TABLE 2-7 -- Examples of Diseases with Granulomatous Inflammations

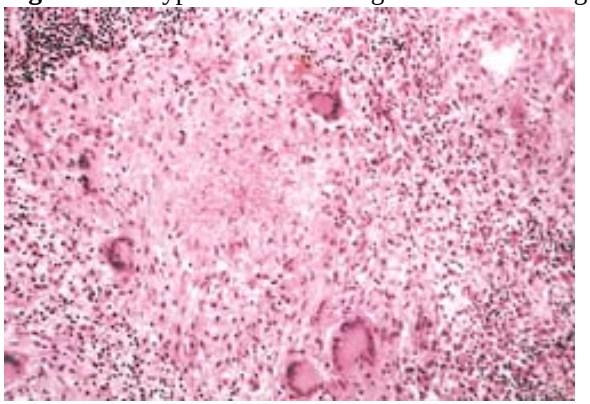
Disease	Cause	Tissue Reaction
Tuberculosis	*Mycobacterium tuberculosis*	Noncaseating tubercle (granuloma prototype): a focus of epithelioid cells, rimmed by fibroblasts, lymphocytes, histiocytes, occasional Langhans giant cell; caseating tubercle: central amorphous granular debris, loss of all cellular detail; acid-fast bacilli
Leprosy	*Mycobacterium leprae*	Acid-fast bacilli in macrophages; non-caseating granulomas
Syphilis	*Treponema pallidum*	Gumma: microscopic to grossly visible lesion, enclosing wall of histiocytes; plasma cell infiltrate; central cells are necrotic without loss of cellular outline
Cat-scratch disease	Gram-negative bacillus	Rounded or stellate granuloma containing central granular debris and recognizable neutrophils; giant cells uncommon

fibroblasts and connective tissue. Frequently, epithelioid cells fuse to form *giant cells* in the periphery or sometimes in the center of granulomas. These giant cells may attain diameters of 40 to 50 µm. They have a large mass of cytoplasm containing 20 or more small nuclei arranged either peripherally (Langhans-type giant cell) or haphazardly (foreign body-type giant cell) (Fig. 2-33). There is no known functional difference between these two types of giant cells, a fact that does not deter students from remembering the morphologic differences!

There are two types of granulomas, which differ in their pathogenesis. *Foreign body granulomas* are incited by relatively inert foreign bodies. Typically, foreign body granulomas form when material such as talc (associated with intravenous drug abuse) (Chapter 9), sutures, or other fibers are large enough to preclude phagocytosis by a single macrophage and do not incite any specific inflammatory or immune response. Epithelioid cells and giant cells form and are apposed to the surface and encompass the foreign body. The foreign material can usually be identified in the center of the granuloma, particularly if viewed with polarized light, in which it appears refractile.

Immune granulomas are caused by insoluble particles, typically microbes, that are capable of inducing a cell-mediated immune response (Chapter 6). This type of immune response does not necessarily produce granulomas but it does so when

Figure 2-33 Typical tuberculous granuloma showing an area of central necrosis, epithelioid cells, multiple Langhans-type giant cells, and lymphocytes.



References

1. Diamond J: Guns, Germs and Steel: The Fates of Human Societies. WW Norton, New York.

2. Majno G: The Healing Hand: Man and Wound in the Ancient World. Cambridge, MA: Harvard University Press, 1975.

3. Weissman G: Inflammation: historical perspectives. In Gallin JI, et al (eds.): Inflammation: Basic Principles and Clinical Correlates. New York: Raven Press, 1992, p 5.

4. Hunter J: A Treatise of the Blood, Inflammation, and Gunshot Wounds, Vol. 1. London: J. Nicoli, 1794.

5. Cohnheim J: Lectures in General Pathology (Translated by AD McKee, from the second German edition, Vol 1). London: New Sydenham Society, 1889.

6. Heifets L: Centennial of Metchnikoff's discovery. J Reticuloendothel Soc 31:381, 1982.

7. Majno G, Palade GE: Studies on inflammation: I. the effect of histamine and serotonin on vascular permeability: an electron microscopic study. J Biophys Biochem Cytol 11:571, 1961.

8. McDonald DM, Thurston G, Baluk P: Endothelial gaps as sites for plasma leakage in inflammation. Microcirculation 6:7, 1999.

9. Majno G, et al: Studies on inflammation: II. the site of action of histamine and serotonin along the vascular tree: a topographic study. J Biophys Biochem Cytol 11:607, 1961.

10. Lampugnani MG, Dejana E: Interendothelial junctions: structure, signalling and functional roles. Curr Opin Cell Biol 9:674, 1997.

11. van Hinsbergh VW, van Nieuw Amerongen GP: Intracellular signalling involved in modulating human endothelial barrier function. J Anat 200:549, 2002.

12. Lentsch AB, Ward PA: Regulation of inflammatory vascular damage. J Pathol 190:343, 2000.

13. Dvorak AM, Feng D: The vesiculo-vacuolar organelle (VVO). A new endothelial cell permeability organelle. J Histochem Cytochem 49:419, 2001.

14. Ferrara N: Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Semin Oncol 29:10, 2002.

15. Bates DO, et al: Regulation of microvascular permeability by vascular endothelial growth factors. J Anat 200:581, 2002.

16. Muller WA: Leukocyte-endothelial cell interactions in the inflammatory response. Lab Invest 82:521, 2002.

---

17. Luscinskas FW, et al: Leukocyte transendothelial migration: a junctional affair. Semin Immunol 14:105, 2002.

18. Gonzalez-Amaro R, Sanchez-Madrid F: Cell adhesion molecules: selectins and integrins. Crit Rev Immunol 19:389, 1999.

19. McEver RP: Selectins: lectins that initiate cell adhesion under flow. Curr Opin Cell Biol 14:581, 2002.

20. Hynes RO: Integrins: bidirectional, allosteric signaling machines. Cell 110:673, 2002.

21. Johnston B, Butcher EC: Chemokines in rapid leukocyte adhesion triggering and migration. Semin Immunol 14:83, 2002.

22. Muller WA: Migration of leukocytes across endothelial junctions: some concepts and controversies. Microcirculation 8:181, 2001.

23. Etzioni A, Doerschuk CM, Harlan JM: Of man and mouse: leukocyte and endothelial adhesion molecule deficiencies. Blood 94:3281, 1999.

24. Bunting M, et al: Leukocyte adhesion deficiency syndromes: adhesion and tethering defects involving beta 2 integrins and selectin ligands. Curr Opin Hematol 9:30, 2002.

25. Cotran RS, Mayadas TN: Endothelial adhesion molecules in health and disease. Pathol Biol 46:164, 1998.

26. Cicchetti G, Allen PG, Glogauer M: Chemotactic signaling pathways in neutrophils: from receptor to actin assembly. Crit Rev Oral Biol Med 13:220, 2002.

27. Jones GE: Cellular signaling in macrophage migration and chemotaxis. J Leukoc Biol 68:593, 2000.

28. Stossel TP, et al: Cell crawling two decades after Abercrombie. Biochem Soc Symp 65:267, 1999.

29. Rickert P, et al: Leukocytes navigate by compass: roles of PI3Kγ and its lipid products. Trends Cell Biol 10:466, 2000.

30. Underhill DM, Ozinsky A: Phagocytosis of microbes: complexity in action. Annu Rev Immunol 20:825, 2002.

31. Nathan C, Shiloh MU: Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci U S A 97:8841, 2000.

32. Babior BM, Lambeth JD, Nauseef W: The neutrophil NADPH oxidase. Arch Biochem Biophys 397:342, 2002.

33. Hampton MB, Kettle AJ, Winterbourn CC: Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. Blood 92:3007, 1998.

34. Risso A: Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity. J Leukoc Biol 68:785, 2000.

35. Lehrer RI, Ganz T: Defensins of vertebrate animals. Curr Opin Immunol 14:96, 2002.

36. Jaeschke H, Smith CW: Mechanisms of neutrophil-induced parenchymal injury. J Leukoc Biol 61:647, 1997.

37. Tapper H: The secretion of preformed granules by neutrophils and macrophages. Curr Opin Immunol 59:613, 1996.

38. Stinchcombe JC, Griffiths GM: Regulated secretion from hemopoietic cells. J Cell Biol 147:1, 1999.

39. Ward DM, Shiflett SL, Kaplan J: Chediak-Higashi syndrome: a clinical and molecular view of a rare lysosomal storage disorder. Curr Mol Med 2:469, 2002.

40. Lekstrom-Himes JA, Gallin JI: Immunodeficiency diseases caused by defects in phagocytes. N Engl J Med 343:1703, 2000.

41. Goldblatt D, Thrasher AJ: Chronic granulomatous disease. Clin Exp Immunol 122:1, 2000.

42. Nathan CF: Points of control in inflammation. Nature 420:846, 2002.

43. Tracey KJ: The inflammatory reflex. Nature 420:853, 2002.

44. Repka-Ramirez MS, Baraniuk JN: Histamine in health and disease. Clin Allergy Immunol 17:1, 2002.

45. Barrington R, et al: The role of complement in inflammation and adaptive immunity. Immunol Rev 180:5, 2001.

46. Liszewski MK, et al: Control of the complement system. Adv Immunol 61:201, 1996.

47. Kaplan AP, et al: The intrinsic coagulation/kinin-forming cascade: assembly in plasma and cell surfaces in inflammation. Adv Immunol 66:225, 1997.

48. Couture R, et al: Kinin receptors in pain and inflammation. Eur J Pharmacol 429:161, 2001.

49. Coughlin S: Thrombin signalling and protease-activated receptors. Nature 407:258, 2000.

50. Coughlin SR, Camerer E: PARticipation in inflammation. J Clin Invest. 111:25, 2003.

51. Funk CD: Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294:1871, 2001.

52. Murakami M, Kudo I: Cellular arachidonate-releasing functions of various phospholipase A_2 s. Adv Exp Med Biol 525:87, 2003.

53. Flower RJ: The development of COX2 inhibitors. Nat Rev Drug Discov 2:179, 2003.

54. Serhan CN, et al: Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med 196:1025, 2002.

55. Levy BD, Serhan CN: Polyisoprenyl phosphates: natural antiinflammatory lipid signals. Cell Mol Life Sci 59:729, 2002.

56. Buckingham JC, Flower RJ: Lipocortin 1: a second messenger of glucocorticoid action in the hypothalamo-pituitary-adrenocortical axis. Mol Med Today 62:438, 1997.

57. Serhan CN, Oliw E: Unorthodox routes to prostanoid formation: new twists in cyclooxygenase-initiated pathways. J Clin Invest 107:1481, 2001.

58. Prescott SM, et al: Platelet-activating factor and related lipid mediators. Annu Rev Biochem 69:419, 2000.

59. Mantovani A, Sozzani S, Introna M: Endothelial activation by cytokines. Ann NY Acad Sci 832:93, 1997.

60. Madge LA, Pober JS: TNF signaling in vascular endothelial cells. Exp Mol Pathol 70:317, 2001.

61. Argiles JM, et al: Journey from cachexia to obesity by TNF. FASEB J 11:743, 1997.

62. Rossi D, Zlotnik A: The biology of chemokines and their receptors. Annu Rev Immunol 18:217, 2000.

63. Zlotnik A, Yoshie O: Chemokines: a new classification system and their role in immunity. Immunity 12:121, 2000.

64. Yoshie O, Imai T, Nomiyama H: Chemokines in immunity. Adv Immunol. 78:57, 2001.

65. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373, 1980.

66. Nathan C: Inducible nitric oxide synthase. J Clin Invest 100:2417, 1997.

67. Laroux FS, et al: Role of nitric oxide in inflammation. Acta Physiol Scand 173:113, 2001.

68. Borregaard N, Cowland JB: Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 89:3503, 1997.

69. Belaaouaj A: Neutrophil elastase-mediated killing of bacteria: lessons from targeted mutagenesis. Microbes Infect 4:1259, 2002.

70. Beckman JS, Koppenol WH: Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. Am J Physiol 271:C1424, 1996.

71. Babior BM: Phagocyles and oxidative stress. Am J Med 109:33, 2003.

72. Severini C, et al: The tachykinin peptide family. Pharmacol Rev 54:285, 2002.

73. Harrison S, Geppetti P. Substance P. Int J Biochem Cell Biol 33:555, 2001.

74. Richardson JD, Vasko MR: Cellular mechanisms of neurogenic inflammation. J Pharmacol Exp Ther 302:839, 2002.

75. Cramer T, et al: HIF-1α is essential for myeloid cell-mediated inflammation. Cell 112:645, 2003.

76. Shi Y, Evans JE, Rock KL: Molecular identification of a danger signal that alerts the immune system to dying cells. Nature 425:516, 2003.

77. Majno G: Chronic inflammation: links with angiogenesis and wound healing. Am J Pathol 153:1035, 1998.

78. Luscinskas FW, Gimbrone MAJ: Endothelial-dependent mechanisms in chronic inflammatory leukocyte recruitment. Ann Rev Med 47:413, 1996.

79. Robinson DS, Kay AB, Wardlaw AJ: Eosinophils. Clin Allergy Immunol 16:43, 2002.

80. Suffredini AF, et al: New insights into the biology of the acute phase response. J Clin Immunol 19:203, 1999.

81. Dinarello CA: Cytokines as endogenous pyrogens. J Infect Dis 179 Suppl 2:S294, 1999.

82. Dinarello CA, Gatti S, Bartfai T: Fever: links with an ancient receptor. Curr Biol 9:R147, 1999.

83. Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 340:448, 1999.

84. Ridker PM: Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 107:363, 2003.

85. Cohen J: The immunopathogenesis of sepsis. Nature 420:885, 2002.

86. Hotchkiss RS, Karl IE: The pathophysiology and treatment of sepsis. N Engl J Med 348:138, 2003.

Chapter 3 - Tissue Renewal and Repair: Regeneration, Healing, and Fibrosis

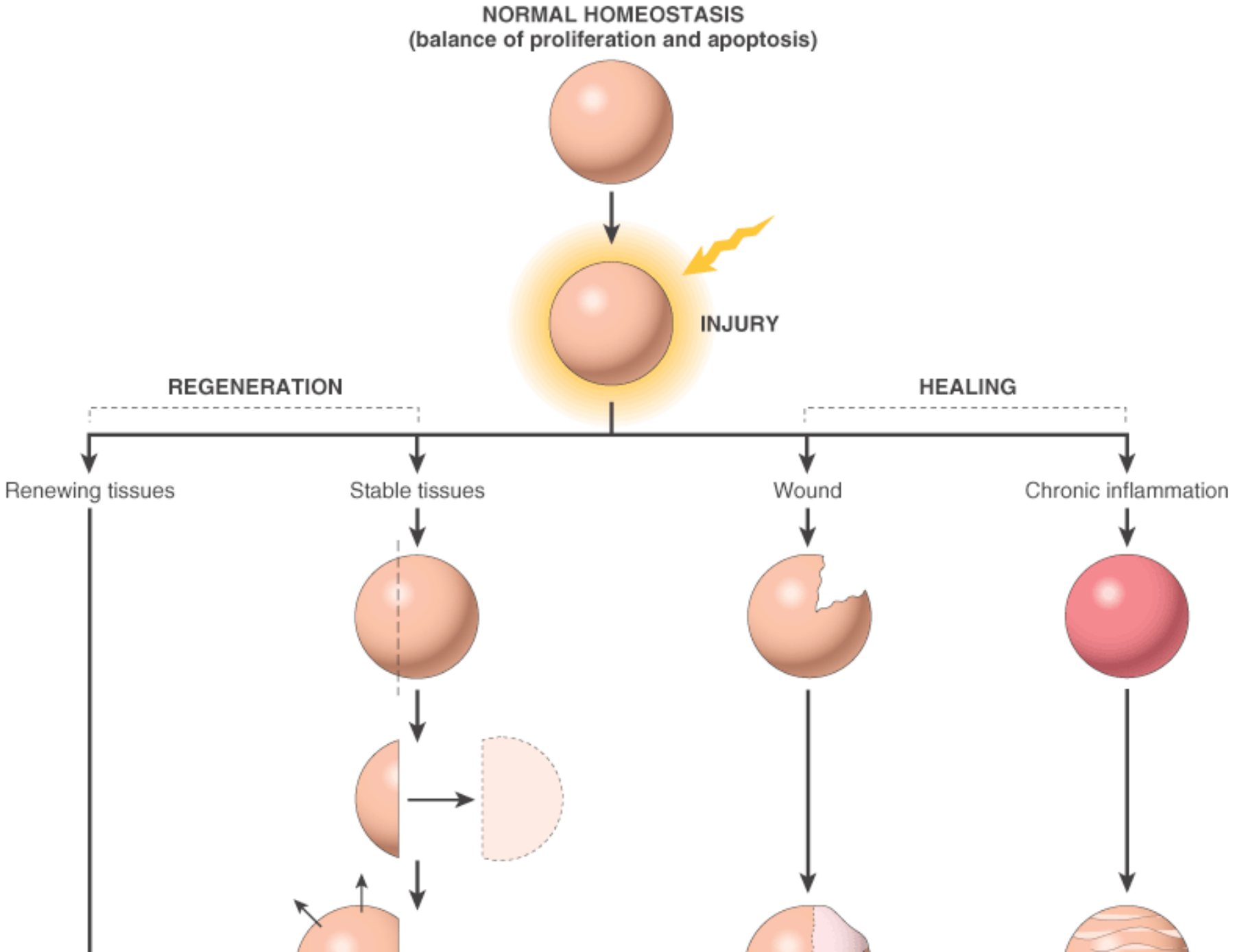
---

The body's ability to replace injured or dead cells and to repair tissues after inflammation is critical to survival. When injurious agents damage cells and tissues, the host responds by setting in motion a series of events that serve to eliminate these agents, contain the damage, and prepare the surviving cells for replication. The repair of tissue damage caused by surgical resection, wounds, and diverse types of chronic injury can be broadly separated into two processes, *regeneration* and *healing* (Fig. 3-1). Regeneration results in restitution of lost tissues; healing may restore original structures but involves collagen deposition and scar formation.

Definitions

The mechanisms of regeneration and healing will be discussed later in this chapter, but it is important from the outset to establish some important distinctions between these processes and to become familiar with terms used to describe them.

Figure 3-1 Tissue response to injury. Repair after injury can occur by regeneration, which restores normal tissue, or by healing, which leads to scar formation and fibrosis.



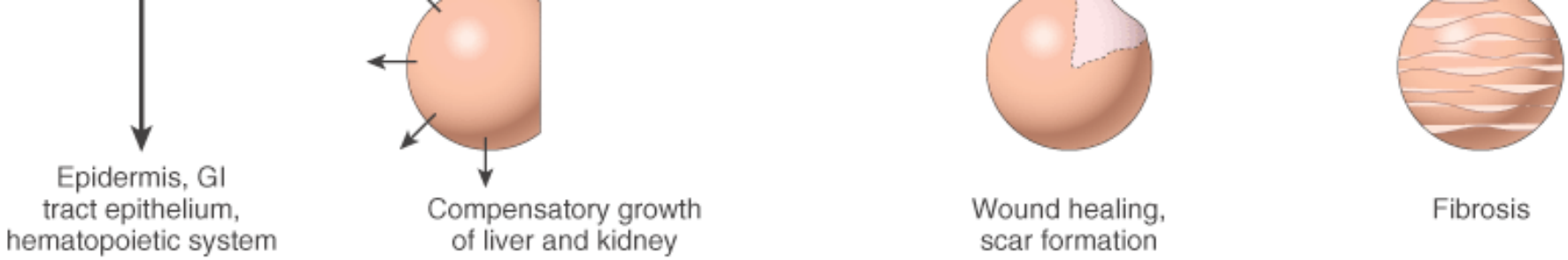


Figure 3-2 Mechanisms regulating cell populations. Cell numbers can be altered by increased or decreased rates of stem cell input, by cell death due to apoptosis, or by changes in the rates of proliferation or differentiation. *(Modified from McCarthy NJ et al: Apoptosis in the development of the immune system: growth factors, clonal selection and bcl-2. Cancer Metastasis Rev 11:157, 1992.)*

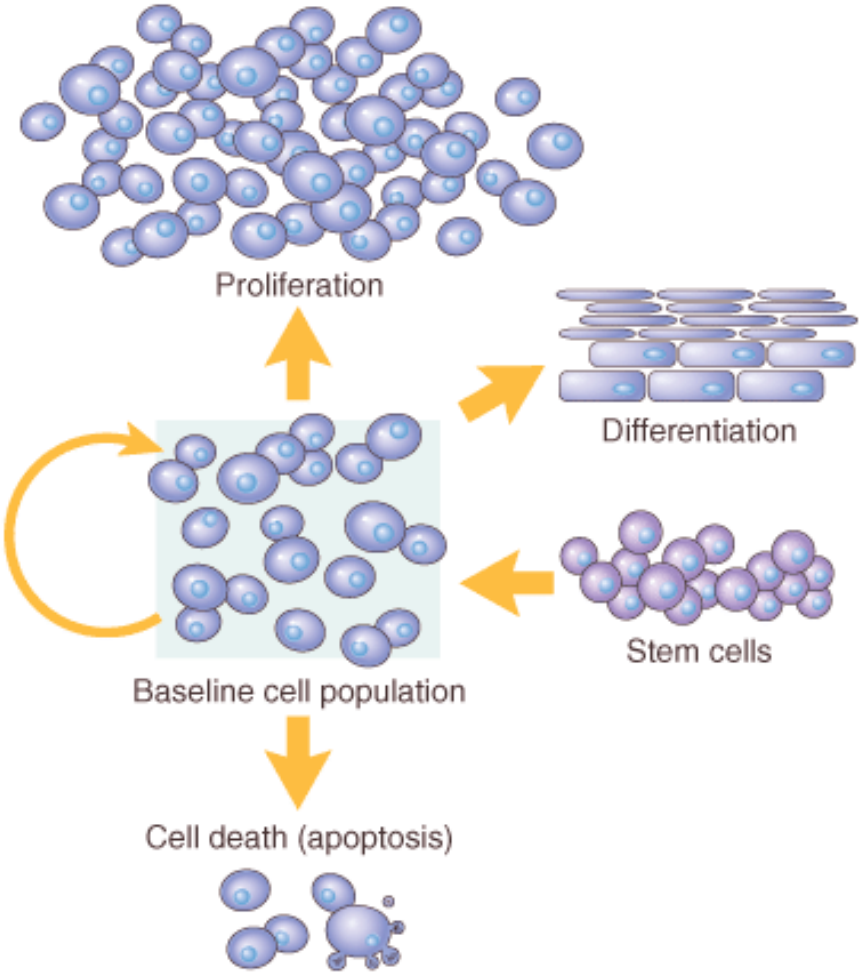


Figure 3-3 Cell-cycle landmarks. The figure shows the cell-cycle phases (G_0 , G_1 , G_2 , S, and M), the location of the G_1 restriction point, and the G_1 /S and G_2 /M cell-cycle checkpoints. Cells from labile tissues such as the epidermis and the gastrointestinal tract may cycle continuously; stable cells such as hepatocytes are quiescent but can enter the cell cycle; permanent cells

such as neurons and cardiac myocytes have lost the capacity to proliferate. *(Modified from Pollard TD and Earnshaw WC: Cell Biology. Philadelphia, Saunders, 2002.)*

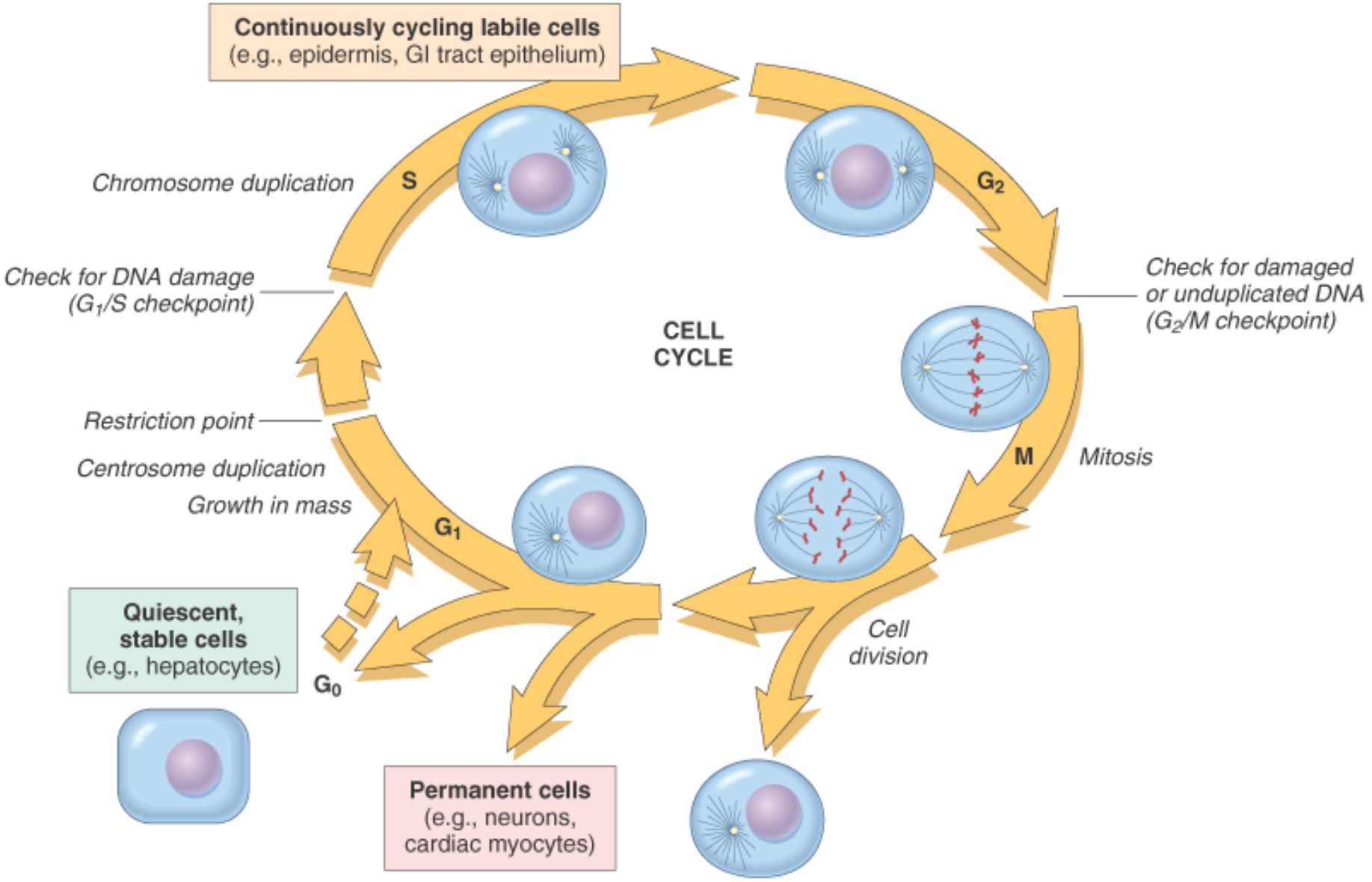
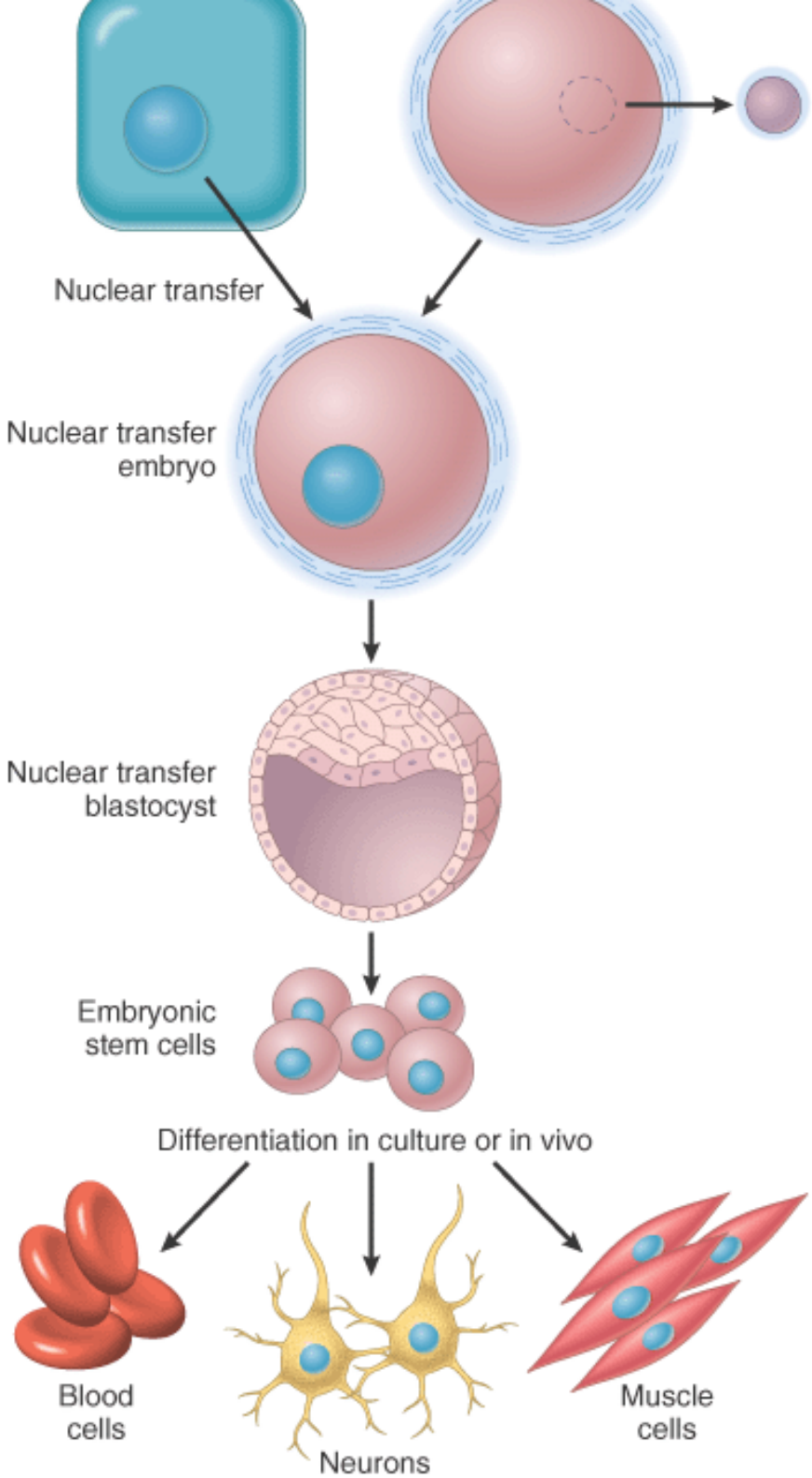


Figure 3-4 Steps involved in therapeutic cloning, using embryonic stem cells (ES cells) for cell therapy. The diploid nucleus of an adult cell from a patient is introduced into an enucleated oocyte. The oocyte is activated, and the zygote divides to become a blastocyst that contains the donor DNA. The blastocyst is dissociated to obtain ES. These cells are capable of differentiating into various tissues, either in culture or after transplantation into the donor. The goal of the procedure is to reconstitute or repopulate damaged organs of a patient, using the cells of the same patient to avoid immunologic rejection. *(Modified from Hochedlinger K, Jaenisch R: Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. N Engl J Med 349:275–286, 2003.)*


Nuclear transfer
Nuclear transfer embryo
Nuclear transfer blastocyst
Embryonic stem cells
Differentiation in culture or in vivo
Blood cells
Neurons
Muscle cells

Neurons

Figure 3-5 Stem-cell niches in various tissues. *A,* Epidermal stem cells located in the bulge area of the hair follicle serve as a stem cells for the hair follicle and the epidermis. *B,* Intestinal stem cells are located at the base of a colon crypt, above Paneth cells. *C,* Liver stem cells (commonly known as oval cells) are located in the canals of Hering *(thick arrow),* structures that connect bile ductules *(thin arrow)* with parenchymal hepatocytes (bile duct and Hering canals are stained for cytokeratin 7; *courtesy of Tania Roskams, M.D., University of Leuven*). *D,* Corneal stem cells are located in the limbus region, between the conjunctiva and the cornea. *(Courtesy of T-T Sun, New York University, New York, NY.)*

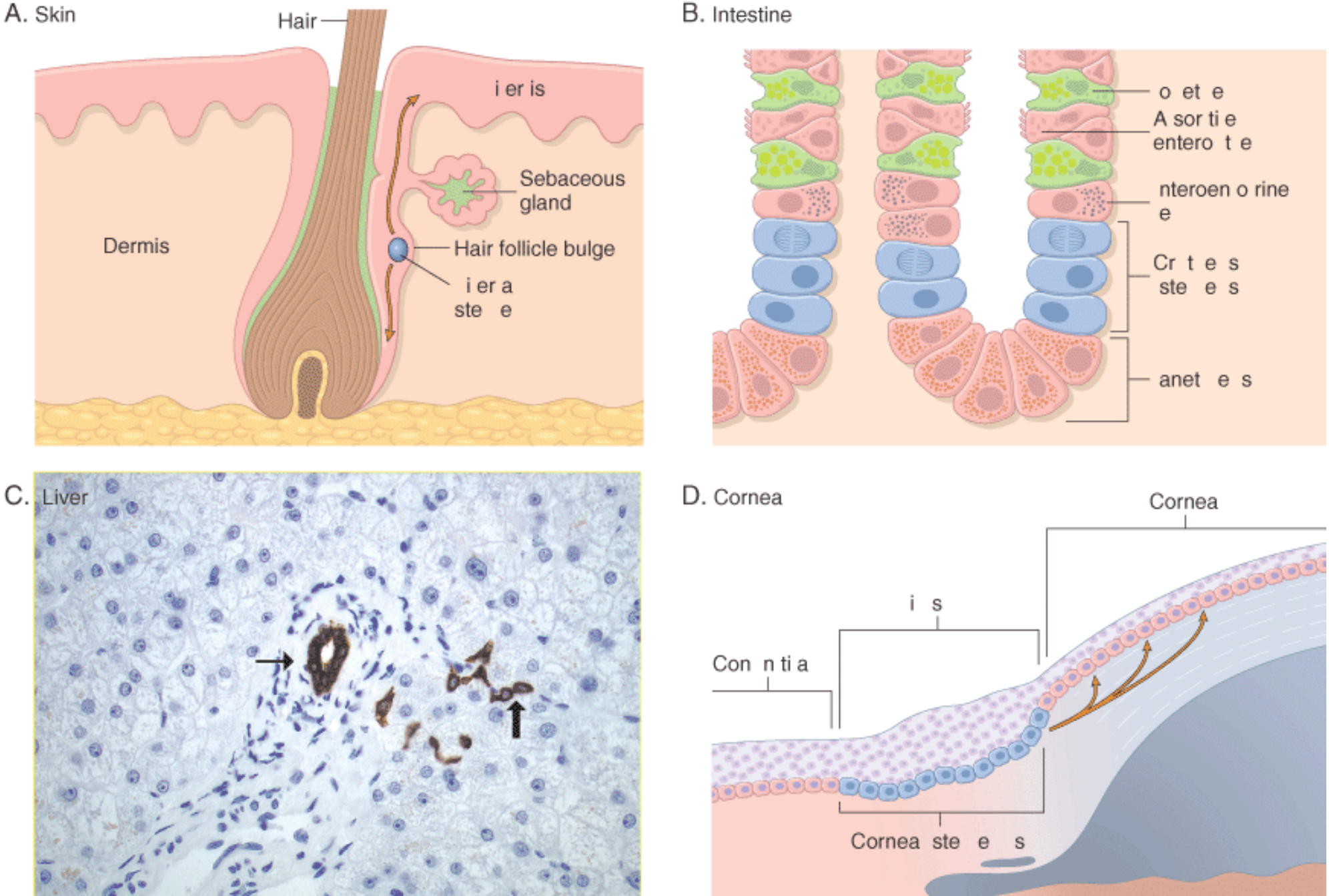


Figure 3-6 Differentiation pathways for pluripotent bone marrow stromal cells. Activation of key regulatory proteins by growth factors, cytokines, or matrix components leads to commitment of stem cells to differentiate into specific cellular lineages. Differentiation of myotubes requires the combined action of several factors (e.g., myoD, myogenin); fat cells require PPARγ, the osteogenic lineage requires CBFA1 (also known as RUNX2), cartilage formation requires Sox9, and endothelial cells require VEGF and FGF-2. *(Adapted and redrawn from Rodan GA, Harada S: The missing bone. Cell 89:677, 1997.)*

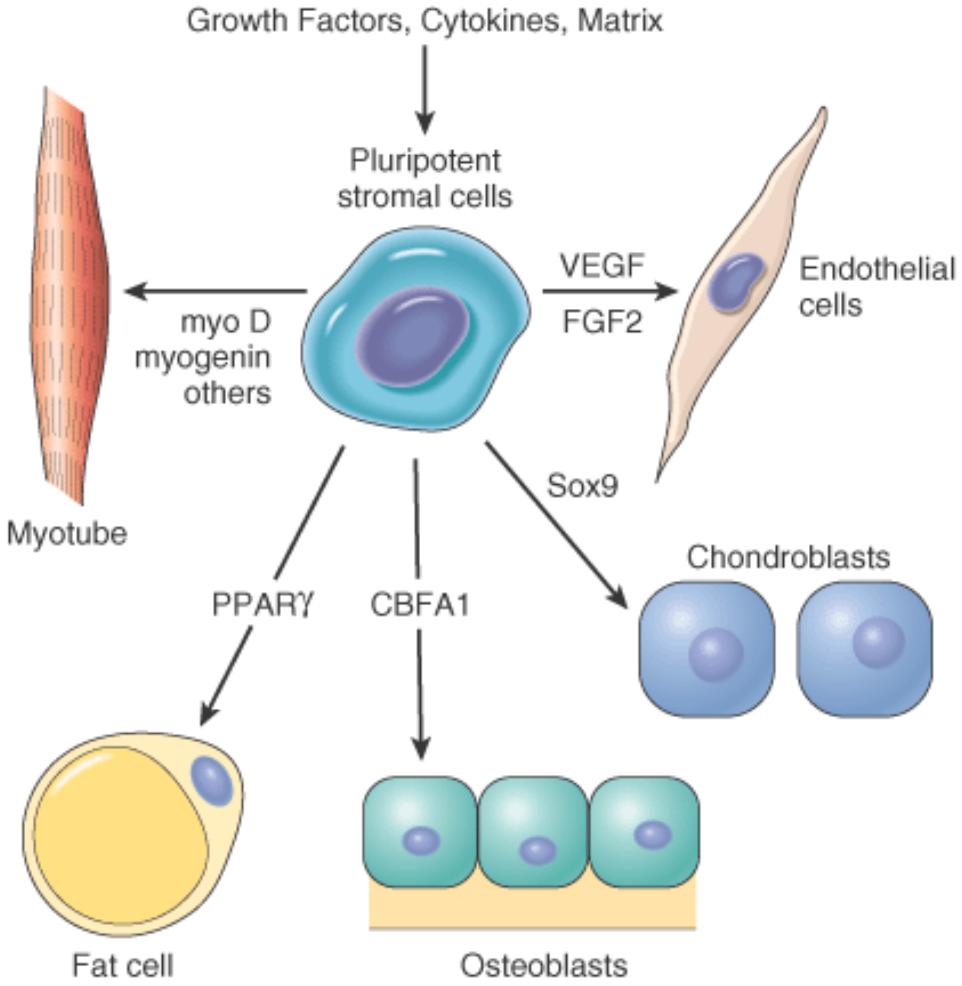


Figure 3-7 Differentiation of embryonic cells and generation of tissue cells by bone marrow precursors. During embryonic development the three germ layers—endoderm, mesoderm, and ectoderm—are formed, generating all tissues of the body. Adult stem cells localized in organs derived from these layers produce cells that are specific for the organs at which they reside. However, some adult bone marrow stem cells, in addition to producing the blood lineages (mesodermal derived), can also generate cells for tissues that originated from the endoderm and ectoderm (indicated by the *red lines*). *(Modified from Korbling M, Estrov Z: Adult stem cells for tissue repair—a new theropeutic concept? N Engl J Med 349:570–582, 2003.)*

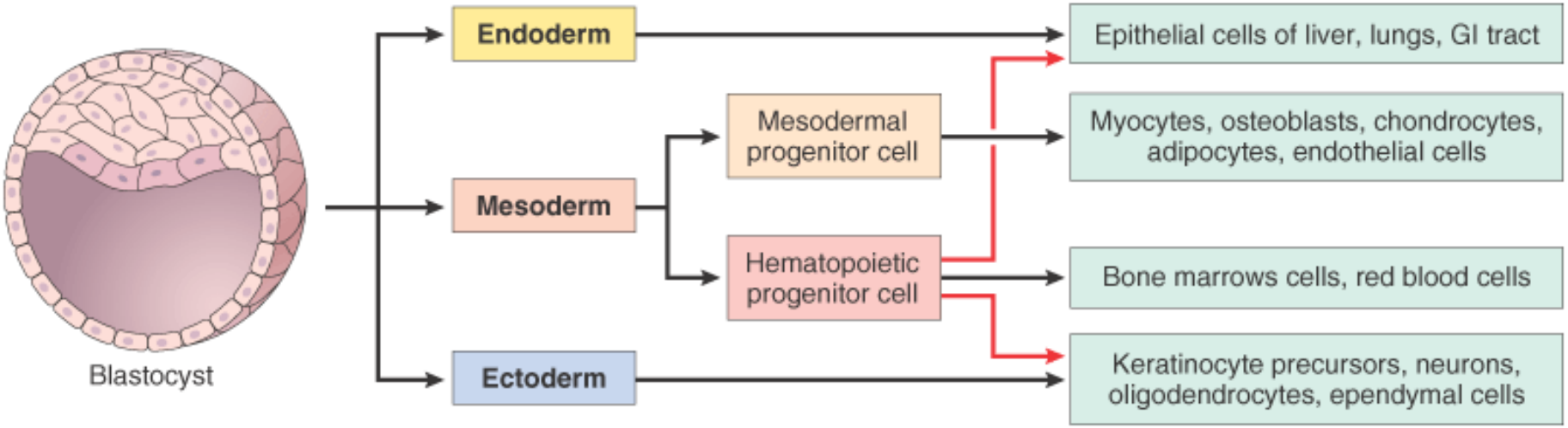


TABLE 3-1 -- Growth Factors and Cytokines Involved in Regeneration and Wound Healing

Cytokine	Symbol	Source	Functions
Epidermal growth factor	EGF	Platelets, macrophages, saliva, urine, milk, plasma	Mitogenic for keratinocytes and fibroblasts; stimulates keratinocyte migration and granulation tissue formation
Transforming growth factor alpha	TGF-α	Macrophages, T lymphocytes, keratinocytes, and many tissues	Similar to EGF; stimulates replication of hepatocytes and certain epithelial cells
Hepatocyte growth factor/scatter factor	HGF	Mesenchymal cells	Enhances proliferation of epithelial and endothelial cells, and of hepatocytes; increases cell motility
Vascular endothelial cell growth factor (isoforms A, B, C, D)	VEGF	Mesenchymal cells	Increases vascular permeability; mitogenic for endothelial cells (see Table 3-3)
Platelet-derived growth factor (isoforms A, B, C, D)	PDGF	Platelets, macrophages, endothelial cells, keratinocytes, smooth muscle cells	Chemotactic for PMNs, marcrophages, fibroblasts, and smooth muscle cells; activates PMNs, macrophages, and fibroblasts; mitogenic for fibroblasts, endothelial cells, and smooth muscle cells; stimulates production of MMPs, fibronectin, and HA; stimulates angiogenesis and wound contraction; remodeling; inhibits platelet aggregation; regulates integrin expression
Fibroblast growth factor-1 (acidic), -2 (basic) and family	FGF	Macrophages, mast cells, T lymphocytes, endothelial cells, fibroblasts, and many tissues	Chemotactic for fibroblasts; mitogenic for fibroblasts and keratinocytes; stimulates keratinocyte migration, angiogenesis, fam wound contraction and matrix deposition
Transforming growth factor beta (isoforms 1, 2, 3); other members of the family are BMP and activin	TGF-β	Platelets, T lymphocytes, macrophages, endothelial cells, keratinocytes, smooth muscle cells, fibroblasts	Chemotactic for PMNs, macrophages, lymphocytes, fibroblasts, and smooth muscle cells; stimulates TIMP synthesis, keratinocyte migration, angiogenesis, and fibroplasia; inhibits production of MMPs and keratinocyte proliferation; regulates integrin expression and other cytokines; induces TGF-β production
Keratinocyte growth factor (also called FGF-7)	KGF	Fibroblasts	Stimulates keratinocyte migration, proliferation, and differentiation

Insulin-like growth factor-1	IGF-1	Macrophages, fibroblasts and other cells	Stimulates synthesis of sulfated proteoglycans, collagen, keratinocyte migration, and fibroblast proliferation; endocrine effects similar to growth hormone
Tumor necrosis factor	TNF	Macrophages, mast cells, T lymphocytes	Activates macrophages; regulates other cytokines; multiple functions
Interleukins	IL-1, etc.	Macrophages, mast cells, keratinocytes, lymphocytes, and many tissues	Many functions. Some examples: chemotactic for PMNs (IL-1) and fibroblasts (IL-4), stimulation of MMP-1 synthesis (IL-1), angiogenesis (IL-8), TIMP synthesis (IL-6); regulation of other cytokines
Interferons	IFN-α, etc.	Lymphocytes and fibroblasts	Activates macrophages; inhibits fibroblast proliferation and synthesis of MMPs; regulates other cytokines
BMP, bone morphogenetic proteins; PMNs, polymorphonuclear leukocytes; MMPs, matrix metalloproteinases; HA, hyaluronic acid; TIMP, tissue inhibitor of matrix metalloproteinase.			
Modified from Schwartz SI: Principles of Surgery, McGraw Hill, New York, 1999.			

those that have major roles in these processes. Other growth factors are alluded to in various sections of the book.

Epidermal Growth Factor (EGF) and Transforming Growth Factor-α (TGF-α).

