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The Skin

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The **integument** comprises the skin and its appendages (referred to as the **adnexa**), including structures such as hair, glands, digital pads, and claws [1–3]. The adnexal structures are of epidermal origin; they are continuous with the epidermal layer of the skin, supported by the underlying connective tissue.

Skin: The **skin** (**cutis**) is one of the body's largest and most important organs, for it forms a protective layer against the external environment and plays a crucial role in homeostasis. It is composed of three layers [4], the epidermis, dermis, and hypodermis (or subcutis), all firmly attached to each other. Important differences between cats and dogs exist (Table 1.1) and effect the healing properties and treatment options available when managing injuries. Skin transmits various stimuli from the external environment to the central nervous system (CNS). The nerve fibers carrying these stimuli penetrate the tissues (muscles and fascia) underlying the hypodermis and travel to the CNS, often within the fascial planes that ultimately merge with the periosteum of the appendicular and axial skeletal elements (Figure 1.1). Fascial planes form distinct compartments for individual muscles in many regions of the body. The skin's vascular components travel by similar routes and are responsible for maintaining body temperature within physiologic limits and regulating systemic blood pressure.

Epidermis: The epidermis of the skin is avascular and serves as the outermost protective layer of the body (Figure 1.2). It minimizes trans-epidermal water loss, prevents invasion by infectious agents and other harmful substances, absorbs ultraviolet radiation by the melanocytes, and aids in Vitamin D biosynthesis. The basic structure of the epidermis is similar in all domesticated mammals with some minor regional and species differences. The thickness of the epidermis is inversely proportional to the density of the hair coat. In dogs and cats, since most of the skin surface is covered with hair, the epidermis is relatively thin. In the dog, the epidermis consists of two to three layers of living cells increasing to 10 layers [5–7] while in the cat, the epidermis is slightly thinner. The average time for epidermal turnover is 22 days in carnivores regardless of the thickness of the epidermis [8, 9]. Dermal papillae are small fingerlike extensions into the epidermis, surrounded by rete ridges of the epidermis. These two structures interlock with each other, anchoring the epidermis. Epidermal rete ridges are absent in most of the skin in carnivore skin due to the dense haircoat [10, 11]. The hair follicles extend into the dermis, firmly anchoring the epidermis. In sparsely haired regions such as the scrotum, inguinal, and axillary areas, the epidermis is slightly thicker and epidermal rete ridges may be observed. The term **glabrous skin** is applied to areas devoid of hairs, such as the nasal plane, lips, and genitals as well as parts of the limb extremities such as digital pads. These regions may have several layers of living keratinocytes, prominent basement membranes, and form epidermal rete ridges [10].

The epidermal layer rests upon a meshwork of extracellular fibers (**dermal–epidermal junction**) upon which the keratinocytes rest, called the **basement membrane** (or **basal lamina**), which is acellular and avascular (Figure 1.2). If the basement membrane is disrupted, as with a skin wound, other cells (such as activated fibroblasts and neutrophils) will pass through it from beneath to participate in healing processes, forming scars, extending capillary loops, and developing granulation beds. Otherwise, the basal lamina remains impassable. Beneath it is the dermis, the vascularized second layer of the skin.

Nonkeratinocytes: Several cell types are contained in these two major layers of the skin (the epidermis and dermis). The most common cells within the epidermis are **keratinocytes**, making up 85% of all epidermal cells. The nonkeratinocytes account for approximately 15% of the epidermis and include the melanocytes, tactile epithelioid cells (*Merkel cells*), and

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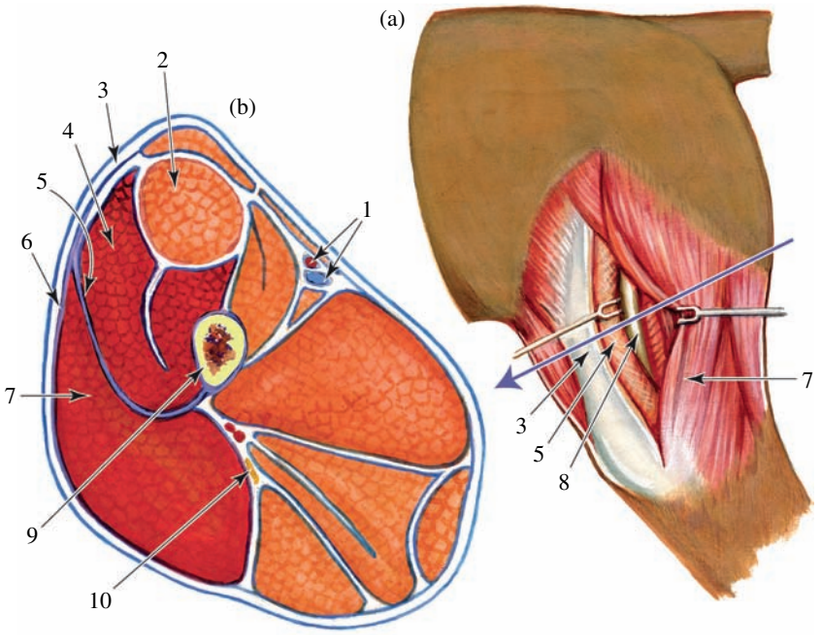

Figure 1.1 (a) Lateral approach to the shaft of the femur is shown. The fascia lata is split along the cranial margin of the biceps femoris m. (b) Cross-sectional view of left thigh. Note the location of nerves and blood vessels between the fascial planes. 1, Femoral artery and vein; 2, Rectus femoris m; 3, Fascia lata; 4, Vastus lateralis m; 5, Deeper lamina of fascia lata which runs between the vastus lateralis and biceps femoris muscles and reaches the shaft of the femur. The biceps femoris and vastus lateralis are therefore separated along this plane to reach the femur shaft; 6, Superficial lamina of fascia lata; 7, Biceps femoris; 8, Lateral shaft of femur; 9, Sciatic nerve.

intraepidermal macrophages (or *Langerhans* cells). **Melanocytes** are derived from neural crest cells. **Dendritic cells** (DCs) are a heterogeneous group of antigen-presenting leukocytes with a common origin that play an important role in the activation of the immune system. These cells have potent antigen-presenting capabilities with characteristic dendritic morphology. Three main cutaneus DC populations have been described: intraepidermal Langerhans cells (LCs), dermal myeloid DCs, and dermal plasmacytoid DCs (pDCs). The **intraepidermal macrophages** (***Langerhans cells***) are interspersed among the much more numerous keratinocytes (Figure 1.3) and act as antigen-presenting cells [13]. The LCs are one type of antigen-presenting DCs involved in cutaneus hypersensitivity reactions. They are capable of inducing

Table 1.1 Summary of skin differences between dogs and cats.

