bind antibodies to tissue (Farr, *1981*).

Neurohormonal peptides such as ACTH and vasoactive intestinal polypeptide have binding affinity for several reagents, including peroxidase-labeled immunoglobulin, Protein A, and streptavidin. Preincubation with excess ACTH(1-24) or use of poly-L-lysine in diluents will be corrective (Scopsi, *1986*b).

We have not suffered these sources of false positivity, which can be controlled for, in part, by using an irrelevant antibody as a positive control, and minimized with high dilutions of antibodies.

Endogenous Label Activity

Peroxidase. Hemoproteins with an iron porphyrin prosthetic group have peroxidase activity, which is highly variable and affected by factors that can be readily controlled in immunoperoxidase staining. The peroxidase activity of peroxidases in epithelial cells (mammary gland and secretory endometrium), megakaryocytes, and mast cells, catalase in liver, cytochrome C, myoglobin, and hemoglobin is suppressible by routine fixation and dehydration of tissues (Fahimi, *1979*; Escribano, *1987*).

The peroxidase activity in red cells, neutrophils, eosinophils, basophils, and histiocytes can be suppressed by multiple techniques that destroy or inactivate enzymatic activity (True, *1981*). (Fig. 1.35).

TUMORS IMMUNOSTAINED WITH ANTI-FACTOR VIII-RELATED ANTIGEN ANTIBODY

	Intensity of Immunostain		
Type of Tumor	**Strong**	**Weak**	**Absent**
Angiosarcoma	0	2	1
Squamous-cell carcinoma	3	3	0
Renal-cell carcinoma	2	0	1

Figure 1.34. *Number of cases of respective tumors immunostained with an anti-Factor VIII-related antigen antibody that also had anti-keratin activity. (From Wilson,* 1984.)

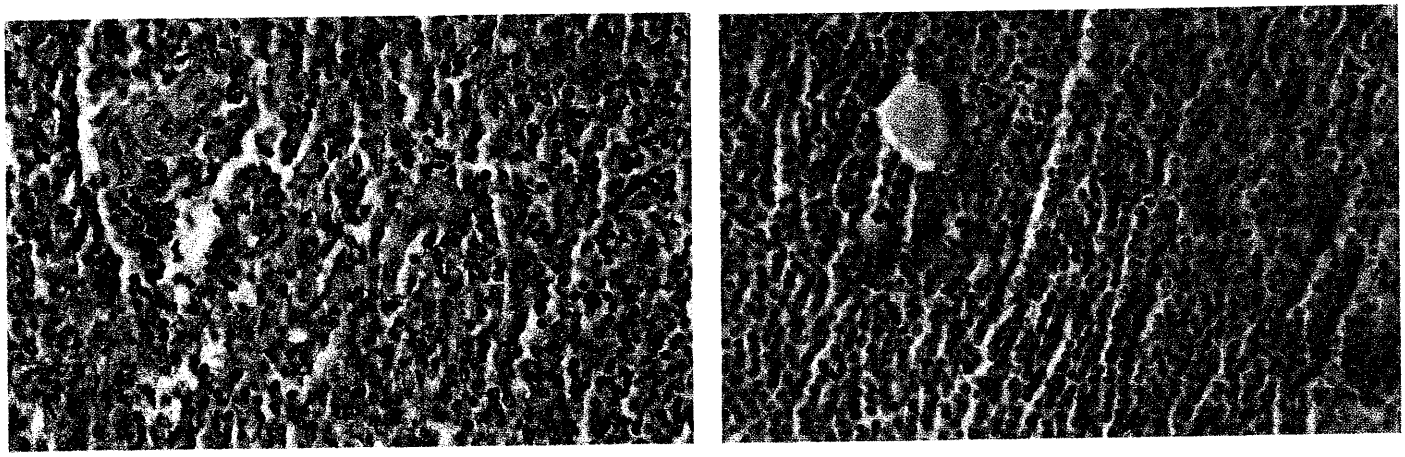


Figure 1.35. (Left) *Section of spleen incubated with antikeratin antibody. Reaction product is seen associated with red cells and neutrophils, because endogenous peroxidase activity was not suppressed.* (DAB-PO; H-counterstained.) (Right) *After endogenous peroxidase activity in an adjacent section of spleen is suppressed, red cells and neutrophils exhibit virtually no staining.* (DAB-PO; H-counterstained.)