These two factors belong to the EGF family and share a common receptor. EGF was discovered by its ability to cause precocious tooth eruption and eyelid opening in newborn mice. EGF is mitogenic for a variety of epithelial cells, hepatocytes, and fibroblasts. It is widely distributed in tissue secretions and fluids, such as sweat, saliva, urine, and intestinal contents. In healing wounds of the skin, EGF is produced by keratinocytes, macrophages, and other inflammatory cells that migrate into the area. EGF binds to a receptor (EGFR) with intrinsic tyrosine kinase activity, triggering the signal transduction events described later. TGF-α was originally extracted from sarcoma

virus-transformed cells and is involved in epithelial cell proliferation in embryos and adults and malignant transformation of normal cells to cancer. TGF-α has homology with EGF, binds to EGFR, and produces most of the biologic activities of EGF. The "EGF receptor" is actually a family of membrane tyrosine kinase receptors that respond to EGF, TGF-α, and other ligands of the EGF family.[44] The main EGFR is referred to as EGFR1, or ERB B1. The ERB B2 receptor (also known as HER-2/Neu) has received great attention because it is overexpressed in breast cancers and is a therapeutic target.

Hepatocyte Growth Factor (HGF).

HGF was originally isolated from platelets and serum. Subsequent studies demonstrated that it is identical to a previously identified growth factor known as *scatter factor* (HGF is also referred to as HGF/scatter factor). It has mitogenic effects in most epithelial cells, including hepatocytes and cells of the biliary epithelium in the liver, and epithelial cells of the lungs, mammary gland, skin, and other tissues.[45] Besides its mitogenic effects, HGF acts as a morphogen in embryonic development and promotes cell scattering and migration. This factor is produced by fibroblasts, endothelial cells, and liver nonparenchymal cells. The receptor for HGF is the product of the proto-oncogene c-*MET*, which is frequently overexpressed in human tumors. HGF signaling is required for survival during embryonic development, as demonstrated by the lethality of knockout mice lacking c-*MET*.

Vascular Endothelial Growth Factor (VEGF).

VEGF is a family of peptides that includes VEGF-A (referred throughout as VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor. VEGF is a potent inducer of blood vessel

formation in early development *(vasculogenesis)* and has a central role in the growth of new blood vessels *(angiogenesis)* in adults (see Table 3-3). [46] It promotes angiogenesis in tumors, chronic inflammation, and healing of wounds. Mice that lack a single allele of the gene (heterozygous VEGF knockout mice) die during embryonic development with defective vasculogenesis and hematopoiesis. VEGF family members signal through three tyrosine kinase receptors: VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-2 is located in endothelial cells and is the main receptor for the vasculogenic and angiogenic effects of VEGF. The role of VEGFR-1 is less well understood, but it may facilitate the mobilization of endothelial stem cells and has a role in inflammation. VEGF-C and VEGF-D bind to VEGFR-3 and act on lymphatic endothelial cells to induce the production of lymphatic vessels (lymphangiogenesis). VEGF-B binds exclusively to VEGFR-1. It is not required for vasculogenesis or angiogenesis, but may play a role in maintenance of myocardial function.

Platelet-Derived Growth Factor (PDGF).

PDGF is a family of several closely related proteins, each consisting of two chains designated *A* and *B*. All three isoforms of PDGF (AA, AB, and BB) are secreted and are biologically active. Recently, two new isoforms—PDGF-C and PDGF-D—have been identified. PDGF isoforms exert their effects by binding to two cell-surface receptors, designated PDGFR α and β, which have different ligand specificities.[47] PDGF is stored in platelet α granules and is released on platelet activation. It can also be produced by a variety of other cells, including activated macrophages, endothelial cells, smooth muscle cells, and many tumor cells. PDGF causes migration and proliferation of fibroblasts, smooth muscle cells, and monocytes, as demonstrated by defects in these functions in mice deficient in either the A or the B chain of PDGF. It also participates in the activation of hepatic stellate cells in the initial steps of liver fibrosis (Chapter 18).

Fibroblast Growth Factor (FGF).

This is a family of growth factors containing more than 10 members, of which acidic FGF (aFGF, or FGF-1) and basic FGF (bFGF, or FGF-2) are the best characterized. FGF-1 and FGF-2 are made by a variety of cells. Released FGFs associate with heparan sulfate in the ECM, which can serve as a reservoir for storing inactive factors. FGFs are recognized by a family of cell-surface receptors that have intrinsic tyrosine kinase activity. A large number of functions are attributed to FGFs, including the following:

- *New blood vessel formation (angiogenesis):* FGF-2, in particular, has the ability to induce the steps necessary for new blood vessel formation both in vivo and in vitro (see below).
- *Wound repair:* FGFs participate in macrophage, fibroblast, and endothelial cell migration in damaged tissues and migration of epithelium to form new epidermis.
- *Development:* FGFs play a role in skeletal muscle development and in lung maturation. For example, FGF-6 and its receptor induce myoblast proliferation and suppress myocyte differentiation, providing a supply of proliferating myocytes. FGF-2 is also thought to be involved in the generation of angioblasts during embryogenesis. FGF-1 and FGF-2 are involved in the specification of the liver from endodermal cells.[48]
- *Hematopoiesis:* FGFs have been implicated in the differentiation of specific lineages of blood cells and development of bone marrow stroma.

TGF-β and Related Growth Factors.

TGF-β belongs to a family of homologous polypeptides that includes three TGF-β isoforms (TGF-β1, TGF-β2, TGF-β3) and factors with wide-ranging functions, such as bone morphogenetic proteins (BMPs), activins, inhibins, and mullerian inhibiting substance.[49] TGF-β1 has the most widespread distribution in mammals and will be referred to as TGF-β. It is a homodimeric protein produced by a variety of different cell types, including platelets, endothelial cells, lymphocytes, and macrophages. Native TGF-βs are synthesized as precursor proteins, which are secreted and then proteolytically cleaved to yield the biologically active growth factor and a second *latent* component. Active TGF-β binds to two cell surface receptors (types I and II) with serine/threonine kinase activity and triggers the phosphorylation of cytoplasmic transcription factors called *Smads*. [50] TGF-β first binds to a type II receptor, which then forms a complex with a type I receptor, leading to the phosphorylation of Smad 2 and 3. Phosphorylated Smad2 and 3 form heterodimers with Smad4, which enter the nucleus and associate with other DNA-binding proteins to activate or inhibit gene transcription. TGF-β has multiple and often opposing effects depending on the tissue and the type of injury. Agents that have multiple effects are called pleiotropic; because of the large diversity of TGF-β effects, it has been said that TGF-β is pleiotropic with a vengeance.

- *TGF-β is a growth inhibitor for most epithelial cell types and for leukocytes.*[51] It blocks the cell cycle by increasing the expression of cell-cycle inhibitors of the Cip/Kip and INK4/ARF families (see Chapter 7). Loss of TGF-β receptors

frequently occurs in human tumors, providing a proliferative advantage to tumor cells.

- The effects of TGF-β on mesenchymal cells depend on concentration and culture conditions, it generally *stimulates the proliferation of fibroblasts and smooth muscle cells*.
- *TGF-β is a potent fibrogenic agent* that stimulates fibroblast chemotaxis, enhances the production of collagen, fibronectin, and proteoglycans. It inhibits collagen degradation by decreasing matrix proteases and increasing protease inhibitor activities. TGF-β is involved in the development of fibrosis in a variety of chronic inflammatory conditions particularly in the lungs, kidney, and liver.
- *TGF-β has a strong anti-inflammatory effect.* Knockout mice lacking the *TGF-β1* gene have widespread inflammation and abundant lymphocyte proliferation, presumably because of unregulated T-cell proliferation and macrophage activation.

Cytokines.

Cytokines have important functions as mediators of inflammation and immune responses (Chapter 6). Some of these proteins can be placed into the larger functional group of polypeptide growth factors because they have growth-promoting activities for a variety of cells. These are discussed in the appropriate chapters.

SIGNALING MECHANISMS IN CELL GROWTH

All growth factors function by binding to specific receptors, which deliver signals to the target cells. These signals have two general effects: (1) they stimulate the transcription of many genes that were silent in the resting cells, and (2) several of these genes regulate the entry of the cells into the cell cycle and their passage through the various stages of the cell cycle. In this section we review the process of receptor-initiated signal transduction as it applies to growth factors and signaling molecules in general, and their role in regulating the cell cycle.

Cell proliferation is a tightly regulated process that involves a large number of molecules and interrelated pathways. The first event that initiates cell proliferation is, usually, the binding of a signaling molecule, the *ligand*, to a specific cell *receptor*. As we shall see, typical ligands are growth factors and proteins of the ECM. We describe different classes of receptor molecules and the pathways by which receptor activation initiates a cascade of events leading to expression of specific genes. We end this section with brief comments about transcription factors.

Based on the source of the ligand and the location of its receptors—in the same, adjacent, or distant cells—three general modes of signaling, named *autocrine*, *paracrine*, and *endocrine*, can be distinguished (Fig. 3-8).

- *Autocrine signaling:* Cells respond to the signaling molecules that they themselves secrete, thus establishing an *autocrine loop*. Several polypeptide growth factors and cytokines act in this manner. Autocrine growth regulation plays a role in liver regeneration, proliferation of antigen-stimulated lymphocytes, and the growth of some tumors. Tumors frequently overproduce growth factors and their receptors, thus stimulating their own proliferation through an autocrine loop.
- *Paracrine signaling:* One cell type produces the ligand, which then acts on adjacent target cells that express the appropriate receptors. The responding cells are in close proximity to the ligand-producing cell and are generally of a different type. Paracrine stimulation is common in connective tissue repair of healing wounds, in which a factor produced by one cell type (e.g., a macrophage) has its growth effect on adjacent cells (e.g., a fibroblast). Paracrine signaling is also necessary for hepatocyte replication during liver regeneration (see below). A special type of paracrine signaling, called *juxtacrine*, occurs when the signaling molecule (e.g., tumor necrosis factor, TGF-α, and heparin-binding epidermal growth factor) is anchored in the cell membrane and binds a receptor in the plasma membrane of another cell. In this type of signaling, receptor-ligand

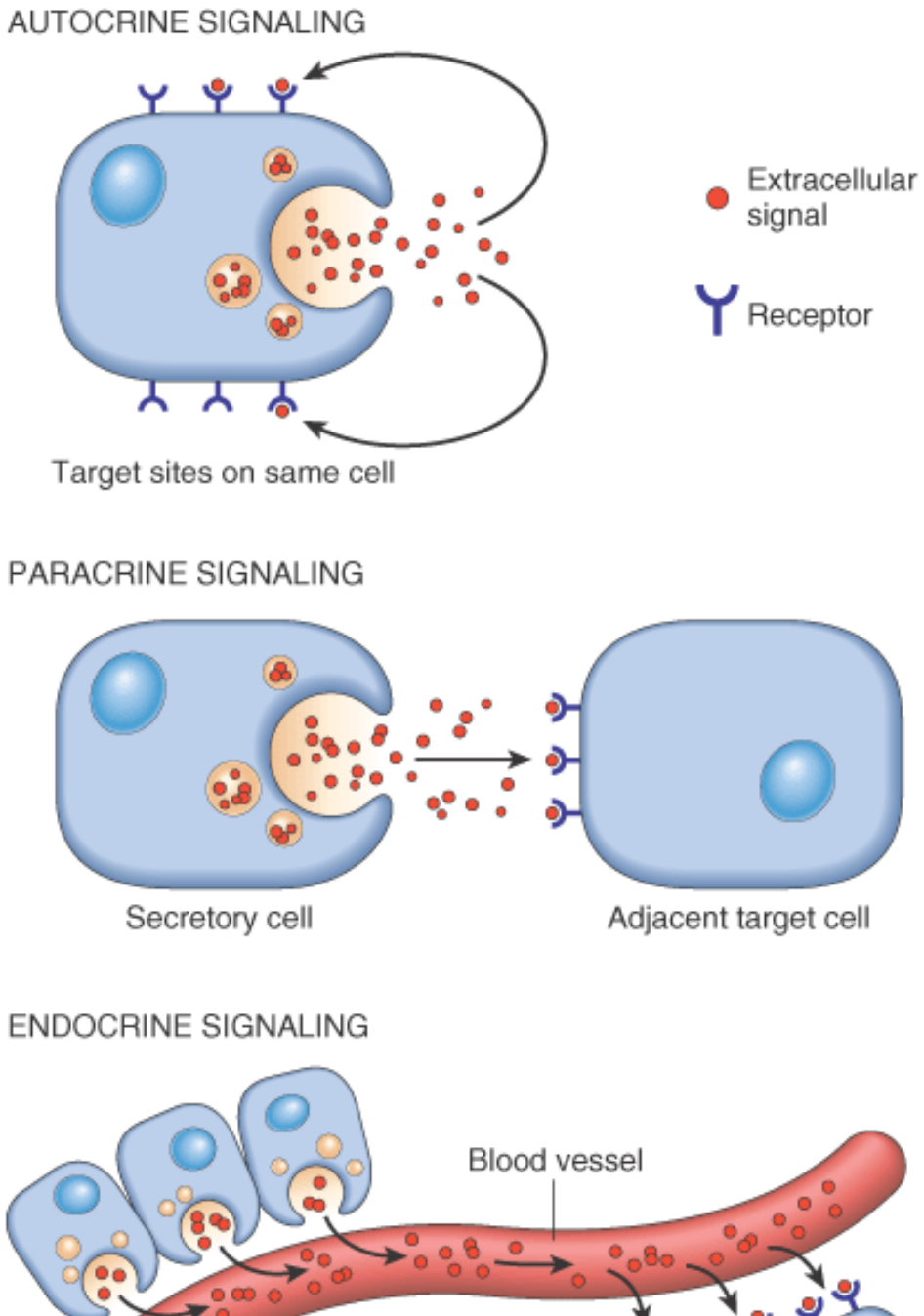
---

interaction is dependent on and promotes cell-cell adhesion.

- *Endocrine signaling:* Hormones are synthesized by cells of endocrine organs and act on target cells distant from their site of synthesis, being usually carried by the blood. Growth

factors may also circulate and act at distant sites, as is the case for HGF. Several cytokines, such as those associated with the systemic aspects of inflammation discussed in Chapter 2 , also act as endocrine agents.

Figure 3-8 General patterns of intercellular signaling demonstrating autocrine, paracrine, and endocrine signaling (see text). *(Modified from Lodish H, et al. [eds]: Molecular Cell Biology, 3rd ed. New York, WH Freeman, 1995, p. 855. © 1995 by Scientific American Books. Used with permission of WH Freeman and Company.)*



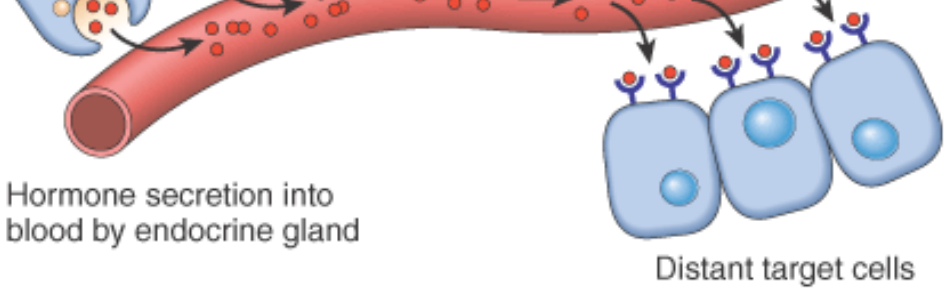
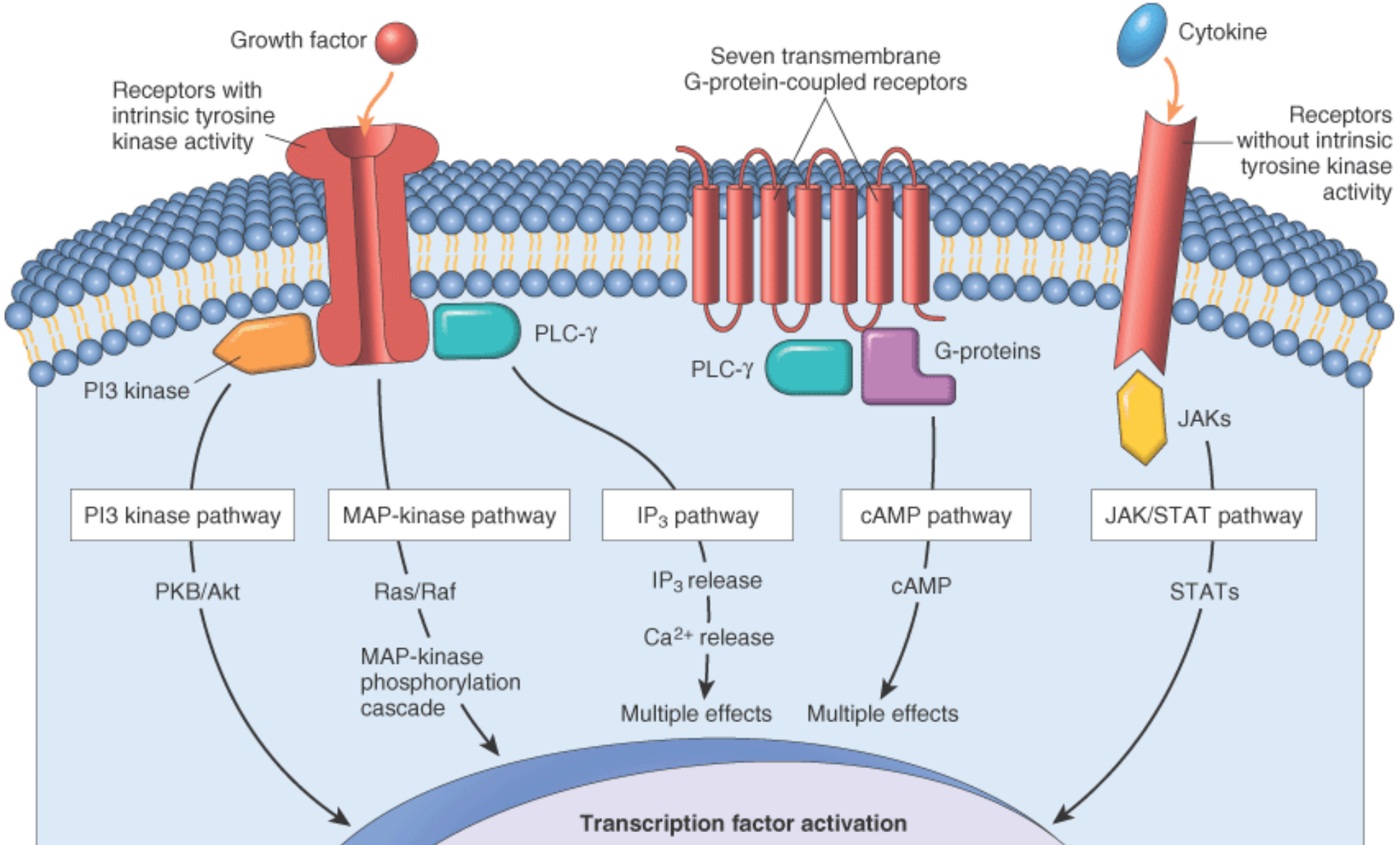


Figure 3-9 Examples of signal transduction systems that require cell-surface receptors. Shown are receptors with intrinsic tyrosine kinase activity, seven transmembrane G-protein-coupled receptors, and receptors without intrinsic tyrosine kinase activity. The figure also shows important signaling pathways transduced by the activation of these receptors through ligand binding.



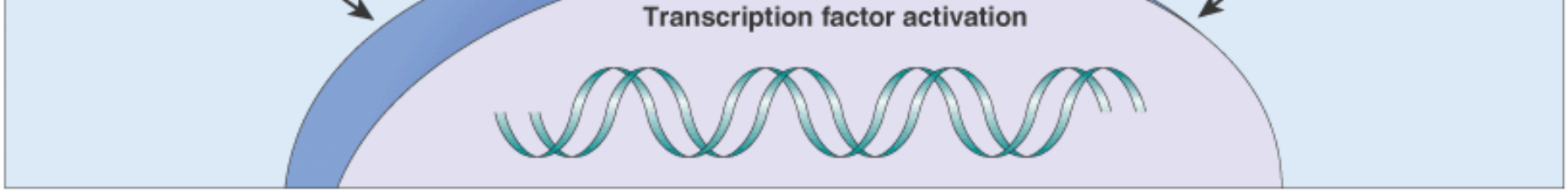


Figure 3-10 Signaling from tyrosine kinase receptors. Binding of the growth factor (ligand) causes receptor dimerization and autophosphorylation of tyrosine residues. Attachment of adapter (or bridging) proteins (e.g., GRB2 and SOS) couples the receptor to inactive RAS. Cycling of RAS between its inactive and active forms is regulated by GAP. Activated RAS interacts with and activates RAF (also known as MAP kinase kinase kinase). This kinase then phosphorylates a component of the MAP kinase signaling pathway, MEK (also known as MAP kinase kinase), which then phosphorylates ERK (MAP kinase). Activated MAP kinase phosphorylates other cytoplasmic proteins and nuclear transcription factors, generating cellular responses. The phosphorylated tyrosine kinase receptor can also bind other components, such as PI-3 kinase, which activates distinct signaling systems.

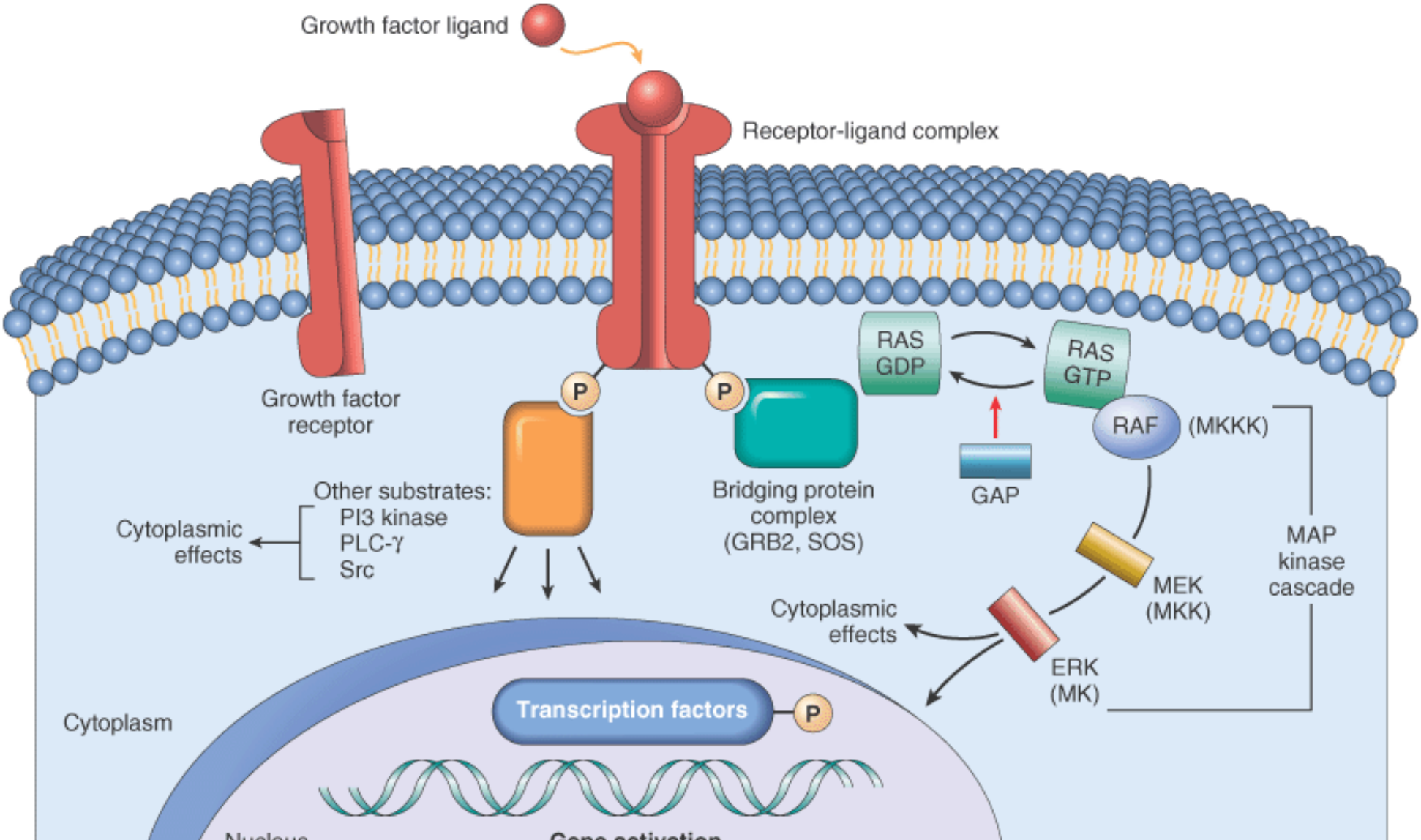
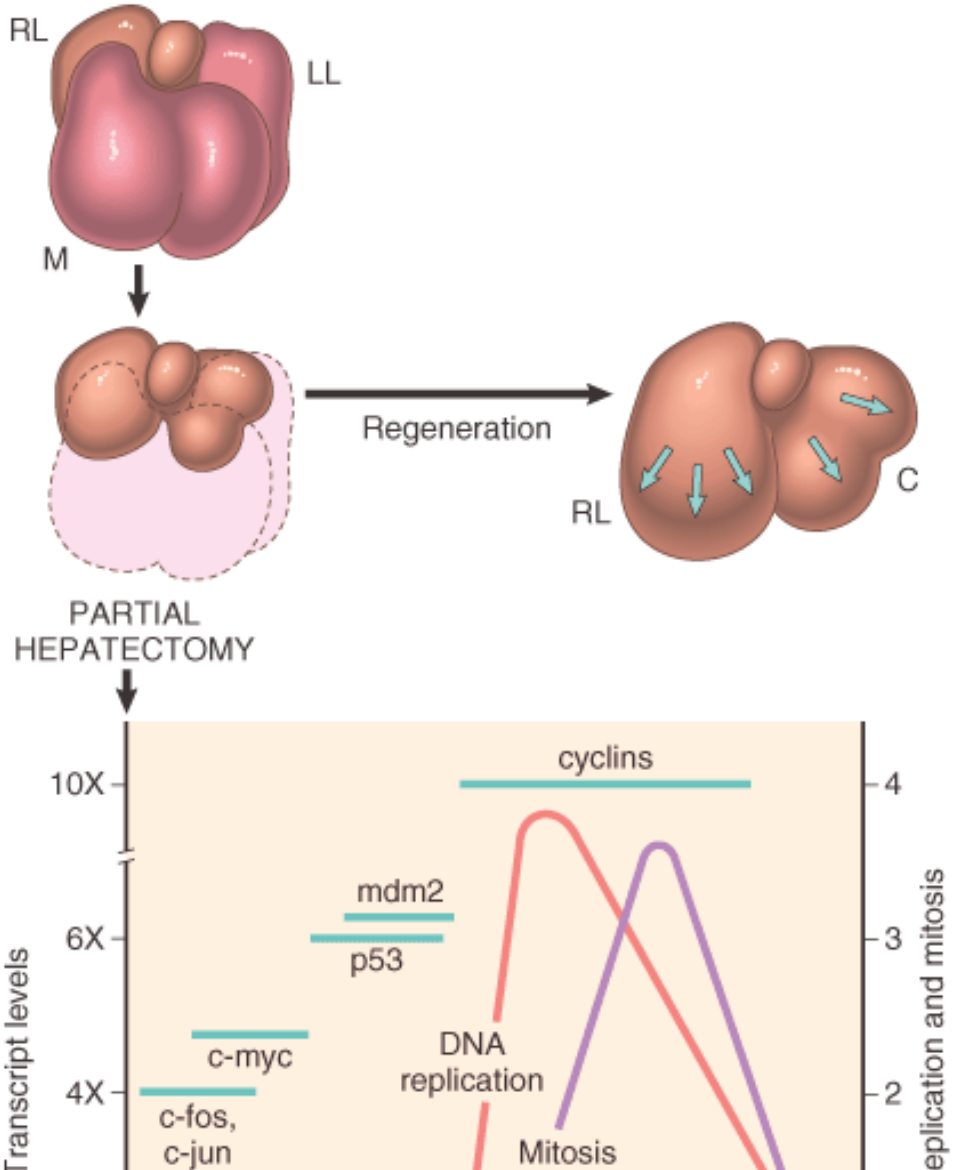


Figure 3-11 Liver regeneration after partial hepatectomy. *Upper panel,* The lobes of the liver of a rat are shown (M, median; RL and LL, right and left lateral lobes; C, caudate lobe). Partial hepatectomy removes two thirds of the liver (median and left lateral lobes), and only the right lateral and caudate lobes remain. After 3 weeks, the right lateral and caudate lobes enlarge to reach a mass equivalent to that of the original liver. Note that there is no regrowth of the median and left lateral lobes removed after partial hepatectomy. *(From Goss RJ: Regeneration versus repair. In Cohen IK, Diegelman RF, Lindblad WJ (eds): Wound Healing. Biochemical and Clinical Aspects. Philadelphia, W. B. Saunders Co., 1992, pp. 20–39.) Lower panel,* Timing of hepatocyte DNA replication, hepatocyte mitosis, and expression of messenger RNAs during liver regeneration. DNA replication is shown as the incorporation of tritiated thymidine × 10^{-4} *(right-side scale)*. Mitosis presented as the percentage of hepatocytes undergoing mitosis *(right-side scale)*. The expression of some of the many mRNAs in the regenerating rat liver is presented as fold elevation above normal *(left-side scale)*. Expression of the proto-oncogenes c-*fos*, c-*jun*, and c-*myc* corresponds to the immediate early gene phase of gene expression during liver regeneration.



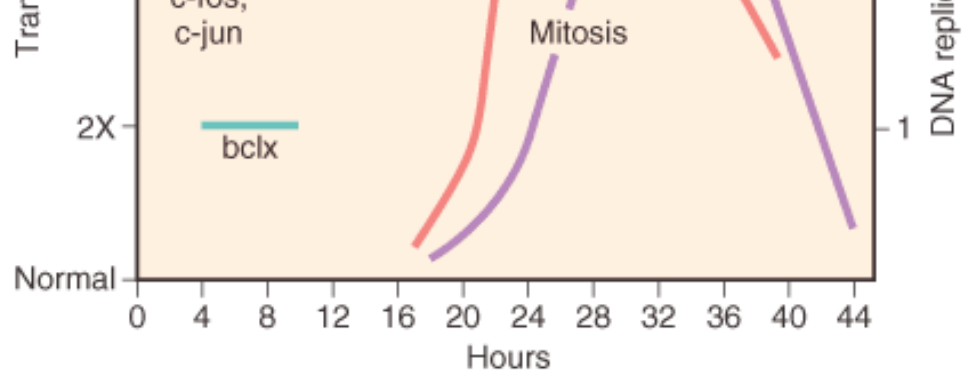


Figure 3-12 Regeneration of human liver. Computed tomography (CT) scans of the donor liver in living-donor hepatic transplantation. *Upper panel,* The liver of the donor before the operation. The right lobe, which will be used as a transplant, is outlined. *Lower panel,* A scan of the liver 1 week after performance of partial hepatectomy to remove the right lobe. Note the great enlargement of the left lobe (outlined in the panel) without regrowth of the right lobe *(Courtesy of R. Troisi, M.D. Ghent University city; reproduced in part from Fausto; Liver Regeneration. In Arias, et al: The Liver: Biology and Pathobiology, 4th ed. Philadelphia, Lippincott Williams & Wilkins, 2001.)*

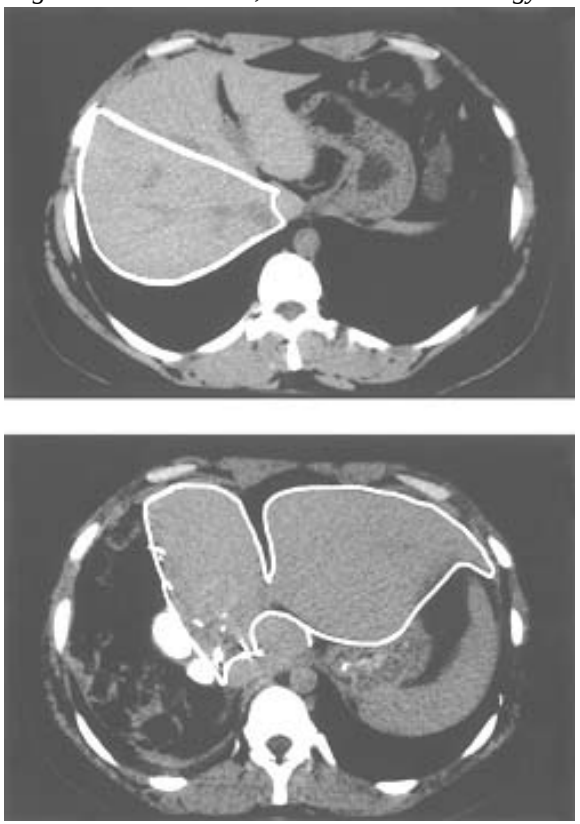


Figure 3-13 Priming and cell-cycle progression in hepatocyte replication during liver regeneration. Quiescent hepatocytes become competent to enter the cell cycle through a priming phase mostly mediated by the cytokines TNF and IL-6 *(upper panel)*. Growth factors, mainly HGF and TGF-α, act on primed hepatocytes to make them progress through the cell cycle and undergo DNA replication *(lower panel)*. Norepinephrine, insulin, thyroid hormone, and growth hormone act as adjuvants for liver regeneration. The factors that determine the termination of cell replication are not known but are likely to involve cell cycle inhibitors, shut-off of growth factor production, and decreased metabolic demand on the liver.

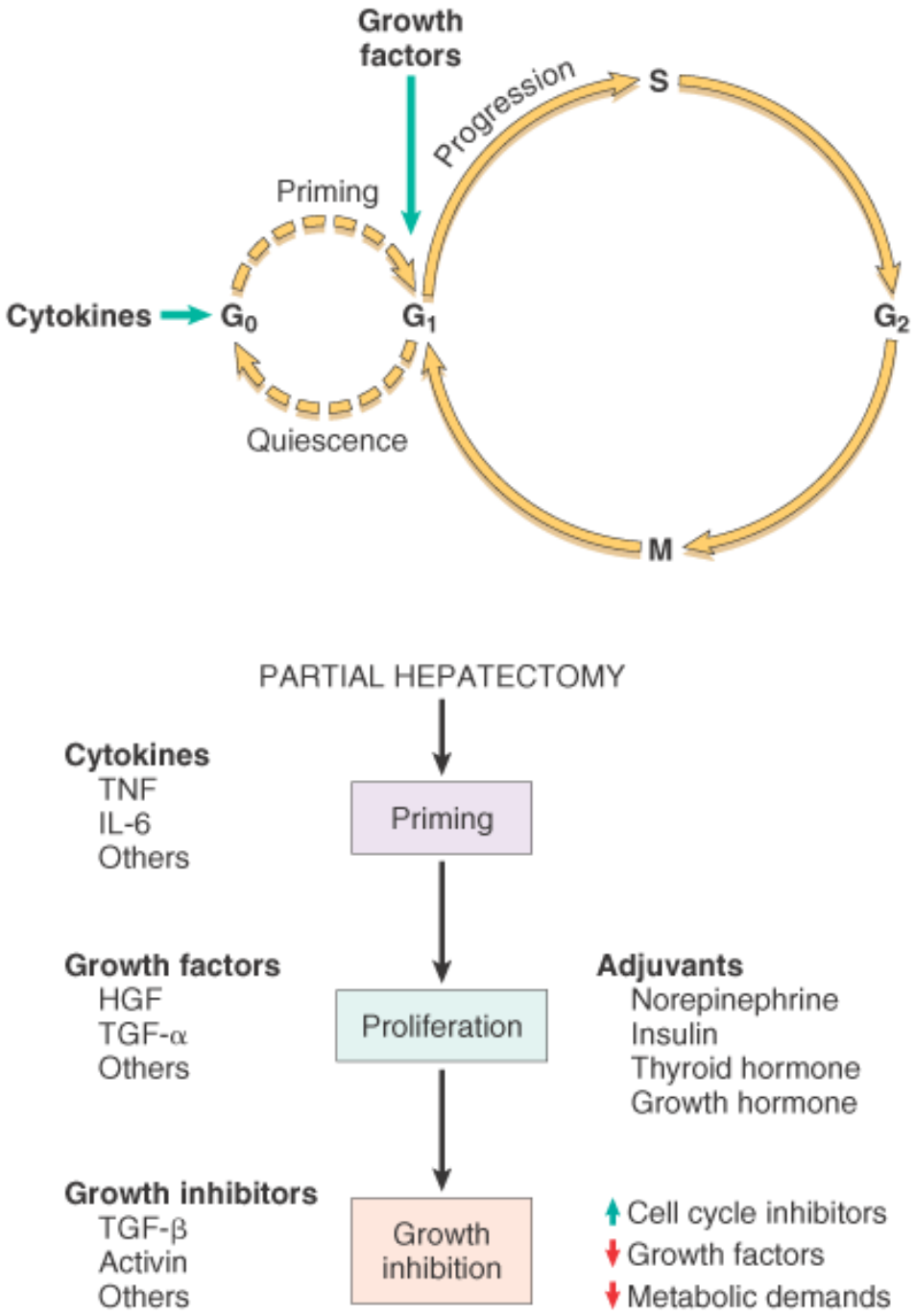


Figure 3-14 Major components of the extracellular matrix (ECM), including collagens, proteoglycans, and adhesive glycoproteins. Both epithelial and mesenchymal cells (e.g., fibroblasts) interact with ECM via integrins. To simplify the diagram, many ECM components (e.g., elastin, fibrillin, hyaluronan, syndecan) are not included.

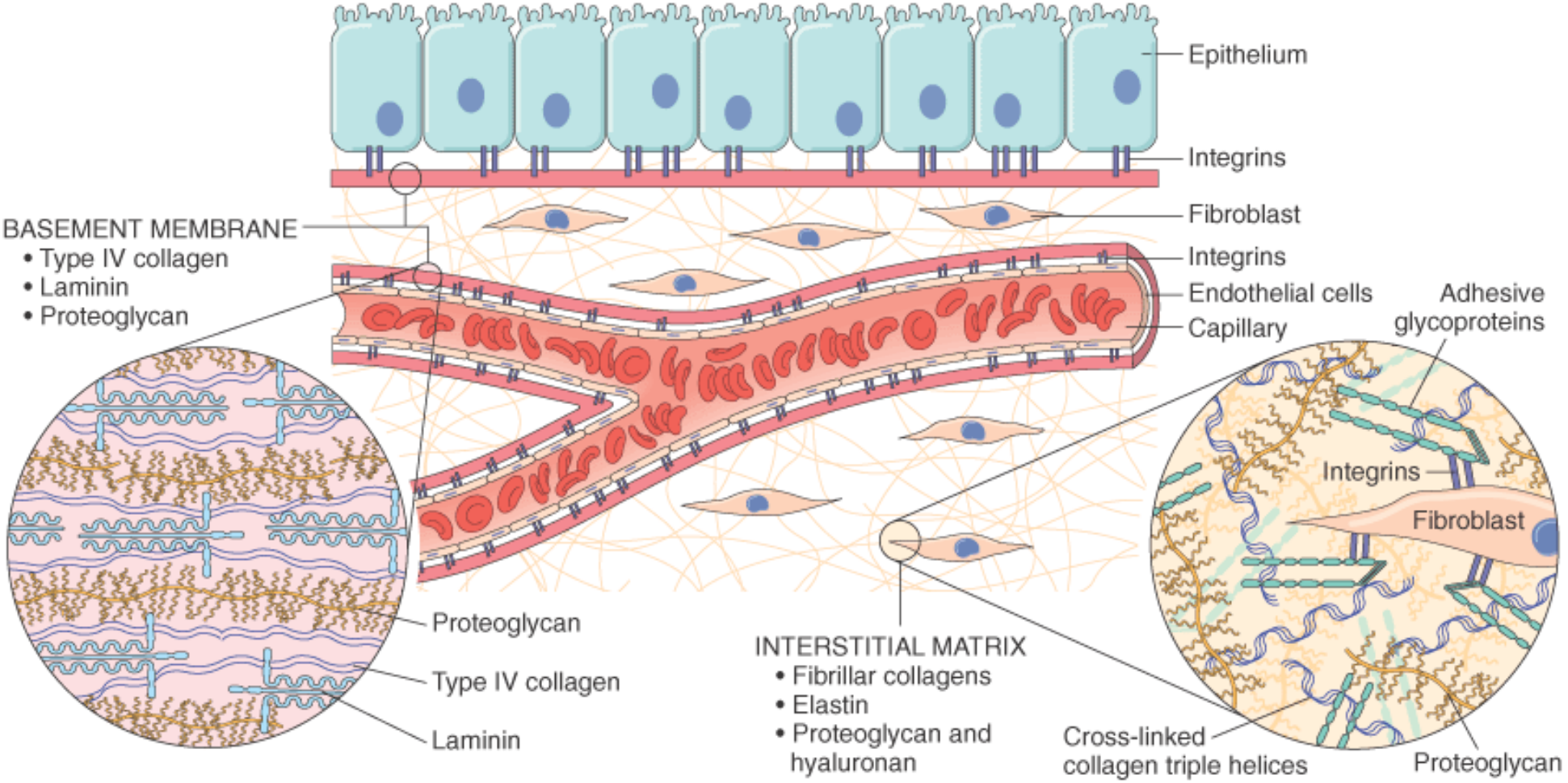


TABLE 3-2 -- Main Types of Collagens, Tissue Distribution, and Genetic Disorders

Collagen Type	Tissue Distribution	Genetic Disorders
Fibrillar Collagens		
I	Ubiquitous in hard and soft tissues	Osteogenesis Imperfecta
		Ehlers-Danlos syndrome—arthrochalasias type
II	Cartilage, intervertebral disk, vitreous	Achondrogenesis type II, spondyloepiphyseal dysplasia syndrome
III	Hollow organs, soft tissues	Vascular Ehlers-Danlos syndrome
V	Soft tissues, blood vessels	Classical Ehlers-Danlos syndrome
IX	Cartilage, vitreous	Stickler syndrome
Basement Membrane Collagens		

IV	Basement membranes	Alport syndrome
Other Collagens		
VI	Ubiquitous in microfibrils	Bethlem myopathy
VII	Anchoring fibrils at dermal-epidermal junctions	Dystrophic epidermolysis bullosa
IX	Cartilage, intervertebral disks	Multiple epiphyseal dysplasias
XVII	Transmembrane collagen in epidermal cells	Benign atrophic generalized epidermolysis bullosa
XV and XVIII	Endostatin-forming collagens, endothelial cells	Knobloch syndrome (type XVIII collagen)
Courtesy of Dr. Peter H. Byers, Department of Pathology, University of Washington, Seattle, WA		

original size after release of the tension. Morphologically, elastic fibers consist of a central core made of elastin, surrounded by a peripheral network of microfibrils. Substantial amounts of elastin are found in the walls of large blood vessels, such as the aorta, and in the uterus, skin, and ligaments. The peripheral microfibrillar network that surrounds the core consists largely of *fibrillin*, a 350-kD secreted glycoprotein, which associates either with itself or with other components of the ECM. The microfibrils serve as scaffolding for deposition of elastin and the assembly of elastic fibers. Inherited defects in fibrillin[86] result in formation of abnormal elastic fibers in a fairly common familial disorder, Marfan syndrome, manifested by changes in the cardiovascular system (aortic dissection) and the skeleton (Chapter 5).

CELL ADHESION PROTEINS

Most adhesion proteins, also called CAMs (cell adhesion molecules), can be classified into four main families: immunoglobulin family CAMs, cadherins, integrins, and selectins. These proteins are located in the cell membrane, where they function as receptors, or they are stored in the cytoplasm. As receptors, CAMs can bind to similar or different molecules in other cells, providing for interaction between the same cells (homotypic interaction) or different cell types (heterotypic interaction). Cadherins are generally involved in calcium-dependent homotypic interactions, while immunoglobulin family CAMs, because of the types of ligands they can bind, participate in both homotypic and heterotypic cell-to-cell interactions. The integrins have broader ligand specificity and are responsible for many events involving cell adhesion.[87] [88]

Integrins bind both to matrix proteins such as fibronectin and laminin, mediating adhesiveness between cells and ECM, as well as to adhesive proteins in other cells, establishing cell-to-cell contacts (see Box 2-1 , Chapter 2). *Fibronectin* is a larger protein that binds to many molecules, such as collagen, fibrin, proteoglycans, and cell-surface receptors. It consists of two glycoprotein chains, held together by disulfide bonds. Fibronectin mRNA has two splice forms, giving rise to tissue fibronectin and plasma fibronectin. The tissue fibronectin forms fibrillar aggregates at wound healing sites. The plasma form binds to fibrin, forming the provisional blood clot that

Figure 3-15 Steps in collagen synthesis (see text).