Item	Dog	Cat
Number of living epidermal cell layers	2–3 in most places, up to 10 layers in some places	Fewer layers of living epidermal cells
Merkel cell carcinoma	Relatively benign	Relatively aggressive
Melanocytes	More numerous	Fewer
Angiosomes (cutaneus perforating vessels)	Greater number of cutaneus perforating vessels in dermis/hypodermis	Fewer number of cutaneus perforating vessels in dermis/hypodermis
The density of tertiary and higher-order vessels	Higher density of tertiary and higher-order vessels	A lower density of tertiary and higher-order vessels
Hairs	Fewer secondary hairs (~9)	More secondary hairs (~12)
Wool (secondary) hairs	No medulla	Contains a medulla
Scrotal skin	Usually, sparse hair	Hairy
Facial sebaceous glands	Fewer on the face	More numerous on the face
Tail glands (modified sebaceous glands)	Located caudal to Ca 7 vertebra	Located at the baser of the tail
Dermis/hypodermis collagen production	May have a higher rate of collagen production	May have a lower rate of collagen synthesis ability
Wound repair	Rapid granulation tissue production	Delayed granulation tissue production

antiviral-specific immune responses *in vivo* [14]. The LCs survey the epithelium constantly for pathogens and migrate to the lymph nodes where they present microbial antigens to T-cells. This results in developing tolerance and maintaining tissue homeostasis [15]. Langerhans cells in the skin are continuously replenished from circulating bone marrow precursors [16, 17]. There is steady-state migration of LCs to skin-draining lymph nodes, perhaps to induce and maintain tolerance to cutaneus antigens. Their number in the epidermis is small compared to keratinocytes, and they are largely present in the upper stratum spinosum.

Tactile epithelioid cells (***Merkel* cells**) are in the stratum basale of hairless and hairy skin and are numerous in the nasal plane of carnivores. These cells, in association with sensory nerve endings, function as epidermal mechanoreceptors (Figure 1.3) that transmit tactile sensations (touch) through cutaneus nerves [18]. Merkel cells are neurosecretory cells thought to be derived from neural crest cells [19, 20]. However, recent studies in mice and humans indicate they may be derivatives of epidermal cells [21, 22]. They are slowly adapting cutaneus mechanoreceptors located in the basal layer of the epidermis. Merkel cell afferents are gentle touch receptors activated by steady skin indentation [21]. In humans, Merkel cell carcinoma is a rare cutaneus neuroendocrine carcinoma that is a highly malignant skin cancer most often associated with the presence of Merkel cell poliovirus genes (MCPyV) [22–24]. Originally thought to arise from Merkel cells, recent studies indicate in humans, the cancer cell origin is from primitive epidermal stem cells, early B-cells, or dermal fibroblasts [25–27]. In canine and feline Merkel cell carcinoma, MCPyV genes were not detected, indicating a different etiology for cancer [28]. In addition, Merkel cell carcinoma appears to be more benign in dogs but more aggressive in cats [29–31].

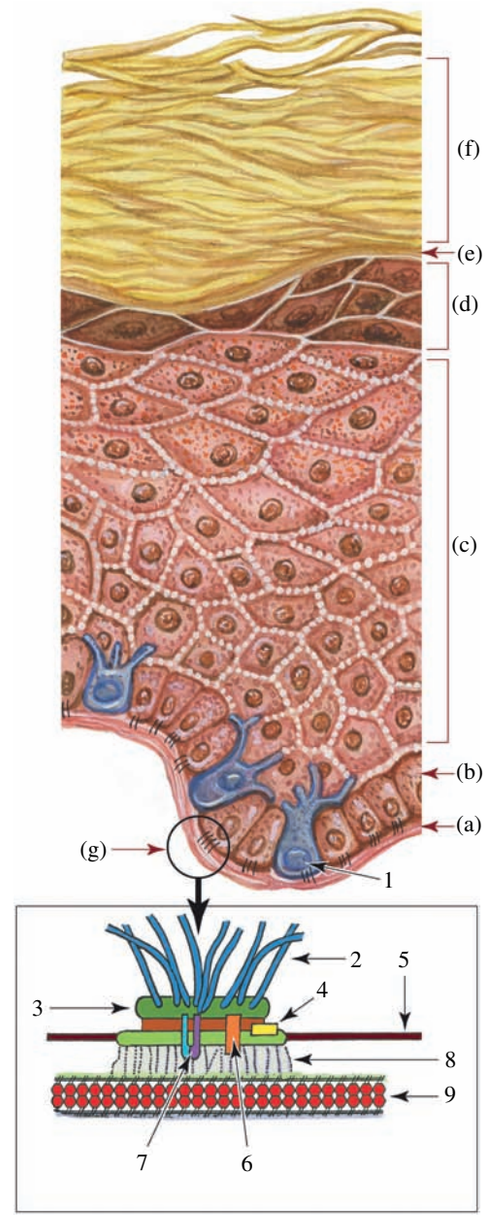

Figure 1.2 Layers of epidermis. (a) Basement membrane. (b) Stratum basale. (c) Stratum spinosum. (d) Stratum granulosum. (e) Stratum lucidum. (f) Stratum corneum. (g) Cells of stratum basale are anchored to the basement membrane by hemidesmosomes. 1, Melanocyte; 2, Keratin filaments (tonofilaments); 3, Plate; 4, Bullous pemphigoid antigen-1; 5, Plasma membrane; 6, Bullous pemphigoid antigen-2; 7, Integrin; 8, Anchoring filaments; 9, Basal lamina.

Cutaneus Immune Barrier

Skin, as an immunologic organ, is present at the critical junction between the host and the environment. The most important function is to guard against potentially damaging agents such as microbes, toxins, and radiation. This is effectively accomplished by the presence of anatomical, biochemical, and immunologic barriers. The anatomical barrier consists of the tight cell-to-cell junctions and associated skeletal proteins of the stratum corneum. This barrier is enhanced by sebaceous gland secretions. Biochemical barriers include hydrolytic enzymes, acids, lipids, and antimicrobial proteins. The immunologic barrier is composed of cellular and humoral constituents of the immune system. Within this barrier exist cooperative arms of innate and adaptive immunity [32]. The **innate immune system** is a primitive defense mechanism comprised immune cells such as macrophages, neutrophils, and LCs, and their associated inflammatory mediators such as cytokines and chemokines. To mount a defensive reaction against the invading pathogen, the innate immune system must discriminate between "self" and "non-self." Several molecules exist in pathogens absent in the host, collectively known as pathogen-associated molecular patterns (PAMPs). Innate immune cells use pattern recognition receptors (PRRs) such as toll-like receptors (TLRs), and peptidoglycan receptors (PGNs) to identify PAMPs in pathogens. Identification of pathogens triggers a cascade of inflammatory reactions including the secretion of cytokines and chemokines. These mediators further enhance the offensive assault on pathogens. Some of