Alkaline Phosphatase. The alkaline phosphatase activity present most intensely in epithelia of bladder, renal tubules, and small bowel, placental trophoblasts, neutrophils, and mast cells is suppressible with techniques that differ with the type of alkaline phosphatase. Because these methods may not suppress all activity, peroxidase may be preferred (Ponder, *1981*).

Biotin. Kidney, pancreas, and liver contain high concentrations of biotin, which may cause false localization of avidin–biotin complexes (Fig. 1.36). Preincubation with free avidin and biotin can block such binding (Wood, *1981*).

Label-Binding Activity

Peroxidase. Horseradish peroxidase has an affinity for cell membranes by two apparent mechanisms: a mannose-specific affinity and a calcium-dependent affinity for a cell-surface glycosyltransferase. These bindings are fixation-sensitive (Straus, *1987*).

Nonimmunologic binding of peroxidase to the hepatitis B virus may also depend on affinity between the carbohydrate components of both substances (Omata, *1980*). Furthermore, binding of cationic peroxidase conjugates to anionic sites in extracellular matrix is preventable by digestion of the negatively charged sites from the tissue or by neutralization of the positive charge of peroxidase (Pino, *1985*).

Avidin. Ionic binding of the basic residues of avidin to sulfate groups of heparin may give false localization of avidin–biotin complexes to mast cells (Fig. 11.37). Because this binding is dependent on a neutral environment, conducting the reaction in a high-pH environment will decrease or abolish such mast-cell granule binding.

Avidin also has an affinity for nuclei, which can be blocked with nonfat dry milk (Duhamel, *1985*). Avidin furthermore exhibits affinity for immunoblotted proteins, suppressible with high-salt buffers (Clark, *1986*).

Protein A. Protein A may bind to tissue immunoglobulins that retain Protein A-binding activity. Thus, Protein A is a poor label for immune complex localization.

Miscellaneous

Other localizations are poorly understood, such as localization to the edge of tissues, to necrotic tissue, and to stroma (Figs. 1.38 and 1.39).

Endogenous pigment may be confused with label, particularly lipochromes or melanin with DAB. A chromogen of contrasting color may be used instead of DAB (Figs. 1.40 and 1.41). Futhermore, the negative control section will demonstrate the distribution of such endogenous pigment.

The determination of specificity of antibody binding is a

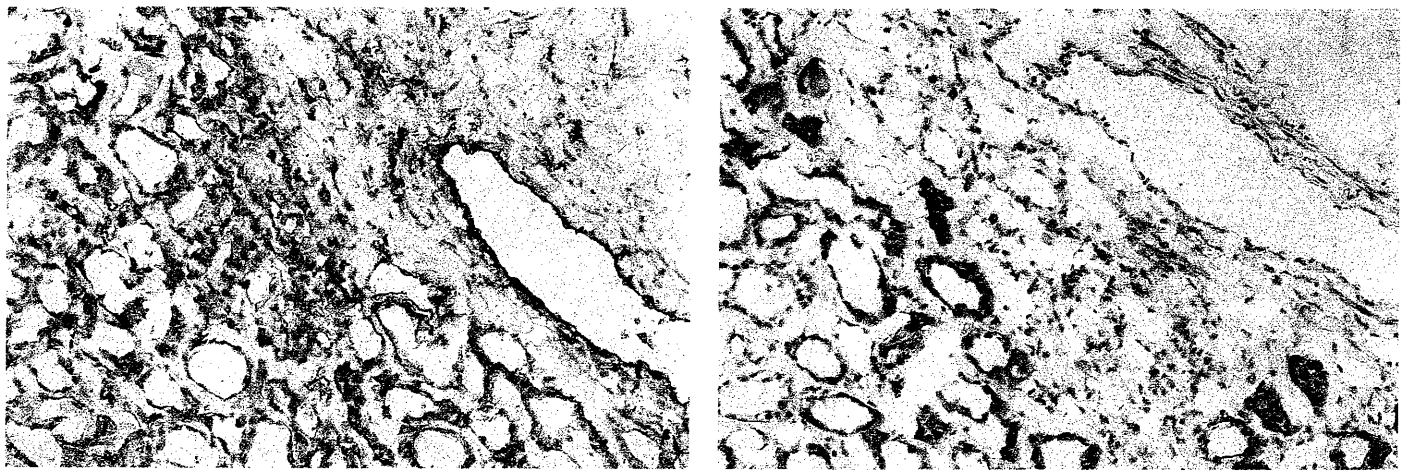
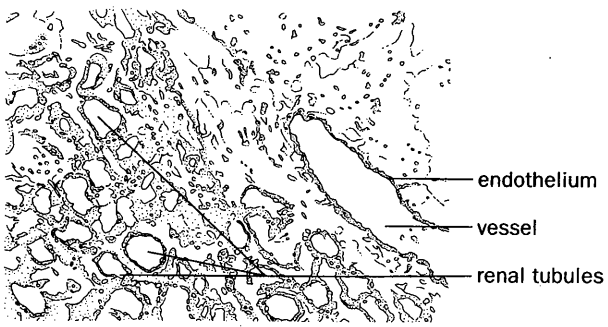