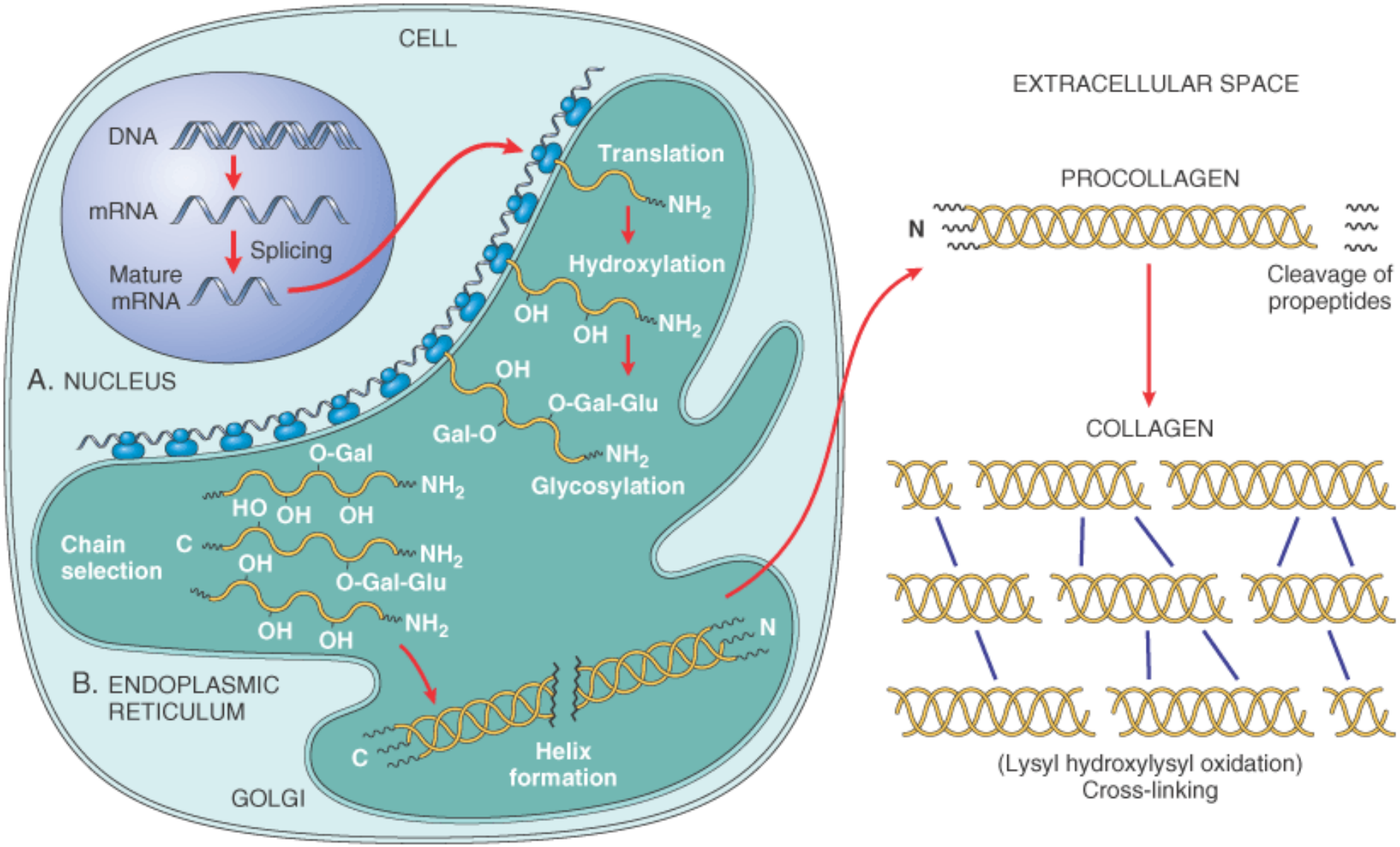
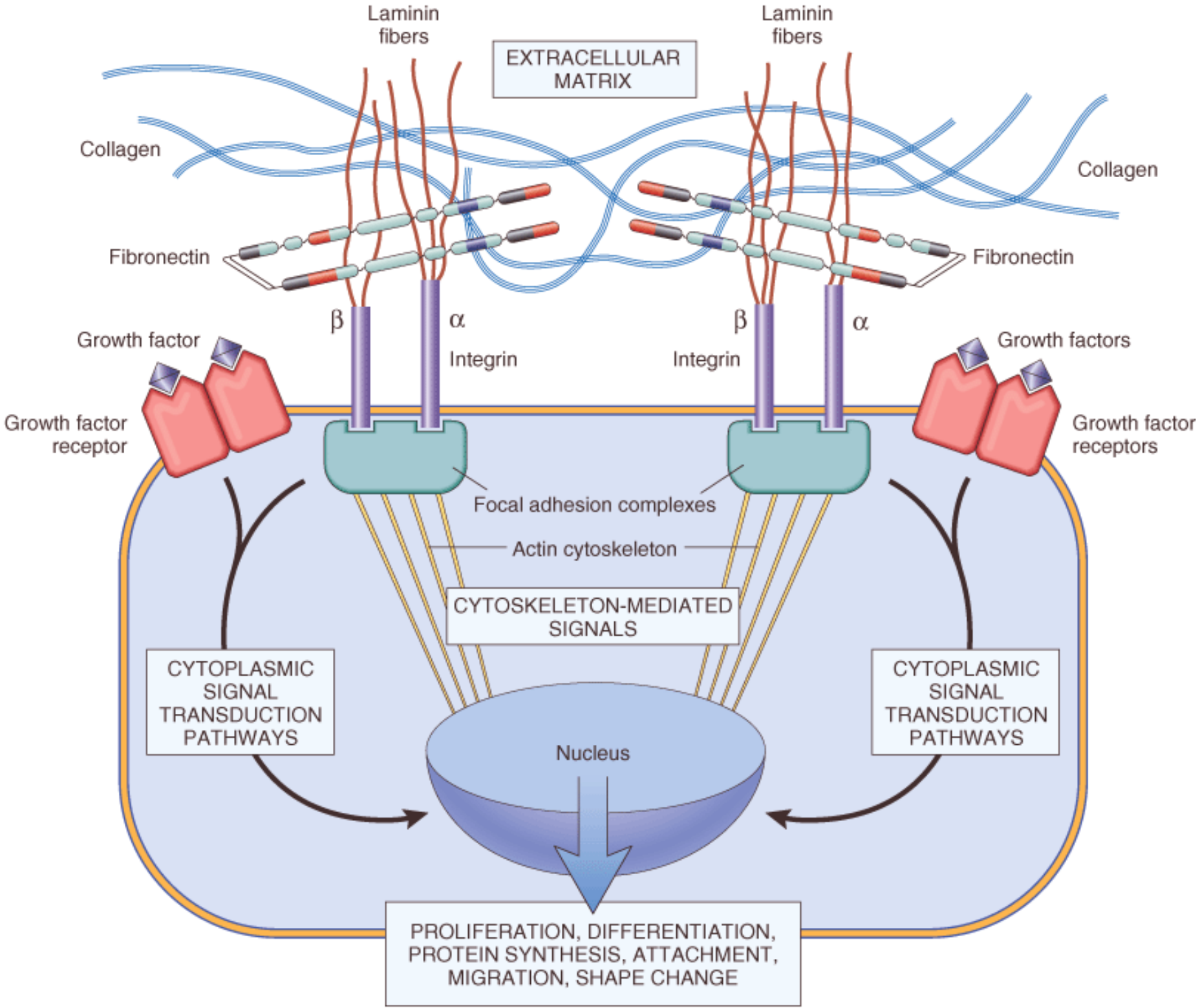


Figure 3-16 Mechanisms by which ECM (e.g., fibronectin and laminin) and growth factors can influence cell growth, motility, differentiation, and protein synthesis. Integrins bind ECM components and interact with the cytoskeleton at focal adhesion complexes (protein aggregates that include vinculin, α-actin, and talin). This can initiate the production of intracellular messengers or can directly mediate nuclear signals. Cell-surface receptors for growth factors may activate signal transduction pathways that overlap with those activated by integrins. Collectively, these are integrated by the cell to yield various responses, including changes in cell growth, locomotion, and differentiation.


Laminin fibers
EXTRACELLULAR MATRIX
Laminin fibers
Collagen
Collagen
Fibronectin
Fibronectin
β
α
Integrin
β
α
Integrin
Growth factor
Growth factors
Growth factor receptor
Growth factor receptors
Focal adhesion complexes
Actin cytoskeleton
CYTOSKELETON-MEDIATED SIGNALS
CYTOPLASMIC SIGNAL TRANSDUCTION PATHWAYS
CYTOPLASMIC SIGNAL TRANSDUCTION PATHWAYS
Nucleus
PROLIFERATION, DIFFERENTIATION, PROTEIN SYNTHESIS, ATTACHMENT, MIGRATION, SHAPE CHANGE

MIGRATION, SHAPE CHANGE

Figure 3-17 *A*, Granulation tissue showing numerous blood vessels, edema, and a loose ECM containing occasional inflammatory cells. This is a trichrome stain that stains collagen blue; minimal mature collagen can be seen at this point. *B*, Trichrome stain of mature scar, showing dense collagen, with only scattered vascular channels.

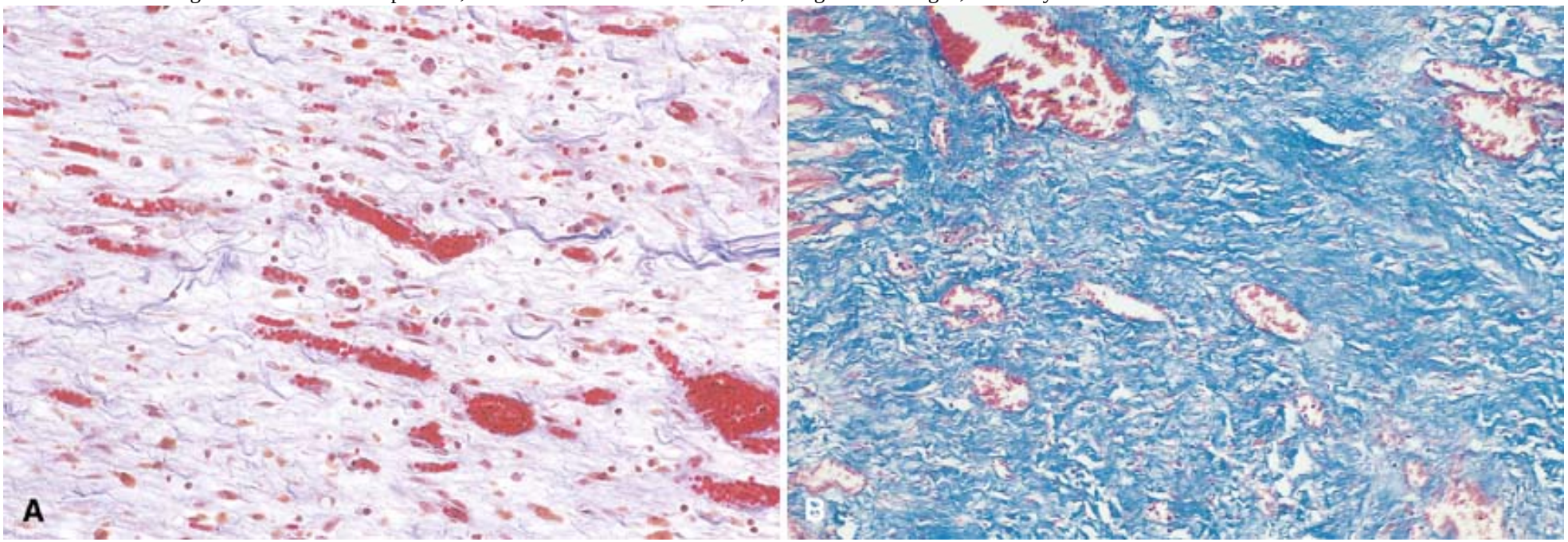


Figure 3-18 Angiogenesis by mobilization of endothelial precursor cells (EPCs) from the bone marrow and from pre-existing vessels (capillary growth). EPCs are mobilized from the bone marrow and may migrate to a site of injury or tumor growth *(upper panel)*. The homing mechanisms have not yet been defined. At these sites, EPCs differentiate and form a mature network by linking with existing vessels. In angiogenesis from pre-existing vessels, endothelial cells from these vessels become motile and proliferate to form capillary sprouts *(lower panel)*. Regardless of the initiating mechanism, vessel maturation (stabilization) involves the recruitment of pericytes and smooth muscle cells to form the periendothelial layer. *(Modified from Conway EM, Collen D, Carmeliet P: Molecular mechanisms of blood vessel growth. Cardiovasc Res 49:507, 2001.)*

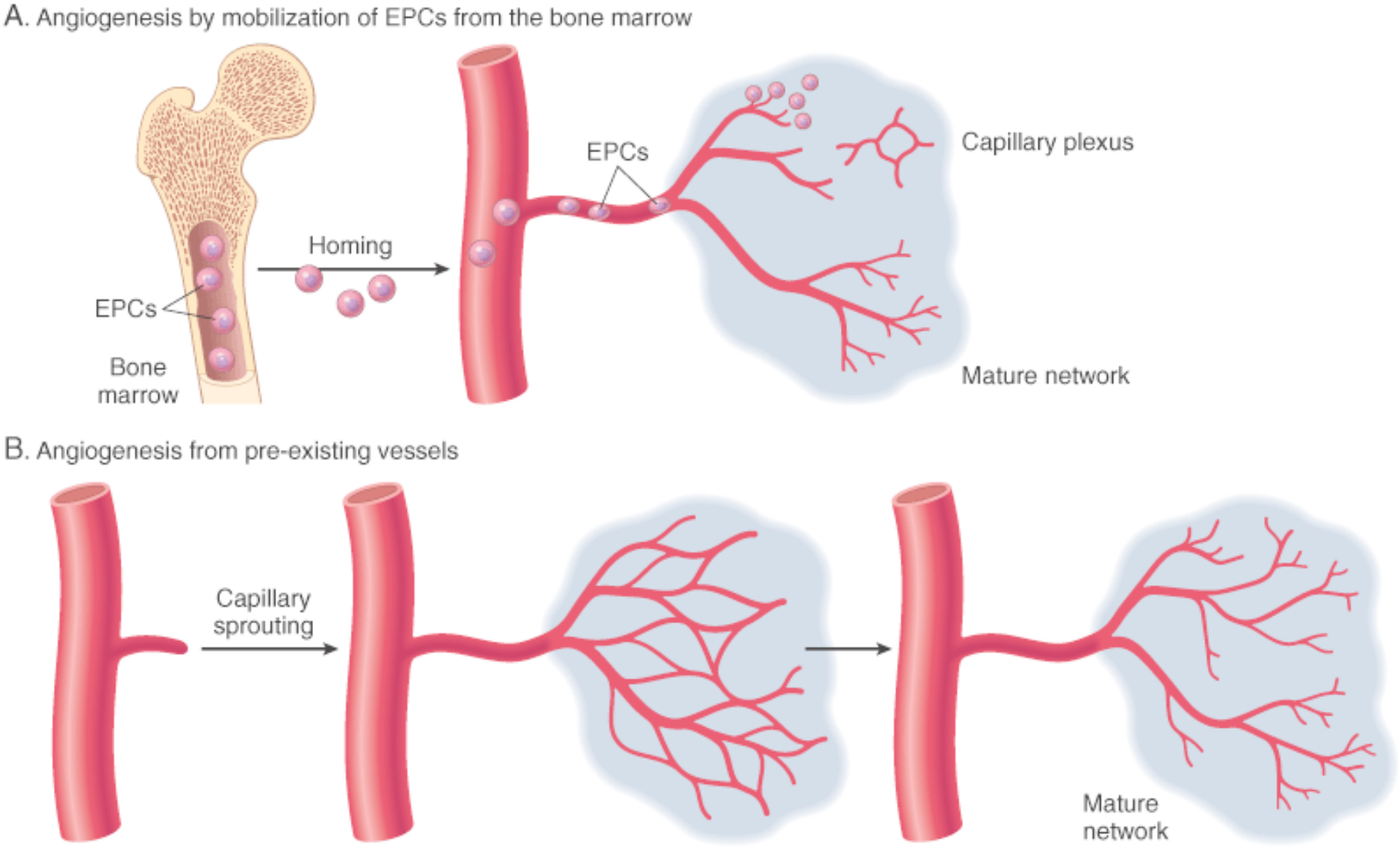


TABLE 3-3 -- Vascular Endothelial Growth Factor (VEGF)

Proteins	Family members: VEGF (VEGF-A), VEGF-B, VEGF-C, VEGF-D
	Dimeric glycoprotein with multiple isoforms
	Targeted mutations in VEGF result in defective vasculogenesis and angiogenesis
Production	Expressed at low levels in a variety of adult tissues and at higher levels in a few sites, such as podocytes in the glomerulus and cardiac myocytes
Inducing Agents	Hypoxia
	TGF-β

	PDGF
	TGF-α
Receptors	VEGFR-1
	VEGFR-2 (restricted to endothelial cells)
	VEGFR-3 (lymphatic endothelial cells)
	Targeted mutations in the receptors result in lack of vasculogenesis
Functions	Promotes angiogenesis
	Increases vascular permeability
	Stimulates endothelial cell migration
	Stimulates endothelial cell proliferation
	VEGF-C selectively induces hyperplasia of lymphatic vasculature
	Up-regulates endothelial expression of plasminogen activator, plasminogen activator inhibitor-1, tissue factor, and interstitial collagenase

in the absence of VEGF, more responsive to inhibitors of angiogenesis. A telling proof of the importance of these molecules is the existence of a genetic disorder caused by mutations in *Tie2* that is characterized by venous malformations.[103] Both physiologic and pathologic angiogenesis can be influenced by agents or conditions that stimulate VEGF expression, such as certain cytokines and growth factors (e.g., TGF-β, PDGF, TGF-α) and, notably, tissue hypoxia, which has long been associated with angiogenesis (see Table 3-3).

Despite the diversity of factors that may participate at various steps in angiogenesis, VEGF emerges as the most important growth factor in adult tissues undergoing physiologic angiogenesis (e.g., proliferating endometrium) as well as pathologic angiogenesis seen in chronic inflammation, wound healing, tumors, and diabetic retinopathy.

ECM Proteins as Regulators of Angiogenesis

A key component of angiogenesis is the motility and directed migration of endothelial cells, required for the formation of new blood vessels. These processes are controlled by several classes of proteins, including (1) *integrins*, especially $\alpha_v \beta_3$, which is critical for the formation and maintenance of newly formed blood vessels,[104] (2) *matricellular proteins*, including thrombospondin 1, SPARC, and tenascin C, which destabilize cell-matrix interactions and therefore promote angiogenesis,[105] and (3) *proteinases*, such as the plasminogen activators and *matrix metalloproteinases*, which are important in tissue remodeling during endothelial invasion. Additionally, these proteinases cleave extracellular proteins, releasing matrix-bound growth factors such as VEGF and FGF-2 that stimulate angiogenesis. Proteinases can also release inhibitors such as endostatin, a small fragment of collagen that inhibits endothelial proliferation and angiogenesis.[106] $\alpha_v \beta_3$ integrin expression in endothelial cells is stimulated by hypoxia. This integrin has multiple effects on angiogenesis: it directly interacts with a metalloproteinase (MMP-2, discussed below), it binds to and regulates the activity of VEGFR-2, and it mediates adhesion to ECM components such as fibronectin, thrombospondin, and osteopontin. [104]

SCAR FORMATION

Growth factors and cytokines released at the site of injury induce fibroblast proliferation and migration into the granulation tissue framework of new blood vessels and loose ECM that initially forms at the repair site. We discuss three processes that participate in the formation of a scar: (1) *emigration and proliferation of fibroblasts in the site of injury*, (2) *deposition of ECM*, and (3) *tissue remodeling*.

Fibroblast Migration and Proliferation

Granulation tissue contains numerous newly formed blood vessels. As discussed previously, VEGF promotes angiogenesis but is also responsible for a marked increase in vascular permeability (VEGF was first named vascular permeability factor).[107] The latter activity leads to exudation and deposition of plasma proteins, such as fibrinogen and plasma fibronectin, in the ECM and provides a provisional stroma for fibroblast and endothelial cell ingrowth. *Migration* of fibroblasts to the site of injury and their subsequent *proliferation* are triggered by multiple growth factors, including TGF-β, PDGF, EGF, FGF, and the cytokines IL-1 and TNF (see Table 3-5). The sources of these growth factors and cytokines include platelets, a variety of inflammatory cells (notably macrophages), and activated endothelium. Macrophages are important cellular constituents of granulation tissue, clearing extracellular debris, fibrin, and other foreign material at the site of repair. These cells also elaborate TGF-β, PDGF, and FGF and there-fore promote fibroblast migration and proliferation.[108] If the appropriate chemotactic stimuli are present, mast cells, eosinophils, and lymphocytes may also accumulate. Each of these cells can contribute directly or indirectly to fibroblast migration and proliferation. Of the growth factors involved in inflammatory fibrosis, TGF-β appears to be the most important because of the multitude of effects that favor fibrous tissue deposition. TGF-β is produced by most of the cells in granulation tissue and causes *fibroblast migration and proliferation, increased synthesis of collagen and fibronectin, and decreased degradation of ECM by metalloproteinases* (discussed later). TGF-β is also chemotactic for monocytes and causes angiogenesis in vivo, possibly by inducing macrophage influx. TGF-β expression is increased in tissues in a number of chronic fibrotic diseases in humans and experimental animals.

ECM Deposition and Scar Formation

As repair continues, the number of proliferating endothelial cells and fibroblasts decreases. Fibroblasts progressively deposit increased amounts of ECM. Fibrillar collagens form a major portion of the connective tissue in repair sites and are important for the development of strength in healing wounds. As described later in the discussion of cutaneous wound healing, collagen synthesis by fibroblasts begins within 3 to 5 days after injury and continues for several weeks, depending on the size of wound. Many of the same growth factors that regulate fibroblast proliferation also stimulate ECM synthesis (see Table 3-4 (Table Not Available)). For example, collagen synthesis is enhanced by several factors, including growth factors (PDGF, FGF, TGF-β) and cytokines (IL-1, IL-13), which are secreted by leukocytes and fibroblasts in healing wounds. *Net collagen accumulation, however, depends not only on increased collagen synthesis but also on decreased degradation.* Ultimately, the granulation tissue scaffolding is converted into a scar composed of spindle-shaped fibroblasts, dense collagen, fragments of elastic tissue, and other ECM components. As the scar matures, vascular regression continues, eventually transforming the richly vascularized granulation tissue into a pale, avascular scar.

Tissue Remodeling

The replacement of granulation tissue with a scar involves transitions in the composition of the ECM. Some of the growth factors that stimulate synthesis of collagen and other connective tissue molecules also modulate the synthesis and activation of metalloproteinases, enzymes that degrade these ECM components. The balance between ECM synthesis and degradation results in *remodeling* of the connective tissue framework—an important feature of both chronic inflammation and wound repair.

Degradation of collagen and other ECM proteins is achieved by a family of matrix metalloproteinases (MMPs), which are dependent on zinc ions for their activity[109] [110] (Fig. 3-19). This

Figure 3-19 Matrix metalloproteinase regulation. Four mechanisms are shown: (1) regulation of synthesis by growth factors or cytokines, (2) inhibition of synthesis by corticosteroids or TGF-β, (3) regulation of the activation of the secreted but inactive precursors, and (4) blockage of the enzymes by specific tissue inhibitors of metalloproteinase (TIMPs). *(Modified from Matrisian LM: Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet 6:122, 1990, with permission from Elsevier Science.)*

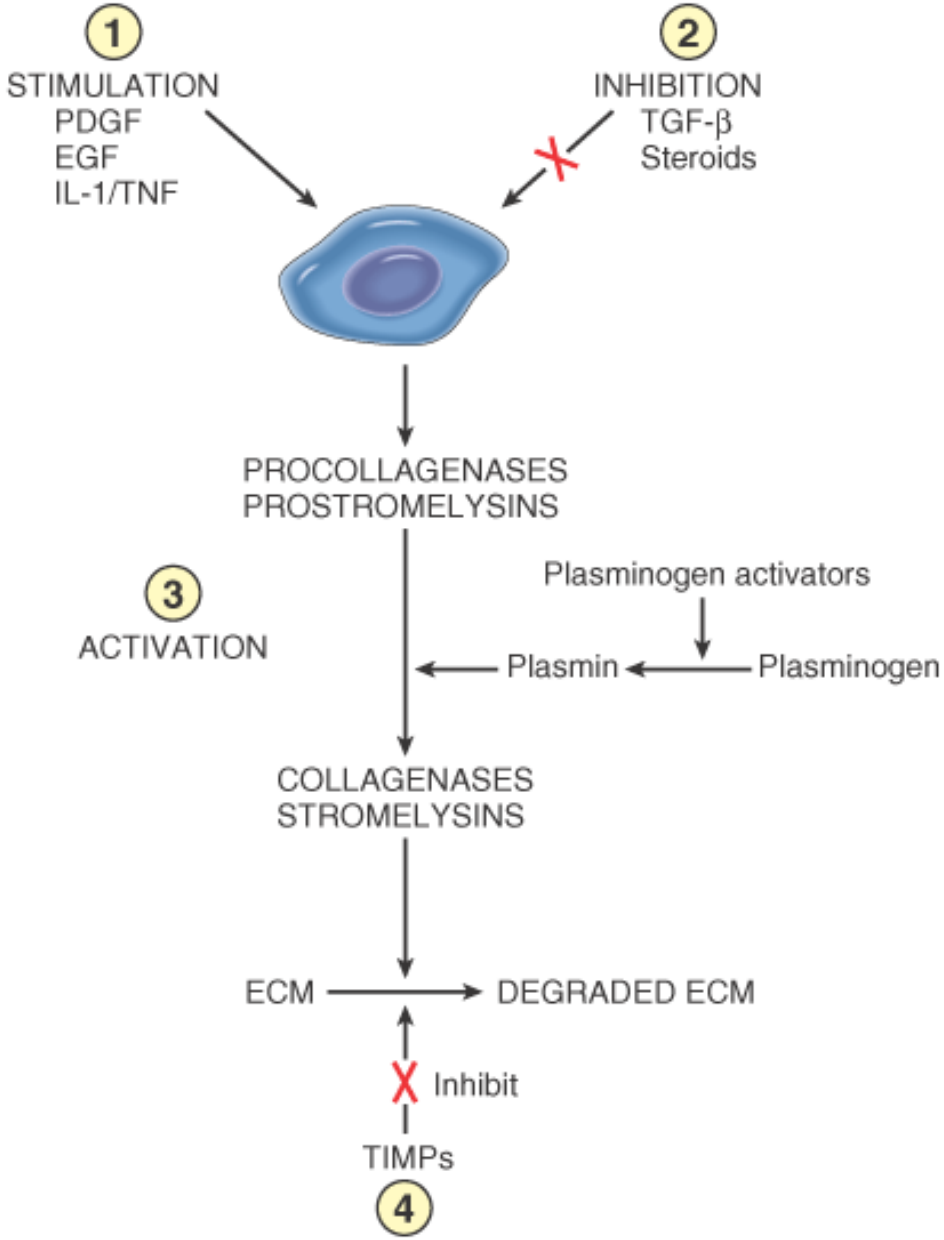


Figure 3-20 Phases of wound healing. *(Modified from Clark RAF: Wound repair. In Clark RAF (ed): The molecular and cellular biology of wound repair, 2nd ed, New York, Plenum Press, 1996, p. 3.)*

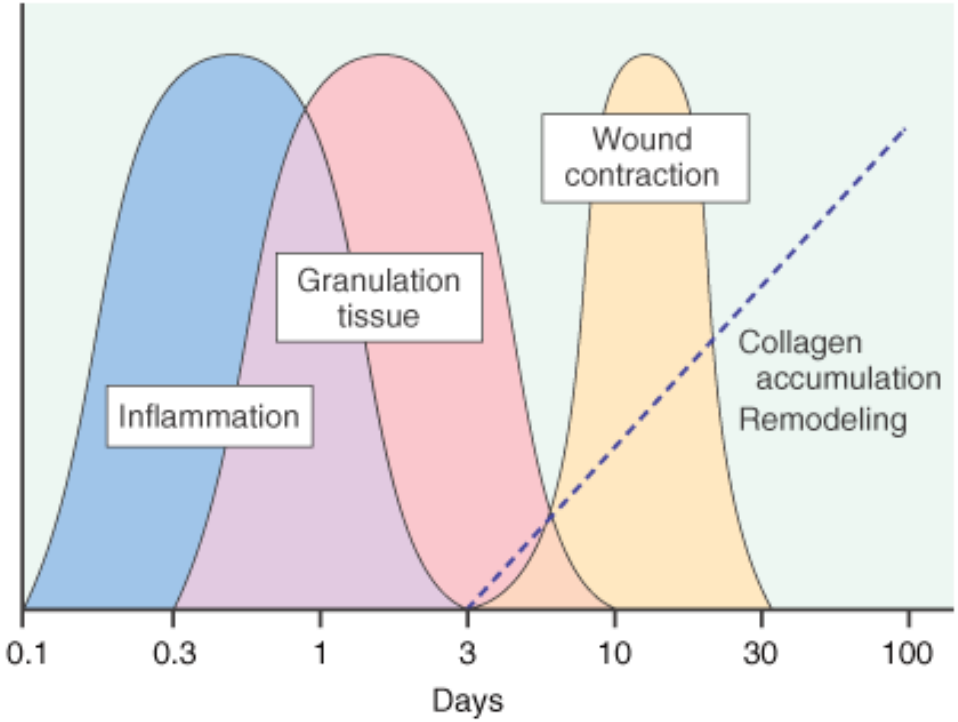
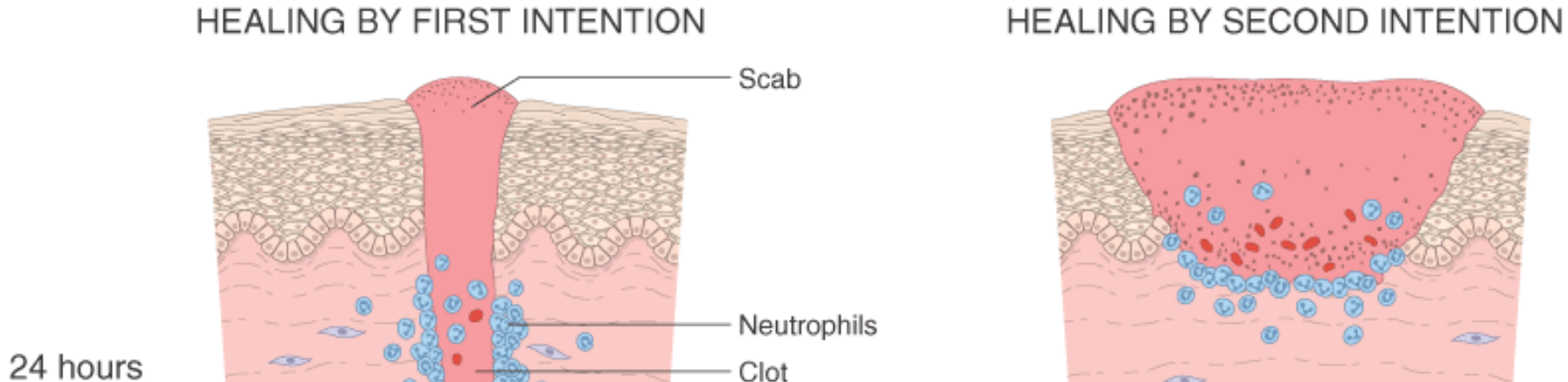


TABLE 3-4 -- Growth Factors and Cytokines Affecting Various Steps in Wound Healing

(Not Available)

Figure 3-21 Steps in wound healing by first intention *(left)* and second intention *(right)*. Note large amounts of granulation tissue and wound contraction in healing by second intention.



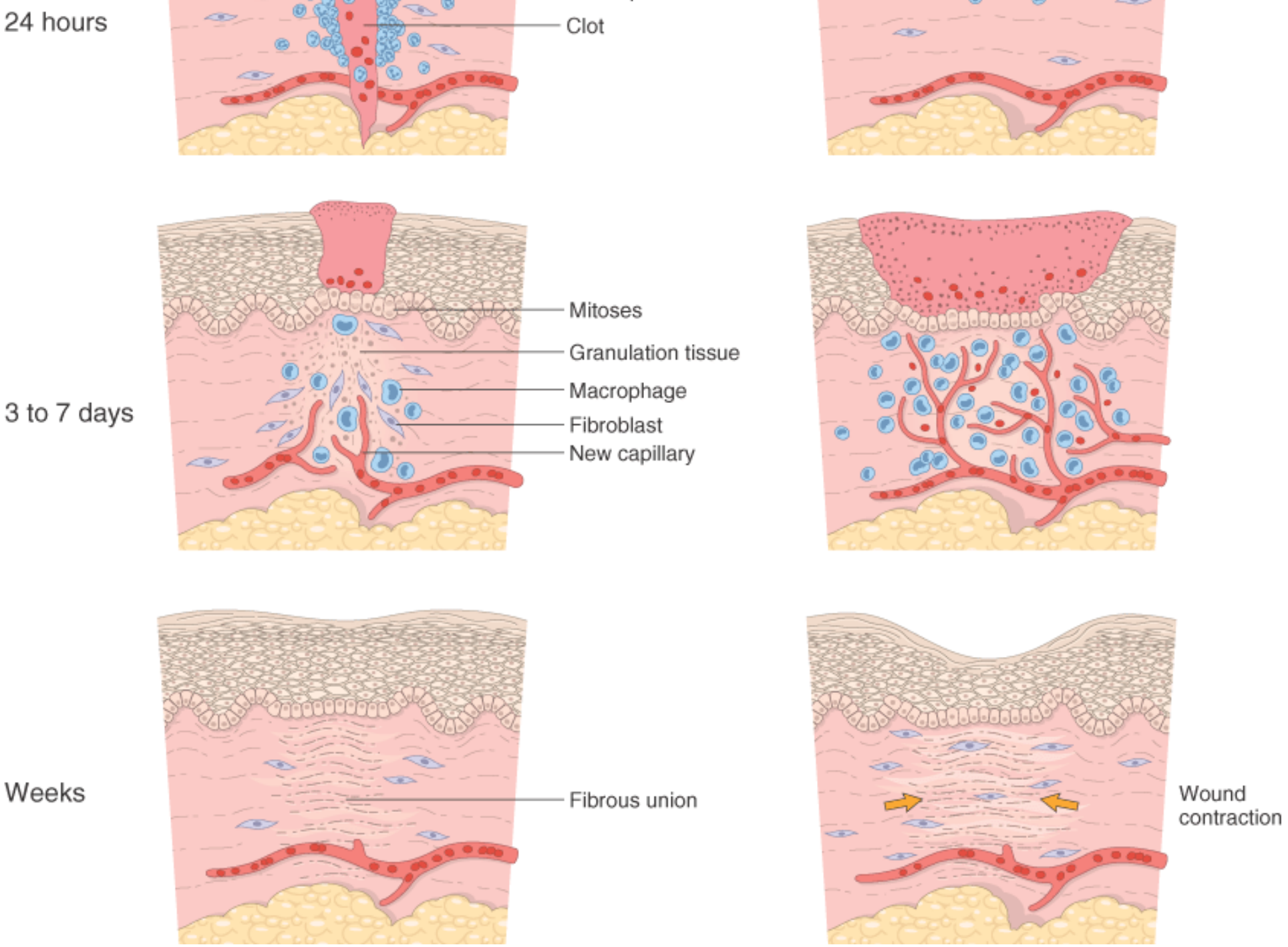


Figure 3-22 Healing of skin ulcers. *A*, Pressure ulcer of the skin, commonly found in diabetic patients. The histology slides show *B*, a skin ulcer with a large gap between the edges of the lesion; *C*, a thin layer of epidermal reepithelialization and extensive granulation tissue formation in the dermis; and *D*, continuing reepithelialization of the epidermis and wound contraction. *(Courtesy of Z. Argenyi, M.D., University of Washington.)*

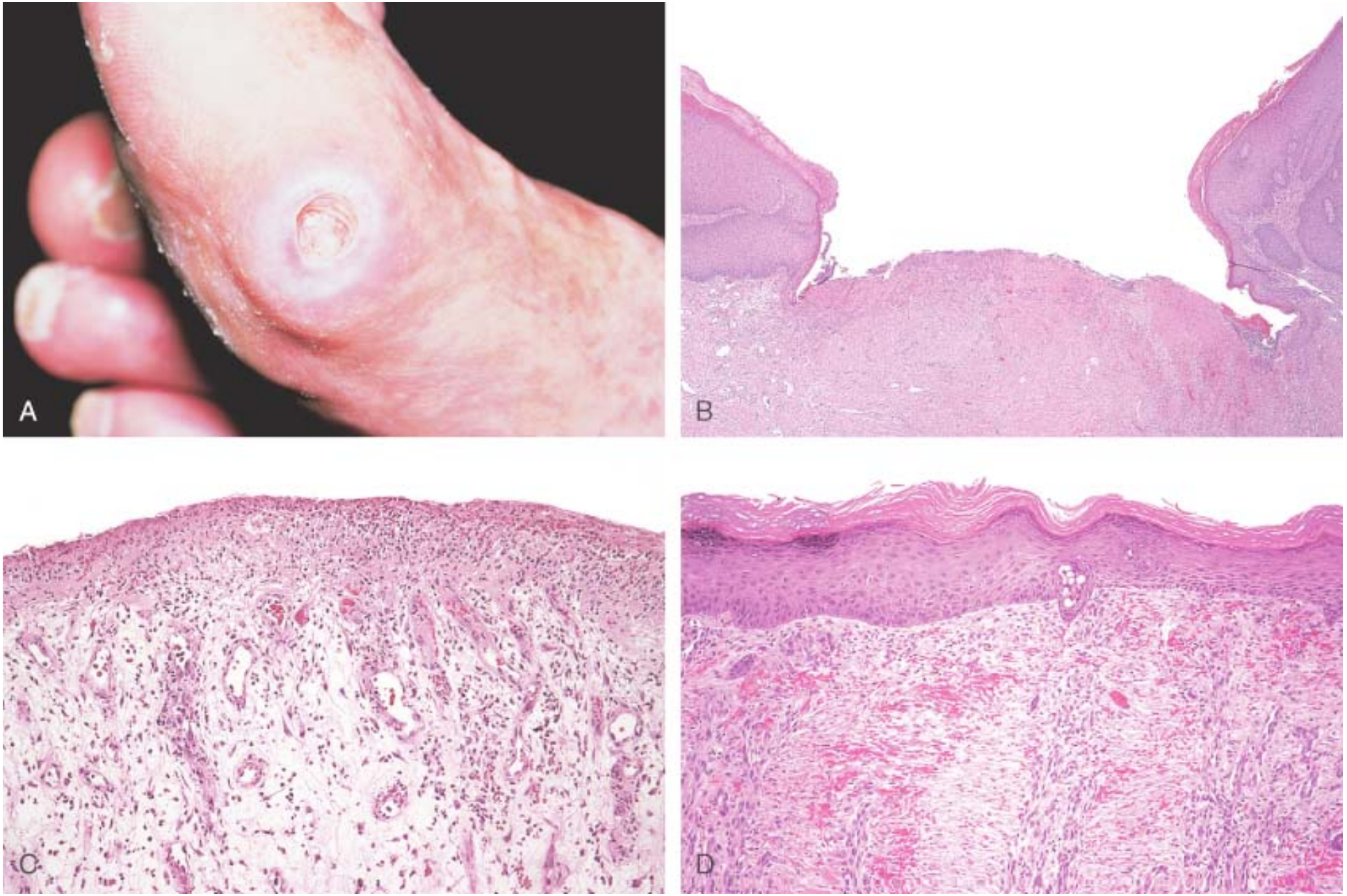


TABLE 3-5 -- Factors That Retard Wound Healing

Local Factors	
Blood supply	Mechanical stress
Denervation	Necrotic tissue

Local infection	Protection (dressings)
Foreign body	Surgical techniques
Hematoma	Type of tissue
Systemic Factors	
Age	Malnutrition
Anemia	Obesity
Drugs (steroids, cytotoxic medications, intensive antibiotic therapy)	Systemic infection
	Temperature
	Trauma, hypovolemia, and hypoxia
Genetic disorders (osteogenesis imperfecta, Ehlers-Danlos syndromes, Marfan syndrome)	
	Uremia
	Vitamin deficiency (vitamin C)
Hormones	Trace metal deficiency (zinc, copper)
Diabetes	
Malignant disease	
Adapted from Schwartz SI: Principles of Surgery. New York, McGraw Hill, 1999.	

- *Infection* is the single most important cause of delay in healing because it results in persistent tissue injury and inflammation.
- *Mechanical factors*, such as early motion of wounds, can delay healing, by compressing blood vessels and separating the edges of the wound.
- *Foreign bodies*, such as unnecessary sutures or fragments of steel, glass, or even bone, constitute impediments to healing.
- *Size, location, and type of wound influence healing.* Wounds in richly vascularized areas, such as the face, heal faster than those in poorly vascularized ones, such as the foot. As we have discussed, small incisional injuries heal faster and with less scar formation than large excisional wounds or wounds caused by blunt trauma.

SUMMARY OF CUTANEOUS WOUND HEALING

Various stages in the healing of an ulcer of the skin are shown in Figure 3-22 . The healing wound, as a prototype of tissue repair, is a dynamic and changing process. The early phase is one of inflammation, followed by formation of granulation tissue and subsequent tissue remodeling and scarring. Simple cutaneous incisional wounds heal by first intention. Large cutaneous wounds heal by second intention, generating a significant amount of scar tissue. Different mechanisms occurring at different times trigger the release of chemical signals that modulate the orderly migration, proliferation, and differentiation of cells and the synthesis and degradation of ECM proteins. These proteins, in turn, directly affect cellular events and modulate cell responsiveness to soluble growth factors. The magic behind the precise orchestration of these events under normal conditions remains beyond our grasp. It almost certainly lies in the regulation of specific soluble and membrane-anchored mediators and their receptors on particular cells, cell-matrix interactions, and the effect of physical factors, including ECM remodeling forces generated by changes in cell shape.

COMPLICATIONS IN CUTANEOUS WOUND HEALING

Complications in wound healing can arise from abnormalities in any of the basic components of the repair process. These aberrations can be grouped into three general categories: (1)

deficient scar formation, (2) *excessive formation of the repair components*, and (3) *formation of contractures*.

Inadequate formation of granulation tissue or assembly of a scar can lead to two types of complications: *wound dehiscence* and *ulceration*. Dehiscence or rupture of a wound is most common after abdominal surgery and is due to increased abdominal pressure. This mechanical stress on the abdominal wound can be generated by vomiting, coughing, or ileus. Wounds can ulcerate because of inadequate vascularization during healing. For example, lower extremity wounds in individuals with atherosclerotic peripheral vascular disease typically ulcerate (Chapter 11). Nonhealing wounds also form in areas devoid of sensation. These neuropathic ulcers are occasionally seen in patients with diabetic peripheral neuropathy (Chapter 24 and Chapter 27).

Excessive formation of the components of the repair process can also complicate wound healing. Aberrations of growth may occur even in what may begin initially as normal wound healing. The accumulation of excessive amounts of collagen may give rise to a raised scar known as a *hypertrophic scar*; if the scar tissue grows beyond the boundaries of the original wound and does not regress, it is called a *keloid* (Fig. 3-23). Keloid formation appears to be an individual predisposition, and for unknown reasons this aberration is somewhat more common in African-Americans. The mechanisms of keloid formation are still unknown. Another deviation in wound healing is the formation of excessive amounts of granulation tissue, which protrudes above the level of the surrounding skin and blocks re-epithelialization. This has been called *exuberant granulation* (or, with more literary fervor, *proud flesh*). Excessive granulation must be removed by cautery or surgical excision to permit restoration of the continuity of the epithelium. Finally (fortunately rarely), incisional scars or traumatic injuries may be followed by exuberant proliferation of fibroblasts and other connective tissue elements that may, in fact, recur after excision. Called *desmoids*, or *aggressive fibromatoses*, these lie in the interface between benign proliferations and malignant (though low-grade) tumors. The line between the benign hyperplasias characteristic of repair and neoplasia is frequently finely drawn (Chapter 7).

Contraction in the size of a wound is an important part of the normal healing process. An exaggeration of this process is called a *contracture* and results in deformities of the wound and the surrounding tissues. Contractures are particularly prone to develop on the palms, the soles, and the anterior aspect of the thorax. Contractures are commonly seen after serious burns and can compromise the movement of joints. Impaired wound contraction occurs in stromelysin-1 (MMP3)—deficient mice, suggesting that proteolysis by this metalloproteinase is required for the assembly of fibroblasts containing actin filaments, needed for the contraction of early wounds. [120]

Figure 3-23 *A*, Keloid. Excess collagen deposition in the skin forming a raised scar known as keloid. *(From Murphy GF, Herzberg AJ: Atlas of Dermatopathology. Philadelphia, Saunders, W.B. 1996, p. 219.) B*, Note the thick connective tissue deposition in the dermis. *(Slide courtesy of Z. Argenyi, M.D., University of Washington, Seattle, WA.)*

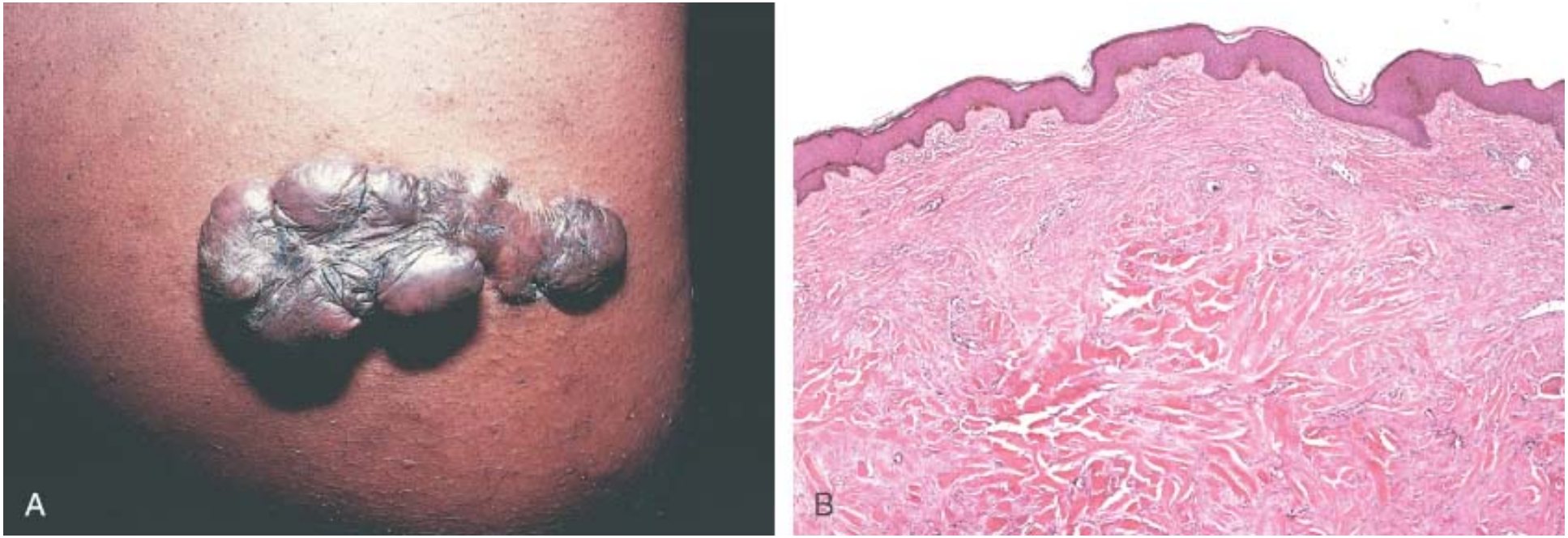


Figure 3-24 Development of fibrosis in chronic inflammation. The persistent stimulus of chronic inflammation activates macrophages and lymphocytes, leading to the production of growth factors and cytokines, which increase the synthesis of collagen. Deposition of collagen is enhanced by decreased activity of metalloproteinases.