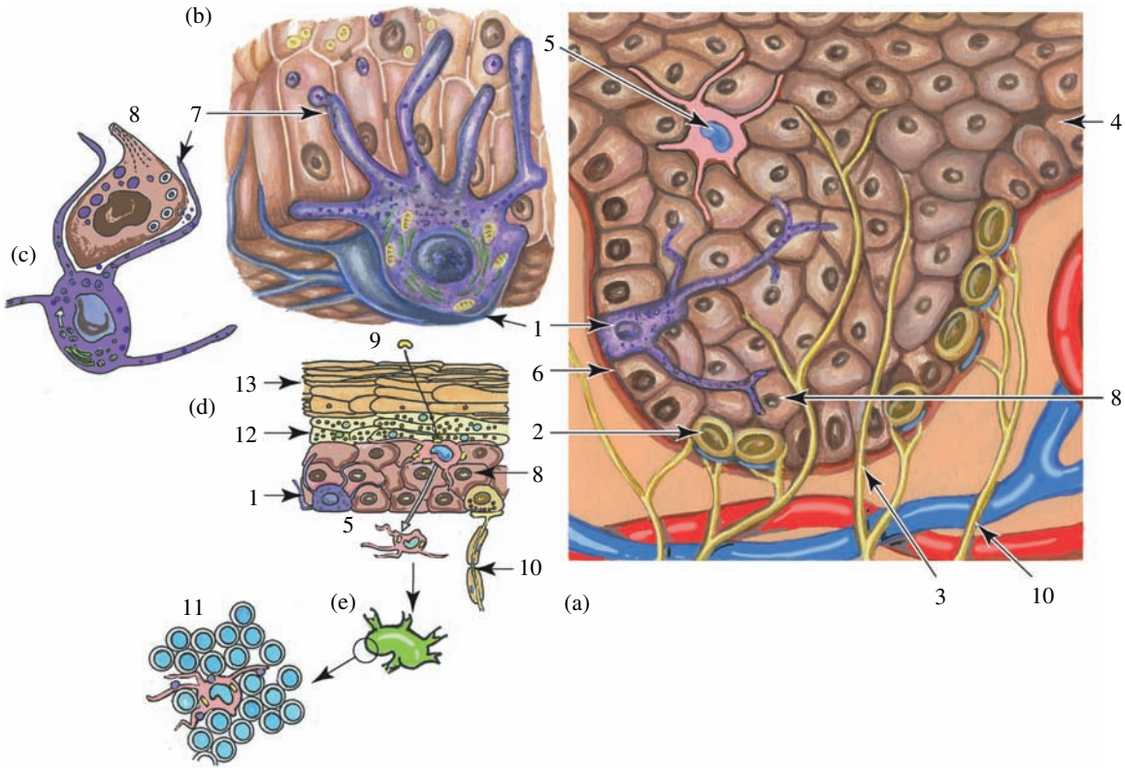

Figure 1.3 Keratinocytes and nonkeratinocytes of the epidermis. (a) Epidermis showing a melanocyte (1), Langerhans cell (5) and keratinocytes (8). (b) A melanocyte shown in-situ with its dendritic processes. (c) An isolated melanocyte shown transferring melanosomes along its cytoplasmic processes to an adjacent keratinocyte. (d) A Langerhans cell ingests an antigen by phagocytosis. It will then migrate from the epidermis to local lymph nodes; (e) Once the Langerhans cells reach a lymph node and transform into dendritic cells, they stimulate T lymphocytes. 1, Melanocyte; 2, Tactile epithelioid (*Merkel*) cells are mechanoreceptors derived from the neural crest. They are shown with their sensory nerve endings exhibiting a broad nerve plate (*Merkel* cell-neurite complex or the tactile hair disc, shown in blue); 3, Unmyelinated nerve fibers that penetrate the epidermis. These nerve endings mainly detect temperature and pain sensations; 4, Stratum basale; 5, A Langerhans cell, which belongs to the immune system. It detects foreign antigens and presents them to T-lymphocytes; 6, Basement membrane; 7, Cytoplasmic processes of a melanocyte; 8, A keratinocyte adjacent to a melanocyte receiving melanosomes; 9, Antigens that penetrate the epidermis are detected by the Langerhans cells; 10, A sensory nerve fiber terminating on a Merkel cell; 11, T-lymphocytes activated by Langerhans cell in the lymph node; 12, Stratum granulosum; 13, Stratum corneum. *Source:* Concept Adapted from Kierszenbaum [12].

the primary players such as lymphocytes and DCs mediate and augment **adaptive immunity** (humoral immunity), which is more evolved and allows immunologic memory.

Melanocytes (Figure 1.3): Melanocytes are derived from the neural crest and are present mainly in the epidermal stratum germinativum and in hair follicles. **Melanophores** (Chromatophores) are found in lower vertebrates (fishes and amphibians) and differ from melanocytes in how they transfer melanin pigment to adjacent areas. Unlike melanocytes which can produce only **eumelanin** (brown/black) or **pheomelanin** (red/yellow), melanophores can synthesize several pigments [33, 34]. Melanophores are also derived from the neural crest and their main function is pigment aggregation in the center of the cell or dispersion throughout the cytoplasm, allowing the animal to effect color changes important for camouflage and social interactions. In mammals, melanocytes transfer **melanosomes** to adjacent keratinocytes of the basal layer via dendritic processes, protecting deeper layers of the skin from ultraviolet radiation (which is particularly damaging to cells during mitosis). Although melanocytes maintain close contact with keratinocytes via dendritic processes, they have a slower turnover rate than keratinocytes. In the dog, on average, one melanocyte exists for every 10–20 keratinocytes, while in the cat, there are fewer melanocytes [35]. The melanocytes, unlike the keratinocytes, are a stable population of cells living many years without undergoing cell division, while keratinocytes divide actively and live only a few days. If melanocytes decide to divide, the consequences are usually serious due to the development of malignant tumors [36].

Pigmentation

Melanocyte and melanosome activities are largely regulated by the pituitary hormone, alpha-melanocyte-stimulating hormone [37, 38] (α-**MSH**, also called **intermedin**). In some mammalian species (rat, rabbit, ox, etc.), the pars intermedia of the pituitary is well-defined and contains large amounts of α-MSH, but in other mammals (and birds) it is practically vestigial, and so α-MSH is thought to originate from the adenohypophysis. In the carnivores, the pars intermedia secretes α-MSH. Alpha-MSH shares an amino acid sequence with another pituitary hormone, adrenocorticotrophic hormone (**ACTH**). The adrenocorticotrophic hormone is composed of 39 amino acids, of which the first 13 represent α-MSH. Because of the common amino acid sequence of these two hormones, hyperpigmentation has been described in some animals with pituitary-dependent hyperadrenocorticism [39], and in others with Addison's-like disease where ACTH levels are increased.

Coat Color and Temperature: The color gene, the dominant C gene, codes for the enzyme tyrosinase (TYR), which is involved in the first step of melanin pigment production. Mutations in the TYR gene result in temperature-sensitive pigment production, producing *Burmese* and *Siamese* colors. Mutation of the TYR gene is also associated with the inhibition of fur pigmentation when temperatures rise above a certain level. When Siamese and Himalayan kittens are born, they are uniformly white due to the reasonably constant temperature of the intrauterine environment. Soon after birth, cooler segments of the body, mainly the extremities, begin to develop pigmentation. In older Siamese cats, the fur darkens as the entire body becomes slightly cooler, due to age-related reductions in cutaneus blood flow. Removal of fur in these cats' results in darker pigmentation of exposed growing hair due to a decrease in surface temperature. For this reason, unnecessary clipping of hair should be avoided in show cats of these breeds.

Alpha-MSH (and ACTH) cause melanosome dispersion, and thus skin darkening, while melatonin (from the pineal gland) and catecholamines cause melanosome concentration (and thus skin pallor). The term **melanoderma** is used to refer to increased melanin pigmentation of the skin, whereas the term **leukoderma** (Vitiligo) refers to a loss of this pigmentation [40]. Autoimmune dermatoses affecting melanocytes result in vitiligo in humans. Dogs and cats are also susceptible to this condition [41]. In dogs and cats, depigmentation mainly affects the face, including eyelashes, nasal planum, oral cavity, ears, and muzzle, but also noticed on footpads, scrotum, nails/claws. White coat color in cats is associated with blue (occasionally orange) eyes, and these animals sometimes exhibit a genetic predisposition to deafness. Calico coat color is sex-linked in cats [42] and is associated with females with XX chromosomes, or males with an extra X chromosome (Klinefelter's Syndrome, with XXY).