Figure 1.36. **(Left)** *Frozen section of kidney immunoreacted with anti-HLA-DR exhibits staining of both endothelial cells and tubules.* (DAB-PO; H-counterstained.) **(Right)** *An adjacent control frozen section in which the primary antibody has been omitted exhibits tubule staining. This negative control indicates that only the endothelial cells contained HLA-DR. The tubules stain because the endogenous biotin binds the avidin–biotin–peroxidase complex.*



very important aspect of assessing the results of an immune reaction. The following criteria of specificity should be satisfied (Swaab, *1975*; Petrusz, *1976*; Swaab, *1977*; Childs, *1983*; Petrusz, *1983*).

Preabsorption of the antibody with the antigen of interest should abolish immunoreactivity. This condition is insufficient because the immunogen may have contained impurities, the antigen may not fully neutralize antibody activity, or the antibody may cross-react with other molecules.

Substitution of non-immune-specific agents (ie, buffer or irrelevant antibody) for any of the immune reactions should abolish reactivity. This type of negative control will also identify nonimmunologic reactivity.

The immune reaction should not be sensitive to small changes in the physical–chemical environment of reaction (ie, changes in pH, salt concentration, temperature), duration of incubation steps, or type of embedding medium. This is a relative criterion, because antigens differ in their sensitivity to alterations in these conditions.

Antigenicity should be demonstrable by a different immunologic technique, such as an analysis of tissue homogenates by immunoassay.

Proof that a given molecule is present in a certain tissue ultimately rests on proof that the functional activity of that molecule is present.

One of the current limitations of immunohistochemistry as it is now practiced in diagnostic laboratories is the inability to satisfy all of these criteria of specificity. Therefore, labs must rely upon the suppliers of antibodies to supply only antibodies that are specific to the specified antigen or to supply data that detail nonspecific reactivity in the expected conditions.

The consequence of not controlling for nonspecificity has been exemplified by the reported nonspecific binding of anti-p21 antibodies (Samowitz, *1987*).

INTERPRETATION

Interpretation of an immunohistochemical stain takes into account the sources of false positivity and negativity discussed above. Good analysis of immunostains depends upon a final set of considerations.

Site of Synthesis

Localization of a substance to a given cell usually, but not always, indicates site of synthesis. For example, immunoreactive myoglobin can be localized to macrophages in the region of muscle necrosis (Eusebi, *1984*). Localization at the electron microscopic level to endoplasmic reticulum is strongly suggestive of site of synthesis, once the observer is satisfied there has not been artifactual displacement of reaction product (Novikoff, *1972*). Demonstration of uptake and incorporation of a radiolabeled amino acid into the substance by specific cell antigen, followed by release into the culture medium, is further important evidence.

Stable Expression

The assumption that cells of a certain histogenesis stably express certain antigens is not necessarily true. For example, the types of keratins synthesized depend upon the stage in the cell cycle (Franke, *1983*). Furthermore, mesothelial cells decrease their synthesis of certain keratins and increase their synthesis of vimentin and of other keratins under conditions of rapid growth, or when grown in suspension, either in ascites or in culture (Connell, *1983*; LaRocca, *1984*).

Homogeneity

The assumption that all cells of a tumor produce the same amount of a given antigen is inaccurate. For example, 1% to 100% of cells in squamous carcinomas of the lung contain identifiable immunoreactive keratin (Ramaekers, *1985*). Functional endocrine tumors are typically composed of a heterogeneous cell population, although usually only one hormone is produced in excess (Mukai, *1982*).