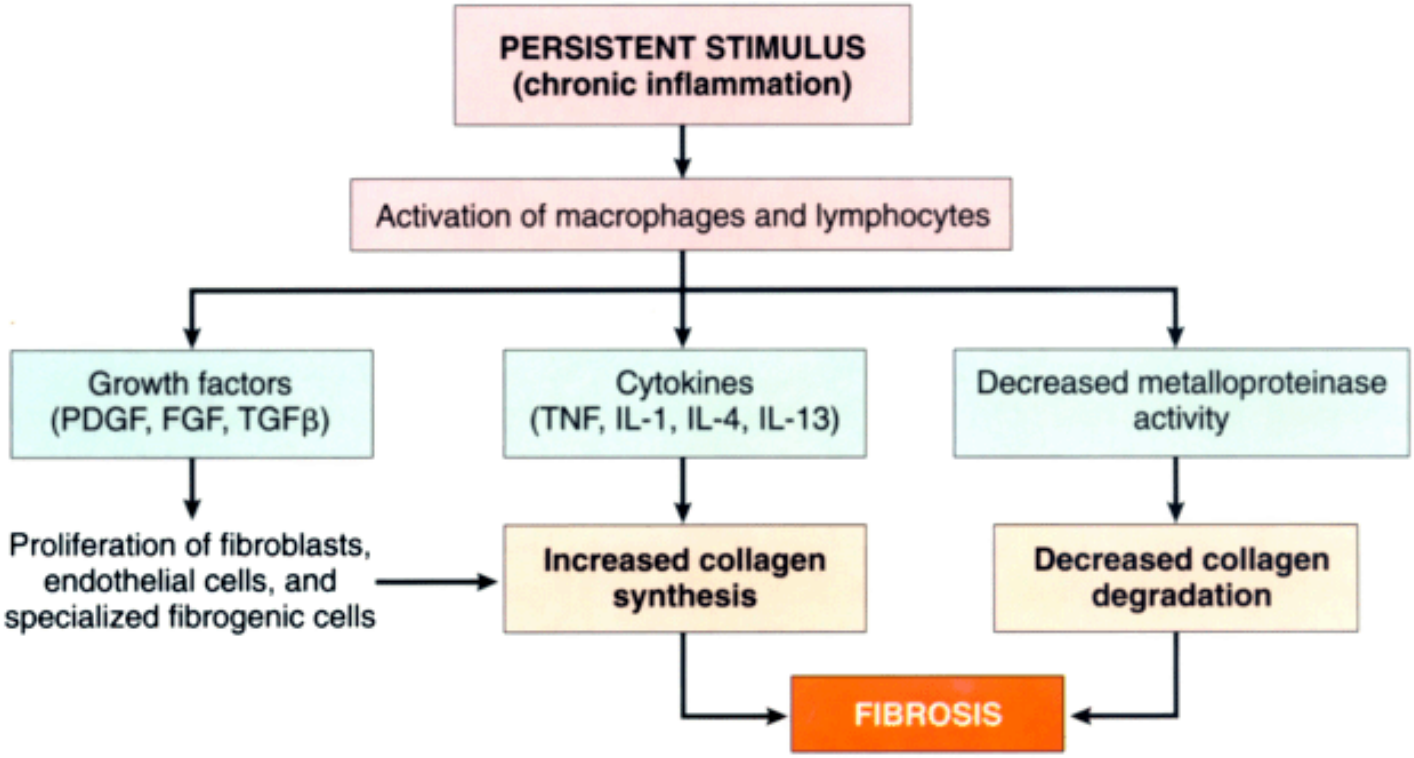
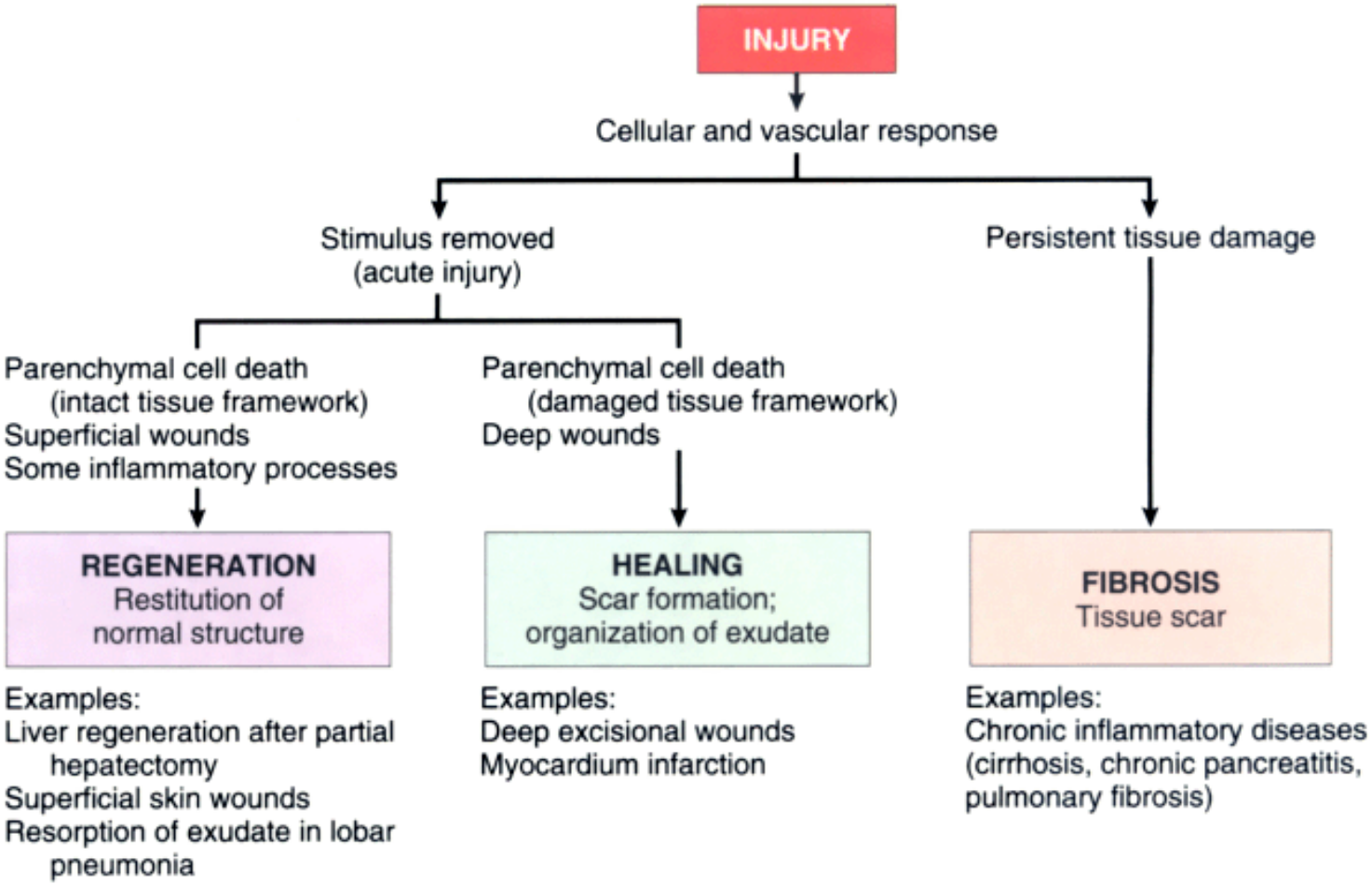


Figure 3-25 Repair responses after injury and inflammation. Repair after acute injury has several outcomes, including normal tissue restitution and healing with scar formation. Healing in chronic injury involves scar formation and fibrosis (see text).



References

1. Goss RJ: Regeneration versus repair. In Cohen IK, Diegelman RF, Lindblad WJ (eds): Wound Healing. Biochemical and Clinical Aspects. Philadelphia, W.B. Saunders, 1992, pp 20–39.

2. Clark RAF: Wound repair. In Clark RAF (ed): The Molecular and Cellular Biology of Wound Repair, 2nd ed. New York, Plenum Press, 1996, pp 3–50.

3. Fausto N: Liver regeneration, in Arias I. M., et al (eds): The Liver: Biology and Pathobiology, 4th ed. Philadelphia, Lippicott, Williams & Wilkins, 2001, pp 591–610.

4. Mullauer L, et al: Mutations in apoptosis genes: a pathogenetic factor for human disease. Mutat Res 488:211, 2001.

5. Hanahan D, Weinberg RA: The hallmarks of cancer. Cell 100:57, 2000.

6. Rosenthal N: Prometheus's vulture and the stem-cell promise. N Engl J Med 349:267, 2003.

7. Korbling M, Estrov Z: Adult stem cells for tissue repair—a new therapeutic concept? N Engl J Med 349:570, 2003.

8. Weissman IL, Anderson DJ, Gage F: Stem and progenitor cells: origins, phenotypes, lineage commitments, and transdifferentiations. Annu Rev Cell Dev Biol 17:387, 2001.

9. Tsai RY, Kittappa R, McKay RD: Plasticity, niches, and the use of stem cells. Dev Cell 2:707, 2002.

10. Fuchs E, Segre JA: Stem cells: a new lease on life. Cell 100:143–155, 2000.

11. Weissman I: Stem cells: units of development, units of regeneration, and units in evolution. Cell 100:157, 2000.

12. Martin GR: Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 78:7634, 1981.

13. Hadjantonakis A, Papaioannou V: The stem cells of early embryos. Differentiation 68:159, 2001.

14. Murray P, Edgar D: The regulation of embryonic stem cell differentiation by leukaemia inhibitory factor (LIF). Differentiation 68:227, 2001.

15. Cavaleri F, Scholer HR: Nanog: a new recruit to the embryonic stem cell orchestra. Cell 113:551, 2003.

15A. Sato N, et al: Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nature Med 10:55, 2004.

16. Hochedlinger K, Jaenisch R: Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. N Engl J Med 349:275, 2003.

17. Watt FM, Hogan BL: Out of Eden: stem cells and their niches. Science 287:1427, 2000.

18. Marshman E, Booth C, Potten CS: The intestinal epithelial stem cell. Bioessays 24:91, 2002.

19. Lavker RM, Sun TT: Epidermal stem cells: properties, markers, and location. Proc Natl Acad Sci USA 97:13473, 2000.

20. Alonso L, Fuchs E: Stem cells in the skin: waste not, Wnt not. Genes Dev 17:1189, 2003.

21. Tseng SCG, Sun-T-T: Stem cells: ocular surface maintenance. In Brightbill FS (ed): Corneal Surgery: Theory, Techniques and Tissue, 3rd ed. New York, Mosby, 1999, pp 9–18.

22. Verfaillie CM: Hematopoietic stem cells for transplantation. Nat Immunol 3:314, 2002.

23. Orkin SH, Morrison SJ: Stem-cell competition. Nature 418:25, 2002.

24. Lagasse E, et al: Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 6:1229, 2000.

25. LaBarge MA, Blau HM: Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. Cell 111:589, 2002.

26. Laflamme MA, et al: Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. Circ Res 90:634, 2002.

27. Wagers AJ, et al: Little evidence for developmental plasticity of adult hematopoietic stem cells. Science 297:2256, 2002.

28. Lemischka I: A few thoughts about the plasticity of stem cells. Exp Hematol 30:848, 2002.

29. Wang X, et al: Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 422:897, 2003.

30. Vassilopoulos G, Wang PR, Russell DW: Transplanted bone marrow regenerates liver by cell fusion. Nature 24:901, 2003.

31. Jiang Y, et al: Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41, 2002.

32. Jiang Y, et al: Multipotent progenitor cells can be isolated from postnatal murine bone marrow, muscle, and brain. Exp Hematol 30:896, 2002.

33. Toma JG, et al: Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 3:778, 2001.

34. Dorshkind K: Stem cells and lineage plasticity: the challenge to existing paradigms. Immunol Rev 187:5, 2002.

35. Fausto N, Campbell JS: The role of hepatocytes and oval cells in liver regeneration and repopulation. Mech Dev 120:117, 2003.

36. Libbrecht L, Roskams T: Hepatic progenitor cells in human liver diseases. Semin Cell Dev Biol 13:389, 2002.

37. Taupin P, Gage FH: Adult neurogenesis and neural stem cells of the central nervous system in mammals. J Neurosci Res 69:745, 2002.

38. Lois C, Alvarez-Buylla A: Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Virology 90:2074, 1993.

39. Pagano SF, et al: Isolation and characterization of neural stem cells from the adult human olfactory bulb. Stem Cells 18:295, 2000.

40. Panchision DM, McKay RD: The control of neural stem cells by morphogenic signals. Curr Opin Genet Dev 12:478, 2002.

41. van Praag H, et al: Functional neurogenesis in the adult hippocampus. 415:1030, 2002.

42. Zammit P, Beauchamp J: The skeletal muscle satellite cell: stem cell or son of stem cell? Differentiation 68:193, 2001.

43. Urbanek K, et al: Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. Proc Natl Acad Sci USA, 100:10440, 2003.

44. Carpenter G: The EGF receptor: a nexus for trafficking and signaling. Bioessays 22:697–707, 2000.

45. Zarnegar R, DeFrances MC, Michalopoulos GK: Hepatocyte growth factor: its role in hepatic growth and pathobiology. In Arias IM, et al (eds): The Liver: Biology and Pathobiology, 4th ed. Philadelphia, Lippincott, Williams & Wilkins, 2001, pp 611–629.

46. Ferrara N, Gerber HP, LeCouter J: The biology of VEGF and its receptors. Nat Med 9:669, 2003.

47. Heldin CH, Eriksson U, Ostman A: New members of the platelet-derived growth factor family of mitogens. Arch Biochem Biophys 398:284, 2002.

48. Zaret KS: Hepatocyte differentiation: from the endoderm and beyond. Curr Opin Genet Dev 11:568, 2001.

49. Massague J: How cells read TGF-β signals. Nature 1:169, 2000.

50. Attisano L, Wrana JL: Signal transduction by the TGF-β superfamily. Science 296:1646, 2002.

51. Moustakas A, et al: Mechanisms of TGF-β signaling in regulation of cell growth and differentiation. Immunol Lett 82:85, 2002.

52. Chang L, Karin M: Mammalian MAP kinase signalling cascades. Nature 410:37, 2001.

53. Garrington TP, Johnson GL: Organization and regulation of mitogen-activated protein kinase signaling pathways. Curr Opin Cell Biol 11:211, 1999.

54. Cantley LC: The phosphoinositide 3-kinase pathway. Science 296:1655, 2002.

55. Lawlor MA, Alessi DR: PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? J Cell Sci 114:2903, 2001.

56. Wilkinson MG, Millar JB: Control of the eukaryotic cell cycle by MAP kinase signaling pathways. FASEB J 14:2147, 2000.

57. Aaronson DS, Horvath CM: A road map for those who don't know JAK-STAT. Science 296:1653, 2002.

58. Levy DE, Darnell JEJ: Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol 3:651, 2002.

59. Neves SR, Ram PT, Iyengar R: G protein pathways. Science 296:1636, 2002.

60. Pierce KL, Premont RT, Lefkowitz RJ: Seven-transmembrane receptors. Nat Rev Mol Cell Biol 3:639, 2002.

61. Berridge MJ, Lipp P, Bootman MD: The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 1:11, 2000.

62. Bootman MD, Berridge MJ, Roderick HL: Calcium signalling: more messengers, more channels, more complexity. Curr Biol 12:R563, 2002.

63. Farfel Z, Bourne HR, Iiri T: The expanding spectrum of G protein diseases. N Engl J Med 340:1012, 1999.

64. Beato M, Klug J: Steroid hormone receptors: an update. Hum Reprod Update 6:225, 2000.

65. McDonnell DP, Norris JD: Connections and regulation of the human estrogen receptor. Science 296:1642, 2002.

---

66. Qi C, Zhu Y, Reddy JK: Peroxisome proliferator-activated receptors, coactivators, and downstream targets. Cell Biochem Biophys 32:187, 2000.

67. Ryan KM, Phillips AC, Vousden KH: Regulation and function of the p53 tumor suppressor protein. Curr Opin Cell Biol 13:332, 2001.

68. Shaulian E, Karin M: AP-1 as a regulator of cell life and death. Nat Cell Biol 4:E131, 2002.

69. Lundberg AS, Weinberg RA: Control of the cell cycle and apoptosis. Eur J Cancer 35:1886, 1999.

70. Pietenpol JA, Stewart ZA: Cell cycle checkpoint signaling: cell cycle arrest versus apoptosis. Toxicology 181–182:475, 2002.

71. Agami R, Bernards R: Convergence of mitogenic and DNA damage signaling in the G_1 phase of the cell cycle. Cancer Lett 177:111, 2002.

72. Ekholm SV, Reed SI: Regulation of G_1 cyclin-dependent kinases in the mammalian cell cycle. Curr Opin Cell Biol 12:676, 2000.

73. Bartek J, Lukas J: Mammalian G_1 - and S-phase checkpoints in response to DNA damage. Curr Opin Cell Biol 13:738, 2001.

74. Walworth NC: Cell-cycle checkpoint kinases: checking in on the cell cycle. Curr Opin Cell Biol 12:697, 2000.

75. Brockes JP, Kumar A: Plasticity and reprogramming of differentiated cells in amphibian regeneration. Nat Rev Mol Cell Biol 3:566, 2002.

76. Tanaka EM: Regeneration: if they can do it, why can't we? Cell 113:559, 2003.

77. Bonner-Weir S: Life and death of the pancreatic beta cells. Trends Endocrinol Metab 11:375, 2000.

78. Kritzik MR, et al: Transcription factor expression during pancreatic islet regeneration. Mol Cell Endocrinol 164:99, 2000.

79. Fausto N: Liver regeneration. J Hepatol 32:19, 2000.

80. Su AI, et al: Gene expression during the priming phase of liver regeneration after partial hepatectomy in mice. Proc Natl Acad Sci USA 99:11181, 2002.

81. Michalopoulos GK, DeFrances MC: Liver regeneration. Science 276:60, 1997.

82. Sanes JR: The basement membrane/basal lamina of skeletal muscle. J Biol Chem 278:12601, 2003.

83. Byers PH: Disorders of collagen biosynthesis and structure. In Scriver CR, et al (eds): The Metabolic & Molecular Basis of Inherited Disease. New York, McGraw-Hill, 2001, pp. 5241–5249.

84. Myllyharju J, Kivirikko KI: Collagens and collagen-related diseases. Ann Med 33:7, 2001.

85. Milewicz DM, Urban Z, Boyd C: Genetic disorders of the elastic fiber system. Matrix Biol 19:471, 2000.

86. Robinson PN, Booms P: The molecular pathogenesis of the Marfan syndrome. Cell Mol Life Sci 58:1698, 2001.

87. Hynes R: Integrins: bidirectional, allosteric signaling machines. Cell 110:673, 2002.

88. Stupack DG, Cheresh DA: Get a ligand, get a life: integrins, signaling and cell survival. J Cell Sci 115:3729, 2002.

89. Bradshaw AD, Sage EH: SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J Clin Invest 107:1049, 2001.

90. Sodek J, et al: Novel functions of the matricellular proteins osteopontin and osteonectin/SPARC. Connect Tissue Res 43:308, 2002.

91. Sugahara K, Kitagawa H: Recent advances in the study of the biosynthesis and functions of sulfated glycosaminoglycans. Curr Opin Struct Biol 10:518, 2000.

92. Toole BP, Wight TN, Tammi MI: Hyaluronan-cell interactions in cancer and vascular disease. J Biol Chem 277:4593, 2002.

93. Ponta H, Sherman L, Herrlich PA: CD44: from adhesion molecules to signalling regulators. Nat Rev Mol Cell Biol 4:33, 2003.

94. Carmeliet P: Angiogenesis in health and disease. Nat Med 9:653, 2003.

95. Conway EM, Collen D, Carmeliet P: Molecular mechanisms of blood vessel growth. Cardiovasc Res 49:507, 2001.

96. Kubo H, Alitalo K: The bloody fate of endothelial stem cells. Genes Dev 17:322, 2003.

97. Rafii S, et al: Contribution of marrow-derived progenitors to vascular and cardiac regeneration. Semin Cell Dev Biol 13:61, 2002.

98. Reyes M, et al: Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 109:337, 2002.

99. Hill JM, et al: Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 348:593, 2003.

100. Blasi F, Carmeliet P: uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol 3:932, 2002.

101. Jain RK: Molecular regulation of vessel maturation. Nat Med 9:685, 2003.

102. Folkman J: Role of angiogenesis in tumor growth and metastasis. Semin Oncol 29:15, 2002.

103. Vikkula M, Boon LM, Mulliken JB: Molecular genetics of vascular malformations. Matrix Biol 20:327, 2001.

104. Iivanainen E, Kahari VM, Heino J, et al: Endothelial cell-matrix interactions. Microsc Res Tech 60:13, 2003.

105. Bornstein P, Sage EH: Matricellular proteins: extracellular modulators of cell function. Curr Opin Cell Biol 14:608, 2002.

106. O'Reilly MS, et al: Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 88:277, 1997.

107. Dvorak HF: VPF/VEGF and the angiogenic response. Semin Perinatol 24:75, 2000.

108. Schnaper HW, et al: TGF-β signal transduction and mesangial cell fibrogenesis. Am J Physiol Renal Physiol 284:F243, 2003.

109. McCawley LJ, Matrisian LM: Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol 13:534, 2001.

110. Vu TH, Werb Z: Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 14:2123, 2000.

111. Kheradmand F, Werb Z: Shedding light on sheddases: role in growth and development. Bioessays 24:8, 2002.

112. O'Kane S: Wound remodelling and scarring. J Wound Care 11:296, 2002.

113. McCallion RL, Ferguson MWJ: Fetal wound healing and the development of antiscarring therapies for adult wound healing. In Clark RAF (ed): The Molecular and Cellular Biology of Wound Repair, 2nd ed. New York, Plenum Press, 1996, pp 561–600.

114. Werner S, Grose R: Regulation of wound healing by growth factors and cytokines. Physicl Rev 83:835–870, 2003.

115. Cross KJ, Mustoe TA: Growth factors in wound healing. Surg Clin North Am 83:531–545, vi, 2003.

116. Henry G, Garner WL: Inflammatory mediators in wound healing. Surg Clin North Am 83:483–507, 2003.

117. Yates S, Rayner TE: Transcription factor activation in response to cutaneous injury: role of AP-1 in reepithelialization. Wound Repair Regen 10:5, 2002.

118. Serini G, Gabbiani G: Mechanisms of myofibroblast activity and phenotypic modulation. Exp Cell Res 250:273, 1999.

119. Tomasek JJ, et al: Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 3:349, 2002.

120. Bullard KM, et al: Impaired wound contraction in stromelysin-1-deficient mice. Ann Surg 230:260, 1999.

Chapter 4 - Hemodynamic Disorders, Thromboembolic Disease, and Shock

Richard N. Mitchell MD, PhD *

The health of cells and organs critically depends on an unbroken circulation to deliver oxygen and nutrients and to remove wastes. However, the well-being of tissues also requires normal fluid balance; abnormalities in vascular permeability or hemostasis can result in injury even in the setting of an intact blood supply. This chapter will describe major disturbances involving hemodynamics and the maintenance of blood flow, including edema, hemorrhage, thrombosis, embolism, infarction, and shock. *Normal fluid homeostasis encompasses maintenance of vessel wall integrity as well as intravascular pressure and osmolarity within certain physiologic ranges.* Changes in vascular volume, pressure, or protein content, or alterations in endothelial function, all affect the net movement of water across the vascular wall. Such water extravasation into the interstitial spaces is called *edema* and has different manifestations depending on its location. In the lower extremities, edema mainly causes swelling; in the lungs, edema causes water to fill alveoli, leading to difficulty in breathing. *Normal fluid homeostasis also means maintaining blood as a liquid until such time as injury necessitates clot formation.* Clotting at inappropriate sites *(thrombosis)* or migration of clots *(embolism)* obstructs blood flow to tissues and leads to cell death *(infarction)*. Conversely, inability to clot after vascular injury results in *hemorrhage*; local bleeding can compromise regional tissue perfusion, while more extensive hemorrhage can result in hypotension *(shock)* and death.

Some of the failures of fluid homeostasis reflect a *primary* pathology in a discrete vascular bed (e.g., hemorrhage due to local trauma) or in systemic coagulation (thrombosis due to hypercoagulability disorders); others may represent a

*The contributions of the late Dr. Ramzi Cotran to this chapter in previous editions are gratefully acknowledged.

secondary manifestation of some other disease process. Thus, pulmonary edema due to increased hydrostatic pressure may be a terminal complication of ischemic or valvular heart disease. Similarly, shock may be the fatal sequela of infection. Overall, disturbances in normal blood flow are major sources of human morbidity and mortality; thrombosis, embolism, and infarction underlie three of the most important causes of pathology in Western society—myocardial infarction, pulmonary embolism, and cerebrovascular accident (stroke). Thus, the hemodynamic disorders described in this chapter are important in a wide spectrum of human disease.

Edema

Approximately 60% of lean body weight is water; two thirds of this water is intracellular, and the remainder is found in the extracellular space, mostly as interstitial fluid (only about 5% of total body water is in blood plasma). The term *edema* signifies increased fluid in the interstitial tissue spaces. In addition, depending on the site, fluid collections in the different body cavities are variously designated *hydrothorax, hydropericardium,* and *hydroperitoneum* (the last is more commonly called *ascites*). *Anasarca* is a severe and generalized edema with profound subcutaneous tissue swelling.

Table 4-1 lists the pathophysiologic categories of edema. The mechanisms of inflammatory edema are largely related to

TABLE 4-1 -- Pathophysiologic Categories of Edema

Increased Hydrostatic Pressure
Impaired venous return
••Congestive heart failure
••Constrictive pericarditis
••Ascites (liver cirrhosis)
••Venous obstruction or compression
••••Thrombosis
••••External pressure (e.g., mass)
••••Lower extremity inactivity with prolonged dependency
Arteriolar dilation
••Heat
••Neurohumoral dysregulation
Reduced Plasma Osmotic Pressure (Hypoproteinemia)
Protein-losing glomerulopathies (nephrotic syndrome)
Liver cirrhosis (ascites)
Malnutrition
Protein-losing gastroenteropathy
Lymphatic Obstruction
Inflammatory
Neoplastic
Postsurgical
Postirradiation
Sodium Retention
Excessive salt intake with renal insufficiency
Increased tubular reabsorption of sodium
Renal hypoperfusion
Increased renin-angiotensin-aldosterone secretion
Inflammation
Acute inflammation
Chronic inflammation

Angiogenesis

Modified from Leaf A, Cotran RS: Renal Pathophysiology, 3rd ed., New York, Oxford University Press, 1985, p 146. Used by permission of Oxford Press, Inc.

local increases in vascular permeability and are discussed in Chapter 2 . The *noninflammatory causes of edema* are described in further detail below. Because of increased vascular permeability, inflammatory edema is a protein-rich *exudate*, with a specific gravity usually over 1.020. Conversely, the edema fluid occurring in hydrodynamic derangements is typically a protein-poor *transudate*, with a specific gravity below 1.012.

In general, the opposing effects of vascular hydrostatic pressure and plasma colloid osmotic pressure are the major factors that govern movement of fluid between vascular and interstitial spaces. Normally the exit of fluid into the interstitium from the arteriolar end of the microcirculation is nearly balanced by inflow at the venular end; a small residuum of excess interstitial fluid is drained by the lymphatics. *Either increased capillary pressure or diminished colloid osmotic pressure can result in increased interstitial fluid* (Fig. 4-1). As extravascular fluid accumulates, the increased tissue hydrostatic pressure and plasma colloid osmotic pressure eventually achieve a new equilibrium, and water reenters the venules. Any excess interstitial edema fluid is typically removed by lymphatic drainage, ultimately returning to the bloodstream via the thoracic duct (see Fig. 4-1); clearly, lymphatic obstruction (e.g., due to scarring or tumor) will also impair fluid drainage and result in edema. Finally, a primary retention of sodium (and its obligatory associated water) in renal disease also leads to edema.

Increased Hydrostatic Pressure.

Local increases in hydrostatic pressure may result from impaired venous outflow. For example, *deep venous thrombosis* in the lower extremities leads to edema, which is restricted to the affected leg. *Generalized increases* in venous pressure, with resulting systemic edema, occur most commonly in *congestive heart failure* (Chapter 12) affecting right ventricular cardiac function.

Figure 4-1 Factors affecting fluid balance across capillary walls. Capillary hydrostatic and osmotic forces are normally balanced so that there is no *net* loss or gain of fluid across the capillary bed. However, *increased* hydrostatic pressure or *diminished* plasma osmotic pressure leads to a net accumulation of extravascular fluid *(edema)*. As the interstitial fluid pressure increases, tissue lymphatics remove much of the excess volume, eventually returning it to the circulation via the thoracic duct. If the ability of the lymphatics to drain tissue is exceeded, persistent tissue edema results.

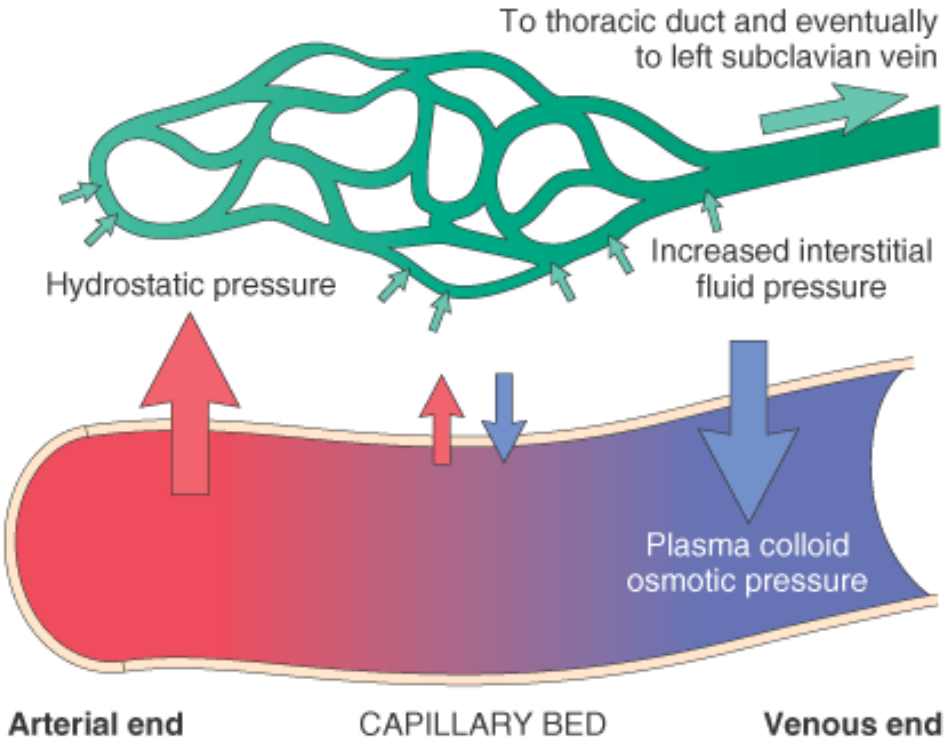


Figure 4-2 Sequence of events leading to systemic edema due to primary heart failure, primary renal failure, or reduced plasma osmotic pressure (as in malnutrition, diminished hepatic protein synthesis, or loss of protein owing to the nephrotic syndrome). ADH, antidiuretic hormone; GFR, glomerular filtration rate.

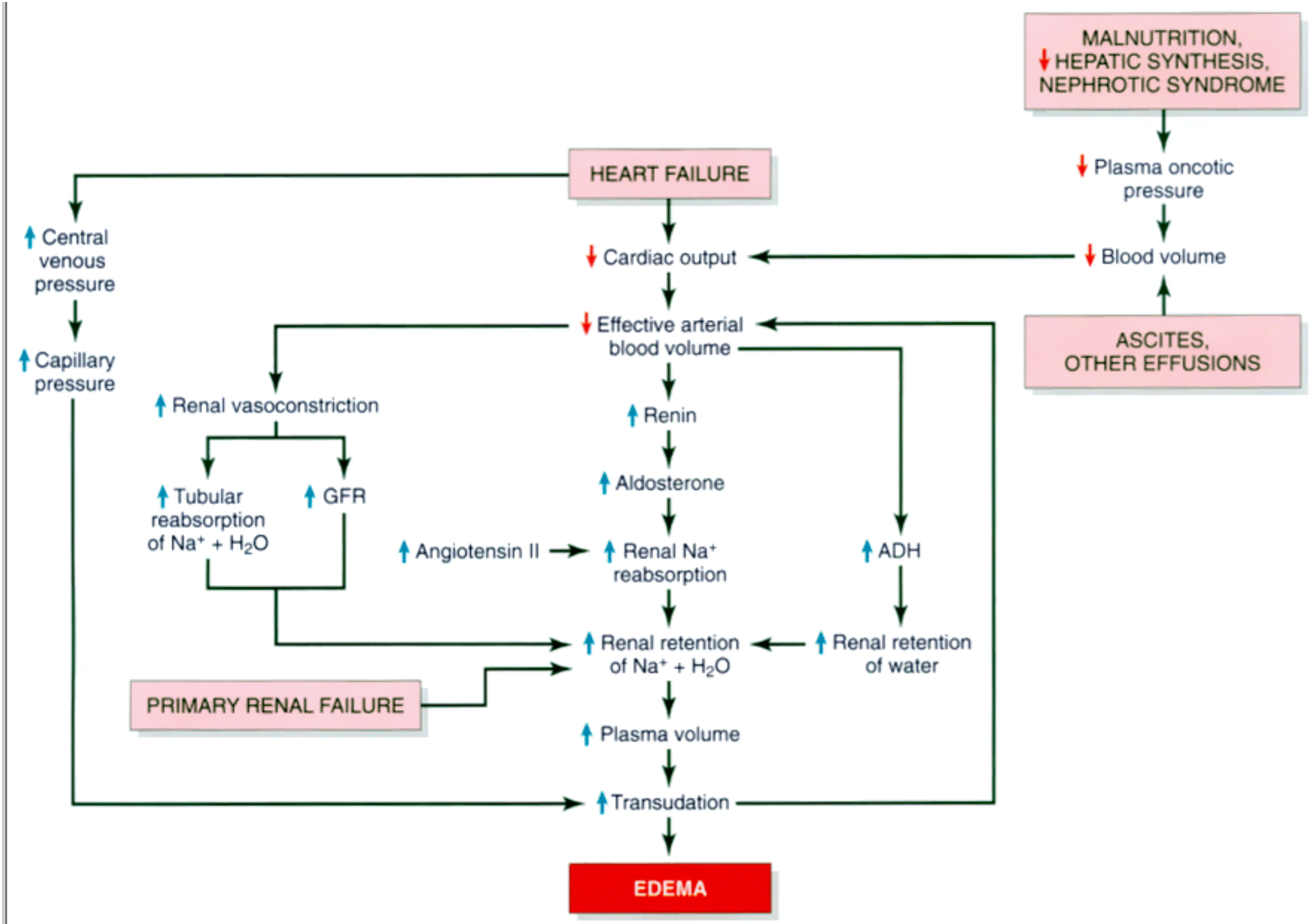


Figure 4-3 Hyperemia versus congestion. In both cases there is an increased volume and pressure of blood in a given tissue with associated capillary dilation and a potential for fluid extravasation. In hyperemia, increased inflow leads to engorgement with oxygenated blood, resulting in *erythema*. In congestion, diminished outflow leads to a capillary bed swollen with

deoxygenated venous blood and resulting in *cyanosis*.

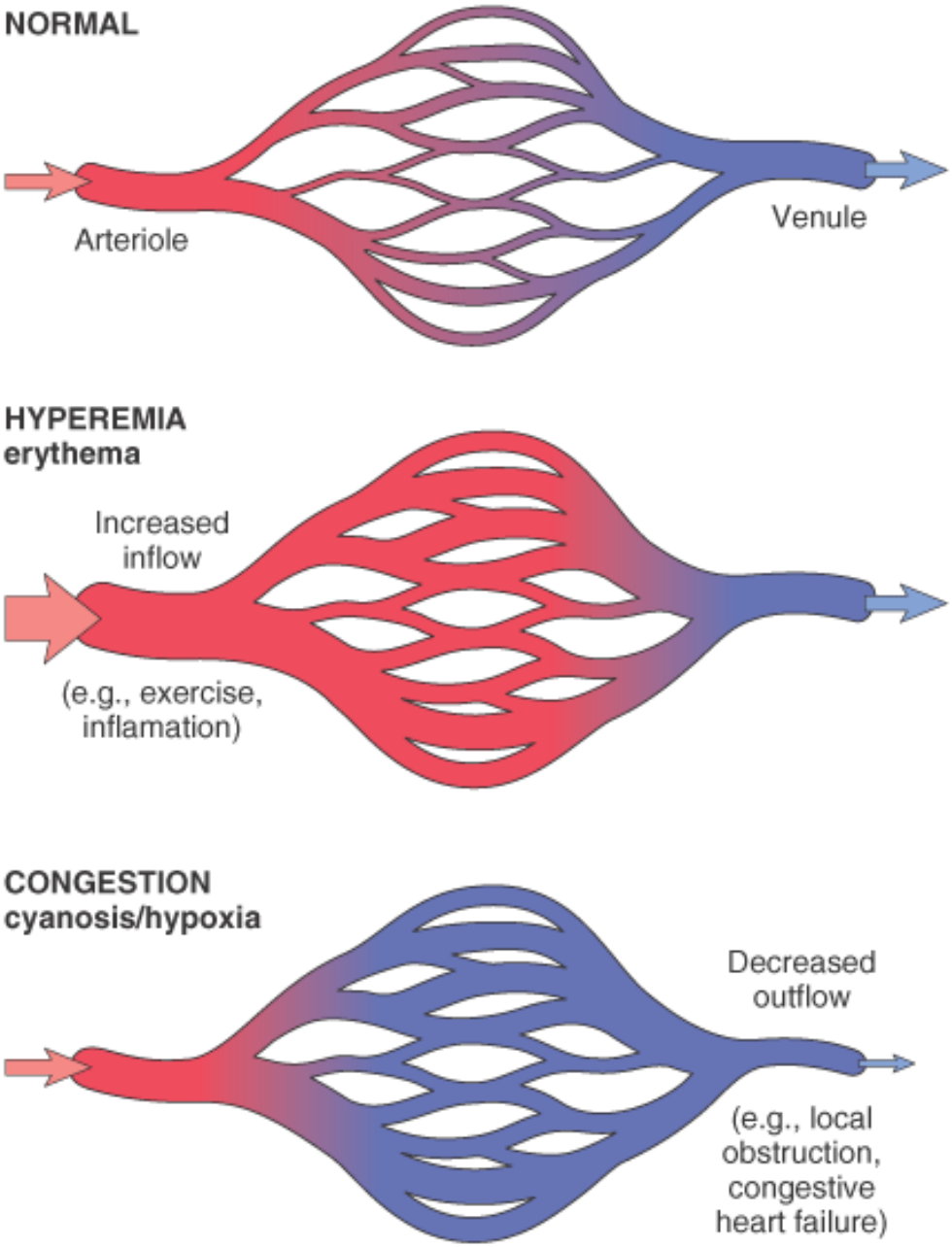


Figure 4-4 Liver with chronic passive congestion and hemorrhagic necrosis. *A*, Central areas are red and slightly depressed compared with the surrounding tan viable parenchyma, forming the so-called "nutmeg liver" pattern. *B*, Centrilobular necrosis with degenerating hepatocytes, hemorrhage, and sparse acute inflammation. *(Courtesy of Dr. James Crawford, Department of Pathology, University of Florida, Gainesville, FL.)*

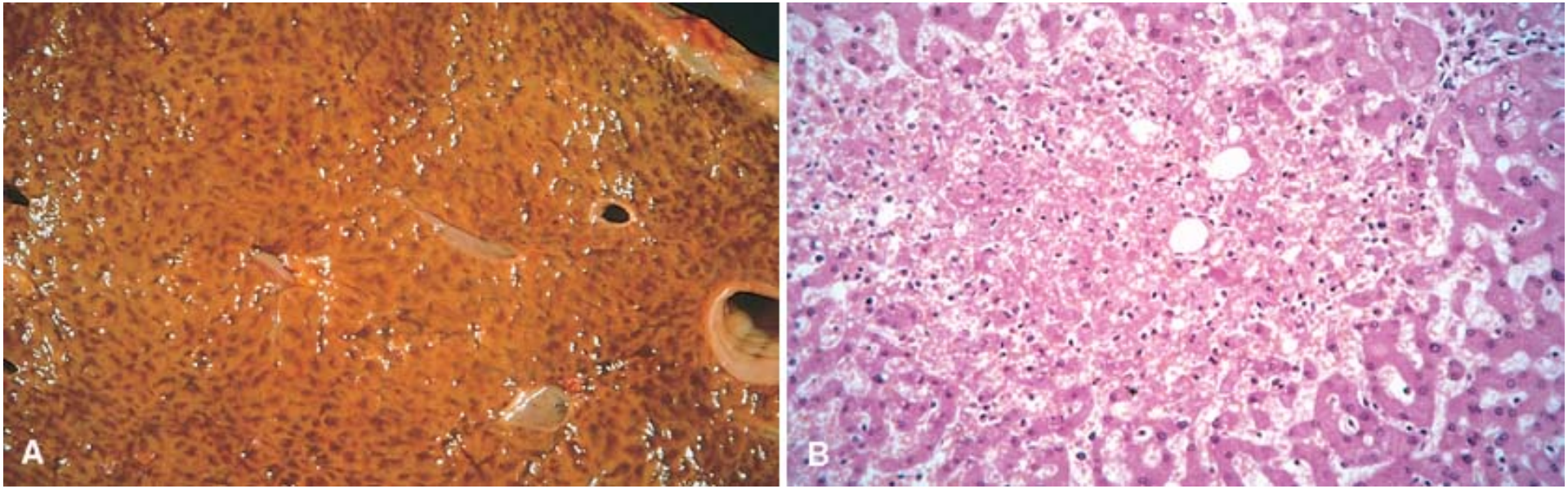


Figure 4-5 *A*, Punctate petechial hemorrhages of the colonic mucosa, seen here as a consequence of thrombocytopenia. *B*, Fatal intracerebral bleed. Even relatively inconsequential volumes of hemorrhage in a critical location, or into a closed space (such as the cranium), can have fatal outcomes.

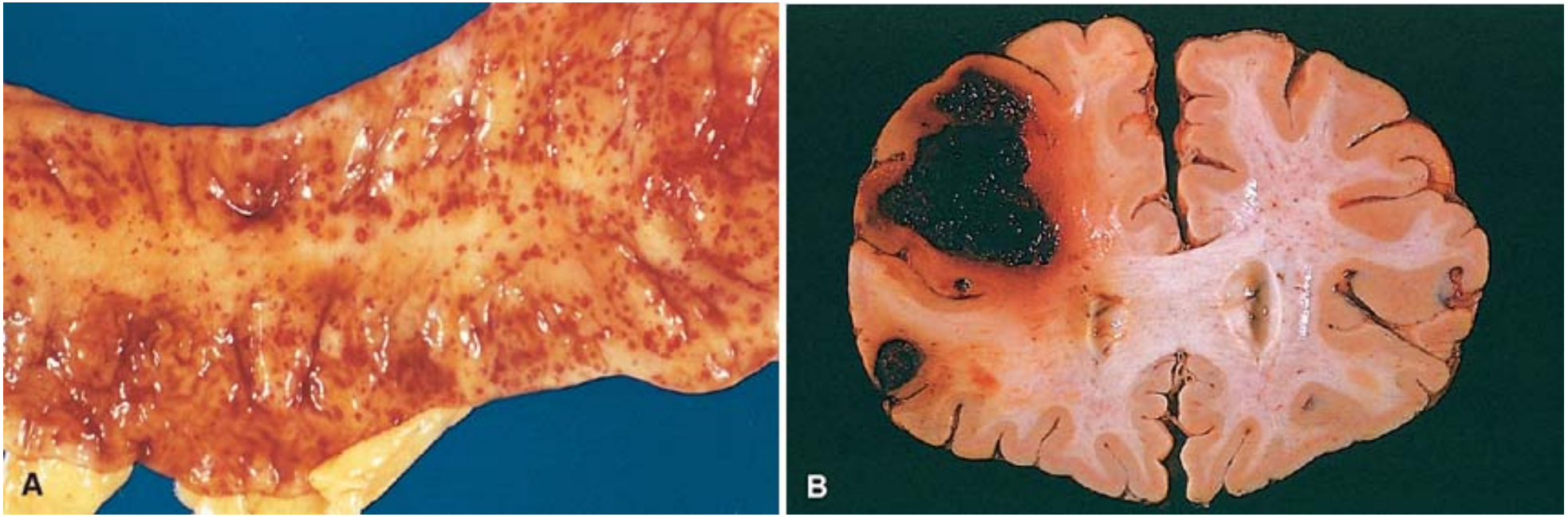
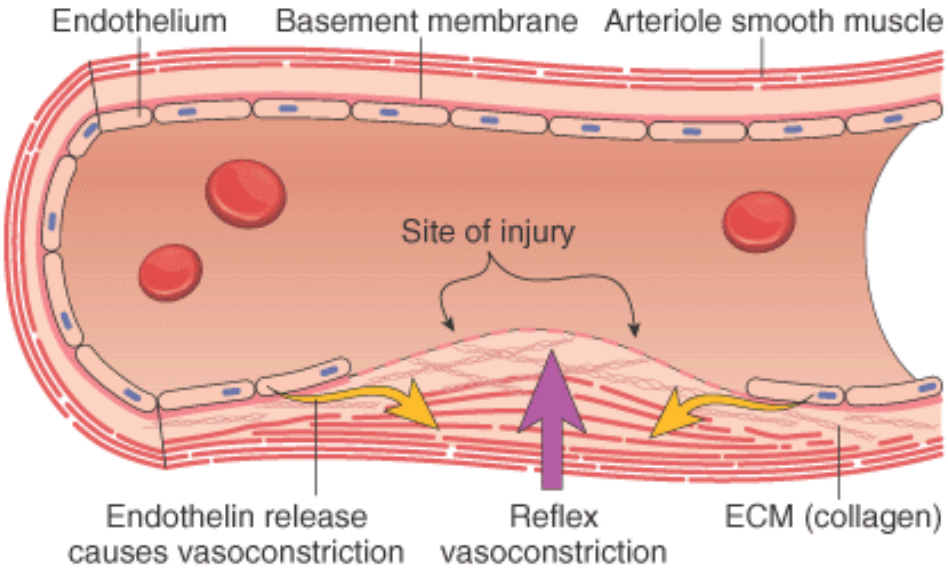


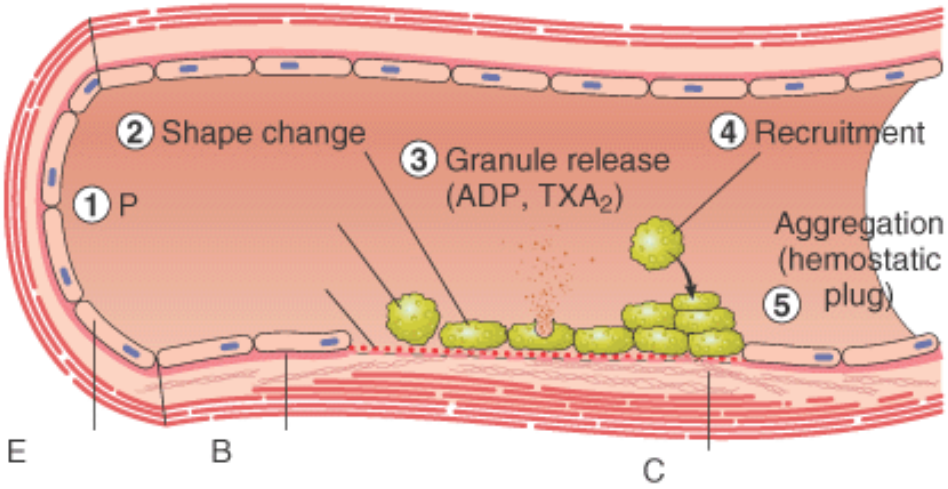
Figure 4-6 Diagrammatic representation of the normal hemostatic process. *A*, After vascular injury, local neurohumoral factors induce a transient vasoconstriction. *B*, Platelets adhere to exposed extracellular matrix (ECM) via von Willebrand factor (vWF) and are activated, undergoing a shape change and granule release; released adenosine diphosphate (ADP) and thromboxane A_2 (TxA_2) lead to further platelet aggregation to form the primary hemostatic plug. *C*, Local activation of the coagulation cascade (involving tissue factor and platelet phospholipids) results in fibrin polymerization, "cementing" the platelets into a definitive secondary hemostatic plug. *D*, Counter-regulatory mechanisms, such as release of tissue type

plasminogen activator (t-PA) (fibrinolytic) and thrombomodulin (interfering with the coagulation cascade), limit the hemostatic process to the site of injury.