The **dermal–epidermal junction**: The dermal–epidermal junction is known as the **basement membrane zone** (Figure 1.2) consisting of the **basement membrane**. The basement membrane has a gate-keeping function controlling the bi-directional traffic of cells and bioactive molecules [43]. The basement membrane region is crucial to stabilizing epidermal attachment to the dermis, and it also acts as a barrier and filter zone. However, nutrients and water can freely diffuse through the basement membrane zone from the dermal side of the skin toward the epidermis. Although the terms "**basal lamina**" and "basement membrane" are used interchangeably, the term basal lamina is usually employed with electron microscopic descriptions, while the term "basement membrane" is generally used with light microscopy. The basement membrane zone is continuous along the entirety of the dermal–epidermal junction and the dermal intersection between hair follicles and skin glands. Based upon electron microscopic studies of this junction, two components of the basal lamina have been described, the **lamina lucida** (40 nm electron-lucent zone, mainly containing the glycoprotein laminin) and the **lamina densa** (50 nm electron-dense zone, composed mainly of collagen). The basement membrane core structural components include collagen IV, laminins, nidogens, and heparin sulfate proteoglycans [44]. The mechanical stability of the basement membrane depends largely on collagen IV scaffold [45]. The stratum basale cells of the epidermis are anchored to the basement membrane zone and dermis through specialized attachments called **hemidesmosomes** (so named because of their appearance as half-desmosomes, Figure 1.2). The outer layer of the hemidesmosomes interfaces with the plasma membrane while its inner layer interfaces with intermediate filaments. Anchoring filaments from hemidesmosome span across the lamina lucida to join lamina densa [46]. This junction is also often associated with numerous disease processes. Protein components such **bullous pemphigoid antigen** is a component of hemidesmosomes [47]. Bullous pemphigoid (BP) is an autoimmune disease in both humans and in animals, associated with antibody formation against the bullous pemphigoid antigen [48–50]. Transient cells also pass through the basement membrane, such as neoplastic cells in certain cancers, neutrophils, and other leukocytes during inflammation. Apparently, invading cells secrete proteolytic enzymes to dissolve the basement membrane [51, 52].

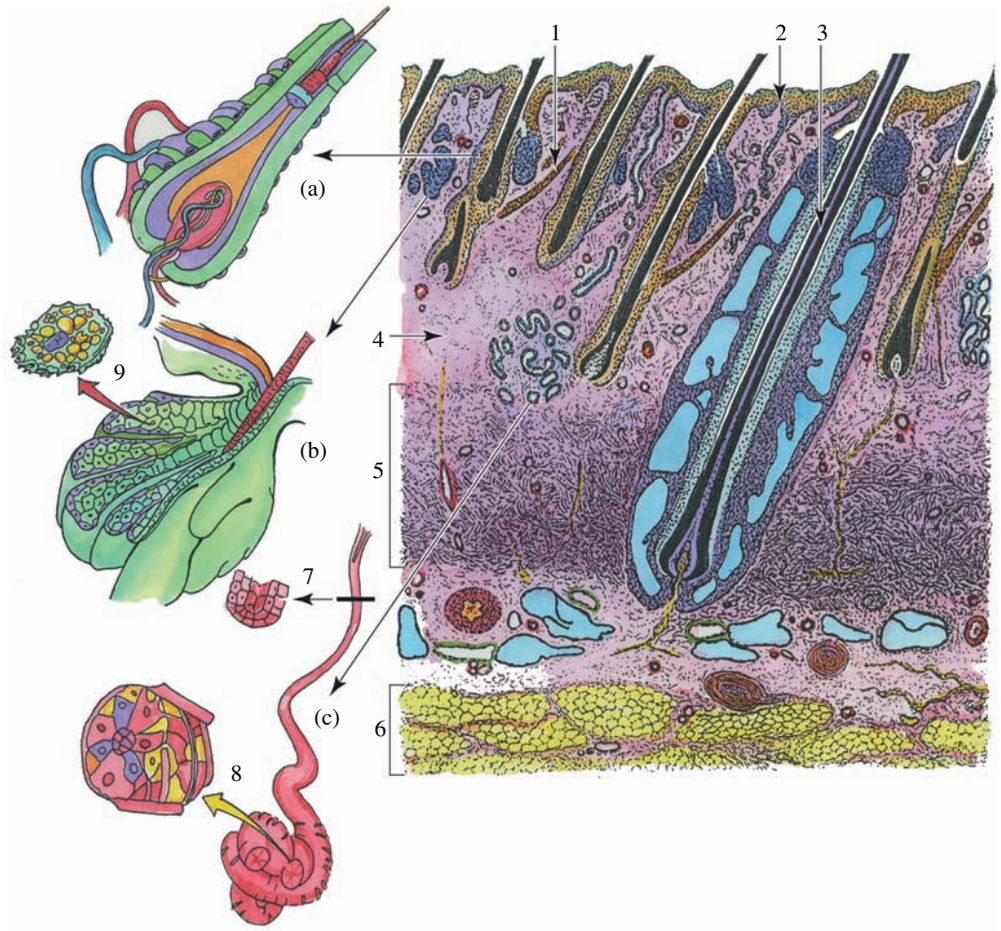

Figure 1.4 Schematic diagram of skin showing hairs and associated structures. (a) Simple hair follicle. (b) Sebaceous gland. (c) An eccrine sweat gland. 1, Arrector pili m; 2, Papillary layer of the dermis; 3, Sinus hair; 4, Dermis; 5, Reticular layer of the dermis; 6, Hypodermis with fat cells; 7, Cross sectional view of a sweat gland excretory duct; 8, Schematic view of the secretory portion of a sweat gland; 9, A sebaceous gland cell. *Source:* Adapted from Konig [53].

Other Structures: Among the epidermal adnexa of the skin are other structures such as hair follicles and sweat and sebaceous glands (Figure 1.4), all of which leave the confines of the epithelial layer and penetrate, some quite deeply, into the dermis [1–3]. Hair follicles are important not only for generating the hairs that insulate the body; but also, for serving as reservoirs for various stem cell populations, including keratinocyte precursors. The follicular bulb regions of the epidermal external root sheaths of some primary hair shafts (Figure 1.9) are associated with smooth muscles (*arrector pili* mm). These structures penetrate the deep dermal region, sometimes even to the hypodermis (Figure 1.5), and are distally attached to fibers in the superficial region of the dermis. These muscles raise the hairs to trap air and insulate the animal when its core body temperature falls.

Skin thickness varies widely in different regions of the body, with mean skin thickness in dogs varying from 0.5 to 5.0 mm and 0.4 to 2 mm in cats [5, 54, 55], depending on a variety of physiological variables including breed, anatomical region of the body, sex, age, and degree of skin hydration [56]. A report indicates a significant negative correlation between age and skin thickness in dogs [55]. The breed of the dog also influences skin thickness, Labrador retrievers exhibited thicker skin than other breeds [55]. The skin is extremely thick in areas prone to abrasions (e.g., footpads), yet thin in other regions (e.g., the flank). The stratum corneum is highly cornified where the skin is subjected to mechanical damage, as in the digital pads. Thick and thin skins are differentiated primarily based on the relative thickness of the stratum corneum, the outermost of the five layers of the epidermis. Hyperadrenocorticism results in thinning of the skin as a physical sign exposed to glucocorticoids [57].