Nomenclature

"Positivity" for, say, keratin implies that all cells contain keratin. As pointed out above, keratin distribution may be heterogeneous. Furthermore, "keratin" is not a single protein or epitope but is, instead, a family of over 19 intermediate filament proteins. Thus, failure to detect keratin does not denote the absence of keratin but denotes, instead, the absence of the epitope detectable by the particular detection system. Proof of absence or nondetectability requires multiple methods, with consideration for the limitations of all methods.

The Set of Cells is Known

Assumptions that the specificity of a given substance for a given set of cells is known are not necessarily accurate. Lysozyme, α_1-antitrypsin, and α_1-antichymotrypsin are often referred to as markers of macrophages. Yet, many epithelial cells synthesize these proteins (see Chapter 7). And Leu-7, described as an anti-NK killer lymphocyte antibody, also reacts with prostate duct cells (Rusthoven, *1985*).

Even the significance of intermediate filament expression in histogenesis is uncertain. The assumptions that mesenchymal cells express vimentin and that epithelial cells express keratin are not always accurate. The spindle-cell component of some squamous carcinomas (Ellis, *1987*), and "epithelial" cells of some thyroid (Henzen-Logmans, *1987*), lung (Upton, *1986*), and renal-cell carcinomas (Herman, *1983*), may express vimentin. Furthermore, some subserosal stromal cells (Bolen, *1986*) and various sarcomas, eg, synovial sarcomas (Miettinen, *1984*), leiomyosarcomas (Miettenen, *1988*), and rhabdomyosarcomas (Colindre, *1988*), express keratin (Miettinen, *1984*).

Significance

A final question, yet unanswered, is whether tumor cells so morphologically undifferentiated as to be characterized only

REFERENCES

Amit AG et al: Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution. *Science* 1986;233:747–53.

Avrameas S: Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry* 1969;6:43–52.

Banks-Schlegal SP et al: Keratin proteins in human lung carcinomas. Combined use of morphology, keratin immunocytochemistry, and keratin immunoprecipitation. *Am J Pathol* 1984;114:273–86.

Battifora H, Kopinski MI: Distinction of mesothelioma from adenocarcinoma. *Cancer* 1985;55:1679–85.

Battifora H, Kopinski M: The influence of protease digestion and duration of fixation on the immunostaining of keratins. *J Histochem Cytochem* 1986;34:1095–1100.

Benno RH et al: Quantitative immunocytochemistry of tyrosine hydroxylase in rat brain. I. Development of a computer assisted method using peroxidase-antiperoxidase technique. *Brain Res* 1982;246:225–36.

Berson SA, Yalow RS: Kinetics of reaction between insulin and insulin-binding antibody. *J Clin Invest* 1957;36:873.

Bigbee JW et al: Effects of primary antiserum dilution on staining of "antigen-rich" tissues with peroxidase–antiperoxidase technique. *J Histochem Cytochem* 1977;25:443–47.

Bolen JW et al: Reactive and neoplastic serosal tissue. A light-microscopic, ultrastructural, and immunocytochemical study. *Am J Surg Pathol* 1986;10:34–7.

Bosman FT et al: Efficiency and sensitivity of indirect immunoperoxidase methods. *Histochemistry* 1983;77:185–94.

Bosman FT, Kruseman ACN: Clinical applications of the enzyme labeled antibody method. *J Histochem Cytochem* 1979;27:1140–7.

Brandon C: Improved immunocytochemical staining through the use of Fab fragments of primary antibody, Fab-specific second antibody, and Fab-horseradish peroxidase. *J Histochem Cytochem* 1985;33:715–9.

Brandtzaeg P: Prolonged incubation time in immunohistochemistry: Effects on fluorescence staining of immunoglobulins and epithelial components in ethanol- and formaldehyde-fixed paraffin-embedded tissues. *J Histochem Cytochem* 1981;29:1302–15.

Buffa R et al: Complement-mediated binding of immunoglobulins to some endocrine cells of the pancreas and gut. *J Histochem Cytochem* 1979;27:1279–80.

Bullock GR: The current status of fixation for electron microscopy. A review. *J Microsc* 1984;133:1–15.

Childs GV: The use of multiple methods to validate immunocytochemical stains. *J Histochem Cytochem* 1983;31:168–76.