A. VASOCONSTRICTION

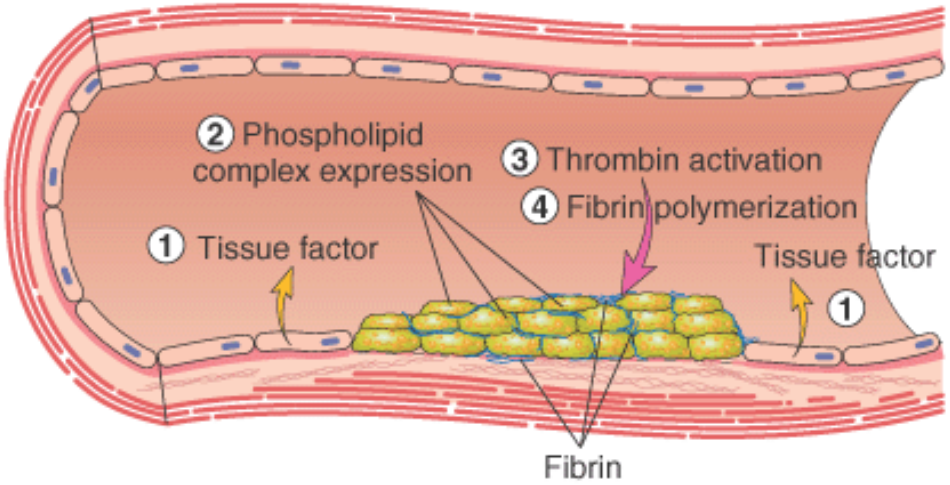


B. PRIMARY HEMOSTASIS



C. SECONDARY HEMOSTASIS

C. SECONDARY HEMOSTASIS



D. THROMBUS AND ANTITHROMBOTIC EVENTS

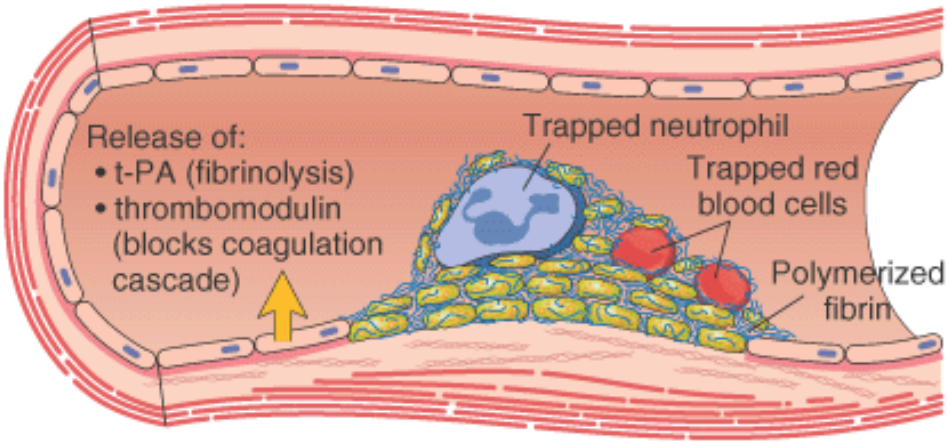


Figure 4-7 Schematic illustration of some of the pro- and anticoagulant activities of endothelial cells. Not shown are the pro- and antifibrinolytic properties. vWF, von Willebrand factor; PGI_2 , prostacyclin; NO, nitric oxide; t-PA, tissue plasminogen activator. Thrombin receptor is referred to as protease activated receptor (PAR; see text).

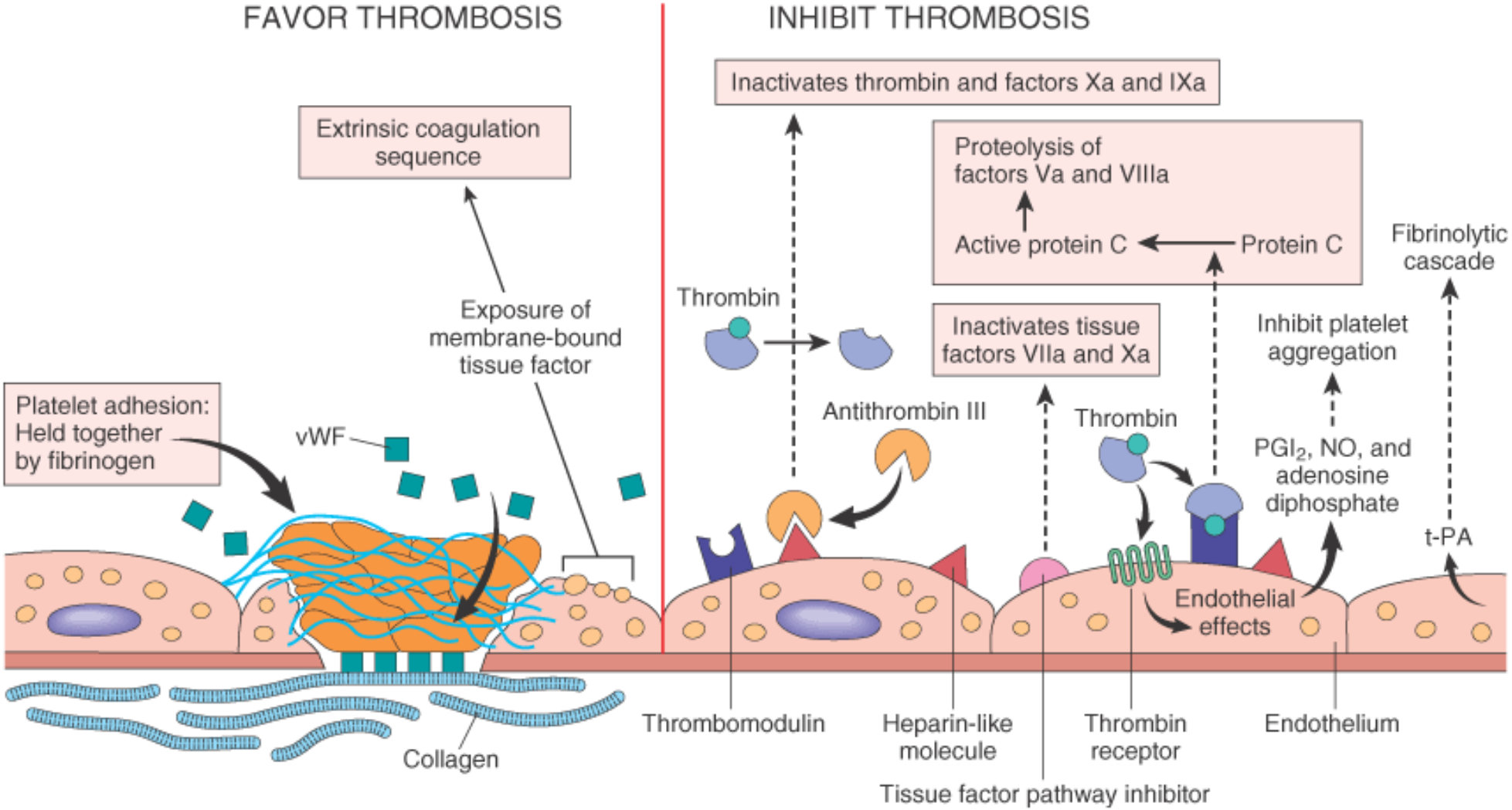


Figure 4-8 Platelet adhesion and aggregation. von Willebrand factor functions as an adhesion bridge between subendothelial collagen and the GpIb platelet receptor complex (the functional complex is composed of GpIb in association with factors V and IX). Aggregation involves linking platelets via fibrinogen bridges bound to the platelet GpIIb-IIIa receptors.

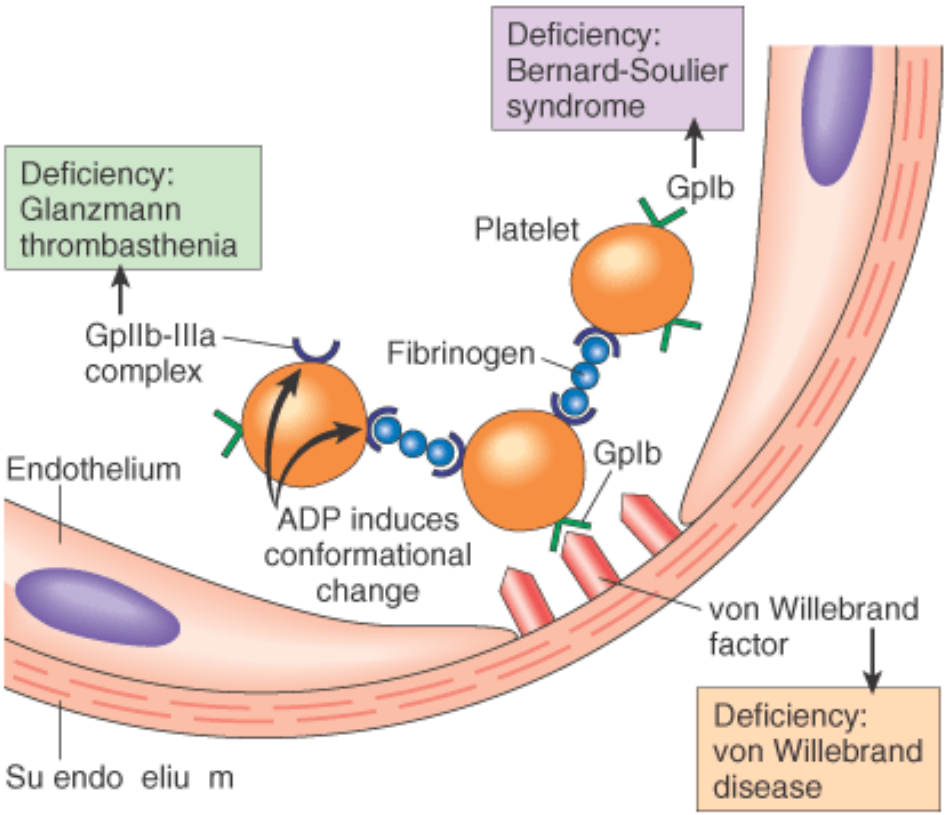
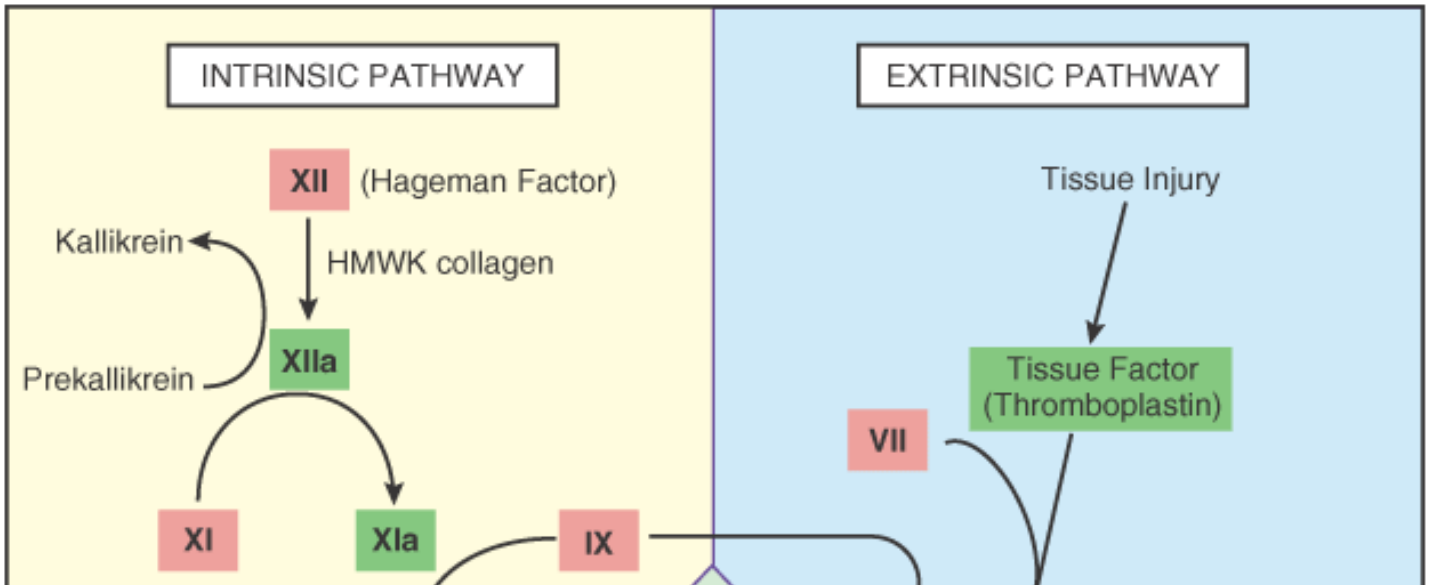


Figure 4-9 The coagulation cascade. Note the common link between the intrinsic and extrinsic pathways at the level of factor IX activation. Factors in red boxes represent inactive molecules; activated factors are indicated with a lower case "a" and a green box. PL, phospholipid surface; HMWK, high-molecular-weight kininogen. Not shown are the anticoagulant inhibitory pathways (see Fig. 4-7 and Fig. 4-12).



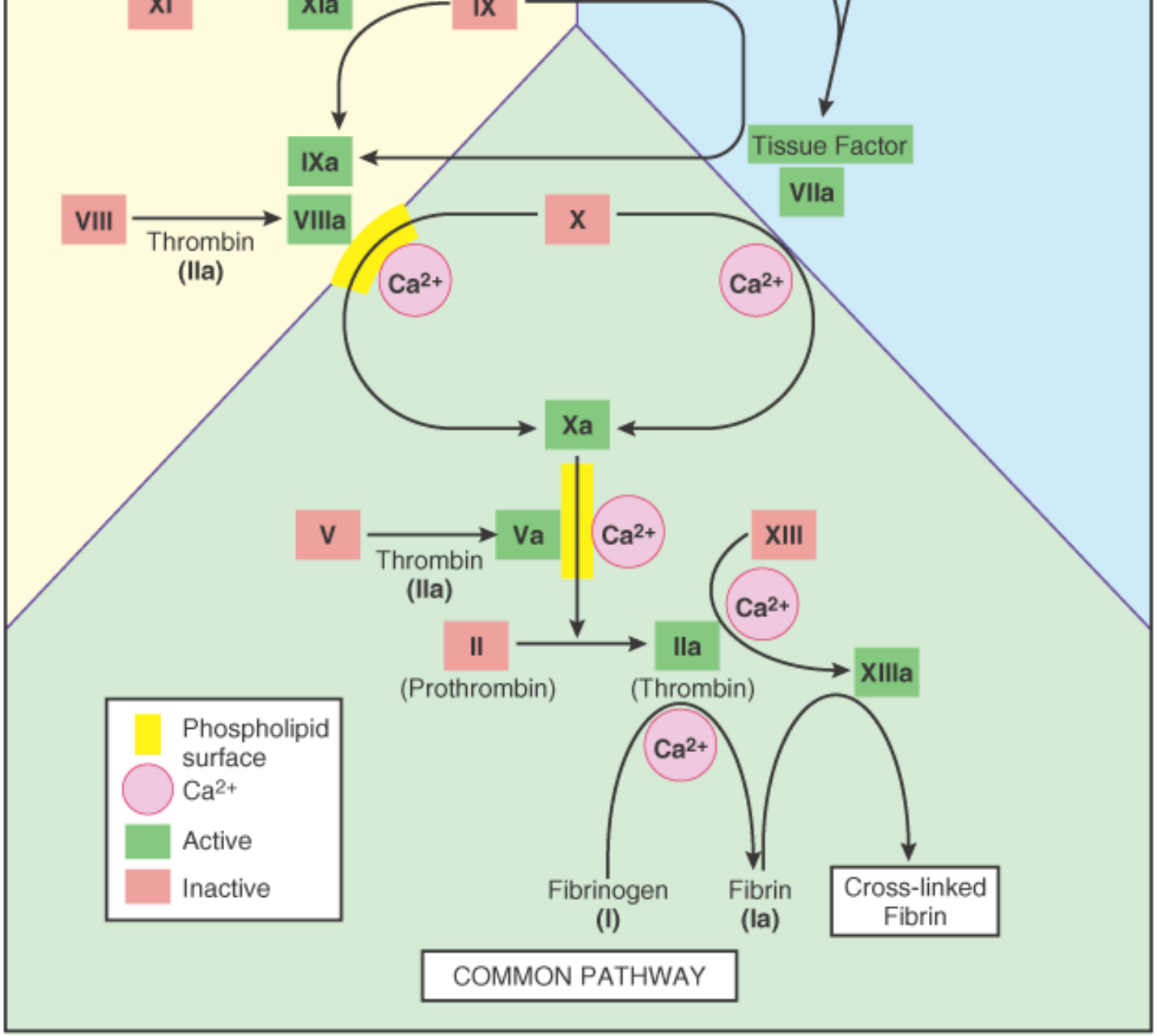


Figure 4-10 Schematic illustration of the conversion of factor X to factor Xa, which in turn converts factor II (prothrombin) to factor IIa (thrombin). The initial reaction complex consists of an enzyme (factor IXa), a substrate (factor X), and a reaction accelerator (factor VIIIa), which are assembled on the phospholipid surface of platelets. Calcium ions hold the assembled components together and are essential for reaction. Activated factor Xa then becomes the enzyme part of the second adjacent complex in the coagulation cascade, converting the prothrombin substrate (II) to thrombin (IIa), with the cooperation of the reaction accelerator factor Va. *(Modified from Mann KG: Clin Lab Med 4:217, 1984.)*

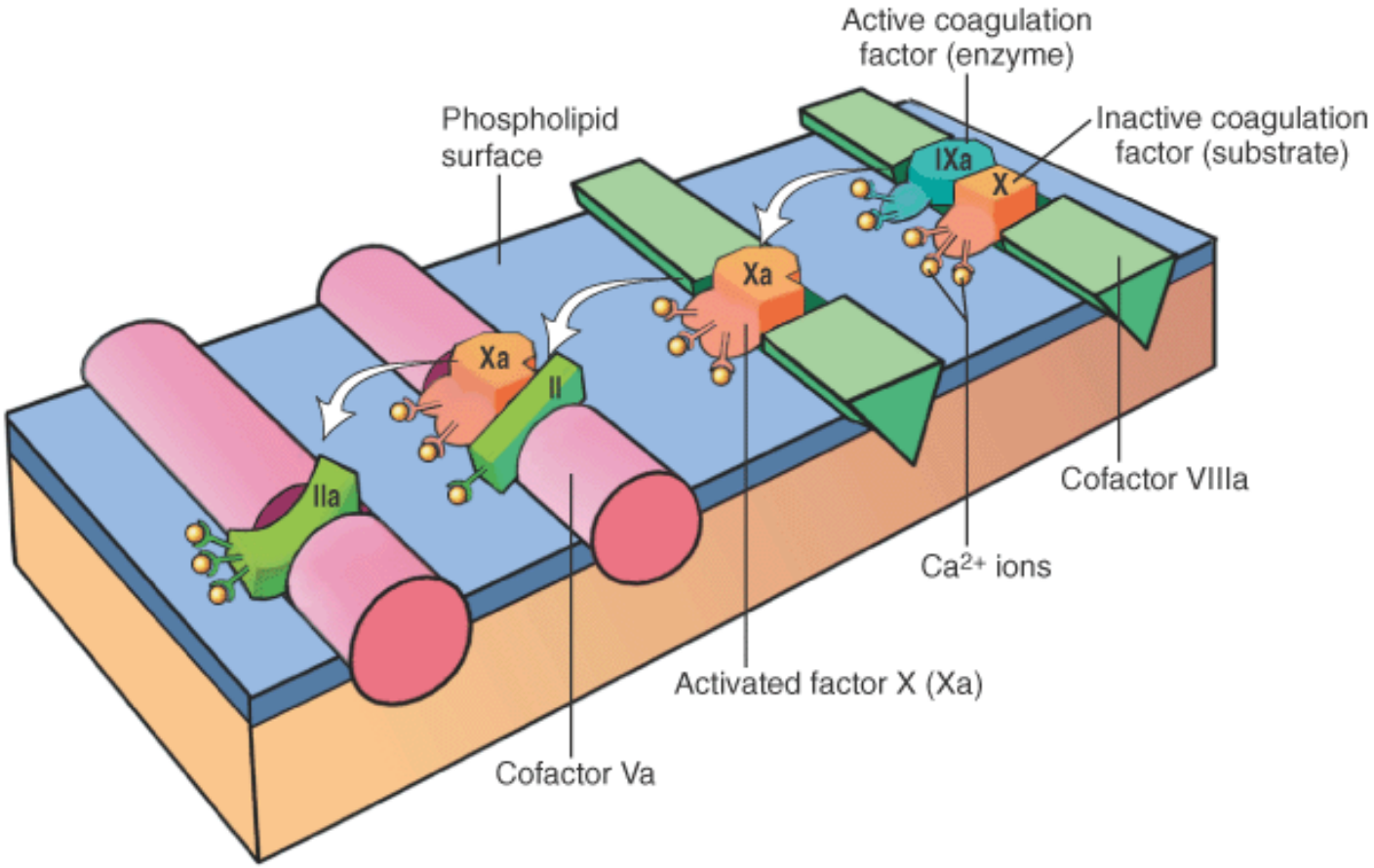


Figure 4-11 The central roles of thrombin in hemostasis and cellular activation. In addition to a critical function in generating cross-linked fibrin (via cleavage of fibrinogen to fibrin and activation of factor XIII), thrombin also directly induces platelet aggregation and secretion (e.g., of TxA_2). Thrombin also activates endothelium to generate leukocyte adhesion molecules and a variety of fibrinolytic (t-PA), vasoactive (NO, PGI_2), or cytokine (PDGF) mediators. Likewise, mononuclear inflammatory cells may be activated by the direct actions of thrombin. ECM, extracellular matrix; NO, nitric oxide; PDGF, platelet-derived growth factor; PGI_2 , prostacyclin; TxA_2 , thromboxane A_2 ; t-PA, tissue type plasminogen activator. See Fig. 4-7 for additional anticoagulant modulators of thrombin activity, such as antithrombin III and thrombomodulin. *(Modified with permission from Shaun Coughlin, MD, PhD, Cardiovascular Research Institute, University of California at San Francisco.)*

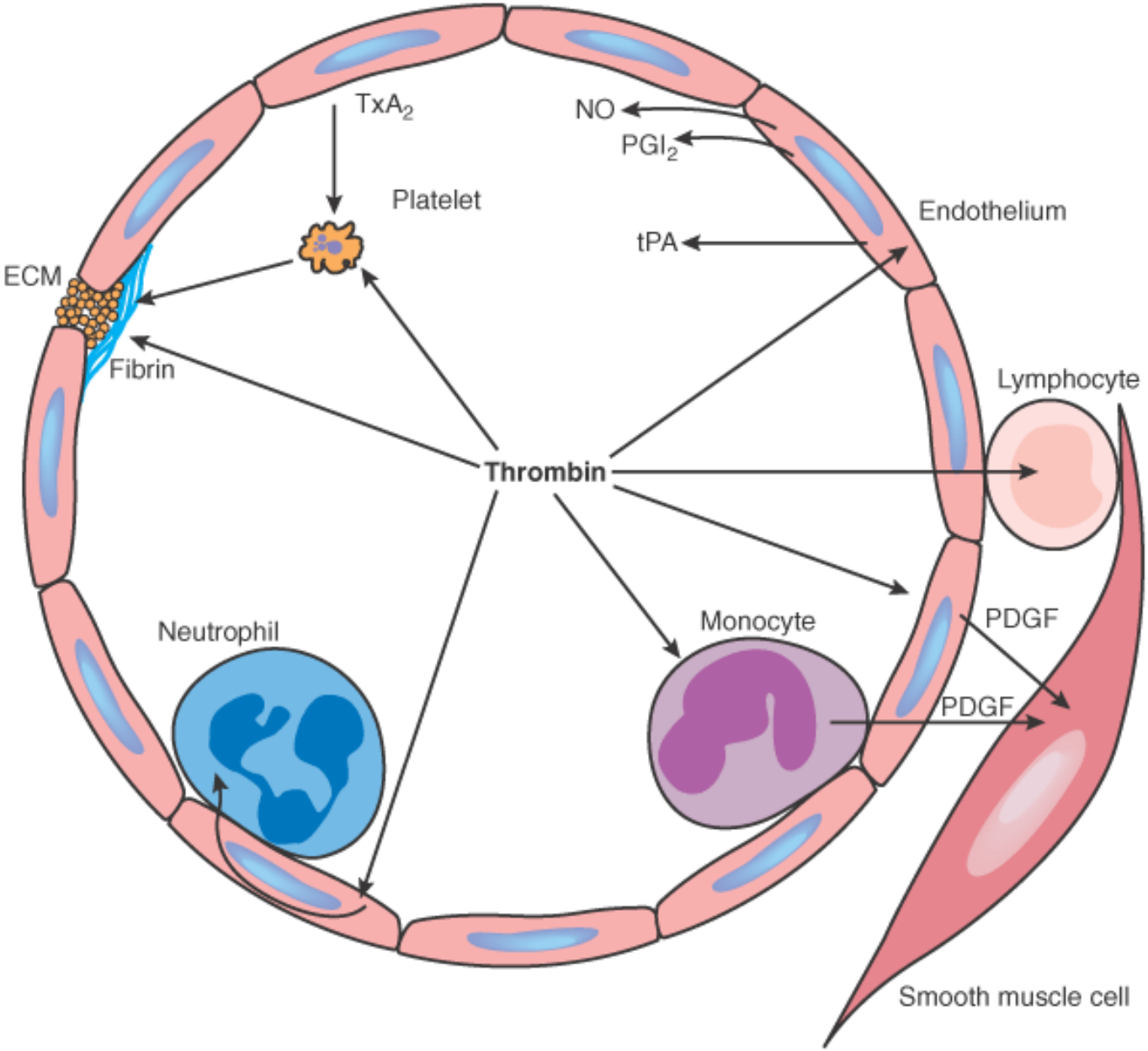


Figure 4-12 The fibrinolytic system, illustrating the plasminogen activators and inhibitors.

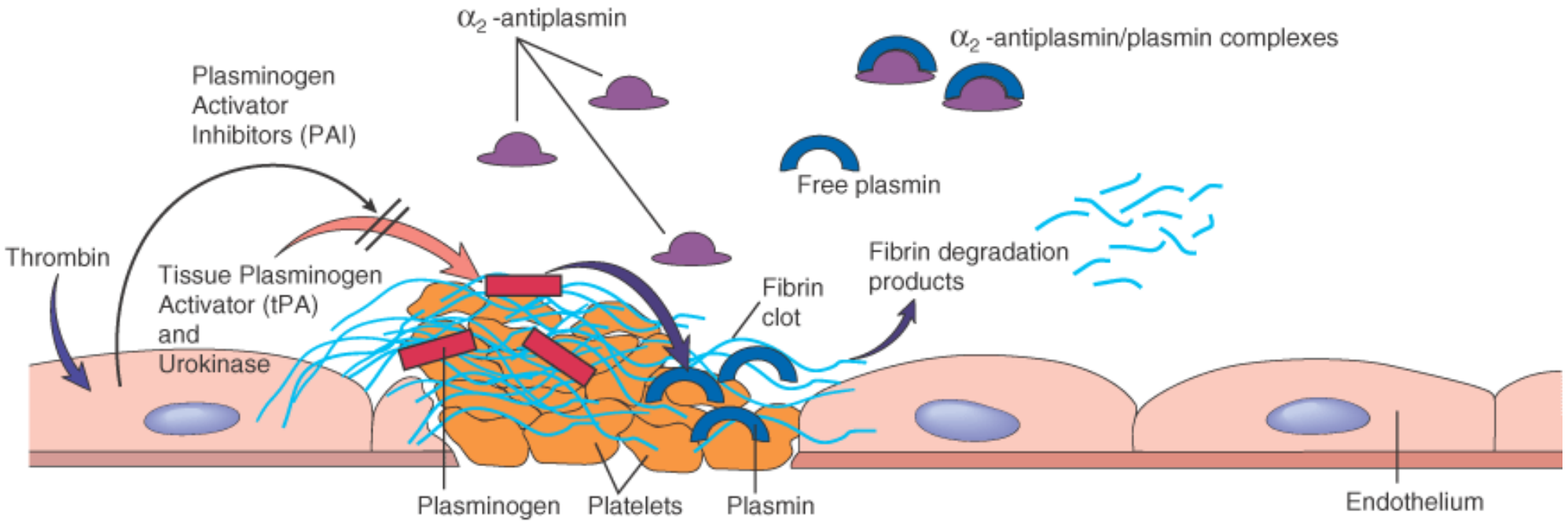


Figure 4-13 Virchow triad in thrombosis. Endothelial integrity is the single most important factor. Note that injury to endothelial cells can affect local blood flow and/or coagulability; abnormal blood flow (stasis or turbulence) can, in turn, cause endothelial injury. The elements of the triad may act independently or may combine to cause thrombus formation.

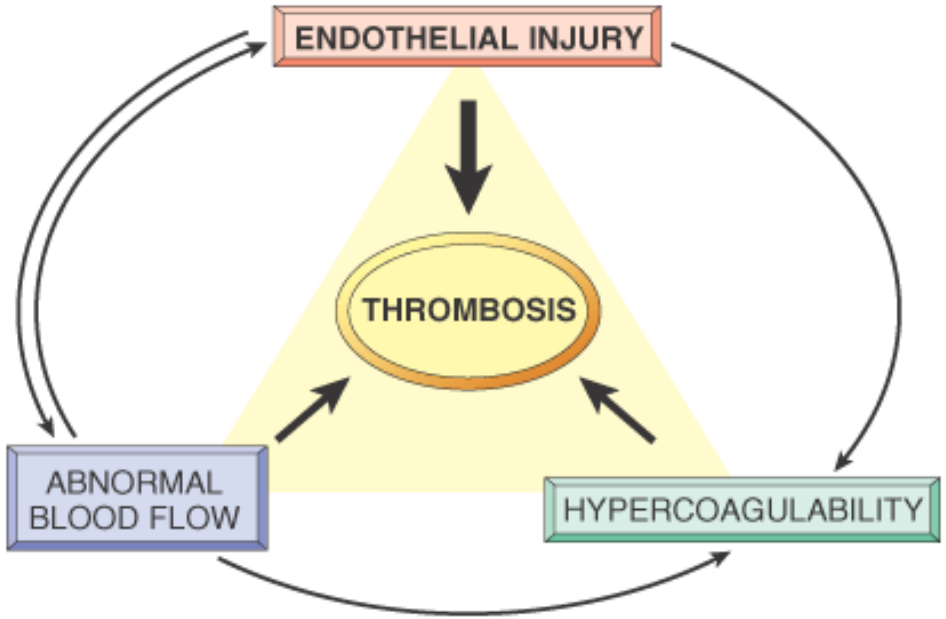


TABLE 4-2 -- Hypercoagulable States

Primary (Genetic)
Common
••Mutation in factor V gene (factor V Leiden)
••Mutation in prothrombin gene
••Mutation in methyltetrahydrofolate gene
Rare
••Antithrombin III deficiency
••Protein C deficiency
••Protein S deficiency
Very rare
••Fibrinolysis defects
Secondary (Acquired)
High risk for thrombosis
••Prolonged bed rest or immobilization
••Myocardial infarction
••Atrial fibrillation
••Tissue damage (surgery, fracture, burns)
••Cancer
••Prosthetic cardiac valves
••Disseminated intravascular coagulation
••Heparin-induced thrombocytopenia
••Antiphospholipid antibody syndrome (lupus anticoagulant syndrome)
Lower risk for thrombosis
••Cardiomyopathy
••Nephrotic syndrome
••Hyperestrogenic states (pregnancy)
••Oral contraceptive use
••Sickle cell anemia
••Smoking

to increased susceptibility to platelet aggregation and reduced PGI_2 release by endothelium. Smoking and obesity promote hypercoagulability by unknown mechanisms.

Among the acquired causes of thrombotic diatheses, the so-called *heparin-induced thrombocytopenia syndrome* and *antiphospholipid antibody syndrome* (previously called the *lupus anticoagulant syndrome*) deserve special mention.

Heparin-induced thrombocytopenia syndrome. [33] [34] Seen in upward of 5% of the population, this syndrome occurs when administration of unfractionated heparin (for purposes of therapeutic anticoagulation) induces formation of antibodies that bind to molecular complexes of heparin and platelet factor 4 membrane protein. This antibody can also bind to similar complexes present on platelet and endothelial surfaces; the result is platelet activation, endothelial injury, and a prothrombotic state. To reduce this problem, specially manufactured low-molecular-weight heparin preparations—which retain anticoagulant activity but do not interact with platelets—are used. These have the additional benefit of a prolonged serum half-life.

Antiphospholipid antibody syndrome. [35] [36] This syndrome has protean clinical presentations, including multiple thromboses; the clinical manifestations are associated with high titers of circulating antibodies directed against anionic phospholipids (e.g., cardiolipin) or, more accurately, against plasma protein epitopes that are unveiled by binding to such phospholipids (e.g., prothrombin). Patients with anticardiolipin antibodies also have a false-positive serologic test for syphilis because the antigen in the standard tests is embedded in cardiolipin. In vitro these antibodies interfere with the assembly of phospholipid complexes and thus inhibit coagulation. However, in vivo, the antibodies induce a *hypercoagulable* state.

Patients with antiphospholipid antibody syndrome fall into two categories. Many have a well-defined autoimmune disease, such as systemic lupus erythematosus (Chapter 6) and have *secondary antiphospholipid syndrome* (such patients previously carried the designation of *lupus anticoagulant syndrome*). The remainder show no evidence of other autoimmune disorder and exhibit only the manifestations of a hypercoagulable state *(primary antiphospholipid syndrome)*. Occasionally the syndrome can occur in association with certain drugs or infections. How antiphospholipid antibodies lead to hypercoagulability is not clear, but possible explanations include direct platelet activation, inhibition of PGI_2 production by endothelial cells, or interference with protein C synthesis or activity. Although antiphospholipid antibodies are associated with thrombotic diatheses, they have also been identified in 5% to 15% of apparently normal individuals and may therefore be necessary but not sufficient to cause full-blown antiphospholipid antibody syndrome.

Individuals with the antiphospholipid antibody syndrome present with an extreme variety of clinical manifestations; these are typically characterized by *recurrent venous* or *arterial thrombi* but also include *repeated miscarriages, cardiac valvular vegetations*, or *thrombocytopenia*. [37] Venous thromboses occur most commonly in deep leg veins, but renal, hepatic, and retinal veins are also susceptible. Arterial thromboses typically occur in the cerebral circulation, but coronary, mesenteric, and renal arterial occlusions have also been described. Depending on the vascular bed involved, the clinical presentations can vary from pulmonary embolism (due to a lower extremity venous thrombus), to pulmonary hypertension (from recurrent subclinical pulmonary emboli), to stroke, bowel infarction, or renovascular hypertension. Fetal loss is attributable to antibody-mediated inhibition of t-PA activity necessary for trophoblastic invasion of the uterus. Antiphospholipid antibody syndrome is also a cause of renal microangiopathy, resulting in renal failure owing to multiple capillary and arterial thromboses (Chapter 20). Patients with antiphospholipid antibody syndrome are at increased risk of a fatal event (upward of 7% in one series of patients with lupus erythematosus, particularly with arterial thromboses or thrombocytopenia). Current treatment includes anticoagulation therapy (aspirin, heparin, and warfarin) and immunosuppression in refractory cases. [35] [37] [38]

Morphology.

Thrombi may develop anywhere in the cardiovascular system: within the cardiac chambers; on valve cusps; or in arteries, veins, or capillaries. They are of variable size and shape, depending on the site of origin and the circumstances leading to their development. Arterial or cardiac thrombi usually begin at a site of endothelial injury (e.g., atherosclerotic plaque) or turbulence (vessel bifurcation); venous thrombi characteristically occur in sites of stasis. An area of attachment to the underlying vessel or heart wall, frequently firmest at the point of origin, is characteristic of all thromboses. Arterial thrombi tend to grow in a retrograde direction from the point of attachment, whereas venous thrombi extend in the direction of blood flow (i.e., toward the heart). The propagating tail may not be well attached and, particularly in veins, is prone to fragmentation, creating an **embolus**.

When formed in the heart or aorta, thrombi may have grossly (and microscopically) apparent laminations, called **lines of Zahn**; these are produced by alternating pale layers of platelets admixed with some fibrin and darker layers containing more red cells. Lines of Zahn are significant only in that they imply thrombosis at a site of blood flow; in veins or in smaller arteries, the laminations are typically not as apparent, and, in fact, thrombi formed in the sluggish flow of venous blood usually resemble statically coagulated blood (similar to blood clotted in a test tube). Nevertheless, careful evaluation generally reveals irregular, somewhat ill-defined laminations.

When arterial thrombi arise in heart chambers or in the aortic lumen, they usually adhere to the wall of the underlying structure and are termed **mural thrombi**. Abnormal myocardial contraction (arrhythmias, dilated cardiomyopathy, or myocardial infarction) leads to cardiac mural thrombi (Fig. 4-14*A*), while ulcerated atherosclerotic plaque and aneurysmal dilation are the precursors of aortic thrombus formation (Fig. 4-14*B*).

Arterial thrombi are usually **occlusive**; the most common sites, in descending order, are coronary, cerebral, and femoral arteries. The thrombus is usually superimposed on an atherosclerotic plaque, although other forms of vascular injury (vasculitis, trauma) may be involved. The thrombi are typically firmly adherent to the injured arterial wall and are gray-white and friable, composed of a tangled mesh of platelets, fibrin, erythrocytes, and degenerating leukocytes.

Venous thrombosis, or **phlebothrombosis**, is almost invariably occlusive; the thrombus often creates a long cast of the vein lumen. Because these thrombi form in a relatively static environment, they tend to contain more enmeshed erythrocytes and are therefore known as **red**, or **stasis**, **thrombi**. Phlebothrombosis most commonly affects the veins of the lower extremities (90% of cases). Less commonly, venous thrombi may develop in the upper extremities, periprostatic plexus, or the ovarian and periuterine veins; under special circumstances, they may be found in the dural sinuses, the portal vein, or the hepatic vein. At autopsy, postmortem clots may be

Figure 4-14 Mural thrombi. *A*, Thrombus in the left and right ventricular apices, overlying a white fibrous scar. *B*, Laminated thrombus in a dilated abdominal aortic aneurysm.

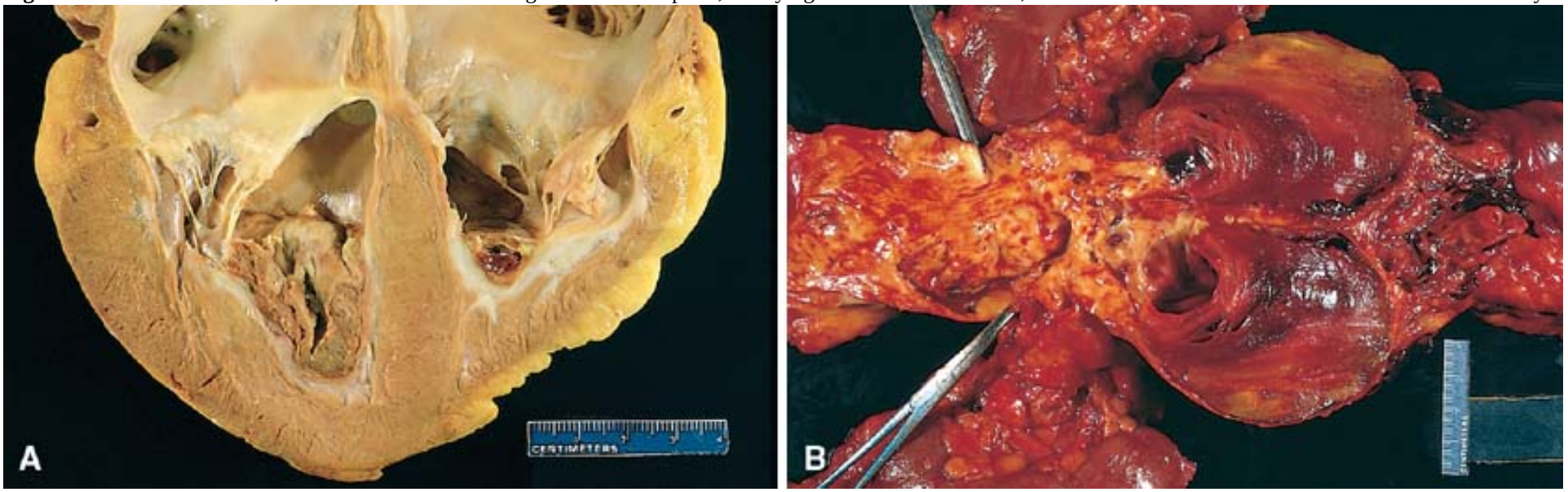


Figure 4-15 Potential outcomes of venous thrombosis.

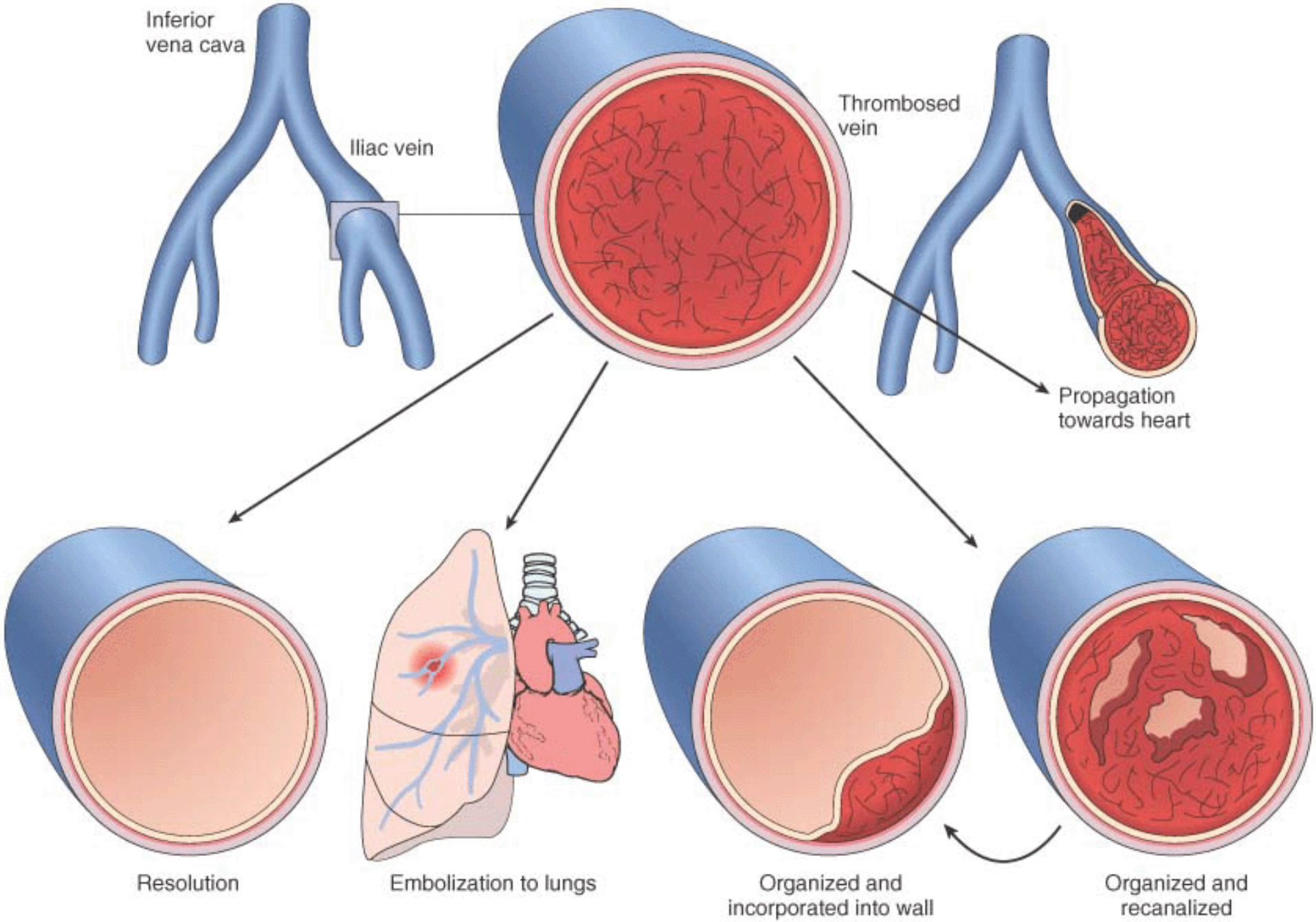


Figure 4-16 Low-power view of a thrombosed artery. *A*, H&E-stained section. *B*, Stain for elastic tissue. The original lumen is delineated by the internal elastic lamina *(arrows)* and is totally filled with organized thrombus, now punctuated by a number of small recanalized channels.