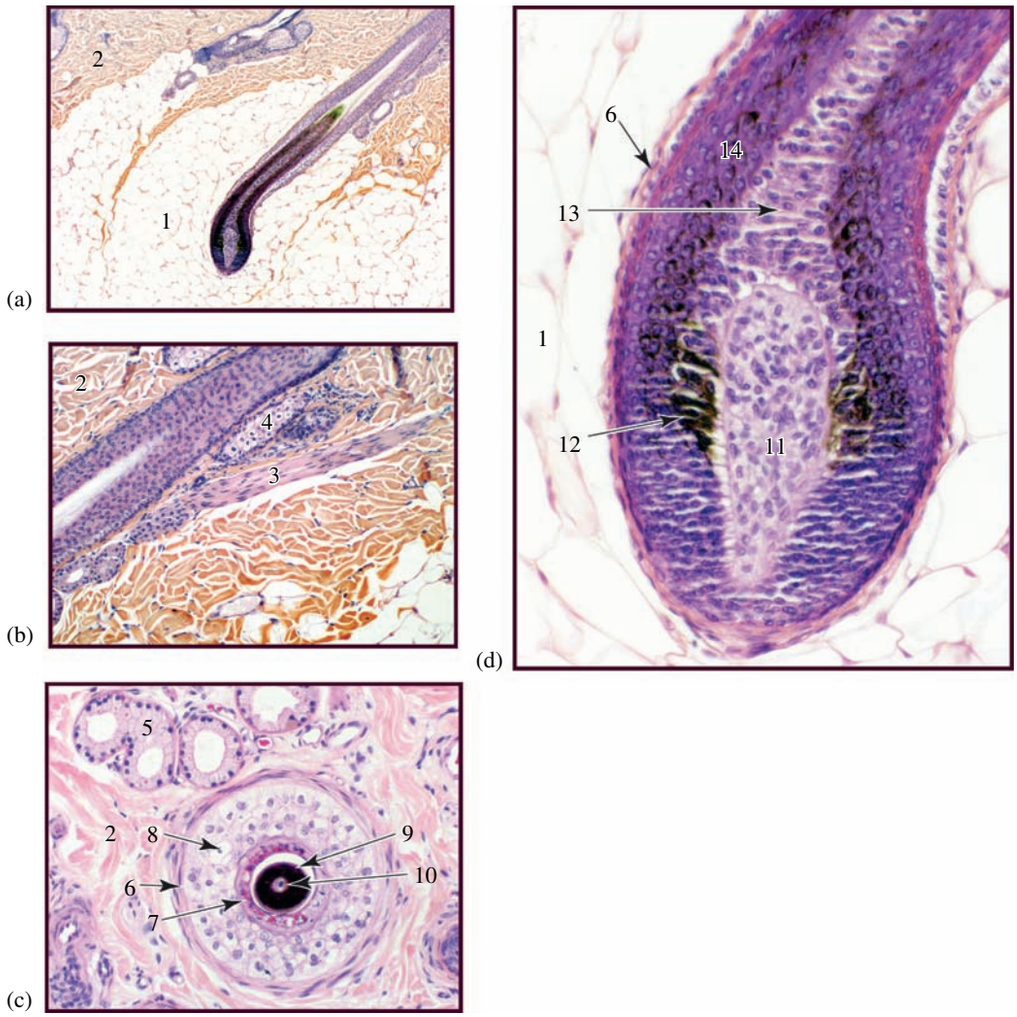

Figure 1.5 Histological features of hair. (a) Hair in the anagen stage (note the characteristic bend; 10×). (b) Arrector pili m. (20×). (c) Cross section of a hair shaft (20×). (d) Hair bulb (40×). 1, Fat cells; 2, Connective tissue of dermis; 3, Arrector pili m.; 4, Sebaceous gland; 5, Sweat gland; 6, Connective tissue around the outer epithelial sheath of the hair shaft; 7, Inner epithelial sheath with cells showing trichohyaline granules; 8, Outer epithelial sheath; 9, Hair cuticle around hair cortex; 10, Medulla of hair; 11, Dermal papilla; 12, Melanin; 13, Cells destined to form the medulla; 14, Cells destined to form the cortex and inner sheath. *Source:* Courtesy of Caceci T, Ph.D., Virginia-Maryland Regional College of Veterinary Medicine.

Epidermal Layers: Thick and thin skins are differentiated primarily based on the relative thickness of the stratum corneum, the outermost of the five layers of the epidermis. These epidermal layers are composed almost entirely of keratinocytes, with some transient cells as well.

- The **stratum basale** is the innermost epidermal layer, resting on the basement membrane. The term "stratum germinativum" is sometimes used to describe this layer, referring to this layer's ability to produce daughter cells by mitosis; but as mitosis is also encountered in the next layer (the stratum spinosum), "stratum basale" is the preferred term in this chapter. Cells of the stratum basale contain water-insoluble keratin assembled into cytoplasmic tonofilaments. The stratum basale itself consists of a single layer of cells in continuous mitosis, maintaining the stem cell population while adding new cells that gradually move to the surface (undergoing the keratinization process) and slough off. Intercellular junctions called **desmosomes** bind cells of the stratum basale at their apical and lateral surfaces (Figure 1.6), while hemidesmosomes anchor these cells to the basement membrane (Figure 1.2). The basal cell layer serves as a progenitor cell layer and contributes to basement membrane formation. The structural components of desmosomes include several