Ciocca DR et al: Intensification of the immunocytochemical reaction by staining both sides of tissue sections. *J Histochem Cytochem* 1987;35:257–60.

Clark CA et al: An unlabeled antibody method using glucose oxidase-antiglucose oxidase complexes (GAG). *J Histochem Cytochem* 1982;30:27–34.

Clark RK et al: Suppression of nonspecific binding of avidin–biotin complex (ABC) to proteins electroblotted to nitrocellulose paper. *J Histochem Cytochem* 1986;34:1509–12.

Colindre J-M et al: Immunohistochemical study of rhabdomyosarcoma. Unexpected staining with S100 protein and cytokeratin. *J Pathol* 1988;155:127–32.

Connell ND, Rheinwald JG: Regulation of the cytoskeleton in mesothelial cells: Reversible loss of keratin and increase in vimentin during rapid growth in culture. *Cell* 1983;34:245–53.

Coons AH: Histochemistry with labeled antibody. *Int Rev Cytol* 1956;5:1–23.

Cooper D et al: Classification of human epithelia and their neoplasms using monclonal antibodies to keratins: Strategies, applications, and limitations. *Lab Invest* 1985;52:243.

Cordell JL et al: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal antialkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984;32:219–29.

Dighiero G et al: High frequency of natural autoantibodies in normal newborn mice. *J Immunol* 1985;134:765–71.

Duhamel RC et al: Use of non-fat dry milk to block non-specific nuclear and membrane staining by avidin corjugates. *J Histochem Cytochem* 1985;33:711–4.

Ellis GL et al: Spindle-cell carcinoma of the aerodigestive tract. *Am J Surg Pathol* 1987;11:335–42.

Escribano LM et al: Endogenous peroxidase activity in human cutaneous and adenoidal mast cells. *J Histochem Cytochem* 1987;35:215–20.

Eusebi V et al: Immunohistochemical localization of myoglobin in non-muscular cells. *Am J Surg Pathol* 1984;8:51–5.

Fahimi HD: An assessment of the DAB methods for cytochemical detection of catalase and peroxidase. *J Histochem Cytochem* 1979;27:1365–6.

Falini B et al: Protein A-peroxidase conjugates for two-stage immunoenzyme staining of intracellular antigens in paraffin-embedded tissues. *J Immunol Meth* 1980;39:111–20.

Feldmann G et al: Morphological aspects of plasma protein synthesis and secretion by the hepatic cells. *Int Rev Cytol* 1985;96:157–89.

Fox CH et al: Formaldehyde fixation. *J Histochem Cytochem* 1985;33:845–53.

Franke WW et al: Change of cytokeratin filament organizaion during the cell cycle: Selective masking of an immunologic determinant in interphase PtK2 cells. *Cell* 1983;97:1255–60.

Gaasbeek Janzen JW et al: Development of the heterogeneous distribution of carbamoyl-phosphate synthetase (ammonia) in rat-liver parenchyma during postnatal development. *J Histochem Cytochem* 1985;33:1205–11.

Getzoff ED et al: Mechanisms of antibody binding to a protein. *Science* 1987;235:1191–6.

Geysen HM et al: Chemistry of antibody binding to a protein. *Science* 1987;235:1184–90.

Goding JW: Use of staphylococcal protein A as an immunological reagent. *J Immunol Meth* 1978;20:241–53.

Goudswaard J et al: Protein A reactivity of various mammalian immunoglobulins. *Scand J Immunol* 1978;8:21–8.

Grace MP et al: Keratin expression in normal esophageal epithelium and squamous cell carcinoma of the esophagus. *Cancer Res* 1985;45:841–6.

Graham RC, Karnovsky MJ: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. *J Histochem Cytochem* 1966;14:291–302.

Griffiths G et al: Quantitation in immunocytochemistry: Correlation of immunogold labeling to absolute number of membrane antigens. *J Histochem Cytochem* 1986;34:1389–98.

Gross DS, Rothfeld JM: Quantitative immunocytochemistry of hypothalamic and pituitary hormones: Validation of an automated, computerized image analysis system. *J Histochem Cytochem* 1985;33:11–20.

Grube D: Immunoreactivities of gastrin (G-) cells. II. Non-specific binding of immunoglobulins to G-cells by ionic interactions. *Histochemistry* 1980;66:149–67.

Guesdon JL et al: The use of avidin-biotin interaction in immunoenzymatic techniques. *J Histochem Cytochem* 1979;27:1131–9.