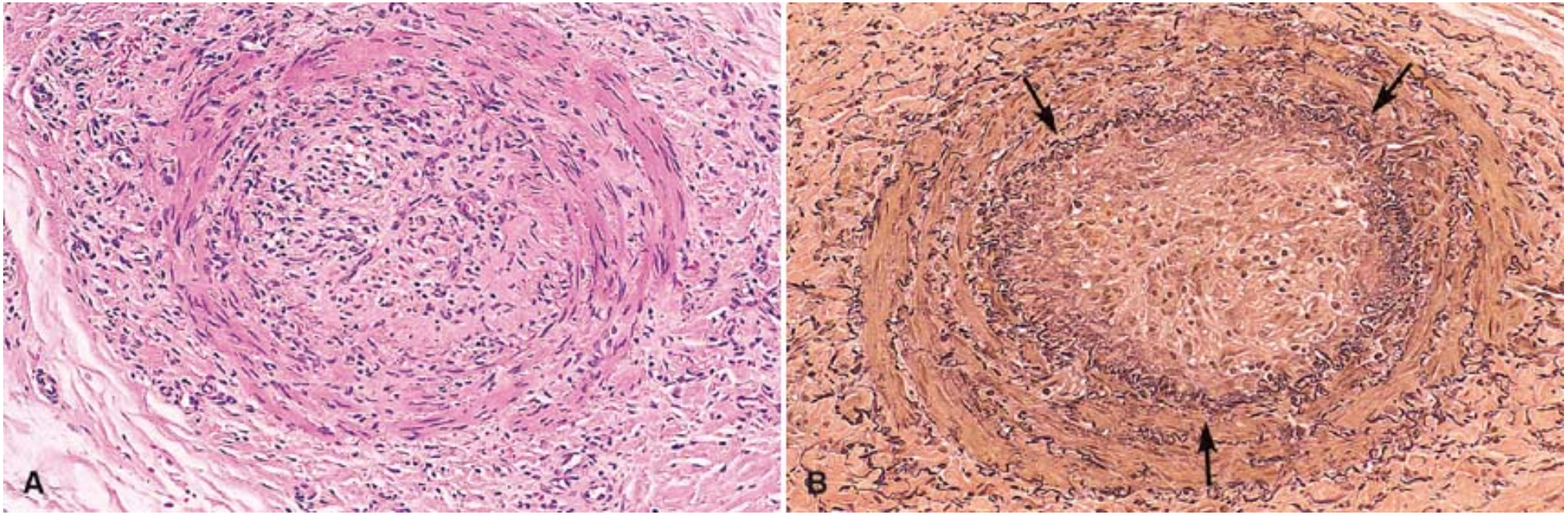


Figure 4-17 Large embolus derived from a lower extremity deep venous thrombosis and now impacted in a pulmonary artery branch.

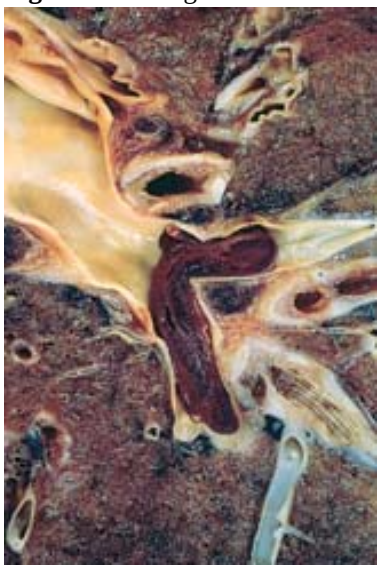


Figure 4-18 Bone marrow embolus in the pulmonary circulation. The cleared vacuoles represent marrow fat that is now impacted in a distal vessel along with the cellular hematopoietic precursors.

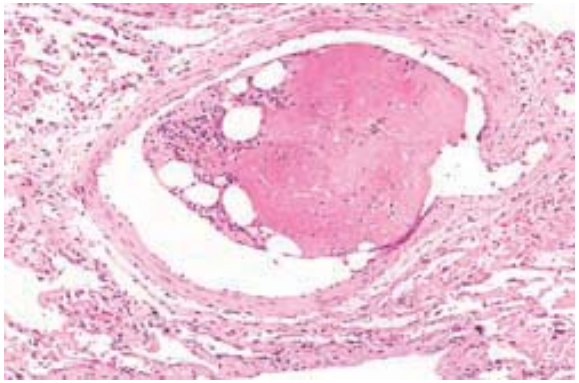


Figure 4-19 Examples of infarcts. *A*, Hemorrhagic, roughly wedge-shaped pulmonary infarct. *B*, Sharply demarcated white infarct in the spleen.

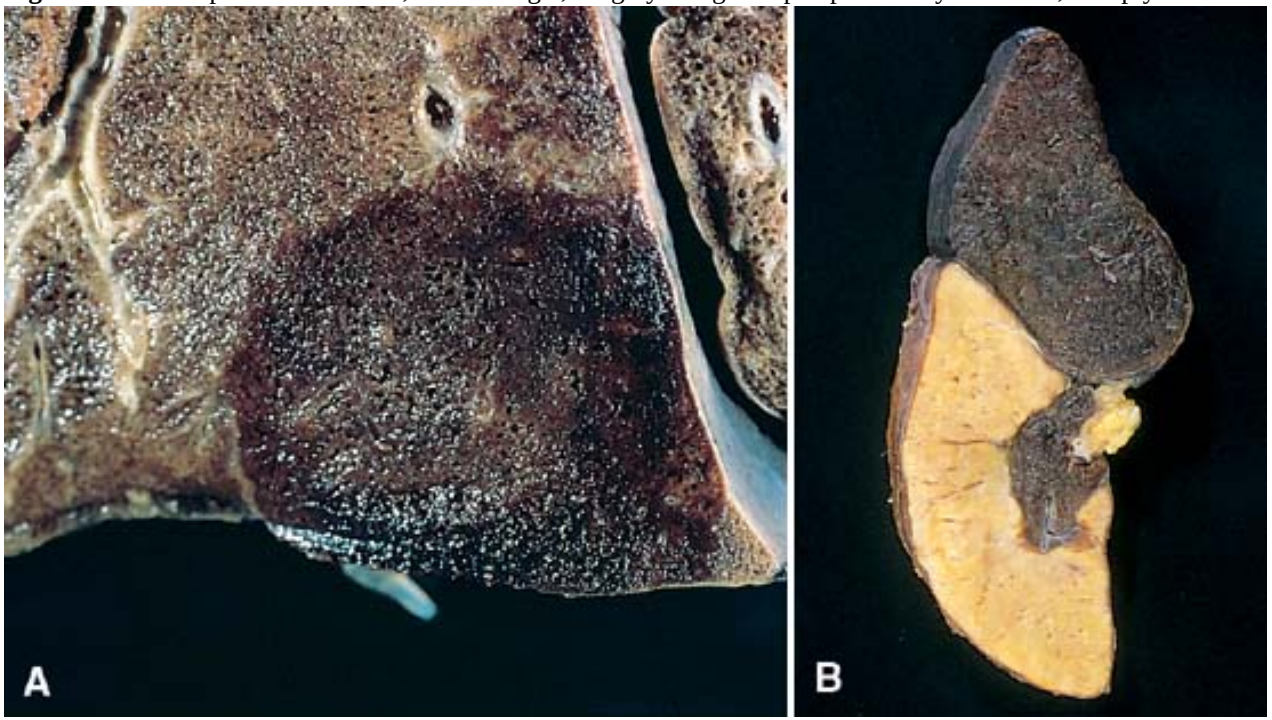


Figure 4-20 Remote kidney infarct, now replaced by a large fibrotic cortical scar.

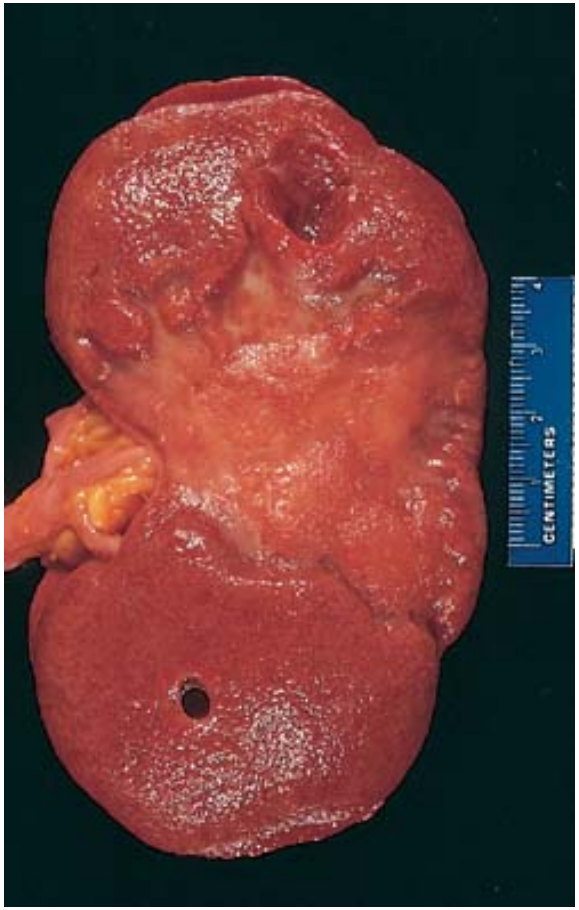


TABLE 4-3 -- Three Major Types of Shock

Type of Shock	Clinical Examples	Principal Mechanisms
Cardiogenic		
	Myocardial infarction	Failure of myocardial pump owing to intrinsic myocardial damage, extrinsic pressure, or obstruction to outflow
	Ventricular rupture	
	Arrhythmia	
	Cardiac tamponade	
	Pulmonary embolism	
Hypovolemic		
	Hemorrhage	Inadequate blood or plasma volume

	Fluid loss, e.g., vomiting, diarrhea, burns, or trauma	
Septic		
	Overwhelming microbial infections	Peripheral vasodilation and pooling of blood; endothelial activation/injury; leukocyte-induced damage; disseminated intravascular coagulation; activation of cytokine cascades
	Endotoxic shock	
	Gram-positive septicemia	
	Fungal sepsis	
	Superantigens	

estimated to account for over 200,000 deaths annually in the United States.[53] Moreover, the reported incidence of sepsis syndromes has increased dramatically in the past two decades, owing to improved life support for high-risk patients, increasing use of invasive procedures, and growing numbers of immunocompromised hosts (secondary to chemotherapy, immunosuppression, or human immunodeficiency virus infection). Septic shock results from spread and expansion of an initially localized infection (e.g., abscess, peritonitis, pneumonia) into the bloodstream.

Most cases of septic shock (approximately 70%) are caused by endotoxin-producing gram-negative bacilli (Chapter 8), hence the term *endotoxic shock*. Endotoxins are bacterial wall lipopolysaccharides (LPSs) that are released when the cell walls are degraded (e.g., in an inflammatory response). LPS consists of a toxic fatty acid *(lipid A)* core and a complex polysaccharide coat (including O antigens) unique to each bacterial species. Analogous molecules in the walls of gram-positive bacteria and fungi can also elicit septic shock.

All of the cellular and resultant hemodynamic effects of septic shock may be reproduced by injection of LPS alone. Free LPS attaches to a circulating LPS-binding protein, and the complex then binds to a cell-surface receptor (called CD14), followed by binding of the LPS to a signal-transducing protein called *mammalian Toll-like receptor protein 4* (TLR-4). (Toll is a *Drosophila* protein involved in fly development; a variety of molecules with homology to Toll [i.e., "Toll-like"] participate in innate immune responses to different microbial components; see Box 6-1 , Chapter 6.) Signals from TLR-4 can then directly activate vascular wall cells and leukocytes or initiate a cascade of cytokine mediators, which propagates the pathologic state. [54] [55] Engagement of TLR-4 on endothelial cells can lead directly to down-regulation of natural anticoagulation mechanisms, including diminished synthesis of tissue factor pathway inhibitor (TFPI) and thrombomodulin. Engagement of the receptor on monocytes and macrophages (even at doses of LPS as minute as 10 picograms/ml) causes profound mononuclear cell activation with the subsequent production of potent effector cytokines such as IL-1 and TNF (Chapter 6). Presumably, this series of responses helps to isolate organisms and to trigger elements of the innate immune system to efficiently eradicate invading microbes. Unfortunately, depending on the dosage and numbers of macrophages that are activated, the secondary effects of LPS release can also cause severe pathologic changes, including fatal shock.

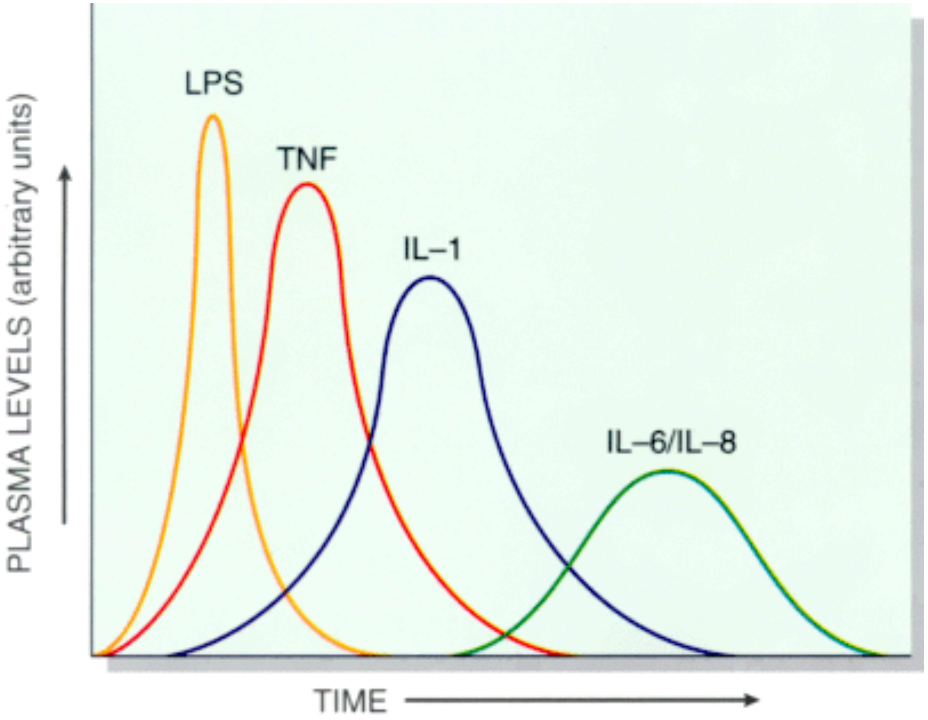
- At low doses, LPS predominantly serves to activate monocytes and macrophages, with effects intended to enhance their ability to eliminate invading bacteria. LPS can also directly activate complement, which likewise contributes to local bacterial eradication. The mononuclear phagocytes respond to LPS by producing cytokines, mainly TNF, IL-1, IL-6, and chemokines. TNF and IL-1 both act on endothelial cells to stimulate the expression of adhesion molecules (Chapter 2 ; Fig. 4-21) and the production of other cytokines and chemokines. Thus, the initial release of LPS results in a circumscribed cytokine cascade doubtless intended to enhance the *local* acute inflammatory response and improve clearance of the infection.
- With moderately severe infections, and therefore with higher levels of LPS (and a consequent augmentation of the cytokine cascade), cytokine-induced secondary effectors (e.g., nitric oxide; Chapter 2) become significant. In addition, systemic effects of the cytokines such as TNF and IL-1 may begin to be seen; these include fever and increased synthesis of acute phase reactants (Chapter 2 ; Fig. 4-21). LPS at higher doses also results in diminished endothelial cell production of thrombomodulin and TFPI, tipping the coagulation cascade toward thrombosis.
- Finally, at still higher levels of LPS, the syndrome of septic shock supervenes (Fig. 4-22); the same cytokines and secondary mediators, now at high levels, result in:
- Systemic vasodilation (hypotension)
- Diminished myocardial contractility

- Widespread endothelial injury and activation, causing systemic leukocyte adhesion and pulmonary alveolar capillary damage (*acute respiratory distress syndrome*; Chapter 15)
- Activation of the coagulation system, culminating in DIC

The hypoperfusion resulting from the combined effects of widespread vasodilation, myocardial pump failure, and DIC induces *multiorgan system failure* affecting the liver, kidneys,

---

Figure 4-21 Cytokine cascade in sepsis. After release of lipopolysaccharide (LPS) from invading gram-negative microorganisms, there are successive waves of tumor necrosis factor (TNF), interleukin-1 (IL-1), and IL-6 secretion. *(Modified from Abbas AK, et al: Cellular and Molecular Immunology, 4th ed. Philadelphia, WB Saunders, 2000.)*



A *progressive stage* characterized by tissue hypoperfusion and onset of worsening circulatory and metabolic imbalances, including acidosis

An *irreversible stage* that sets in after the body has incurred cellular and tissue injury so severe that even if the hemodynamic defects are corrected, survival is not possible.

In the early nonprogressive phase of shock, a variety of *neurohumoral mechanisms* help maintain cardiac output and blood pressure. These include baroreceptor reflexes, release of catecholamines, activation of the renin-angiotensin axis, antidiuretic hormone release, and generalized sympathetic stimulation. The net effect is *tachycardia, peripheral vasoconstriction, and renal conservation of fluid*. Cutaneous vasoconstriction, for example, is responsible for the characteristic coolness and pallor of skin in well-developed shock (although septic shock may initially cause cutaneous *vasodilation* and thus present with warm, flushed skin). Coronary and cerebral vessels are less sensitive to this compensatory sympathetic response and thus maintain relatively normal caliber, blood flow, and oxygen delivery to their respective vital organs.

If the underlying causes are not corrected, shock passes imperceptibly to the progressive phase, during which there is widespread tissue hypoxia. In the setting of persistent oxygen deficit, intracellular aerobic respiration is replaced by anaerobic glycolysis with excessive production of lactic acid. The resultant metabolic *lactic acidosis lowers the tissue pH and blunts the vasomotor response*; arterioles dilate, and blood begins to pool in the microcirculation. Peripheral pooling not only worsens the cardiac output, but also puts endothelial cells at risk for developing anoxic injury with subsequent DIC. With widespread tissue hypoxia, vital organs are affected and begin to fail; *clinically the patient may become confused, and the urine output declines*.

Unless there is intervention, the process eventually enters an irreversible stage. Widespread cell injury is reflected in lysosomal enzyme leakage, further aggravating the shock state. Myocardial contractile function worsens in part because of nitric oxide synthesis. If ischemic bowel allows intestinal flora to enter the circulation, endotoxic shock may be superimposed. At this point, the patient has complete renal shutdown owing to acute tubular necrosis (Chapter 20), and despite heroic measures, the downward clinical spiral almost inevitably culminates in death.

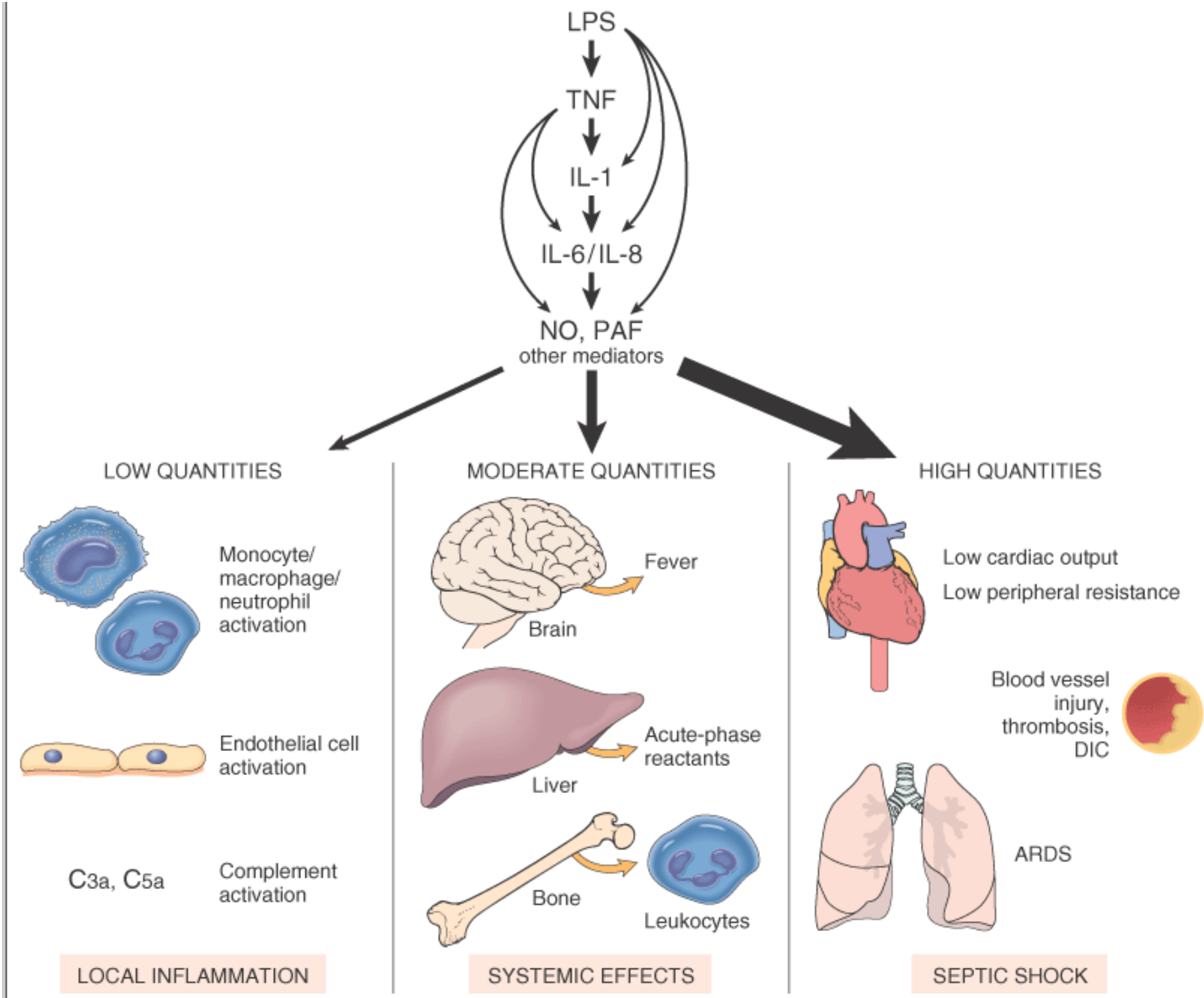
Morphology.

The cellular and tissue changes induced by shock are essentially those of hypoxic injury (Chapter 1); since shock is characterized by **failure of multiple organ systems**, the cellular changes may appear in any tissue. Nevertheless, they are particularly evident in brain, heart, lungs, kidneys, adrenals, and gastrointestinal tract.

The **brain** may develop so-called ischemic encephalopathy, discussed in Chapter 28 . The **heart** may undergo focal or widespread coagulation necrosis or

---

Figure 4-22 Effects of lipopolysaccharide (LPS) and secondarily induced effector molecules. LPS initiates the cytokine cascade described in Figure 4-21 ; in addition, LPS and the various factors can directly stimulate downstream cytokine production, as indicated. Secondary effectors that become important include nitric oxide (NO) and platelet-activating factor (PAF). At low levels, only local inflammatory effects are seen. With moderate levels, more systemic events occur in addition to the local vascular effects. At high concentrations, the syndrome of septic shock is seen. DIC, disseminated intravascular coagulation; ARDS, adult respiratory distress syndrome. *(Modified from Abbas AK, et al: Cellular and Molecular Immunology, 4th ed. Philadelphia, WB Saunders, 2000.)*


LPS
TNF
IL-1
IL-6/IL-8
NO, PAF
other mediators
LOW QUANTITIES
MODERATE QUANTITIES
HIGH QUANTITIES
Monocyte/ macrophage/ neutrophil activation
Endothelial cell activation
C3a, C5a
Complement activation
Fever
Brain
Acute-phase reactants
Liver
Bone
Leukocytes
Low cardiac output
Low peripheral resistance
Blood vessel injury, thrombosis, DIC
ARDS
LOCAL INFLAMMATION
SYSTEMIC EFFECTS
SEPTIC SHOCK

References

1. Schrier R, Abraham W: Hormones and hemodynamics in heart failure. N Engl J Med 341:577, 1999.

2. Bick R, Murano G: Physiology of hemostasis. Clin Lab Med 14:677, 1994.

3. Hoffman M, Monroe DR: A cell-based model of hemostasis. Thromb Haemost 85:958, 2001.

4. Gross P, Aird W: The endothelium and thrombosis. Semin Thromb Hemost 26:463, 2000.

5. Pearson J: Endothelial cell function and thrombosis. Best Pract Res Clin Haematol 12:329, 1999.

6. Michiels C: Endothelial cell functions. J Cell Physiol 196:430, 2003.

7. Esmon C: Protein C anticoagulant pathway and its role in controlling microvascular thrombosis and inflammation. Crit Care Med 29 (Suppl 7):S48, 2001.

8. Kato H: Regulation of functions of vascular wall cells by tissue factor pathway inhibitor: basic and clinical aspects. Arterioscler Thromb Vasc Biol 22:539, 2002.

9. Lijnen H, Collen D: Endothelium in hemostasis and thrombosis. Prog Cardiovasc Dis 39:343, 1997.

10. Denis C: Molecular and cellular biology of von Willebrand factor. Int J Hematol 75:3, 2002.

11. Doshi S, Marmur J: Evolving role of tissue factor and its pathway inhibitor. Crit Care Med 30 (5 Suppl):S241, 2002.

12. Binder B, et al: Plasminogen activator inhibitor 1: physiological and pathophysiological roles. News Physiol Sci 17:56, 2002.

13. Bouchard B, Tracy P: Platelets, leukocytes, and coagulation. Curr Opin Hematol 9:263, 2001.

14. Heemskerk JW, Bevers EM, Lindhout T: Platelet activation and blood coagulation. Thromb Hemost 88:186, 2002.

15. Mann KG, Butenas S, Brummel K: The dynamics of thrombin formation. Atheroscler Throm Vasc Biol 23:17, 2003.

16. Caen J, Rosa J: Platelet-vessel wall interaction: from bedside to molecules. Thromb Haemost 74:18, 1995.

17. Casserly I, Topol E: Glycoprotein IIb/IIIa antagonists — from bench to practice. Cell Mol Life Sci 59:478, 2002.

18. Norris LA: Blood coagulation. Best Pract Res Clin Obstet Gynaecol 17:369, 2003.

19. Semeraro N, Colucci M: Tissue factor in health and disease. Thromb Haemost 78:759, 1997.

20. Coughlin SR, Camerer E: PARticipation in inflammation. J Clin Invest 111:25, 2003.

21. Galino P: The inhibitor of tissue factor: factor VII pathway. Thromb Res 106:V257, 2002.

22. Pearson T, LaCava J, Weil H: Epidemiology of thrombotic-hemostatic factors and their associations with cardiovascular disease. Am J Clin Nutr 65 (Suppl 5):1674S, 1997.

23. Wootton D, Ku D: Fluid mechanics of vascular systems, diseases, and thrombosis. Annu Rev Biomed Eng 1:299, 1999.

24. Emmerich J et al: Combined effect of factor V Leiden and prothrombin 20210A on risk of venous thromboembolism—pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study group for pooled analysis in venous thromboelmbolism. Thromb Haemost 86:809, 2001.

25. Thomas R: Hypercoagulability syndromes. Arch Intern Med 161:2433, 2001.

26. Harpel P, Zhang X, Borth W: Homocysteine and hemostasis: pathogenic mechanisms predisposing to thrombosis. J Nutr 126 (Suppl 4):1285S, 1996.

27. Poort S, et al: A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 88:3698, 1996.

28. Vicente V, et al: The prothrombin gene variant 20210A in venous and arterial thromboembolism. Haematologica 84:356, 1999.

29. Kottke-Marchant K: Genetic polymorphisms associated with venous and arterial thrombosis: an overview. Arch Pathol Lab Med 126:295, 2002.

30. Rosendaal F, Helmerhorst F, Vandenbroucke J: Oral contraceptives, hormone replacement therapy and thrombosis. Thromb Haemost 86:112, 2001.

31. Caine GJ, et al: The hypercoagulable state of malignancy: pathogenesis and current debate. Neoplasia 4:465, 2002.

32. Lip G, Chin B, Blann A: Cancer and the prothrombotic state. Lancet Oncol 3:27, 2002.

33. Januzzi JJ, Jang I: Fundamental concepts in the pathobiology of heparin-induced thrombocytopenia. J Thromb Thrombolysis 10 (Suppl 1):7, 2000.

34. Chong B: Heparin-induced thrombocytopenia. J Thromb Hemost 7:1472, 2003.

35. Rand JH: The antiphospholipid syndrome. Ann Rev Med 54:409, 2003.

36. Honley JG: Antiphospholipid syndrome: an overview. CMAJ 168:1675, 2003.

37. Alving B: Diagnosis and management of patients with the antiphospholipid syndrome. J Thromb Thrombolysis 12:89, 2001.

38. Shapiro S: The lupus anticoagulant/antiphospholipid syndrome. Ann Rev Med 47:533, 1996.

39. Becattini C, Agnelli G: Pathogenesis of venous thromboembolism. Curr Opin Pulmon Med 8:300, 2002.

40. Bick R: Disseminated intravascular coagulation: a review of etiology, pathophysiology, diagnosis, and management: guidelines for care. Clin Appl Thromb Hemost 8:1, 2002.

41. Perrier A: Noninvasive diagnosis of pulmonary embolism. Haematologica 82:328, 1997.

42. Perrier A, Bounameaux H: Diagnosis of pulmonary embolism in outpatients by sequential noninvasive tools. Semin Thromb Hemost 27:25, 2001.

43. Heit J: Venous thromboembolism epidemiology: implications for prevention and management. Semin Thromb Hemost 28 (Suppl 2):3, 2002.

44. Goldhaber S: Pulmonary embolism. N Engl J Med 339:93, 1998.

45. Mellor A, Soni N: Fat embolism. Anaesthesia 56:145, 2001.

46. Parisi DM, Koval K, Egol K: Fat embolism syndrome. Am J Orthop 31:507, 2002.

47. Dudney T, Elliot C: Pulmonary embolism from amniotic fluid, fat, and air. Prog Cardiovasc Dis 36:447, 1994.

48. King M, Harmon K: Unusual forms of pulmonary embolism. Clin Chest Med 15:561, 1994.

49. Madsen J, Hink J, Hyldegaard O: Diving physiology and pathophysiology. Clin Physiol 14:597, 1994.

50. Neuman T: Arterial gas embolism and decompression sickness. News Physiol Sci 17:77, 2002.

51. Davies S: Amniotic fluid embolus: a review of the literature. Can J Anaesth 48:88, 2001.

52. Locksmith GJ: Amniotic fluid embolism. Obstet Gynecol Clin 26:435, 1999.

53. Angus D, et al: Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Crit Care Med 29:1303–1310, 2001.

54. Van Amersfoort ES, VanBerkel TJ, Kuiper J: Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. Clin Microbiol Rev 16:379, 2003.

55. Opal S, Huber C: Bench-to-bedside review: Toll-like receptors and their role in septic shock. Crit Care 6:125, 2002.

56. Glauser M: Pathophysiologic basis of sepsis: considerations for future strategies of intervention. Crit Care Med 28 (9 Suppl):S4, 2000.

57. Hardaway R, Williams C, Vasquez Y: Disseminated intravascular coagulation in sepsis. Semin Thromb Hemost 27:577, 2001.

---

58. Natanson C: Anti-inflammatory therapies to treat sepsis and septic shock: a reassessment. Crit Care Med 25:1095, 1997.

59. Hotchkiss RS, Karl IE: Medical progress: the pathophysiology and treatment of sepsis. N Engl J Med 348:138, 2003.

60. Johnson H, Torres B, Soos J: Superantigens: structure and relevance to human disease. Proc Soc Exp Biol Med 212:99, 1996.

61. Llewelyn M, Cohen J: Superantigens: microbial agents that corrupt immunity. Lancet Infect Dis 2:156, 2002.

62. Stevens D: The toxic shock syndromes. Infect Dis Clin North Am 10:727, 1996.

63. Torres B, Johnson H: Modulation of disease by superantigens. Cur Opin Immunol 10:465, 1998.

Chapter 5 - Genetic Disorders

Genetic disorders are far more common than is widely appreciated. The lifetime frequency of genetic diseases is estimated to be 670 per 1000.[1] Included in this figure are not only the "classic" genetic disorders but also cancer and cardiovascular diseases, the two most common causes of death in the Western world. Both of these have major genetic components. Cardiovascular diseases, such as atherosclerosis and hypertension, result from complex interactions of genes and environment, and most cancers are now known to result from an accumulation of mutations in somatic cells (Chapter 7).

The genetic diseases encountered in medical practice represent only the tip of the iceberg, that is, those with less extreme genotypic errors permitting full embryonic development and live birth. It is estimated that 50% of spontaneous abortuses during the early months of gestation have a demonstrable chromosomal abnormality; there are, in addition, numerous smaller detectable errors and many others still beyond our range of identification. About 1% of all newborn infants possess a gross chromosomal abnormality, and approximately 5% of individuals under age 25 develop a serious disease with a significant genetic component. How many more mutations remain hidden?

The draft sequence of the human genome is complete and much has been learned about the "genetic architecture" of humans. Some of what has been revealed was quite unexpected.[2] For example, we now know that less than 2% of the human genome codes for proteins, whereas more than one half represents blocks of repetitive nucleotide codes whose functions remain mysterious. What was totally unexpected was that humans have a mere 30,000 genes rather than the 100,000 predicted only recently. Quite remarkably, this figure is not much greater than that of the mustard plant, with 26,000 genes! However, it is also known that by alternative splicing, 30,000 genes can give rise to greater than 100,000 proteins. In addition, very recent studies indicate that fully formed proteins can be sliced and stitched together to give rise to peptides that could not have been predicted from the structure of the gene.[2A] Humans are not so poor, after all. With the completion of the human genome project, a new term, called *genomics*, has been added to the medical vocabulary. Whereas genetics is the study of single or a few genes and their phenotypic effects, genomics is the study of all the genes in the genome and their interactions. DNA microarray analysis of tumors (Chapter 7) is an excellent example of genomics in current clinical use.[3] However, the most important contribution of genomics to human health will be in the unraveling of complex multifactorial diseases (discussed later) that arise from the interaction of multiple genes with environmental factors.[4]

Another surprising revelation from the recent progress in genomics is that, on average, any two individuals share 99.9% of their DNA sequences. Thus, the remarkable diversity of humans is encoded in about 0.1% of our DNA. The secrets to disease predisposition and response to environmental agents and drugs must therefore reside within these variable regions. Although small as compared to the total nucleotide sequences, this 0.1% represents about 3 million base pairs. The most common form of DNA variations in the human genome is the single nucleotide polymorphism (SNP). Typically, the SNPs are biallelic (i.e., only two choices exist at a given site within the population), and they may occur anywhere in the genome—within exons, introns, or intergenic regions. Less than 1% of SNPs occur in coding regions. These could of course alter the gene product and give rise to a disease. Much more commonly, however, the SNP is just a marker that is co-inherited with a disease-causing gene, due to physical proximity. Another way of expressing this is to say that the SNP and the genetic factor are in linkage disequilibrium. Much effort is ongoing to make SNP maps of the human genome so that we can decipher genetic determinants of disease.[5] Just as genomics involves the study of all the DNA sequences, *proteomics* concerns itself with the measurement of all proteins expressed in a cell or tissue. Currently, progress in proteomics is lagging behind genomics, because the methodology to identify hundreds of distinct proteins simultaneously is not fully developed, but much effort continues.

Although genomics and proteomics are revealing a treasure-trove of information, our ability to organize and mine such a vast array of data is not yet fully developed. To simultaneously analyze patterns of expression involving thousands of genes and proteins has required the parallel development of computer-based techniques that can manage vast collections of data. In response to this, an exciting new discipline called *bioinformatics* has sprouted. This has involved biologists, computer scientists, physicists, and mathematicians, a true example of a multidisciplinary approach in modern medical practice.[6]

Much of the progress in medical genetics has resulted from the spectacular advances in molecular biology, involving recombinant DNA technology. The details of these techniques are well known and are not repeated here. Some examples, however, of the impact of recombinant DNA technology on medicine are worthy of attention.

- *Molecular basis of human disease:* Two general strategies have been used to isolate and characterize involved genes (Fig. 5-1). The *functional cloning*, or *classic*, approach has been successfully used to study a variety of inborn errors of metabolism, such as phenylketonuria and disorders of hemoglobin synthesis. Common to these genetic diseases is knowledge of the abnormal gene product and the corresponding protein. When the affected protein is known, a variety of methods can be employed to isolate the normal gene, to

clone it, and ultimately to determine the molecular changes that affect the gene in patients with the disorder. Because in many common single-gene disorders, such as cystic fibrosis, there was no clue to the nature of the defective gene product, an alternative strategy called *positional cloning*, or the "candidate gene," approach had to be employed. This strategy initially ignores the biochemical clues from the phenotype and relies instead on mapping the disease phenotype to a particular chromosome location. This mapping is accomplished if the disease is associated with a distinctive cytogenetic change (e.g., fragile-X syndrome) or by linkage analysis. In the latter, the approximate location of the gene is determined by linkage to known "marker genes" or SNPs that are in close proximity to the disease locus. Once the region in which the mutant gene lies has been localized within reasonably narrow limits, the next step is to clone several pieces of DNA from the relevant segment of the genome. Expression of the cloned DNA in vitro, followed by identification of the protein products, can then be used to identify the aberrant protein encoded by the mutant genes. This approach has been used successfully in several diseases, such as cystic fibrosis, neurofibromatosis, Duchenne muscular dystrophy (a hereditary disorder characterized by progressive muscle weakness), polycystic

kidney disease, and Huntington disease. In addition to this step-by-step approach to cloning single genes, cDNA microarray analysis allows simultaneous detection of thousands of genes and their RNA products. When normal and diseased tissues are analyzed in this fashion, changes in the expression levels of multiple genes can be detected, thus providing a more comprehensive profile of genetic alterations in diseased tissues.

- *Production of human biologically active agents:* An array of ultrapure biologically active agents can now be produced in virtually unlimited quantities by inserting the requisite gene into bacteria or other suitable cells in tissue culture. Some examples of genetically engineered products already in clinical use include soluble TNF receptor for blocking TNF in treatment of rheumatoid arthritis, tissue plasminogen activator for the treatment of thrombotic states, growth hormone for the treatment of deficiency states, erythropoietin to reverse several types of anemia, and myeloid growth and differentiation factors (granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor) to enhance production of monocytes and neutrophils in states of poor marrow function.
- *Gene therapy:* The goal of treating genetic diseases by transfer of somatic cells transfected with the normal gene, although simple in concept, has yet to succeed on a large scale. Problems include designing appropriate vectors to carry the gene and unexpected complications resulting from random insertion of the normal gene in the host genome. In recent well-publicized cases, gene therapy in patients with x-linked SCID (severe combined immunodeficiency, Chapter 6) who lack the common γ chain of cytokine receptors had to be put on hold because the transduced gene inserted next to a host gene that controls proliferation of cells. The resulting dysregulation gave rise to T-cell leukemia in the patient.
- *Disease diagnosis:* Molecular probes are proving to be extremely useful in the diagnosis of both genetic and non-genetic (e.g., infectious) diseases. The diagnostic applications of recombinant DNA technology are detailed at the end of this chapter.

Figure 5-1 Schematic illustration of the strategies employed in functional and positional cloning. Functional cloning begins with relating the clinical phenotype to biochemical-protein abnormalities, followed by isolation of the mutant gene. Positional cloning, also called candidate gene approach, begins by mapping and cloning the disease gene by linkage analysis, without any knowledge of the gene product. Identification of the gene product and the mechanism by which it produces the disease follow the isolation of the mutant gene.

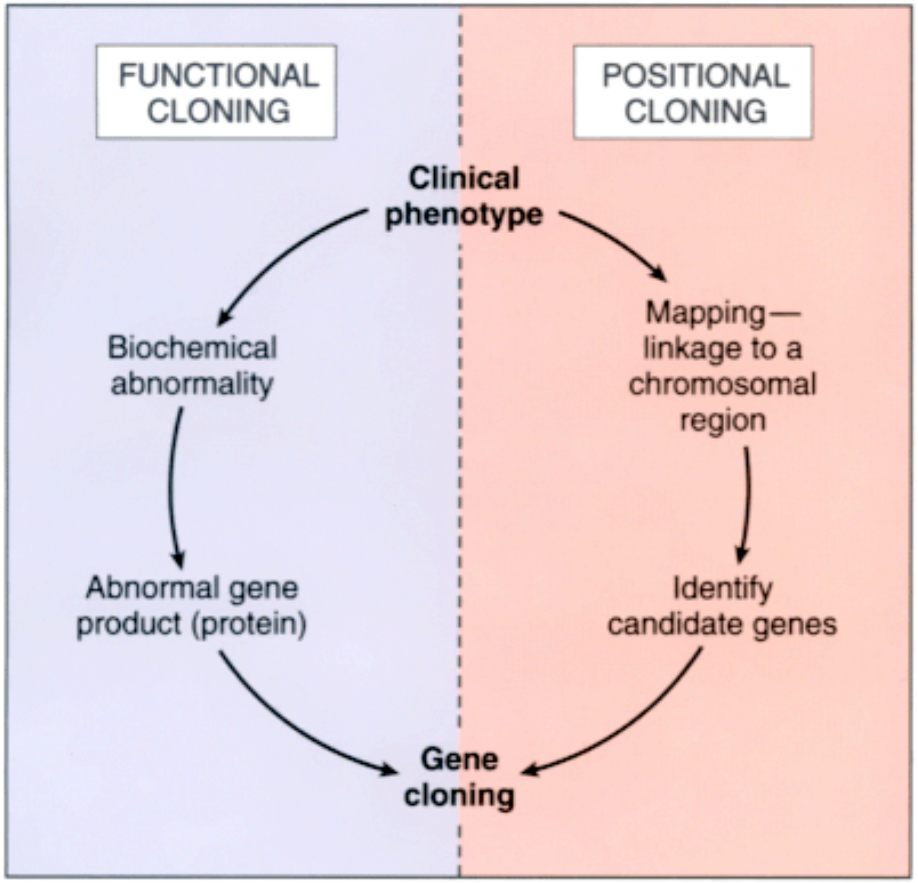


Figure 5-2 Schematic illustration of a point mutation resulting from a single base pair change in the DNA. In the example shown, a CTC to CAC change alters the meaning of the genetic code (GAG to GUG in the opposite strand), leading to replacement of glutamic acid by valine in the polypeptide chain. This change, affecting the sixth amino acid of the normal β-globin (β_A) chain, converts it to sickle β-globin (β_S).

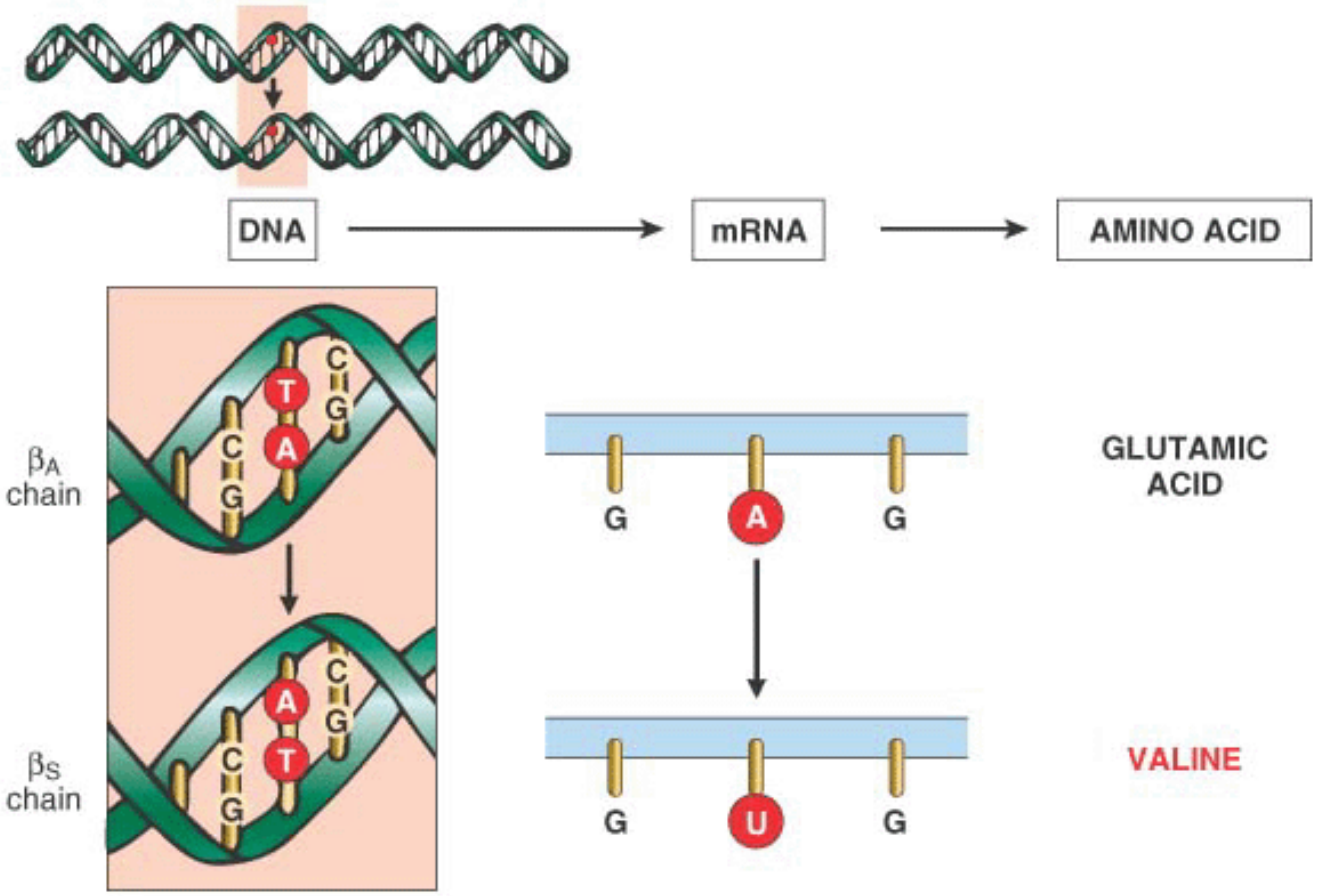


Figure 5-3 Single-base deletion at the ABO (glycosyltransferase) locus, leading to a frameshift mutation responsible for the O allele. *(From Thompson MW, et al: Thompson and Thompson Genetics in Medicine, 5th ed. Philadelphia, WB Saunders, 1991, p 134.)*

ABO A allele

. . . Leu – Val – Val – Thr – Pro . . .