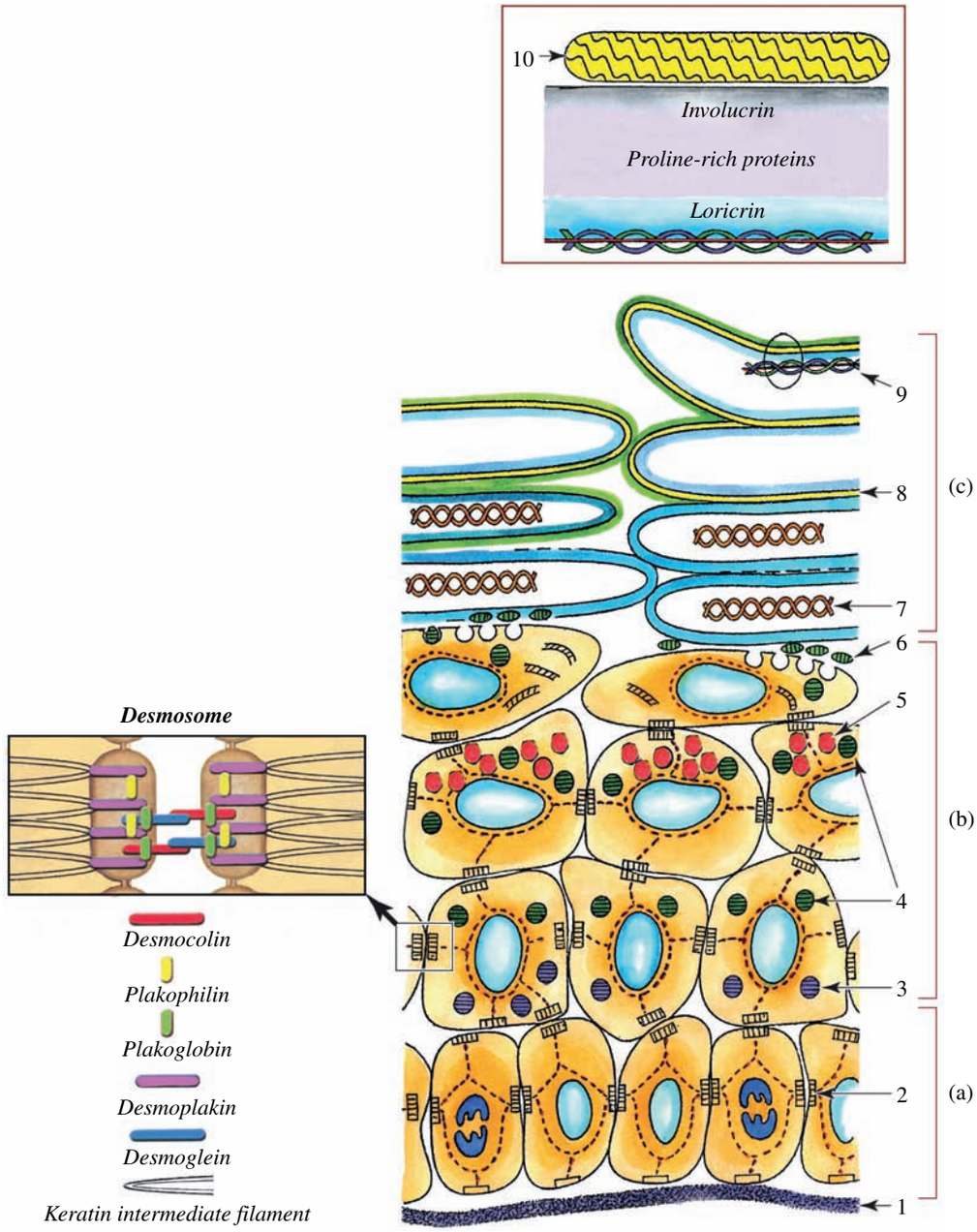

Figure 1.6 Schematic diagram of the epidermis depicting the differentiation of keratinocytes and the formation of the water barrier. (a) Stratum basale. (b) Stratum spinosum and granulosum. (c) Stratum corneum. The stratum basale cells divide by mitosis, with some of the daughter cells forming the stratum spinosum. These cells mature into stratum granulosum, and the type of keratin molecules manufactured changes from *keratins 5,14* (stratum spinosum) to *keratins 1,10*. The cytoplasm of the stratum spinosum cells exhibits *lamellar bodies* (derived from lipids). The cells of the stratum granulosum are flattened nucleated keratinocytes that manufacture more lamellar bodies and *filaggrin*, a non-filamentous protein that induces aggregation of keratins. The keratohyalin granules lose their limiting membranes and are associated with tonofilaments. The products of the lamellar bodies are released into intercellular spaces, adding waxy coats to the stratum corneum cells. This is the principal water-proofing layer. 1, Basal lamina; 2, Desmosome; 3, Melanosome; 4, Lamellar body; 5, Filaggrin granule; 6, Extruded lipids; 7, Keratin filaments; 8, Cell envelope; 9, Keratin-filaggrin complex lining the inner surface of the cell membrane; 10, Multilayered lipid linked to involucrin. *Source:* Adapted from Kierszenbaum [12].

core glycoproteins (desmoglein and desmocollin) and desmosomal plaque proteins (desmoplakin I and II, plakoglobin, and plakophilin). Cats and dogs apparently do not differ in the distribution of core glycoproteins in the skin [58].

- The **stratum spinosum** (or prickle cell layer) is the next layer of the epidermis, and the thickest layer, often consisting of two to four tiers of cells. Extremely thick skin may exhibit more than 40 cell layers. Cells of stratum spinosum display characteristic "spines" under light microscopy. These spines are fixation artifacts at the locations where **desmosomes** hold adjacent keratinocytes together [59, 60] (Figure 1.2). In most resources, they are referred to as "intercellular bridges," but in fact, there is no continuity of cytoplasm; the adjacent plasma membranes remain intact. The desmosomes are anchored to the cells themselves via **intermediate filaments**. Intermediate filaments are a very broad class of fibrous proteins that play an important role as both structural and functional elements of the cytoskeleton of epithelial cells. For example, epidermal cells contain **keratins**, a diverse family of intermediate filaments, one purpose of which is to connect adjacent cells through desmosomes. Keratin filaments (tonofilaments) are more organized in these cells, forming **tonofibrils**. Currently, the term "keratin" covers all intermediate filament-forming proteins with specific physicochemical properties. The best-known function of keratins and keratin filaments is to provide a scaffold (through self-bundling and by forming thicker strands) with which epithelial cells and tissues can sustain mechanical stress, maintain their structural integrity, and ensure mechanical resilience [61].
- The **stratum granulosum** is the next layer, often consisting of two to three layers of flattened epithelial cells with cytoplasm rich in keratohyalin granules. **Keratohyalin granules** are non-membrane-bound aggregates containing highly phosphorylated proteins, which cause the granules to stain with basic dyes (Figure 1.7). Keratohyalin granules are formed and deposited on intermediate filaments during the maturation of keratinocytes. **Profilaggrin** is the main component of these abundant, basophilic keratohyalin granules from which the granular layer of the epidermis derives its name. Profilaggrin is a phosphorylated polymer of high molecular weight (>400 kDa), composed of tandem repeats of filaggrin monomers joined by small linker peptides. During the transition from stratum granulosum to stratum corneum, the conversion of profilaggrin to monomeric **filaggrin** (filament aggregating protein) occurs by site-specific proteolysis and dephosphorylation. Filaggrin is an intermediate filament-associated protein that aids in the packing of keratin filaments during the terminal differentiation of keratinocytes. Filaggrin gene mutation or altered Filaggrin expression disrupts its normal function and may result in skin diseases and accelerated pathogen penetration [62]. Keratohyalin material may not be visible in some types of skin and is often lacking in thin regions. The stratum granulosum usually consists of one to three layers of granular cells that are transcriptionally active and deposit a cornified envelope of cross-linked proteins beneath the plasma membrane. The more distally located cells gradually lose their nuclei and become metabolically inert as they transition into the stratum corneum.
- The **stratum lucidum** is a layer present only on the nose and footpads and as such, generally not recognized as a distinct layer by most histologists. This is an extremely thin and translucent layer, being apparent only in a thick and hairless epidermis. The stratum lucidum contains a thin layer of flattened cells continuing to undergo keratinization. The nuclei and most of the organelles of the cells are undergoing degeneration. The stratum lucidum may not be visible histologically (depending on the type of skin being studied) but the changes that occur in the stratum lucidum must occur in all keratinocytes for them to become fully keratinized. This layer has no apparent cytological features that distinguish it from stratum corneum and is generally considered absent in carnivores [5, 6, 10, 63].
- The **stratum corneum is t**he outermost epidermal layer (Figure 1.7), consisting of several layers of flat, cornified usually a nuclear cells filled with arrays of tonofibrils embedded in a keratohyalin matrix, surrounded by a water-insoluble "cell envelope" [12, 64]. The stratum corneum may be compared with a brick wall, the flattened protein-rich corneocytes representing the bricks, and mortar, and represented by intercellular lipid-rich matrix [65, 66]. The layer provides the barrier against the entry of noxious chemicals and pathogens and against the desiccating effects of the environment [67]. The thickness of this layer of cells depends on the body region where it is found. In general, the stratum corneum is 3–35 μm in cats and 5–150 μm microns in dogs [68]. The stratum corneum in the dog was reported to have 47 cell layers in thickness [6]. The outermost laminae of cornified cells slough off (a process called **desquamation**), being continually replaced by keratinocytes of the inner layer. The rate of desquamation in normal skin normally matches the rate of cell proliferation in the stratum basale (and perhaps in the lower regions of stratum spinosum). The stratum corneum is maintained in perfect equilibrium, with renewal and replacement of desquamated cell layers by well-balanced epidermal proliferation, and progressive differentiation consisting of the synthesis of the lipid-enriched **lamellar bodies** and secretory organelles. The secretion of lamellar bodies before the cornification process promotes lipid accumulation around each corneocyte. Lamellar bodies begin at the suprabasilar epidermal layer and accumulate in significant amounts in the uppermost cells of stratum granulosum, accounting for 20% of the cell volume. The thick corneocyte