Hand PH et al: Monoclonal antibodies of predefined specificity detect activated ras gene expression in human mammary and colon carcinomas. *Proc Natl Acad Sci USA* 1984;81:5227–31.

Hearn SA et al: Immunoelectron microscopic labeling of immunoglobulin in plasma cells after osmium fixation and epoxy

embedding. *J Histochem Cytochem* 1985;33:1212–18.
Henzen-Logmans SC et al: Expression of cytokeratins and vimentin in epithelial cells of normal and pathologic thyroid tissue. *Virchows Arch* [*A*] 1987;410:347–54.
Herman CJ et al: Is renal cell (Grawitz) tumor a carcinosarcoma? *Virchows Arch* [*B*] 1983;44:73–83.
Hopwood D: Some aspects of fixation with glutaraldehyde. *J Anat* 1967;101:83–92.
Hixson DC et al: Evaluation of periodate/lysine/paraformaldehyde fixation as a method for cross-linking plasma membrane glycoproteins. *J Histochem Cytochem* 1981;29:561–6.
Hsu SM et al: The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques. A comparison between ABC and unlabeled antibody PAP procedures. *J Histochem Cytochem* 1981;29:577–80.
Inoue S: *Video Microscopy*. New York & London, Plenum Press, 1986.
Jacobsen M et al: The effect of fixation and trypsinization on the immunohistochemical demonstration of intracellular immunoglobulin in paraffin embedded material. *Acta Pathol Microbiol Scand* [*A*] 1980;88:369–76.
Jasiewicz ML et al: Selective retrieval of biotin-labeled cells using immobolized avidin. *Exp Cell Res* 1976;100:213–7.
Kims S et al: Antibody diversity: Somatic hypermutation of rearranged Vh genes. *Cell* 1981;27:573–81.
King WJ, Greene GL: Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. *Nature* 1984;307:745–7.
Kohler G: Derivation and diversification of monoclonal antibodies. *Science* 1986;233:1281–6.
Kohler G, Milstein C: Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495–7.
Kraehenbuhl J-P et al: Immunocytochemical localization of secretory proteins in bovine pancreatic exocrine cells. *J Cell Biol* 1977; 72:406.
Lafer EM et al: Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J Exp Med* 1981;153:897–909.
LaRocca PJ, Rheinwald JG: Coexpression of simple epithelial keratins and vimentin by human mesothelium and mesothelioma in vivo and in culture. *Cancer Res* 1984;44:2991–9.
Lauriola L et al: Detection of S-100 labelled cells in nasopharyngeal carcinoma. *J Clin Pathol* 1984;37:1235–8.
Lippman SM et al: The prognostic and biological significant of cellular heterogeneity in medullary thyroid carcinoma: A study of calcitonin. 1-dopa decarboxylase, and histaminase. *J Clin Endocrinol Metab* 1982;54:233–40.
Livesey SA et al: Cryofixation taking on a new look. *Nature* 1987; 327:255–6.
Mason DY, Sammons R: Alkaline phosphatase and peroxidase for double immunoenzymatic labelling of cellular constituents. *J Clin Pathol* 1978;31:454–60.
Mays ET et al: Determination of protein loss during aqueous and phase partition fixation using formalin and glutaraldehyde. *J Histochem Cytochem* 1984;32:1107–12.
McCarty KS et al: Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med* 1985;109:716–21.
McKeon FD et al: Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. *Nature* 1986;319:463–8.
Miettinen M: Immunoreactivity for cytokeratin and epithelial membrane antigen in leiomyosarcoma. *Arch Pathol Lab Med* 1988; 112:637–40.
Miettinen M, Virtanen I: Synovial sarcoma—a misnomer. *Am J Pathol* 1984;117:18–25.
Moll R et al: The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumors, and cultured cells. Cell 1982;31:11–24.
Moyle WR et al: Quantitative explanation for increased affinity shown by mixtures of monoclonal antibodies: Importance of a circular complex. *Mol Immunol* 1983;20:439–452.
Mukai K et al: Retrospective study of 77 pancreatic endocrine tumors using the immunoperoxidase method. *Am J Surg Pathol* 1982; 6:387–99.
Nakane P: Recent progress in the peroxidase-labeled antibody method. *Ann NY Acad Sci* 1975;254:203.
Nakane PK: Simultaneous localization of multiple tissue antigens using the peroxidase-labeled antibody method: A study on pituitary glands of the rat. *J Histochem Cytochem* 1968;16:557–60.
Novikoff A et al: Diffusion artifacts in 3, 3'-diaminobenzidine cytochemistry. *J Histochem Cytochem* 1972;20:745–9.
Omata M et al: Nonimmunologic binding of horseradish peroxidase to hepatitis B surface antigen. *Am J Clin Pathol* 1980;73:626–32.
Petergy F et al: Affinity requirements for antibody assays mapped by monoclonal antibodies. *J Immunol* 1983;130:1809–13.
Petrusz P: Essential requirements for the validity of immunocytochemical staining procedures. *J Histochem Cytochem* 1983; 31:176–9.
Petrusz P et al: Specificity in immunocytochemical staining. *J Histochem Cytochem* 1976;10:1110–2.
Pino RM: Binding of Fab-horseradish peroxidase conjugates by charge and not by immunospecificity. *J Histochem Cytochem* 1985;33:55–8.
Ploem JS: A study of filters and light sources in immunofluorescence microscopy. *Ann NY Acad Sci* 1971;177:414–29.
Ponder BA, Wilkinson MM: Inhibition of endogenous tissue alkaline phosphatase with the use of alkaline phosphatase conjugates in immunohistochemistry. *J Histochem Cytochem* 1981;29:981–4.
Posthuma G et al: Usefulness of the immunogold technique in quantitation of a soluble protein in ultra-thin sections. *J Histochem Cytochem* 1987;35:405–10.
Posthuma G et al: Immunocytochemical assays of amylase and chymotrypsinogen in rat pancreas secretory granules. Efficacy of using immunogold-labeled ultrathin cryosections to estimate relative protein concentrations. *J Histochem Cytochem* 1984;32:1028–34.
Ramaekers RCS et al: Intermediate filament proteins in the study of tumor heterogeneity: An in-depth study of tumors of the urinary and respiratory tracts. *Ann NY Acad Sci* 1985;455:614–34.
Rebek J: Model studies in molecular recognition. *Science* 1987; 235:1478–83.
Rennke HG, Venkatachalam MA: Chemical modification of horseradish peroxidase. Preparation and characterization of tracer enzymes with different isoelectric points. *J Histochem Cytochem* 1979;27:1352–3.
Rode J et al: Neuron specific enolase and S-100 protein as possible prognostic indicators in melanoma. *Histopathology* 1984;8:1041–52.
Roitt IM et al: *Immunology*. London, New York, Gower Medical Publishing, 1985.
Rusthoven JJ et al: The natural-killer-cell-associated HNK-1 (Leu-7) antibody reacts with hypertrophic and malignant prostatic epithelium. *Cancer* 1985;56:289–93.
Rygaard J, Olsen W: Determination of characteristics of interference filters. *Ann NY Acad Sci* 1971;177:430–3.
Samowitz WS et al: Reported binding of monoclonal antibody RAP-5 to formalin-fixed tissue sections is not indicative of ras p21 expression. *Hum Pathol* 1988;19:127–32.
Schipper J, Tilders FJH: A new technique for studying specificity of immunocytochemical procedures. Specificity of serotonin immunostaining. *J Histochem Cytochem* 1983;31:12–8.
Scopsi L, Larsson L-I: Increased sensitivity in peroxidase immunocytochemistry. A comparative study of a number of peroxidase visualization methods employing a model system. *Histochemistry* 1986a;84:221–30.