. . . CTC GTG GTG ACC CCT T . . .

ABO O allele

. . . CTC GTG GT– ACC CCT T . . .

. . . Leu – Val – **Val – Pro – Leu** . . .

altered reading frame →

Figure 5-4 Four-base insertion in the hexosaminidase A gene in Tay-Sachs disease, leading to a frameshift mutation. This mutation is the major cause of Tay-Sachs disease in Ashkenazi Jews. *(From Nussbaum, RL, et al: Thompson and Thompson Genetics in Medicine, 6th ed. Philadelphia, WB Saunders, 2001, p. 212.)*

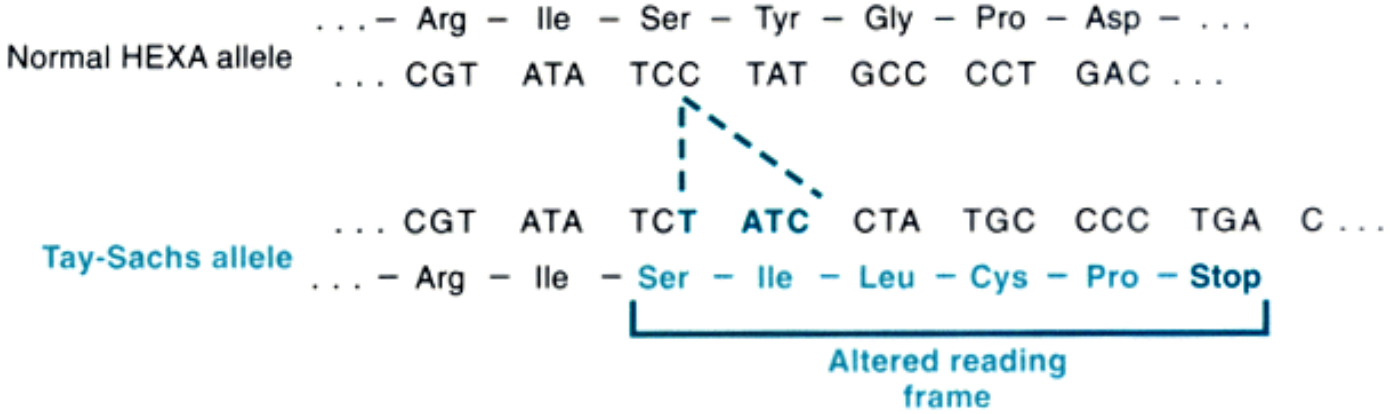


Figure 5-5 Point mutation leading to premature chain termination. Partial mRNA sequence of the β-globin chain of hemoglobin showing codons for amino acids 38 to 40. A point mutation (C→U) in codon 39 changes glutamine (Gln) codon to a stop codon, and hence protein synthesis stops at the 38th amino acid.

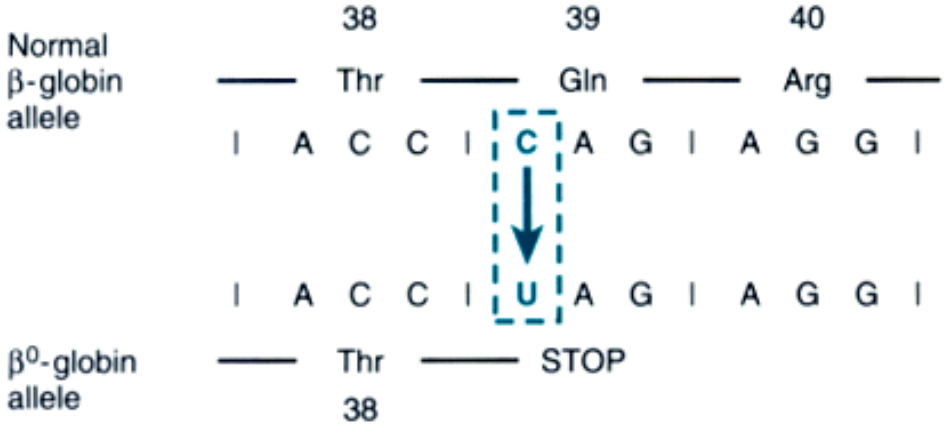


Figure 5-6 Three-base deletion in the common cystic fibrosis (CF) allele results in synthesis of a protein that is missing amino acid 508 (phenylalanine). Because the deletion is a multiple of three, this is not a frameshift mutation. *(From Thompson MW, et al: Thompson and Thompson Genetics in Medicine, 5th ed. Philadelphia, WB Saunders, 1991, p. 135.)*

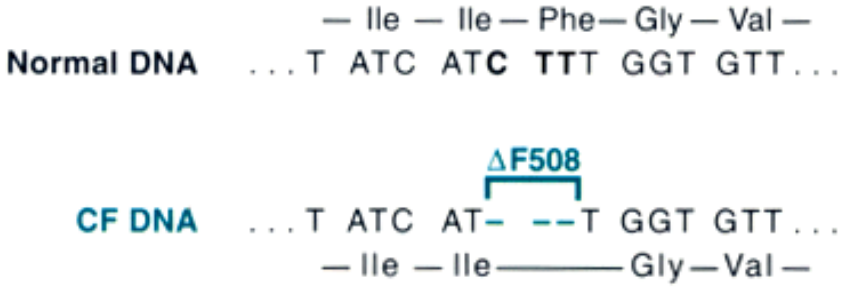


TABLE 5-1 -- Autosomal Dominant Disorders

System	Disorder
Nervous	Huntington disease
	Neurofibromatosis *
	Myotonic dystrophy
	Tuberous sclerosis
Urinary	Polycystic kidney disease
Gastrointestinal	Familial polyposis coli
Hematopoietic	Hereditary spherocytosis
	von Willebrand disease
Skeletal	Marfan syndrome *
	Ehlers-Danlos syndrome (some variants) *
	Osteogenesis imperfecta
	Achondroplasia
Metabolic	Familial hypercholesterolemia *
	Acute intermittent porphyria

*Discussed in this chapter. Other disorders listed are discussed in appropriate chapters of this book.

Autosomal recessive disorders include almost all inborn errors of metabolism. The various consequences of enzyme deficiencies are discussed later. The more common of these conditions are listed in Table 5-2 . Most are presented elsewhere; a few prototypes are discussed later in this chapter.

TABLE 5-2 -- Autosomal Recessive Disorders

System	Disorder
Metabolic	Cystic fibrosis
	Phenylketonuria
	Galactosemia
	Homocystinuria
	Lysosomal storage diseases *
	α_1 -Antitrypsin deficiency

	Wilson disease
	Hemochromatosis
	Glycogen storage diseases *
Hematopoietic	Sickle cell anemia
	Thalassemias
Endocrine	Congenital adrenal hyperplasia
Skeletal	Ehlers-Danlos syndrome (some variants) *
	Alkaptonuria *
Nervous	Neurogenic muscular atrophies
	Friedreich ataxia
	Spinal muscular atrophy

*Discussed in this chapter. Many others are discussed elsewhere in the text.

X-Linked Disorders

All sex-linked disorders are X-linked, almost all X-linked recessive. Several genes are encoded in the "male-specific region of Y"; all of these are related to spermatogenesis.[9] Males with mutations affecting the Y-linked genes are usually infertile, and hence there is no Y-linked inheritance. As discussed later, a few additional genes with homologues on the X chromosome have been mapped to the Y chromosome, but no disorders resulting from mutations in such genes have been described.

X-linked recessive inheritance accounts for a small number of well-defined clinical conditions. The Y chromosome, for the most part, is not homologous to the X, and so mutant genes on the X are not paired with alleles on the Y. Thus, the male is said to be *hemizygous* for X-linked mutant genes, so these disorders are expressed in the male. Other features that characterize these disorders are as follows:

- An affected male does not transmit the disorder to his sons, but all daughters are carriers. Sons of heterozygous women have, of course, one chance in two of receiving the mutant gene.
- The heterozygous female usually does not express the full phenotypic change because of the paired normal allele. Because of the random inactivation of one of the X chromosomes in the female, however, females have a variable proportion of cells in which the mutant X chromosome is active. Thus, it is remotely possible for the normal allele to be inactivated in most cells, permitting full expression of heterozygous X-linked conditions in the female. Much more commonly, the normal allele is inactivated in only some of the cells, and thus the heterozygous female expresses the disorder partially. An illustrative condition is *glucose-6-phosphate dehydrogenase (G6PD) deficiency*. Transmitted on the X chromosome, this enzyme deficiency, which predisposes to red cell hemolysis in patients receiving certain types of drugs (Chapter 13), is expressed principally in males. In the female, a proportion of the red cells may be derived from marrow cells with inactivation of the normal allele. Such red cells are at the same risk for undergoing hemolysis as are the red cells in the

hemizygous male. Thus, the female is not only a carrier of this trait, but also is susceptible to drug-induced hemolytic reactions. Because the proportion of defective red cells in heterozygous females depends on the random inactivation of one of the X chromosomes, however, the severity of the hemolytic reaction is almost always less in heterozygous women than in hemizygous men. Most of the X-linked conditions listed in Table 5-3 are covered elsewhere in the text.

TABLE 5-3 -- X-Linked Recessive Disorders

System	Disease
Musculoskeletal	Duchenne muscular dystrophy
Blood	Hemophilia A and B
	Chronic granulomatous disease
	Glucose-6-phosphate dehydrogenase deficiency
Immune	Agammaglobulinemia
	Wiskott-Aldrich syndrome
Metabolic	Diabetes insipidus
	Lesch-Nyhan syndrome
Nervous	Fragile-X syndrome *

*Discussed in this chapter. Others discussed in appropriate chapters in the book.

There are only a few *X-linked dominant* conditions. They are caused by dominant disease alleles on the X chromosome. These disorders are transmitted by an affected heterozygous female to half her sons and half her daughters and by an affected male parent to all his daughters but none of his sons, if the female parent is unaffected. Vitamin D-resistant rickets is an example of this type of inheritance.

BIOCHEMICAL AND MOLECULAR BASIS OF SINGLE-GENE (MENDELIAN) DISORDERS

Mendelian disorders result from alterations involving single genes. The genetic defect may lead to the formation of an abnormal protein or a reduction in the output of the gene product. As mentioned earlier, mutations may affect protein synthesis by affecting transcription, mRNA processing, or translation. The phenotypic effects of a mutation may result directly, from abnormalities in the protein encoded by the mutant gene, or indirectly, owing to interactions of the mutant protein with other normal proteins. For example, all forms of Ehlers-Danlos syndrome (EDS) are associated with abnormalities of collagen. In some forms (e.g., vascular type), there is a mutation in one of the collagen genes, whereas in others (e.g., kyphoscoliosis type), the collagen genes are normal, but there is a mutation in the gene that encodes lysyl hydroxylase, an enzyme essential for the cross-linking of collagen. In these patients, collagen weakness is secondary to a deficiency of lysyl hydroxylase.

Virtually any type of protein may be affected in single-gene disorders and by a variety of mechanisms (Table 5-4). To some extent, the pattern of inheritance of the disease is related to the kind of protein affected by the mutation, as was discussed earlier and is reiterated subsequently. For the purposes of this discussion, the mechanisms involved in single-gene disorders can be classified into four categories: (1) *enzyme defects and their consequences*; (2) *defects in membrane receptors and transport systems*; (3) *alterations in the structure, function, or quantity of nonenzyme proteins*; and (4) *mutations resulting in unusual reactions to drugs*.

Mutations may result in the synthesis of a defective enzyme with reduced activity or in a reduced amount of a normal enzyme. In either case, the consequence is a metabolic block. Figure 5-7 provides an example of an enzyme reaction in which the substrate is converted by intracellular enzymes, denoted as 1, 2, and 3, into an end product through intermediates 1 and 2. In this model, the final product exerts feedback control on enzyme 1. A minor pathway producing small quantities of M_1 and M_2 also exists. The biochemical consequences of an enzyme defect in such a reaction may lead to three major consequences:

1. *Accumulation of the substrate*, depending on the site of block, may be accompanied by accumulation of one or both intermediates. Moreover, an increased concentration of intermediate 2 may stimulate the minor pathway and thus

---

lead to an excess of $M_1$and M_2. Under these conditions, tissue injury may result if the precursor, the intermediates, or the products of alternative minor pathways are toxic in high concentrations. For example, in galactosemia, the deficiency of galactose-1-phosphate uridyltransferase (Chapter 10) leads to the accumulation of galactose and consequent tissue damage. In phenylketonuria, a deficiency of phenylalanine hydroxylase (Chapter 10) results in the accumulation of phenylalanine. Excessive accumulation of complex substrates within the lysosomes as a result of deficiency of degradative enzymes is responsible for a group of diseases generally referred to as *lysosomal storage diseases*.

2. *An enzyme defect can lead to a metabolic block and a decreased amount of end product* that may be necessary for normal function. For example, a deficiency of melanin may result from lack of tyrosinase, which is necessary for the biosynthesis of melanin from its precursor, tyrosine. This results in the clinical condition called *albinism*. If the end product is a feedback inhibitor of the enzymes involved in the early reactions (in Fig. 5-7 , it is shown that the product inhibits enzyme 1), the deficiency of the end product may permit overproduction of intermediates and their catabolic products, some of which may be injurious at high concentrations. A prime example of a disease with such an underlying mechanism is the Lesch-Nyhan syndrome (Chapter 26).
3. *Failure to inactivate a tissue-damaging substrate* is best exemplified by α_1 -antitrypsin (α_1 -AT) deficiency. Patients who have an inherited deficiency of serum α_1 -AT are unable to inactivate neutrophil elastase in their lungs. Unchecked activity of this protease leads to destruction of elastin in the walls of lung alveoli, leading eventually to pulmonary emphysema (Chapter 15).

TABLE 5-4 -- Biochemical and Molecular Basis of Some Mendelian Disorders

Protein Type/Function	Example	Molecular Lesion	Disease
Enzyme	Phenylalanine hydroxylase	Splice site mutation: reduced amount	Phenylketonuria
	Hexosaminidase	Splice site mutation or frameshift mutation with stop codon: reduced amount	Tay-Sachs disease
	Adenosine deaminase	Point mutations: abnormal protein with reduced activity	Severe combined immunodeficiency
Enzyme Inhibitor	α_1 -Antitrypsin	Missense mutations: impaired secretion from liver to serum	Emphysema and liver disease
Receptor	Low-density lipoprotein receptor	Deletions, point mutations: reduction of synthesis, transport to cell surface, or binding to low-density lipoprotein	Familial hypercholesterolemia
	Vitamin D receptor	Point mutations: failure of normal signaling	Vitamin D-resistant rickets
Transport			

Oxygen	Hemoglobin	Deletions: reduced amount	α-Thalassemia
		Defective mRNA processing: reduced amount	β-Thalassemia
		Point mutations: abnormal structure	Sickle cell anemia
Ions	Cystic fibrosis transmembrane conductance regulator	Deletions and other mutations	Cystic fibrosis
Structural			
Extracellular	Collagen	Deletions or point mutations cause reduced amount of normal collagen or normal amounts of mutant collagen	Osteogenesis imperfecta; Ehlers-Danlos syndromes
	Fibrillin	Missense mutations	Marfan syndrome
Cell membrane	Dystrophin	Deletion with reduced synthesis	Duchenne/Becker muscular dystrophy
	Spectrin, ankyrin, or protein 4.1	Heterogeneous	Hereditary spherocytosis
Hemostasis	Factor VIII	Deletions, insertions, nonsense mutations, and others: reduced synthesis or abnormal factor VIII	Hemophilia A
Growth Regulation	Rb protein	Deletions	Hereditary retinoblastoma
	Neurofibromin	Heterogeneous	Neurofibromatosis type 1

Figure 5-7 Scheme of a possible metabolic pathway in which a substrate is converted to an end product by a series of enzyme reactions. M1, M2, products of a minor pathway.

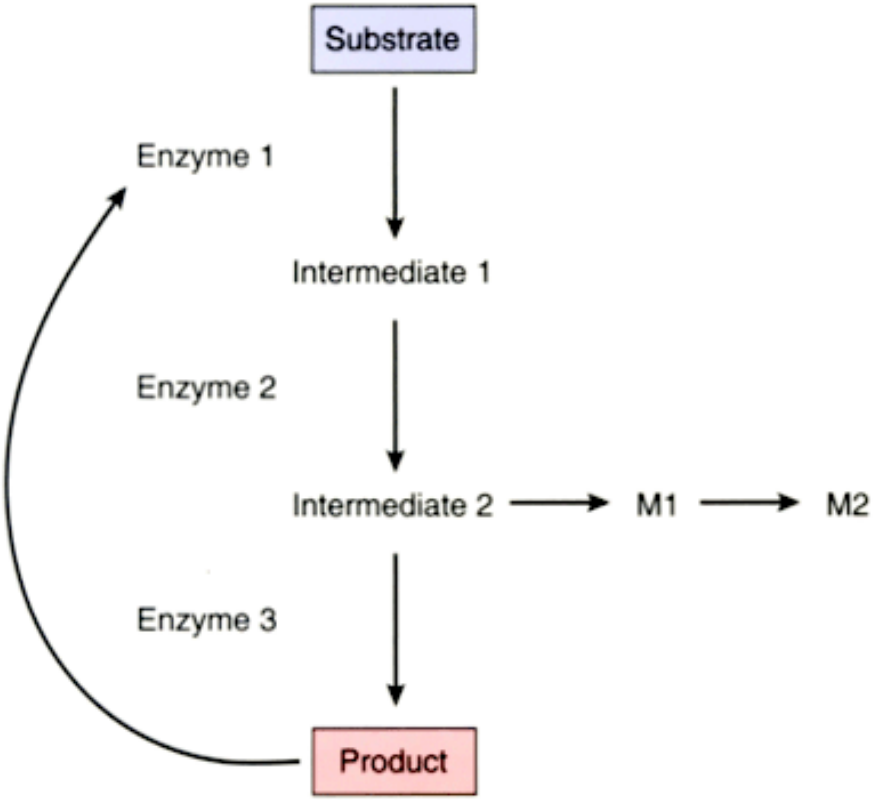


TABLE 5-5 -- Classification of Ehlers-Danlos Syndromes (EDS)

EDS Type *	Clinical Findings	Inheritance	Gene Defects
Classical (I/II)	Skin and joint hypermobility, atrophic scars, easy bruising	Autosomal dominant	COL5A1, COL5A2
Hypermobility (III)	Joint hypermobility, pain, dislocations	Autosomal dominant	Unknown
Vascular (IV)	Thin skin, arterial or uterine rupture, bruising, small joint hyperextensibility	Autosomal dominant	COL3A1
Kyphoscoliosis (VI)	Hypotonia, joint laxity, congenital scoliosis, ocular fragility	Autosomal recessive	Lysyl-hydroxylase
Arthrochalasia (VIIa,b)	Severe joint hypermobility, skin changes mild, scoliosis, bruising	Autosomal dominant	COL1A1, COL1A2
Dermatosparaxsis (VIIc)	Severe skin fragility, cutis laxa, bruising	Autosomal recessive	Procollagen *N*-peptidase

*EDS were previously classified by Roman numerals. Parentheses show previous numerical equivalents.

deficiency of lysyl hydroxylase results in the synthesis of collagen that lacks normal structural stability.

The *vascular type of EDS results from abnormalities of type III collagen*. This form is genetically heterogeneous because at least three distinct types of mutations affecting the *COL3A1* gene for collagen type III can give rise to this variant. Some affect the rate of synthesis of pro α1 (III) chains, others affect the secretion of type III procollagen, and still others lead to the synthesis of structurally abnormal type III collagen. Some mutant alleles behave as dominant negatives (see discussion under autosomal dominant disorders) and thus produce severe phenotypic effects. These molecular studies provide a rational basis for the pattern of transmission and clinical features that are characteristic of this variant. First, because vascular type EDS results from mutations involving a structural protein (rather than an enzyme protein), an autosomal dominant pattern of inheritance would be expected. Second, because blood vessels and intestines are known to be rich in collagen type III, an abnormality of this collagen is consistent with severe defects (e.g., spontaneous rupture) in these organs.

In two forms of EDS—arthrochalasia type and dermatosparaxis type—the fundamental defect is in the conversion of type I procollagen to collagen. This step in collagen synthesis involves cleavage of noncollagen peptides at the N-terminal and C-terminal of the procollagen molecule. This is accomplished by N-terminal-specific and C-terminal-specific peptidases. *The defect in the conversion of procollagen to collagen in the arthrocalasic type has been traced to mutations that affect one of the two type I collagen genes, COL1A1* and *COL1A2*. As a result, structurally abnormal pro α1 (I) or pro α2 (I) chains that resist cleavage of N-terminal peptides are formed. In patients with a single mutant allele, only 50% of the type I collagen chains are abnormal, but because these chains interfere with the formation of normal collagen helices, heterozygotes manifest the disease. By contrast, the related dermatosparaxis type is caused by mutations in the procollagen-N-peptidase genes, essential for the cleavage of collagens. In this case, the enzyme deficiency leads to an autosomal recessive form of inheritance.

Finally, the *classical type of EDS* is worthy of brief mention, since molecular analysis of the variant suggests that genes other than collagen genes may be involved in the pathogenesis of EDS. In 30% to 50% of these cases, mutations in the genes for type V collagen *(COL5A1* and *COL5A1)* have been detected. Surprisingly, despite a phenotype typical of EDS, no other collagen gene abnormalities have been found in these cases. This has led to the speculation that other proteins in the extracellular matrix, such as tenascin-X, may also be involved in regulating collagen synthesis.

To summarize, the common thread in EDS is some abnormality of collagen. These disorders, however, are extremely heterogeneous. At the molecular level, a variety of defects, varying from mutations involving structural genes for collagen to those involving enzymes that are responsible for post-transcriptional modifications of mRNA, have been detected. Such molecular heterogeneity results in the expression of EDS as a clinically heterogeneous disorder with several patterns of inheritance.

DISORDERS ASSOCIATED WITH DEFECTS IN RECEPTOR PROTEINS

Familial Hypercholesterolemia

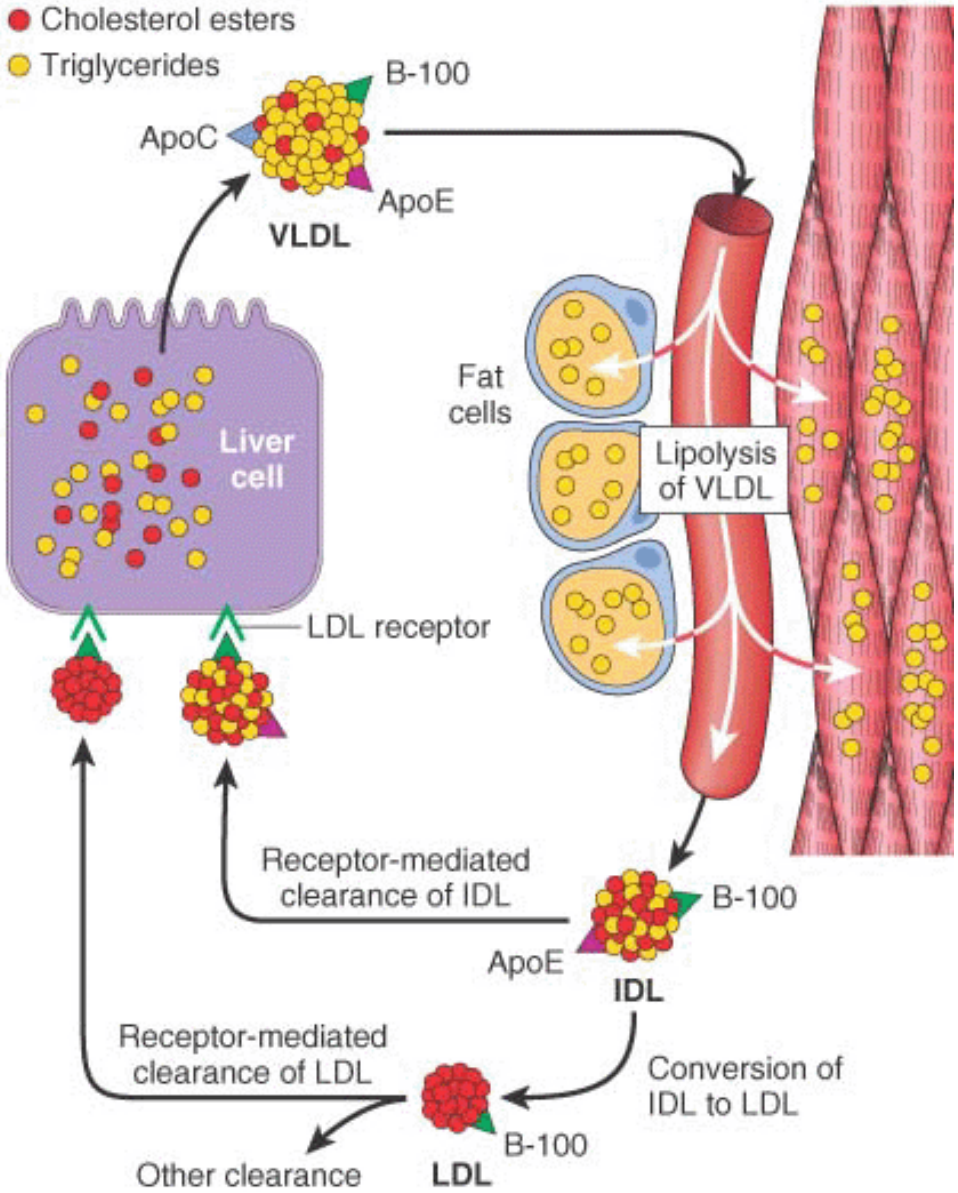
Familial hypercholesterolemia is a "receptor disease" that is the consequence of a *mutation in the gene encoding the receptor for low density lipoprotein (LDL), which is involved in the transport and metabolism of cholesterol*. As a consequence of receptor abnormalities, there is a loss of feedback control and elevated levels of cholesterol that induce premature atherosclerosis, leading to a greatly increased risk of myocardial infarction.[19] [20]

Familial hypercholesterolemia is possibly the most frequent mendelian disorder. Heterozygotes with one mutant gene, representing about 1 in 500 individuals, have from birth a twofold to threefold elevation of plasma cholesterol level, leading to tendinous xanthomas and premature atherosclerosis in adult life (Chapter 11). Homozygotes, having a double dose of the mutant gene, are much more severely affected and may have fivefold to sixfold elevations in plasma cholesterol levels. These individuals develop skin xanthomas and coronary, cerebral, and peripheral vascular atherosclerosis at an early age. Myocardial infarction may develop before age 20. Large-scale studies have found that familial hypercholesterolemia is present in 3% to 6% of survivors of myocardial infarction.

An understanding of this disorder requires that we briefly review the normal process of cholesterol metabolism and transport. Approximately 7% of the body's cholesterol circulates in the plasma, predominantly in the form of LDL. As might be expected, the level of plasma cholesterol is influenced by its synthesis and catabolism and the liver plays a crucial role in both these

processes (Fig. 5-8). The first step in this complex sequence is the secretion of very-low-density lipoproteins (VLDL) by the liver into the bloodstream. VLDL particles are rich in triglycerides, although they do contain lesser amounts of cholesteryl esters. When a VLDL particle reaches the capillaries of adipose tissue or muscle, it is cleaved by lipoprotein lipase, a process that extracts most of the triglycerides. The resulting molecule, called *intermediate-density lipoprotein (IDL)*, is reduced in triglyceride content and enriched in cholesteryl esters, but it retains two of the three apoproteins (B-100 and E) present in the parent VLDL particle (see Fig. 5-8). After release from the capillary endothelium, the IDL particles have one of two fates. Approximately 50% of newly formed IDL is rapidly taken up by the liver through a receptor-mediated transport. The receptor responsible for the binding of IDL to liver cell membrane recognizes both apoprotein B-100 and apoprotein E. It is called the *LDL receptor*, however, because it is also involved in the hepatic clearance of LDL, as described later. In the liver cells,

Figure 5-8 Schematic illustration of low-density lipoprotein (LDL) metabolism and the role of the liver in its synthesis and clearance. Lipolysis of very-low-density lipoprotein (VLDL) by lipoprotein lipase in the capillaries releases triglycerides, which are then stored in fat cells and used as a source of energy in skeletal muscles.



Other clearance LDL

Figure 5-9 The LDL receptor pathway and regulation of cholesterol metabolism.

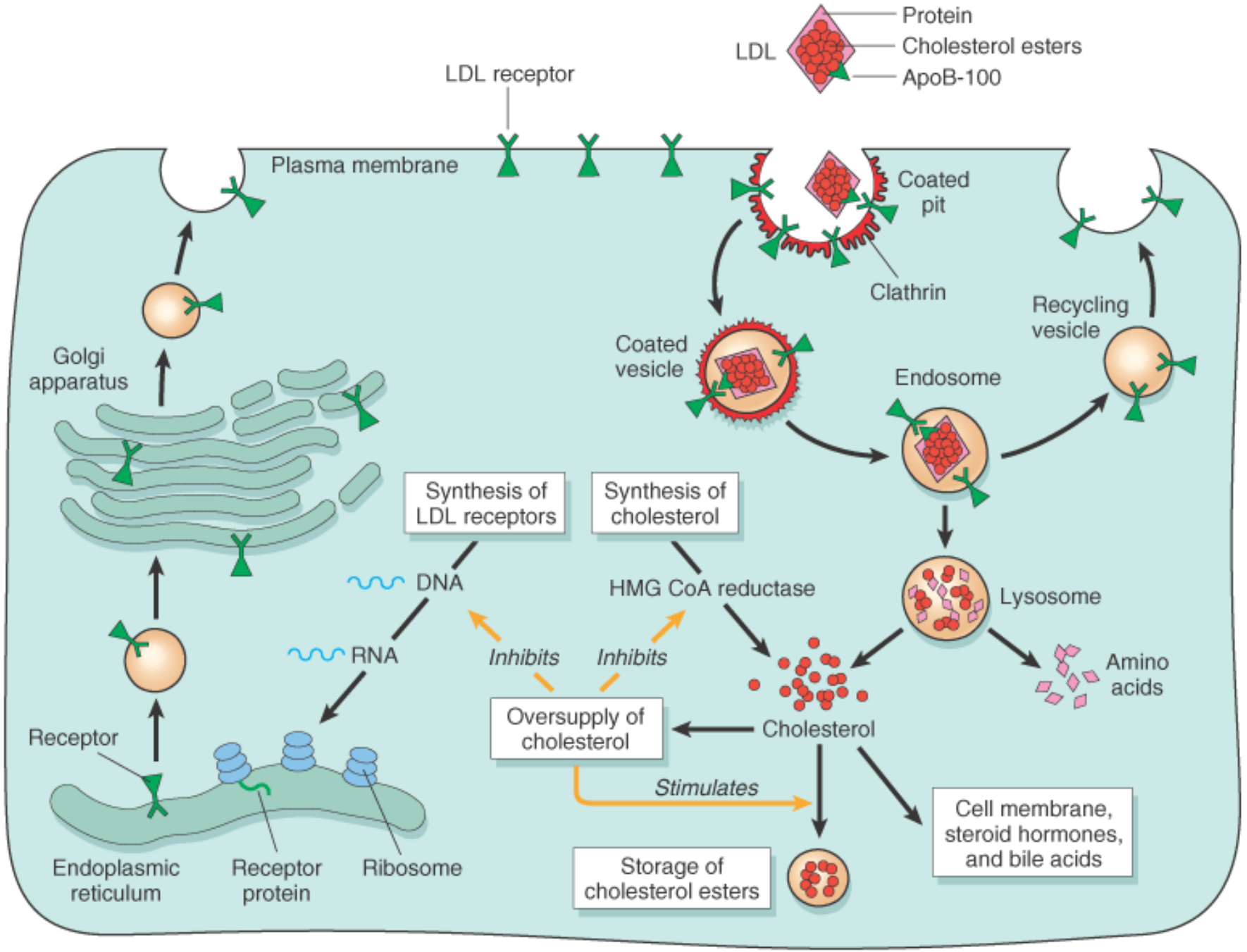
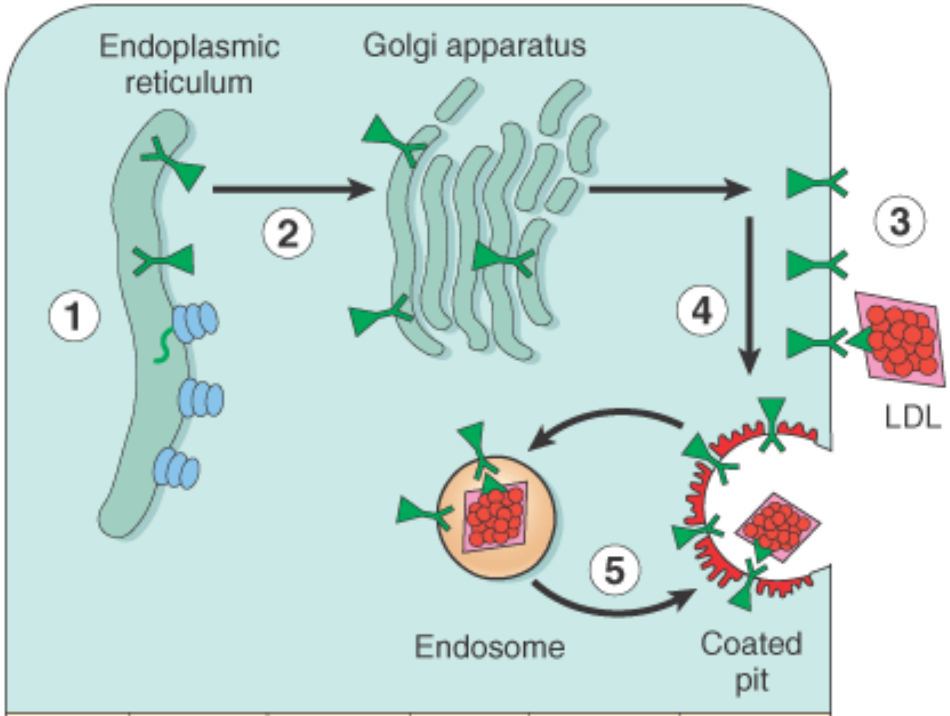


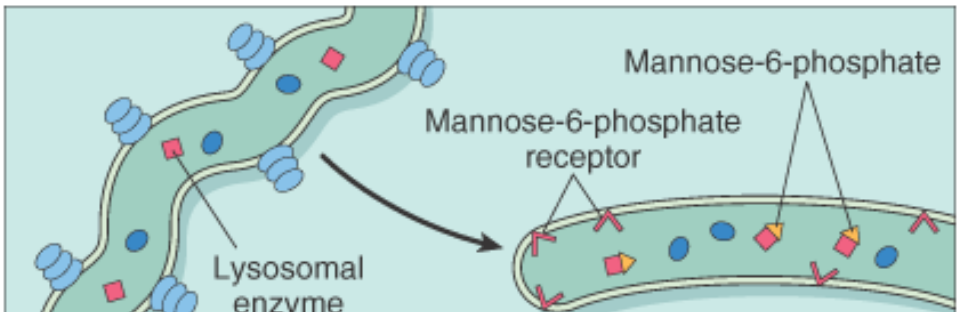
Figure 5-10 Classification of LDL receptor mutations based on abnormal function of the mutant protein. These mutations disrupt the receptor's synthesis in the endoplasmic reticulum,

transport to the Golgi complex, binding of apoprotein ligands, clustering in coated pits, and recycling in endosomes. Each class is heterogeneous at the DNA level. *(Modified with permission from Hobbs HH, et al: The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. Annu Rev Genet 24:133–170, 1990. © 1990 by Annual Reviews.)*



Mutation class	Synthesis	Transport	Binding	Clustering	Recycling
I	X				
II	→	X			
III	→	→	X		
IV	→	→	→	X	
V	→	→	→	→	X

Figure 5-11 Synthesis and intracellular transport of lysosomal enzymes.



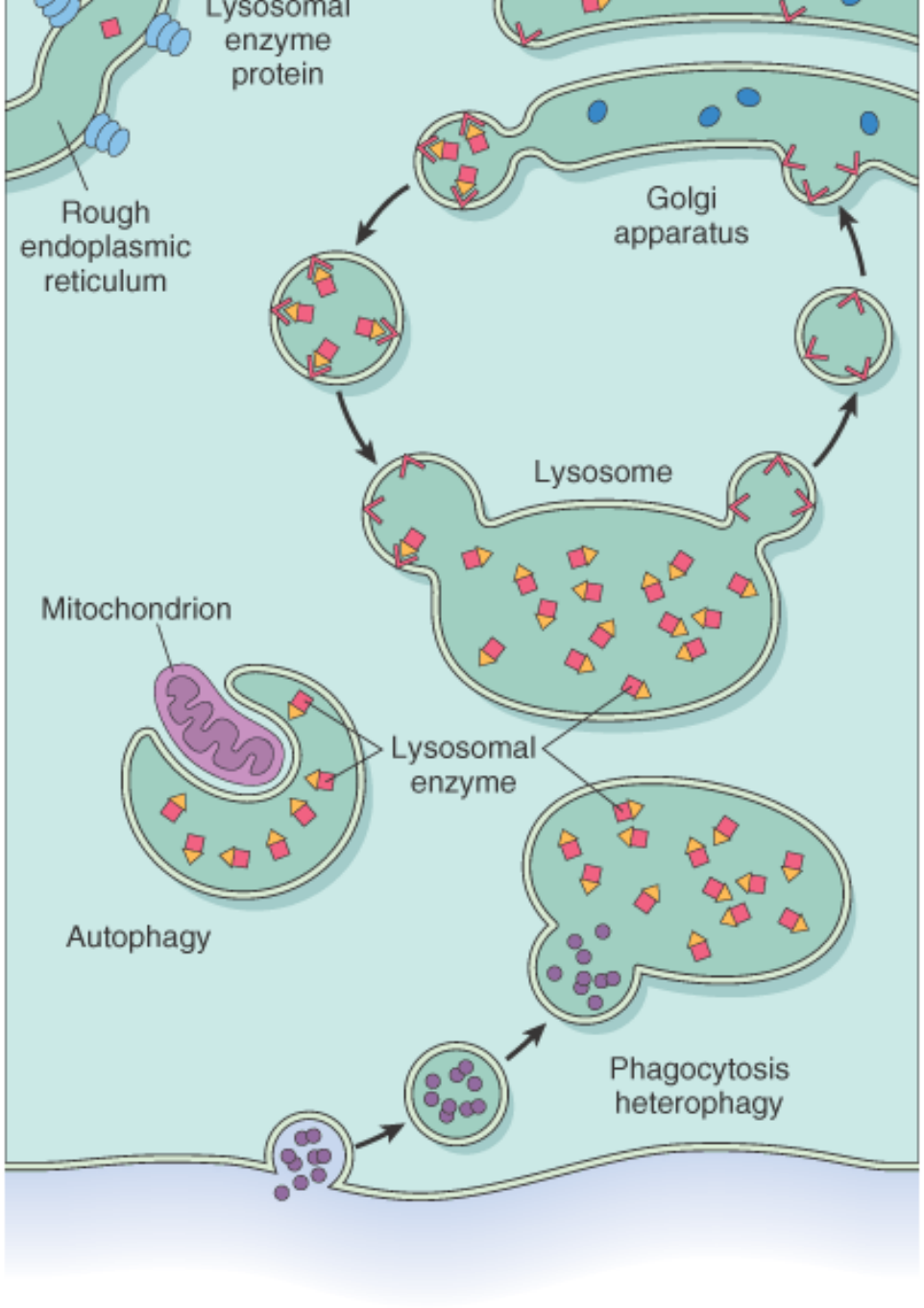


Figure 5-12 Schematic diagram illustrating the pathogenesis of lysosomal storage diseases. In the example shown, a complex substrate is normally degraded by a series of lysosomal enzymes (A, B, and C) into soluble end products. If there is a deficiency or malfunction of one of the enzymes (e.g., B), catabolism is incomplete and insoluble intermediates accumulate in the lysosomes.

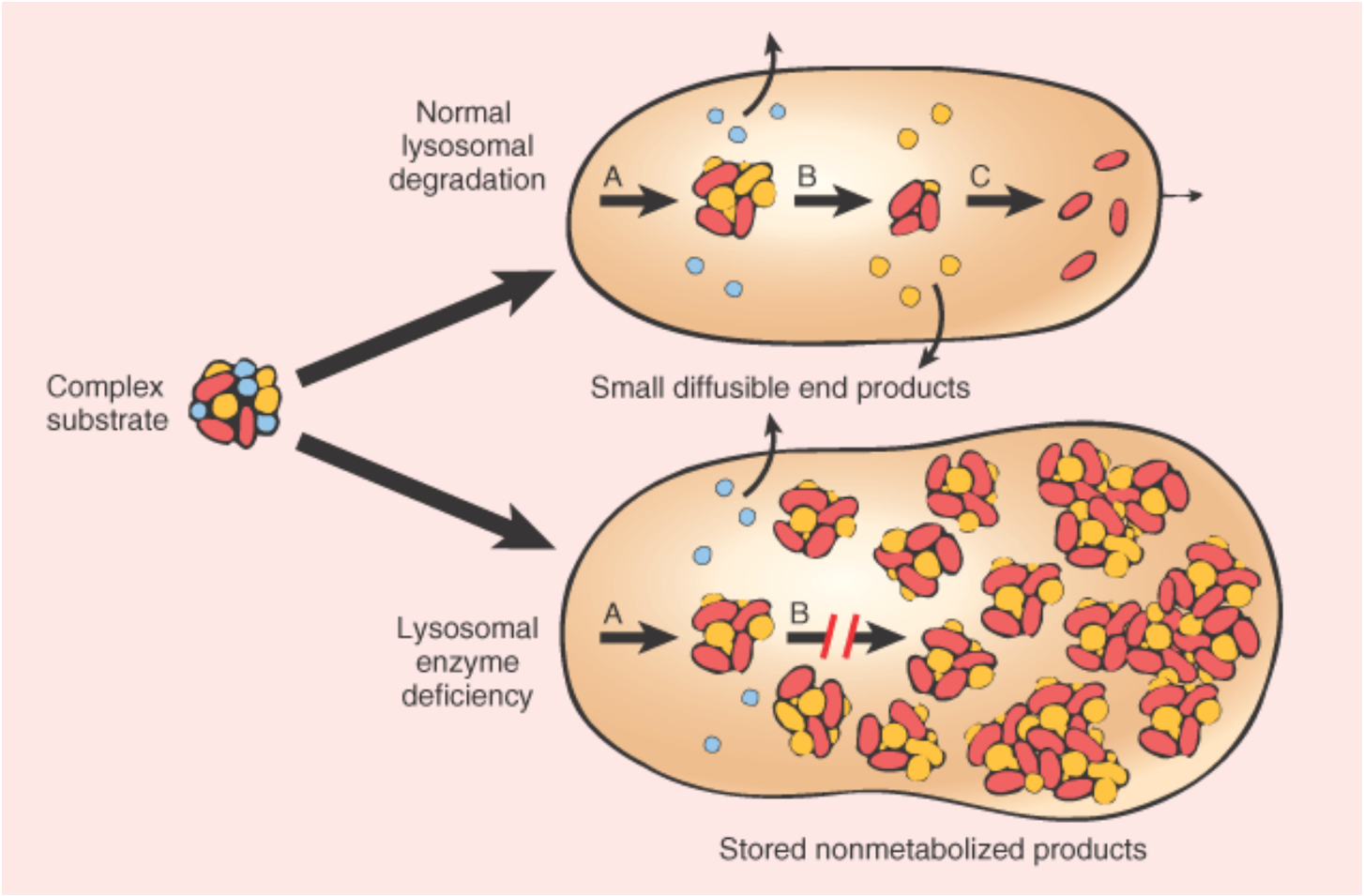


TABLE 5-6 -- Lysosomal Storage Diseases

Disease	Enzyme Deficiency	Major Accumulating Metabolites
Glycogenosis		
Type 2—Pompe disease	α-1,4-Glucosidase (lysosomal glucosidase)	Glycogen
Sphingolipidoses		
GM_1 gangliosidosis	GM_1 ganglioside β-galactosidase	GM_1 ganglioside, galactose-containing oligosaccharides
••Type 1—infantile, generalized		
••Type 2—juvenile		
GM_2 gangliosidosis		

••Tay-Sachs disease	Hexosaminidase-α subunit	GM_2 ganglioside
••Sandhoff disease	Hexosaminidase-β subunit	GM_2 ganglioside, globoside
••GM_2 gangliosidosis, variant AB	Ganglioside activator protein	GM_2 ganglioside
Sulfatidoses		
Metachromatic leukodystrophy	Arylsulfatase A	Sulfatide
Multiple sulfatase deficiency	Arylsulfatases A, B, C; steroid sulfatase; iduronate sulfatase; heparan N-sulfatase	Sulfatide, steroid sulfate, heparan sulfate, dermatan sulfate
Krabbe disease	Galactosylceramidase	Galactocerebroside
Fabry disease	α-Galactosidase A	Ceramide trihexoside
Gaucher disease	Glucocerebrosidase	Glucocerebroside
Niemann-Pick disease: types A and B	Sphingomyelinase	Sphingomyelin
Mucopolysaccharidoses (MPS)		
MPS I H (Hurler)	α-L-Iduronidase	Dermatan sulfate, heparan sulfate
MPS II (Hunter)	L-Iduronosulfate sulfatase	
Mucolipidoses (ML)		
I-cell disease (ML II) and pseudo-Hurler polydystrophy	Deficiency of phosphorylating enzymes essential for the formation of mannose-6-phosphate recognition marker; acid hydrolases lacking the recognition marker cannot be targeted to the lysosomes but are secreted extracellularly	Mucopolysaccharide, glycolipid
Other Diseases of Complex Carbohydrates		
Fucosidosis	α-Fucosidase	Fucose-containing sphingolipids and glycoprotein fragments
Mannosidosis	α-Mannosidase	Mannose-containing oligosaccharides
Aspartylglycosaminuria	Aspartylglycosamine amide hydrolase	Aspartyl-2-deoxy-2-acetamido-glycosylamine
Other Lysosomal Storage Diseases		
Wolman disease	Acid lipase	Cholesterol esters, triglycerides
Acid phosphate deficiency	Lysosomal acid phosphatase	Phosphate esters

of cytoplasmic inclusions can be visualized, the most prominent being whorled configurations within lysosomes composed of onion-skin layers of membranes (Fig. 5-14*B*). In time, there is progressive destruction of neurons, proliferation of microglia, and accumulation of complex lipids in phagocytes within the brain substance. A similar process occurs in the cerebellum as well as in neurons throughout the basal ganglia, brain stem, spinal cord, and dorsal root ganglia and in the neurons of the autonomic nervous system. The ganglion cells in the retina are similarly swollen with GM_2 ganglioside, particularly at the margins of the macula. A **cherry-red spot** thus appears in the macula, representing accentuation of the normal color of the macular choroid

contrasted with the pallor produced by the swollen ganglion cells in the remainder of the retina (Chapter 29). This finding is characteristic of Tay-Sachs disease and other storage disorders affecting the neurons.