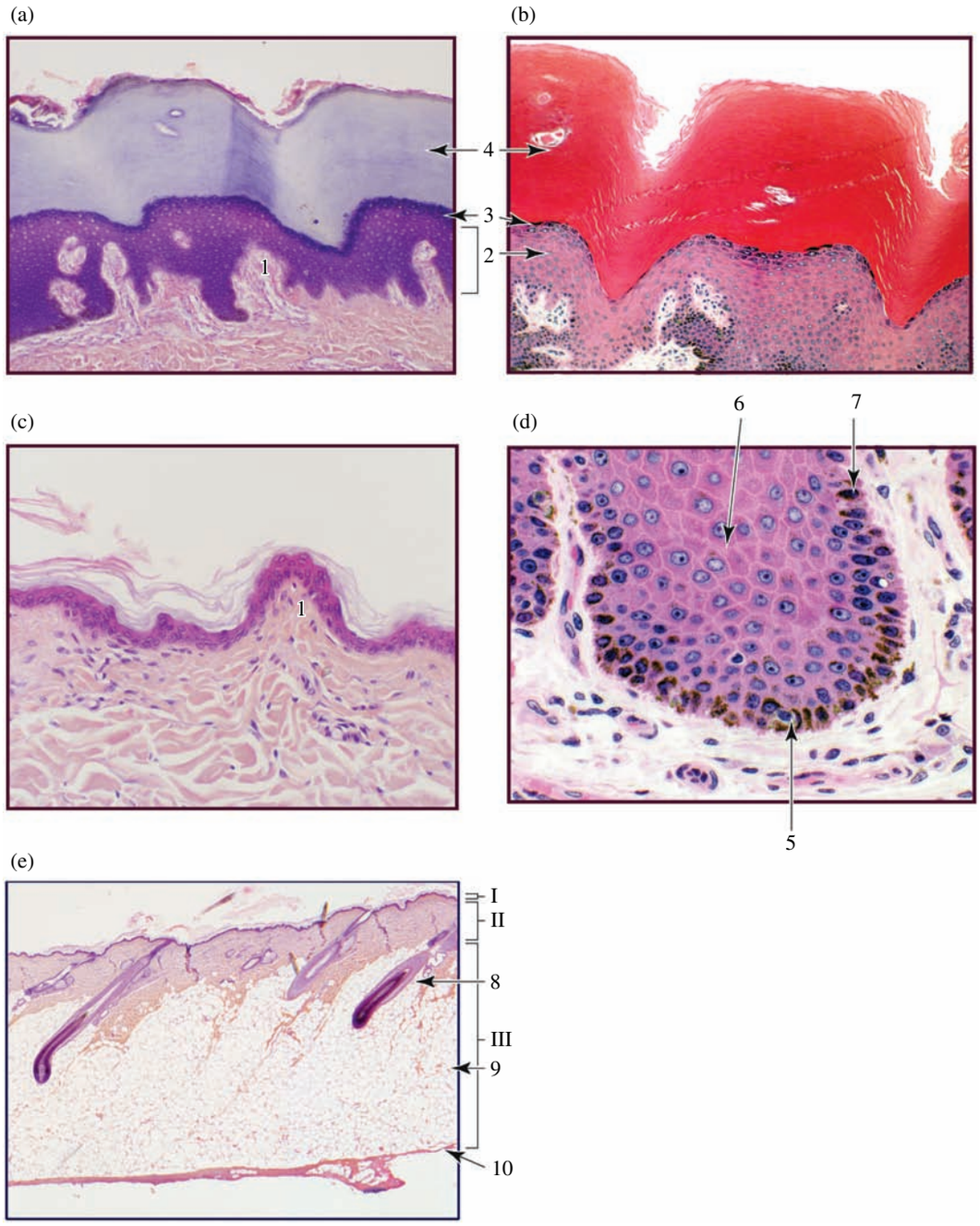

Figure 1.7 Examples of thick and thin skin. (a,b) Thick epidermis, palmar skin (10×). (c) Thin epidermis (20×). (d) Stratum germinativum and stratum spinosum (40×). 1, Dermal projections; 2, Stratum spinosum; 3, Stratum granulosum; 4, Stratum corneum; 5, Melanocyte; 6, "Intercellular bridges" of stratum spinosum; 7, Stratum basale; (e) Low magnification view of the skin showing 3 layers; I, Epidermis; II, Dermis; III, Hypodermis; 8, Hair follicle; 9, Fat cells; 10, Cutaneus muscle. *Source:* Courtesy of Caceci, T Ph.D., Virginia-Maryland College of Veterinary Medicine.

membrane forms from the synthesis and sequestration of keratin protein intracellularly. The entire **cycle of epidermal renewal** takes approximately 15–30 days. The mechanisms responsible for well-regulated desquamation are complex and involve the elimination of corneocyte cohesion by proteolytic degradation of desmosomes. Stratum **corneum chymotryptic enzyme** (SCCE) is a serine protease enzyme [69] that has been identified and characterized as having a specific function in corneocyte desquamation. This enzyme may be responsible for the degradation of the desmosomes

that bind the keratinocytes together. Corneocyte desquamation occurs in a biphasic manner, with an initial phase that begins early after the formation of stratum corneum with already exhibiting extensive corneodesmosomal degradation. This is continued by a second phase of desquamation at the peripheral region of the stratum corneum resulting in the shedding of surface corneocytes [70].

The Normal Keratinization Process

The keratinization (or cornification) process starts with mitotic activity in the stratum basale and terminates with the formation of the stratum corneum (Figure 1.6). These cells contain keratohyalin granules, which stain basophilic with hematoxylin and eosin (H & E) stains. Cell division alters cell mechanics in neighboring cells from overcrowding, resulting in compressive stress, altering cell adhesion, and increasing the probability of delamination (a division of single-layered epidermis into complex stratified epithelia) at the site [71–73]. Mitotic inhibition has been shown to reduce delamination [71]. The proliferating basal layer of cells is tightly packed, attached to both the basement membrane as well as the terminally differentiated postmitotic suprabasal layer which moves outwards by delamination, filling the many layers of the epidermis. During their upward migration, the keratinocytes maintain tight cell–cell junctions. Within the epithelial tissue, desmosomes provide a robust linkage of cytoskeletal elements, with actin cytoskeleton playing a major role in desmosome assembly [74]. Epithelial cell migration in the skin requires tightly regulated desmosome assembly and disassembly [74]. The process of desmosomal disassembly and reassembly also occurs in events such as wound healing or tumor cell migration. Upon reaching the most superficial layer, the keratinocytes lose their nuclei and die, forming the cornified layer [71].

Keratinocytes (a term reserved for epidermal cells destined to form a stratum corneum) produce an intermediate filament protein called **keratin**, which makes the skin and hair pliable and insoluble, thus helping to form an unreactive barrier against the external environment. The dense meshwork of intracellular keratin filaments interconnected between cells by desmosomes provides the basis for the mechanical strength within the epidermis. **Keratohyalin granules** are matrix proteins that increase in concentration within cells of the stratum granulosum layer [75], promoting the assembly of keratin filaments into larger bundles. The complex mixture of fibrous structural proteins in the epidermis is collectively referred to as **keratin proteins** [76]. Historically, keratin proteins stood for all the proteins extracted from horns, hairs, claws, hooves, and skin.