Scopsi L et al: Nonspecific immunocytochemical reactions with certain neurohormonal peptides and basic peptide sequences. *J Histochem Cytochem* 1986b;34:1469–75.
Stefansson K et al: Circulating autoantibodies to the 200,000-dalton protein of neurofilaments in the serum of healthy individuals. *Science* 1985;228:1117–9.
Sternberger LA et al: The unlabeled antibody-enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antiperoxidase). *J Histochem Cytochem* 1970;18:315–33.
Sternberger LA, Petrali JP: Quantitative immunocytochemistry of pituitary receptors for luteinizing hormone-releasing hormone. *Cell Tissue Res* 1975;162:141–76.
Sternberger LA, Sternberger NH: The unlabeled antibody method: Comparison of peroxidase–antiperoxidase with avidin–biotin complex by a new method of quantification. *J Histochem Cytochem* 1986;34:599–605.
Sternberger LA, Sternberger NH: Monclonal antibodies distinguish phosphorylated and nonphosphorylated forms of neurofilaments in situ. *Proc Natl Acad Sci USA* 1983;80:6126–30.
Strauss W: Unusual binding sites for horseradish peroxidase may contribute to "background" adsorption of the enzyme. *J Histochem Cytochem* 1987;35:703–4.
Swaab DF et al: Can specificity ever be proved in immunocytochemical staining? *J Histochem Cytochem* 1977;25:388–91.
Swaab DF, Pool CW: Specificity of oxytocin and vasopressin immunofluorescence. *J Endocrinol* 1975;66:263–72.
Tokuyasu KT: Immunohistochemistry on ultrathin frozen sections. *Histochem J* 1980;12:381–403.
True LD: Quantitative immunohistochemistry: A new tool for surgical pathology? *Am J Clin Pathol* 1988;90:324–5.
True LD, Nakane PK: Immunoelectronmicroscopy, in *Current Trends in Morphological Techniques 1981,* Vol III. *Uniscience Series: Methods in Aging Research:* Boca Raton, FL, CRC Press, pp
Upton MP et al: Expression of vimentin in surgically resected adenocarcinomas and large cell carcinomas of lung. *Am J Surg Pathol* 1986;10:560–7.
Vacca LL et al: Application of immunoperoxidase techniques to localize horseradish peroxidase tracer in the central nervous system. *J Histochem Cytochem* 1975;23:208–15.
Wabl MR: Electron microscopic localization of two proteins on the surface of the 50S ribosomal subunit of Escherichia coli using specific antibody markers. *J Mol Biol* 1974;84:241–7.
Warnke R: Alteration of immunoglobulin-bearing lymphoma cells by fixation. *J Histochem Cytochem* 1979;27:1195–6.
Weisburger EK et al: Testing of twenty-one environmental aromatic amines or derivates for long-term toxicity or carcinogenicity. *J Environ Pathol Toxicol* 1981;2:325–56.
Welinder KG: Amino acid sequence studies of horseradish peroxidase. *Eur J Biochem* 1979;96:483–502.
Wicker R: Comparison of immunofluorescent and immunoenzymatic techniques applied to the study of viral antigens. *Ann NY Acad Sci* 1971;177:490–500.
Wilson AJ: Factor VIII-related antigen staining by immunoperoxidase technic in smaller laboratories: A potential problem. *Am J Clin Pathol* 1984;81:117–20.
Wood GS, Warnke R: Suppression of endogenous avidin-binding activity in tissues and its relevance to biotin-avidin detection systems. *J Histochem Cytochem* 1981;29:1196.