Many alleles have been identified at the α-subunit locus, each associated with a variable degree of enzyme deficiency and hence with variable clinical manifestations. The affected infants appear normal at birth but begin to manifest signs and symptoms at about age 6 months. There is relentless motor and mental deterioration, beginning with motor incoordination, mental obtundation leading to muscular flaccidity, blindness, and increasing dementia. Sometime during the early course of the disease, the characteristic, but not pathognomonic, cherry-red spot appears in the macula of the eye grounds in almost all patients. Over the span of 1 or 2 years, a complete, pathetic vegetative state is reached, followed by death at age 2 to 3 years.

Antenatal diagnosis and carrier detection are possible by enzyme assays and DNA-based analysis.[25] The clinical features of the two other forms of GM_2 gangliosidosis (see Fig. 5-13), Sandhoff disease, resulting from β-subunit defect, and GM_2 activator deficiency, are similar to those of Tay-Sachs disease.

Figure 5-13 The three-gene system required for hexosaminidase A activity and the diseases that result from defects in each of the genes. The function of the activator protein is to bind the ganglioside substrate and present it to the enzyme. *(Modified from Sandhoff K, et al: The GM_2 gangliosidoses. In Scriver CR, et al [eds]: The Metabolic Basis of Inherited Disease, 6th ed. New York, McGraw-Hill, 1989, p. 1824.)*

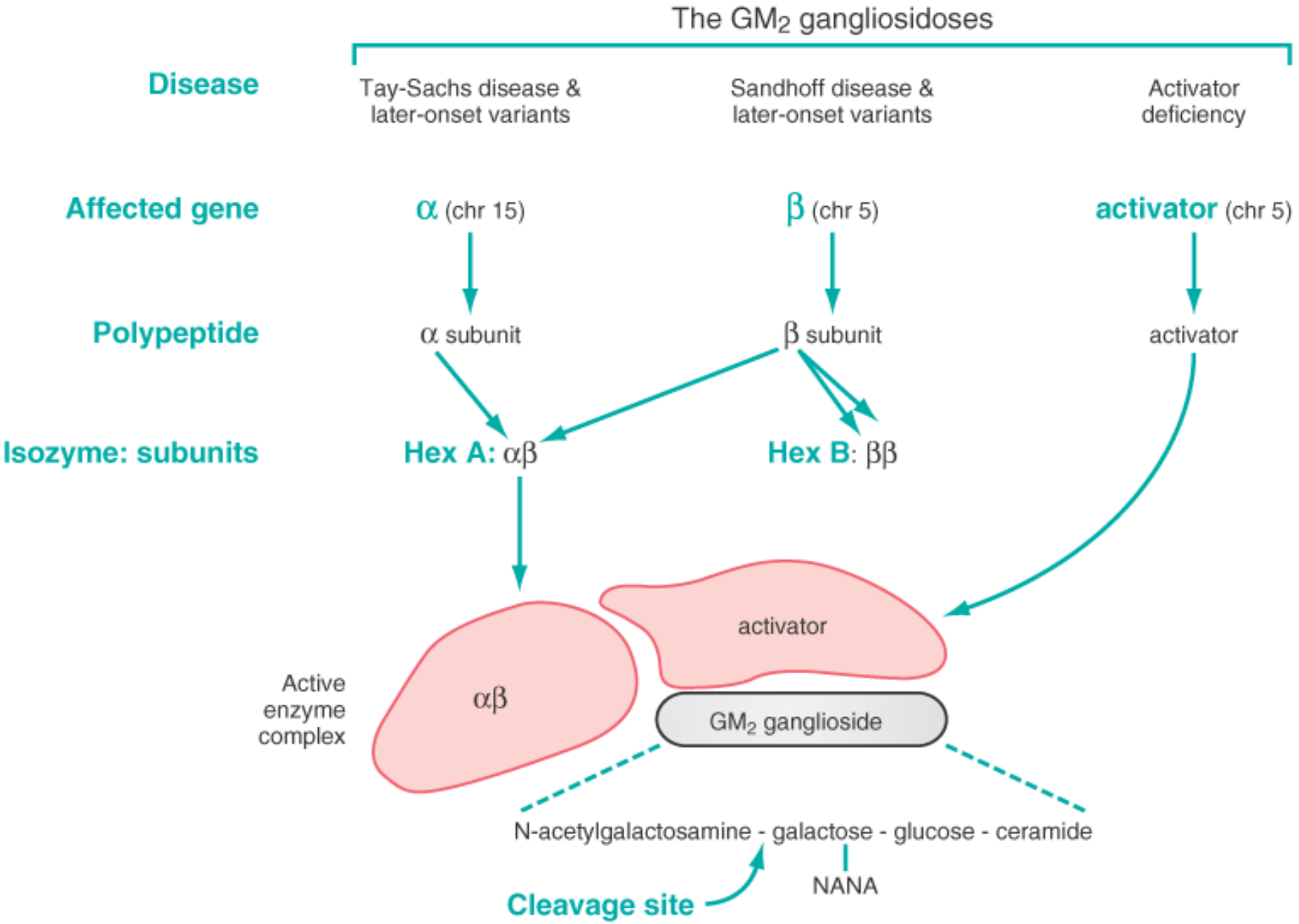


Figure 5-14 Ganglion cells in Tay-Sachs disease. *A*, Under the light microscope, a large neuron has obvious lipid vacuolation. *(Courtesy of Dr. Arthur Weinberg, Department of Pathology, University of Texas Southwestern Medical Center, Dallas.) B*, A portion of a neuron under the electron microscope shows prominent lysosomes with whorled configurations. Part of the nucleus is shown above. *(Electron micrograph courtesy of Dr. Joe Rutledge, University of Texas Southwestern Medical Center, Dallas, TX.)*

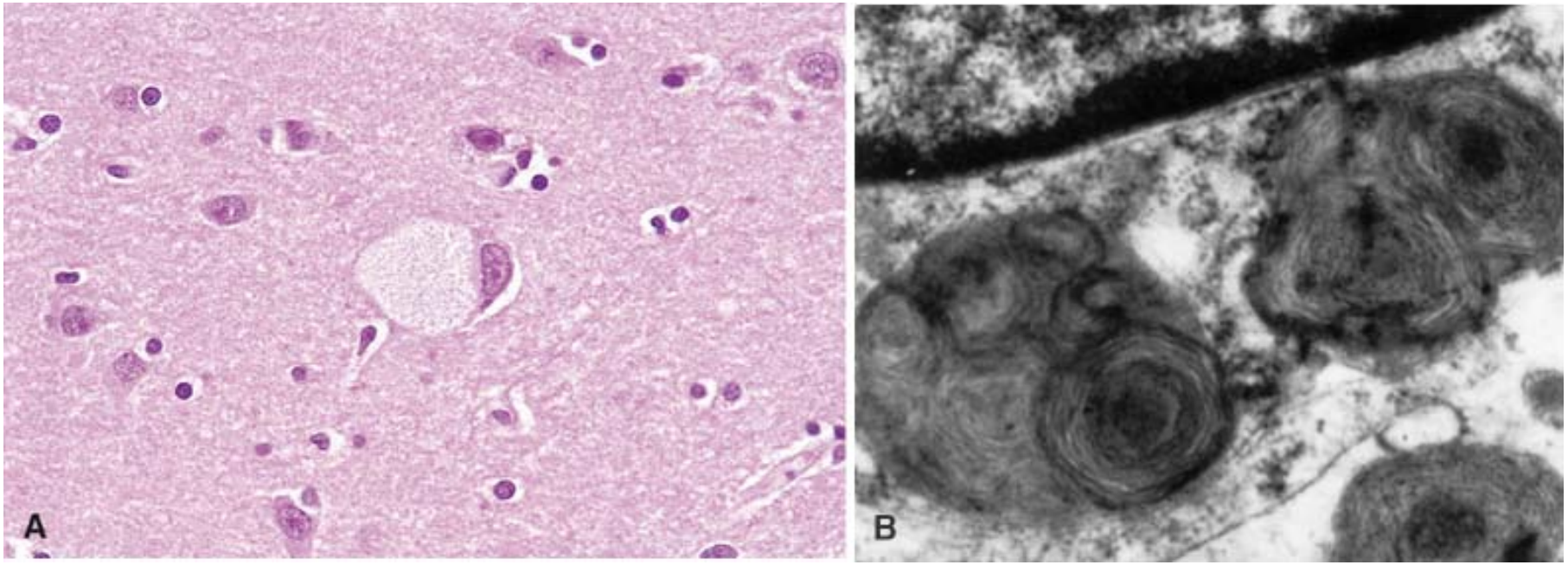


Figure 5-15 Niemann-Pick disease in liver. The hepatocytes and Kupffer cells have a foamy, vacuolated appearance owing to deposition of lipids. *(Courtesy of Dr. Arthur Weinberg, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

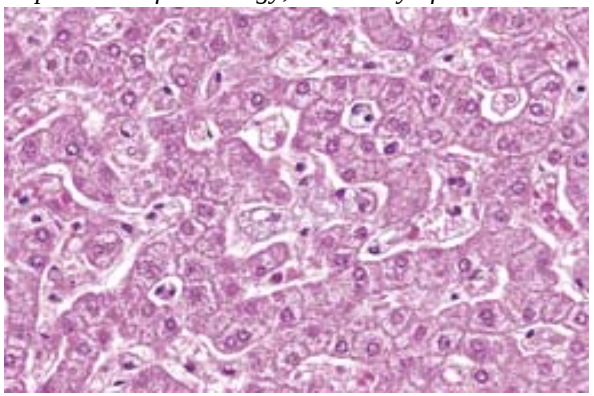


Figure 5-16 Gaucher disease involving the bone marrow. *A*, Gaucher cells with abundant lipid-laden granular cytoplasm. *B*, Electron micrograph of Gaucher cells with elongated distended lysosomes. *(Courtesy of Dr. Matthew Fries, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*

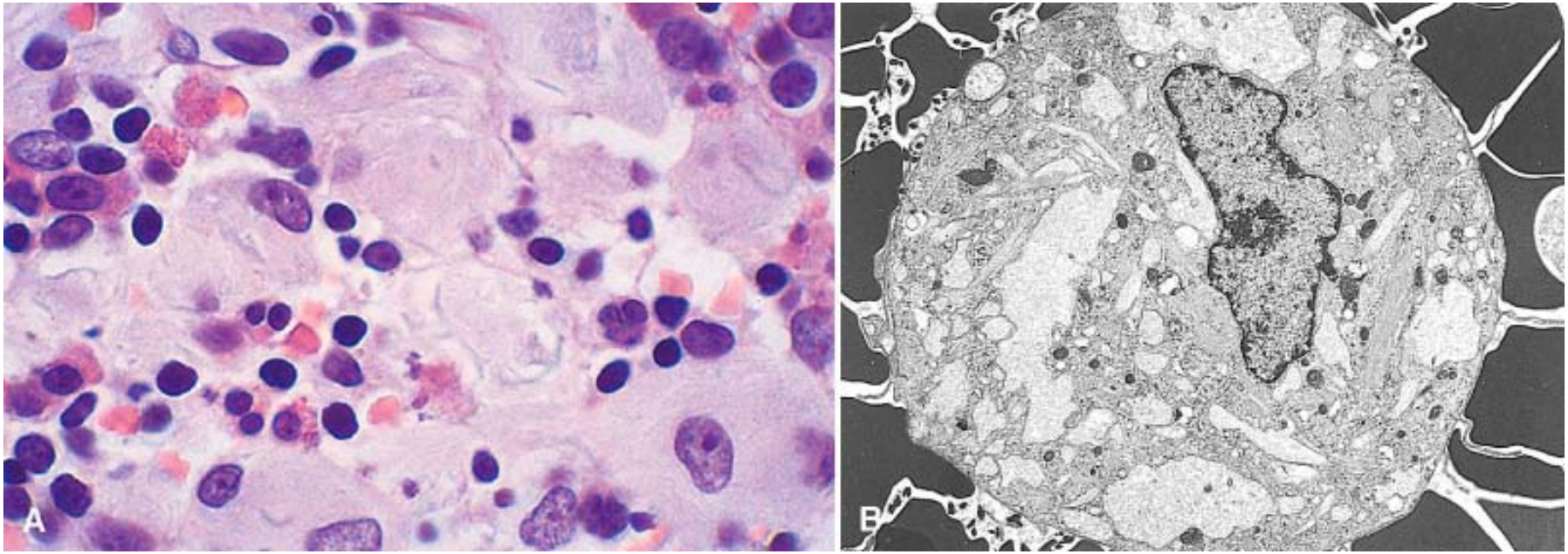
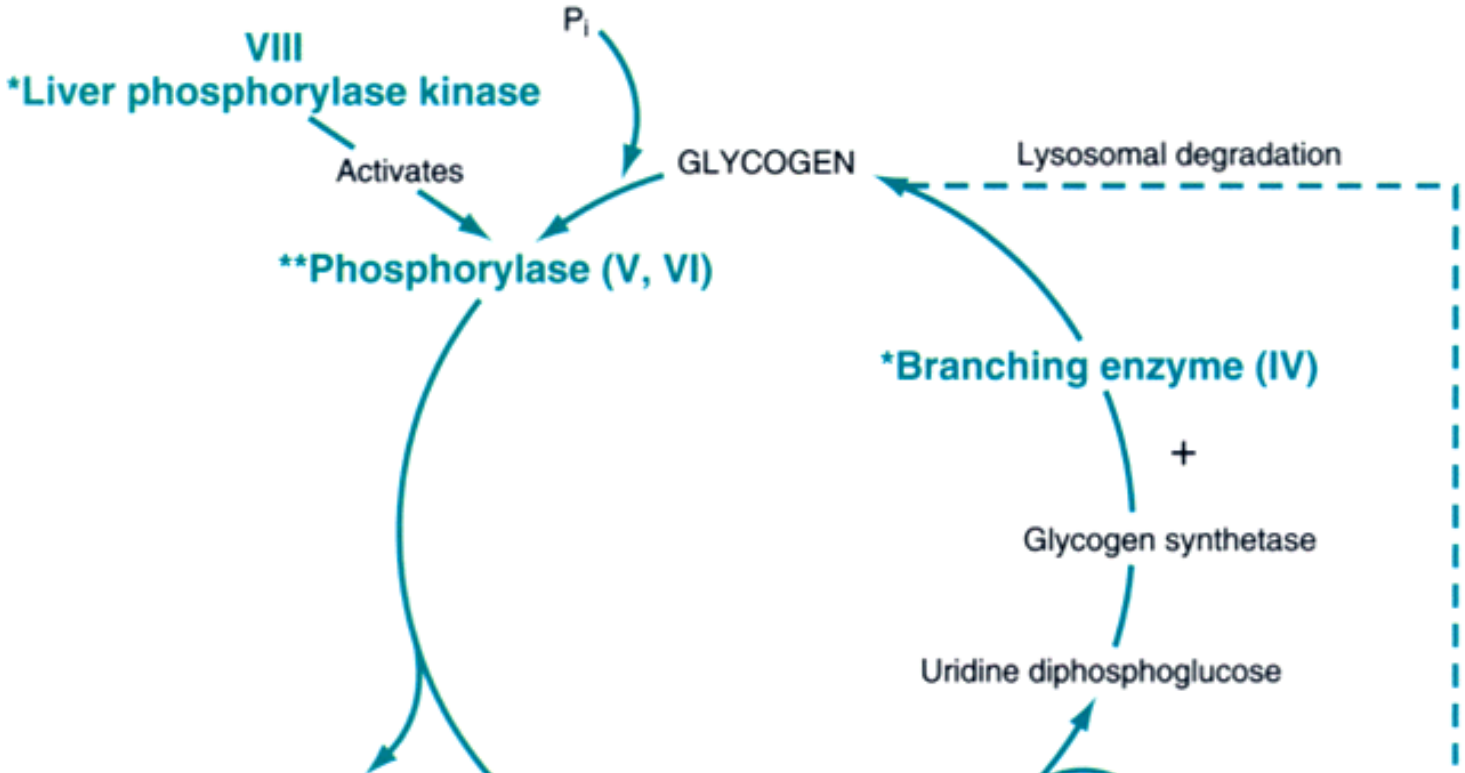


Figure 5-17 Pathways of glycogen metabolism. Asterisks mark the enzyme deficiencies associated with glycogen storage diseases. Roman numerals indicate the type of glycogen storage disease associated with the given enzyme deficiency. Types V and VI result from deficiencies of muscle and liver phosphorylases, respectively. *(Modified from Hers H, et al: Glycogen storage diseases. In Scriver CR, et al [eds]: The Metabolic Basis of Inherited Disease, 6th ed. New York, McGraw-Hill, 1989, p. 425.)*



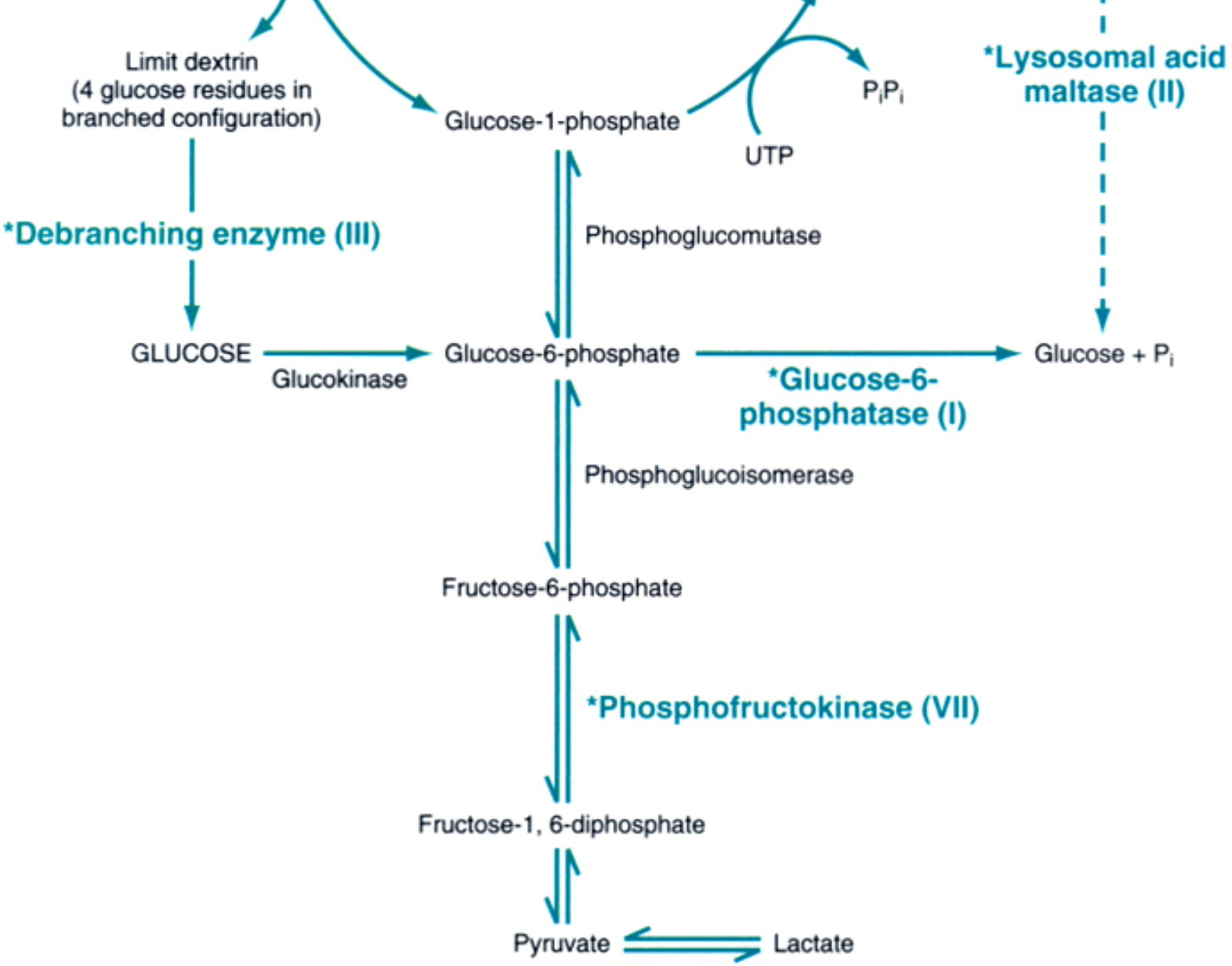


Figure 5-18 *Top*, Simplified schema of normal glycogen metabolism in the liver and skeletal muscles. *Middle*, Effects of an inherited deficiency of hepatic enzymes involved in glycogen metabolism. *Bottom*, Consequences of a genetic deficiency in the enzymes that metabolize glycogen in skeletal muscles.

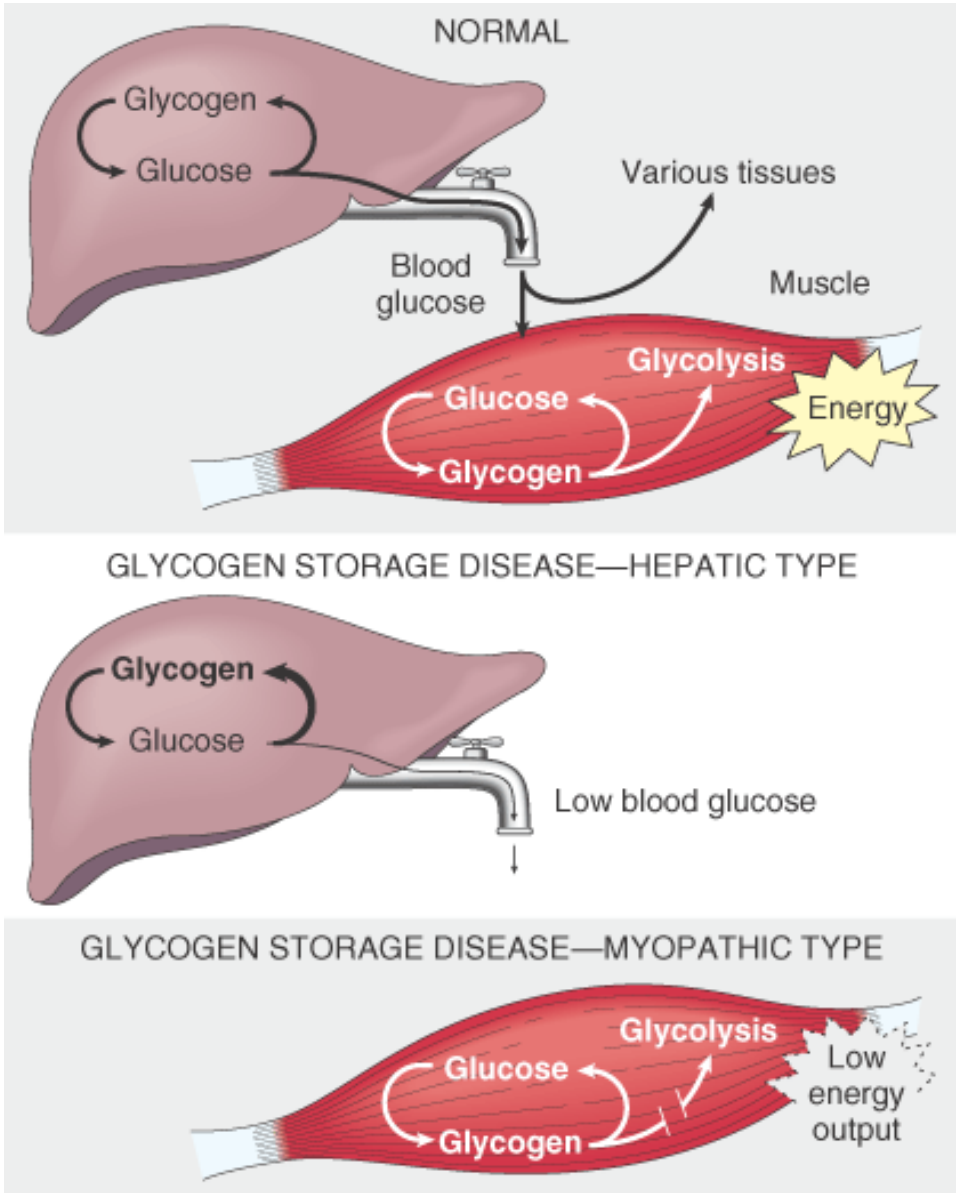
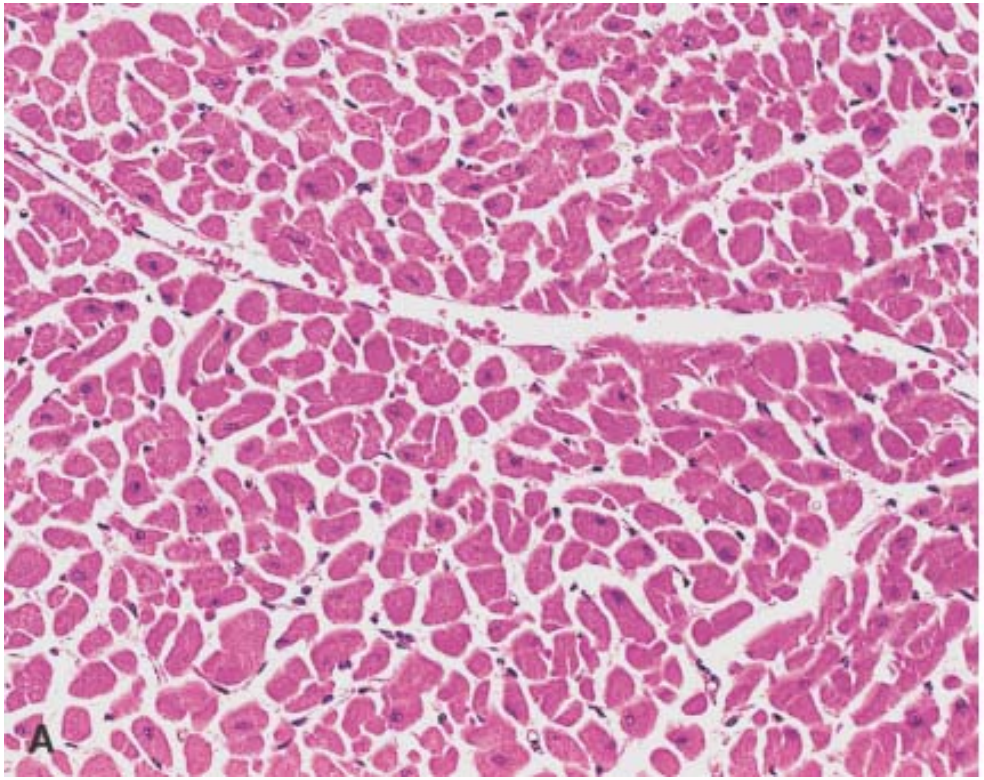


Figure 5-19 Pompe disease (glycogen storage disease type II). *A*, Normal myocardium with abundant eosinophilic cytoplasm. *B*, Patient with Pompe disease (same magnification) showing the myocardial fibers full of glycogen seen as clear spaces. *(Courtesy of Dr. Trace Worrell, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*



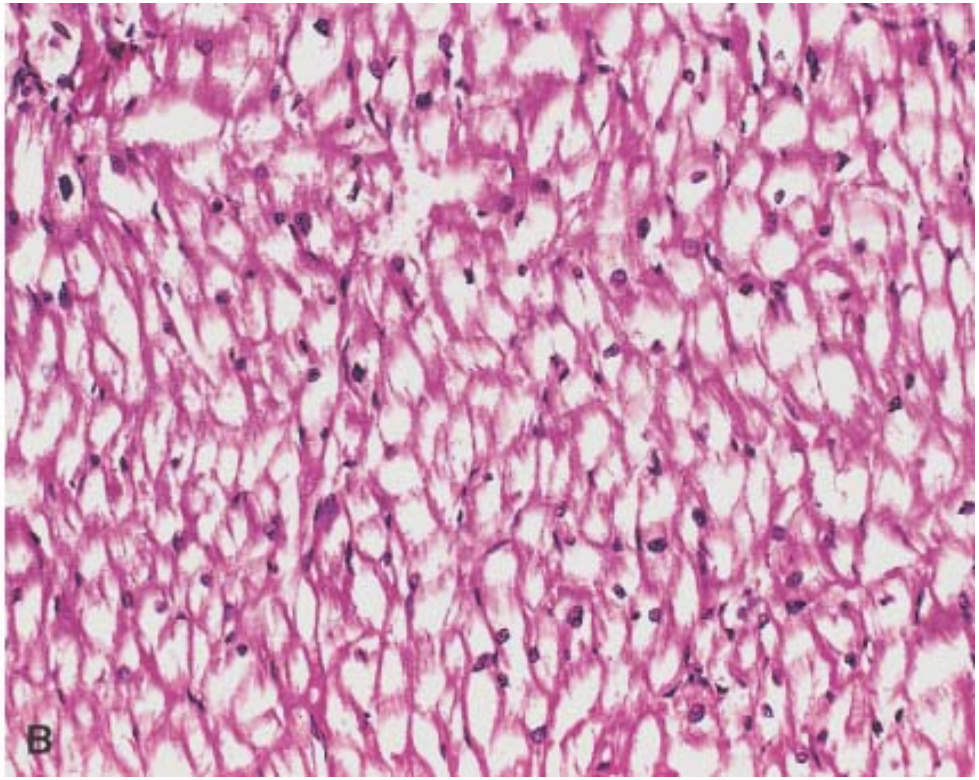


TABLE 5-7 -- Principal Subgroups of Glycogenoses

Clinicopathologic Category	Specific Type	Enzyme Deficiency	Morphologic Changes	Clinical Features
Hepatic Type	Hepatorenal—von Gierke disease (type I)	Glucose-6-phosphatase	Hepatomegaly—intracytoplasmic accumulations of glycogen and small amounts of lipid; intranuclear glycogen	In untreated patients: failure to thrive, stunted growth, hepatomegaly, and renomegaly. Hypoglycemia due to failure of glucose mobilization, often leading to convulsions. Hyperlipidemia and hyperuricemia resulting from deranged glucose metabolism; many patients develop gout and skin xanthomas. Bleeding tendency due to platelet dysfunction. With treatment most survive and develop late complications, e.g., hepatic adenomas
			Renomegaly—intracytoplasmic accumulations of glycogen in cortical tubular epithelial cells	
Myopathic Type	McArdle syndrome (type V)	Muscle phosphorylase	Skeletal muscle only—accumulations of glycogen predominant in subsarcolemmal location	Painful cramps associated with strenuous exercise. Myoglobinuria occurs in 50% of cases. Onset in adulthood (>20 years). Muscular exercise fails to raise lactate level in venous blood. Serum creatine kinase always elevated. Compatible with normal longevity
Miscellaneous Types	Generalized glycogenosis —Pompe disease (type II)	Lysosomal glucosidase (acid maltase)	Mild hepatomegaly—ballooning of lysosomes with glycogen, creating lacy cytoplasmic pattern	Massive cardiomegaly, muscle hypotonia, and cardiorespiratory failure within 2 years. A milder adult form with only skeletal muscle involvement, presenting

			Cardiomegaly—glycogen within sarcoplasm as well as membrane-bound	with chronic myopathy
			Skeletal muscle—similar to changes in heart	

the urine if allowed to stand and undergo oxidation.[38] The gene encoding homogentisic oxidase, mapped to 3q21, was cloned in 1996,[39] 64 years after the initial description of the disease by Garrod.

Morphology.

The retained homogentisic acid selectively binds to collagen in connective tissues, tendons, and cartilage, imparting to these tissues a blue-black pigmentation **(ochronosis)** most evident in the ears, nose, and cheeks. **The most serious consequences of ochronosis, however, stem from deposits of the pigment in the articular cartilages of the joints.** In some obscure manner, the pigmentation causes the cartilage to lose its normal resiliency and become brittle and fibrillated.[40] Wear-and-tear erosion of this abnormal cartilage leads to denudation of the subchondral bone, and often tiny fragments of the fibrillated cartilage are driven into the underlying bone, worsening the damage. The vertebral column, particularly the intervertebral disc, is the prime site of attack, but later the knees, shoulders, and hips may be affected. The small joints of the hands and feet are usually spared.

The metabolic defect is present from birth, but the degenerative arthropathy develops slowly and usually does not become clinically evident until the thirties. Although it is not life-threatening, it may be severely crippling. The disability may be as extreme as that encountered in the severe forms of osteoarthritis (Chapter 26) of the elderly, but in alkaptonuria the arthropathy occurs at a much earlier age.

DISORDERS ASSOCIATED WITH DEFECTS IN PROTEINS THAT REGULATE CELL GROWTH

Normal growth and differentiation of cells is regulated by two classes of genes: proto-oncogenes and tumor-suppressor genes, whose products promote or restrain cell growth (Chapter 7). It is now well established that mutations in these two classes of genes are important in the pathogenesis of tumors. In the vast majority of cases, cancer-causing mutations affect somatic cells and hence are not passed in the germ line. In approximately 5% of all cancers, however, mutations transmitted through the germ line contribute to the development of cancer. Most familial cancers are inherited in an autosomal dominant fashion, but a few recessive disorders have also been described. This subject is discussed in greater detail in Chapter 7 . Here we provide an example of two common familial neoplasms.

Neurofibromatosis: Types 1 and 2

Neurofibromatoses comprise two autosomal dominant disorders, affecting approximately 100,000 people in the United States. They are referred to as *neurofibromatosis type 1* (previously called *von Recklinghausen disease*) and *neurofibromatosis type 2* (previously called *acoustic neurofibromatosis*). Although there is some overlap in clinical features, these two entities are genetically distinct.[41]

Neurofibromatosis type 1 is a relatively common disorder, with a frequency of almost 1 in 3000. Although approximately 50% of the patients have a definite family history consistent

with autosomal dominant transmission, the remainder appear to represent new mutations. In familial cases, the expressivity of the disorder is extremely variable, but the penetrance is 100%.

Neurofibromatosis type 1 has three major features: (1) *multiple neural tumors (neurofibromas) dispersed anywhere on or in the body*; (2) *numerous pigmented skin lesions, some of which are café au lait spots*; and (3) *pigmented iris hamartomas, also called Lisch nodules*. A bewildering assortment of other abnormalities (cited later) may accompany these cardinal manifestations.

Morphology.

The neurofibromas arise within or are attached to nerve trunks anywhere in the skin, including the palms and soles, as well as in every conceivable internal site, including the cranial nerves. Three types of neurofibromas are found in individuals with neurofibromatosis type 1: cutaneous, subcutaneous, and plexiform. **Cutaneous**, or dermal, neurofibromas are soft, sessile, or pedunculated lesions that vary in number from a few to many hundreds. **Subcutaneous neurofibromas** grow just beneath the skin; they are firm, round masses that are often painful. The cutaneous and subcutaneous neurofibromas may be less than 1 cm in diameter; moderate-sized pedunculated lesions; or huge, multilobar pendulous masses, 20 cm or more in greatest diameter. The third variant, referred to as **plexiform neurofibroma**, diffusely involves subcutaneous tissue and contains numerous tortuous, thickened nerves; the overlying skin is frequently hyperpigmented. These may grow to massive proportions, causing striking enlargement of a limb or some other body part. Similar tumors may occur internally, and in general the deeply situated lesions tend to be large. Microscopically, neurofibromas reveal proliferation of all the elements in the peripheral nerve, including neurites, Schwann cells, and fibroblasts. Typically, these components are dispersed in a loose, disorderly pattern, often in a loose, myxoid stroma. Elongated, serpentine Schwann cells predominate, with their slender, spindle-shaped nuclei. The loose and disorderly architecture helps differentiate these neural tumors from schwannomas. The latter, composed entirely of Schwann cells, virtually never undergo malignant transformation, whereas plexiform neurofibromas become malignant in about 5% of patients with neurofibromatosis type 1.[42] Malignant transformation is most common in the large plexiform tumors attached to major nerve trunks of the neck or extremities. The superficial lesions, despite their size, rarely become malignant.

The cutaneous pigmentations, the second major component of this syndrome, are present in more than 90% of patients. Most commonly, they appear as light brown **café au lait** macules, with generally smooth borders, often located over nerve trunks. They are usually round to ovoid, with their long axes parallel to the underlying cutaneous nerve. Although normal individuals may have a few café au lait spots, it is a clinical maxim that when six or more spots greater than 1.5 cm in diameter are present in an adult, the patient is likely to have neurofibromatosis type 1.

Lisch nodules (pigmented hamartomas in the iris) are present in more than 94% of patients age 6 years or older. They do not produce any symptoms but are helpful in establishing the diagnosis.

A wide range of associated abnormalities has been reported in these patients. Perhaps most common (seen in 30% to 50% of patients) are skeletal lesions, which take a variety of forms, including (1) erosive defects owing to contiguity of neurofibromas to bone, (2) scoliosis, (3) intraosseous cystic lesions, (4) subperiosteal bone cysts, and (5) pseudoarthrosis of the tibia. Patients with neurofibromatosis type 1 have a twofold to fourfold greater risk of developing other tumors, especially Wilms tumors, rhabdomyosarcomas, meningiomas, optic gliomas, and pheochromocytomas. Affected children are at increased risk of developing chronic myeloid leukemia.

Although some patients with this condition have normal intelligence quotients (IQs), there is an unmistakable tendency for reduced intelligence. When neurofibromas arise within the gastrointestinal tract, intestinal obstruction or gastrointestinal bleeding may occur. Narrowing of a renal artery by a tumor may induce hypertension. Owing to variable expression of the gene, the range of clinical presentations is almost limitless, but ultimately the diagnosis rests on the concurrence of multiple café au lait spots and multiple skin tumors. The neurofibromatosis type 1 *(NF-1)* gene has been mapped to chromosome 17q11.2. It encodes a protein called *neurofibromin*, which down-regulates the function of the $p21^{Ras}$ oncoprotein (see section on oncogenes, Chapter 7). *NF-1* therefore belongs to the family of tumor-suppressor genes.

Neurofibromatosis type 2 is an autosomal dominant disorder in which patients develop a range of tumors, most commonly bilateral acoustic schwannomas and multiple meningiomas. Gliomas, typically ependymomas of the spinal cord, also occur in these patients. Many individuals with neurofibromatosis type 2 also have non-neoplastic lesions, which include nodular ingrowth of Schwann cells into the spinal cord (schwannosis), meningioangiomatosis (a proliferation of meningeal cells and blood vessels that grows into the brain), and glial hamartia (microscopic nodular collections of glial cells at abnormal locations, often in the superficial and deep layers of the cerebral cortex). Café au lait spots are present, but Lisch nodules in the iris are not found. This disorder is much less common than neurofibromatosis type 1, having a frequency of 1 in 40,000 to 50,000.

The *NF-2* gene, located on chromosome 22q12, is also a tumor-suppressor gene. As further discussed in Chapter 7 , the product of the *NF-2* gene, called *merlin*, shows structural similarity to the ezrin, radixin, moesin (ERM) family of proteins. These cytoskeletal proteins interact with actin on the one hand and membrane proteins on the cell surface on the other hand. It is thought

that *merlin* regulates contact inhibition and proliferation of Schwann cells.[43]

Disorders with Multifactorial Inheritance

As pointed out earlier, the multifactorial disorders result from the combined actions of environmental influences and two or more mutant genes having additive effects. The genetic component exerts a dosage effect—the greater the number of inherited deleterious genes, the more severe the expression of the disease. Because environmental factors significantly influence

---

the expression of these genetic disorders, the term polygenic inheritance should not be used.

A number of normal phenotypic characteristics are governed by multifactorial inheritance, such as hair color, eye color, skin color, height, and intelligence. These characteristics exhibit a continuous variation in population groups, producing the standard bell-shaped curve of distribution. Environmental influences, however, significantly modify the phenotypic expression of multifactorial traits. For example, type II diabetes mellitus has many of the features of a multifactorial disorder. It is well recognized clinically that individuals often first manifest this disease after weight gain. Thus, obesity as well as other environmental influences unmasks the diabetic genetic trait. Nutritional influences may cause even monozygous twins to achieve different heights. The culturally deprived child cannot achieve his or her full intellectual capacity.

The following features characterize multifactorial inheritance. These have been established for the multifactorial inheritance of congenital malformations and, in all likelihood, obtain for other multifactorial diseases.[44]

- The risk of expressing a multifactorial disorder is conditioned by the number of mutant genes inherited. Thus, the risk is greater in siblings of patients having severe expressions of the disorder. For example, the risk of cleft lip in the siblings of an index case is 2.5% if the cleft lip is unilateral but 6% if it is bilateral. Similarly, the greater the number of affected relatives, the higher is the risk for other relatives.
- The rate of recurrence of the disorder (in the range of 2% to 7%) is the same for all first-degree relatives (i.e., parents, siblings, and offspring) of the affected individual. Thus, if parents have had one affected child, the risk that the next child will be affected is between 2% and 7%. Similarly, there is the same chance that one of the parents will be affected.
- The likelihood that both identical twins will be affected is significantly less than 100% but is much greater than the chance that both nonidentical twins will be affected. Experience has proven, for example, that the frequency of concordance for identical twins is in the range of 20% to 40%.
- The risk of recurrence of the phenotypic abnormality in subsequent pregnancies depends on the outcome in previous pregnancies. When one child is affected, there is up to a 7% chance that the next child will be affected, but after two affected siblings, the risk rises to about 9%.
- Expression of a multifactorial trait may be continuous (lack a distinct phenotype, e.g., height) or discontinuous (with a distinct phenotype, e.g., diabetes mellitus). In the latter, disease is expressed only when the combined influences of the genes and environment cross a certain threshold. In the case of diabetes, for example, the risk of phenotypic expression increases when the blood glucose levels go above a certain level.

Assigning a disease to this mode of inheritance must be done with caution. It depends on many factors but first on familial clustering and the exclusion of mendelian and chromosomal modes of transmission. A range of levels of severity of a disease is suggestive of multifactorial inheritance, but, as pointed out earlier, variable expressivity and reduced penetrance of single mutant genes may also account for this

TABLE 5-8 -- Multifactorial Disorders

Disorder	Chapter

Cleft lip or cleft palate (or both)	Chapter 10
Congenital heart disease	Chapter 12
Coronary heart disease	Chapter 12
Hypertension	Chapter 11
Gout	Chapter 27
Diabetes mellitus	Chapter 24
Pyloric stenosis	Chapter 17

phenomenon. Because of these problems, sometimes it is difficult to distinguish between mendelian and multifactorial inheritance.

In contrast to the mendelian disorders, many of which are uncommon, the multifactorial group includes some of the most common ailments to which humans are heir (Table 5-8). Most of these disorders are described in appropriate chapters elsewhere in this book.

Normal Karyotype

As is well known, human somatic cells contain 46 chromosomes; these comprise 22 homologous pairs of autosomes and two sex chromosomes, XX in the female and XY in the male. The study of chromosomes—*karyotyping*—is the basic tool of the cytogeneticist. The usual procedure of producing a chromosome spread is to arrest mitosis in dividing cells in metaphase by the use of mitotic spindle inhibitors (e.g., colcemid) and then to stain the chromosomes. In a metaphase spread, the individual chromosomes take the form of two chromatids connected at the centromere. A karyotype is a standard arrangement of a photographed or imaged stained metaphase spread in which chromosome pairs are arranged in order of decreasing length.

A variety of staining methods that allow identification of each individual chromosome on the basis of a distinctive and reliable pattern of alternating light and dark bands along the length of the chromosome have been developed. The one most commonly employed uses a Giemsa stain and is hence called *G banding*. A normal male karyotype with G banding is illustrated in Figure 5-20 . With G banding, approximately 400 to 800 bands per haploid set can be detected. The resolution obtained by banding techniques can be dramatically improved by obtaining the cells in prophase. The individual chromosomes appear markedly elongated, and up to 1500 bands per karyotype may be recognized. The use of these banding techniques permits certain identification of each chromosome as well as delineation of precise breakpoints and other subtle alterations, to be described later.

Before this discussion of the normal karyotype is concluded, reference must be made to commonly used cytogenetic terminology. Karyotypes are usually described using a shorthand system of notations. The following order is used: Total number of chromosomes is given first, followed by the sex chromosome complement, and finally the description of abnormalities in ascending numerical order. For example, a

Figure 5-20 Normal male karyotype with G banding. *(Courtesy of Dr. Nancy Schneider, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX.)*