ISBN 0-397-44658-6

Library of Congress Cataloging-in-Publication Data
Atlas of diagnostic immunohistopathology / edited by Lawrence D. True ; foreword by Juan Rosai.
p. cm.
Includes bibliographies and index.
ISBN 0-397-44658-6
1. Diagnostic immunohistochemistry—Atlases. I. True, Lawrence. [DNLM: 1. Immunohistochemistry—atlases. 2. Immunologic Techniques—atlases. QY 17 A881]
RB46.6.A85 1990
616.07'583—dc20
DNLM/DLC
for Library of Congress 88-81429
CIP

Editor Sharon Rule, Kristin Robie
Designer Carol Drozdyk
Illustrator Alan Landau
Art Director Jill Feltham

10 9 8 7 6 5 4 3 2 1

Printed in Singapore by Imago Productions (FE) PTE, Ltd.

Distributed in USA and Canada by:
J.B. Lippincott Company
East Washington Square
Philadelphia, PA 19105 USA

Distributed in UK and Continental Europe by:
Harper & Row Ltd.
Middlesex House
34-42 Cleveland Street
London W1P 5FB UK

Distributed in Australia and New Zealand by:
Harper & Row (Australasia) Pty Ltd.
P.O. Box 226
Artarmon, N.S.W. 2064, Australia

Distributed in Southeast Asia, Hong Kong, India and Pakistan by:
Harper & Row Publishers (Asia) Pte Ltd.
37 Jalan Pemimpin 02-01
Singapore 2057

Distributed in Japan by:
Igaku Shoin Ltd.
Tokyo International
P.O. Box 5063
Tokyo, Japan