Calcineurin is a serine/threonine protein phosphatase that is widespread in many tissues, especially in the immune system and neural tissues. In the immune system, inhibition of calcineurin by cyclosporine leads to disruption of T-cell function [77]. Inhibition of calcineurin has been shown to inhibit the proliferation of cultured keratinocytes [78], probably due to inhibition of p21 expression resulting in downregulation of keratinocyte differentiation [79] and survival [80]. Wound healing depends on keratinocyte proliferation, differentiation, and migration. Cell motility/migration depends on calcineurin/nuclear factor of activated T cell (NFAT) signaling pathway [81, 82], induced by calcium–calmodulin complex. Calcium influx into keratinocytes induces their motility, driven by T-plastic (an action-bundling protein) through cross-linking to actin filaments and their synthesis regulated by calcineurin/NFAT pathway [83].

Cells of the stratum spinosum begin forming intermediate filaments into organized bundles (**tonofibrils**), which extend into the cytoplasm, attaching to desmosomes. Keratin is packaged into membrane-bound organelles within the stratum spinosum cells, which are referred to as **lamellar bodies** (also membrane-coating granules or *Odland* bodies) in the more distally located cells of the epidermis. These lamellar bodies are rich in glycosphingolipids, phospholipids, cholesterol, and numerous hydrolases. Keratinocytes become flattened as they continue to migrate toward the surface, forming the next layer, the stratum granulosum. These cells contain numerous keratohyalin granules without a limiting membrane. In addition to the keratohyalin granules, these cells also continue to accumulate more lamellar bodies and begin to release the lamellar glycolipid acylglucosylceramide (a glucocerebroside) into intercellular spaces. This waxy glycolipid forms wide sheets in the intercellular spaces of the stratum granulosum, coating keratinocytes of the upper layers, but principally the stratum corneum, and providing a water barrier for the epidermis. The stratum granulosum and stratum corneum also show nuclear fragmentation (karyorrhexis) and modification of cytoplasmic organelles. Cells of the stratum corneum lack nuclei and other intracellular organelles in normal circumstances, and their cytoplasm contains keratin cross-linked with another matrix protein, filaggrin. The keratin-filaggrin complex lines the inside of the cell membrane, forming the "cell envelope," which is cross-linked with the waxy acylglucosylceramide across the cell membrane. The cell envelope is a complex array of proteins such as periplakin, envoplakin, involucrin, and loricrin, which are cross-linked by transglutaminase-1, providing structural and mechanical integrity to the cells forming a water barrier [84]. The stratum

corneum keratinocytes are rich in sphingolipids (ceramides), which are a major component of the liquid barrier. Thus, an important role of the keratinization process is to produce a stratum corneum, the main barrier system of the epidermis. In addition to aiding the assembly of keratin bundles, hydrolysis of filaggrin is regulated in such a manner as to yield free amino acids in extracellular spaces of the stratum corneum, promoting its water-holding properties.

The Abnormal Keratinization Process

The importance of the stratum corneum is highlighted in burn victims, where large areas of this barrier may be lost, resulting in life-threatening infections. Additionally, repeated application of detergents to the skin, or dietary essential fatty acid deficiencies (e.g., linoleic acid), may result in lipid depletion of the stratum corneum; breakdown of the liquid barrier; and frequently epidermal hyperplasia, producing scaling, roughness, and alopecia. Cytokines released by the epidermis may also initiate acute dermal inflammation. In certain conditions (such as **primary seborrhea** in the cocker spaniel [85]), there is a proliferation abnormality in which the total cell cycle time and the active proliferative pool of basal keratinocytes are increased, resulting in an accumulation of scale, excessively dry or greasy skin [86]. The final step in keratinization is desquamation, and failure to exfoliate dead cells in cats and dogs may result in scaly skin (primary/secondary exfoliative dermatoses).

Pemphigus and **Bullous Pemphigoid** (BP) are chronic autoimmune skin diseases that lead to mucosal and/or epidermal blisters and ulcers [87]. They do so by disrupting either the intraepidermal intercellular attachments of the keratinocytes and mucosal epithelial cells (pemphigus) or the epidermal and mucosal attachments to the basement membrane. Clinically, pemphigus and BP are often indistinguishable. Their lesions are usually apparent on the face (most strikingly so on the planum nasale) and there are accompanying lesions of the feet and/or mouth. Pemphigus and BP have characteristic microscopic lesions that, together with clinical findings, serve as the basis for diagnosis.

Hairs: Hairs (*pili*) (Figures 1.4, 1.5, and 1.8), which are important for thermoregulation, are epidermal derivatives. Small bundles of arrector pili muscles serve to raise hairs from the skin, trapping air between them; as air makes a good insulator, this conserves body temperature. The arrector pili are innervated by postganglionic sympathetic nerve endings, which are sometimes referred to as **pilomotor nerve** endings. Arrector pili are best developed along the dorsal line of the neck, back, and tail [2, 3, 68, 88]. The hair coat consists of **outer hair** (*capilli*, cover hair, primary hair, or guard hair), and **wool hair** (*pili lanei* or secondary hair; Figure 1.8d) situated below the outer coat. The outer hair is thick, consisting of an outer cuticle and cortex, and a central medulla. The primary hair is surrounded by several secondary hairs. Wool hairs are finer and may (in the cat) or may not (in the dog) contain a medulla. Relative proportions of pigment, air bubbles, and the extent of pigment distribution in the cortical/medullary layers of outer hairs determine various hues of coat color, ranging from white (no pigment) to yellow (small amount of pigment), red (more pigment), and black (abundant pigment). Coat color does not necessarily reflect skin color, as a white-coated animal may have dark skin beneath the hair coat. The term **melanotrichia** refers to increased melanin hair pigmentation, whereas the terms **leukotrichia** and **poliosis** refer to a loss of hair pigmentation (graying of hair).

Each hair has two components, the hair follicle, and the hair shaft. The **hair follicle** is a tubular invagination of the epidermis, consisting of a **hair bulb** (i.e., the proximal end of the invaginated hair follicle) resting on a vascularized dermal core called the **dermal papilla** (from which it is separated by a thin basement membrane). Each hair shaft has an inner medulla and an outer cortex [68]. The cortex is sheathed in a cuticle, and each hair follicle is sheathed in a connective tissue coat. The cuticle is formed by a single layer of flat keratinized cells whose free edges overlap like shingles on a roof, all directed toward the distal end of the shaft. The cuticle of the internal root sheath is formed by overlapping keratinized cells like those of the cuticle of the hair, except that the free edges are oriented in the opposite direction toward the hair bulb. This arrangement results in solid implantation of the hair root in the hair follicle and helps to explain why hairs are not easily pulled out as well as why catagen and telogen hairs do not simply fall out.

The arrector pili are attached to the shaft of the hair follicle at a region called the **follicular bulb** (or the **bulge region**, Figure 1.9), which contains stem cells (**clonogenic keratinocytes**). These stem cells can migrate to regenerate hair shafts, form new sebaceous glands, or even contribute to the regeneration of the surface epithelium [89–93] (Figure 1.9). Thus, stem cells are clinically relevant in repairing skin wounds [12, 94, 95], and regenerating interfollicular epidermis [93, 96]. These stem cells may also contribute to tumorigenesis on canine tumors of skin origin, such as trichoblastomas, trichoepitheliomas, and tricholemmomas [97].

Hairs are generally arranged in clusters (hair beds), and these beds in dogs and cats may contain hair follicles opening either independently or through a common opening at the epidermal surface (compound hairs, Figure 1.8). Dorsal and

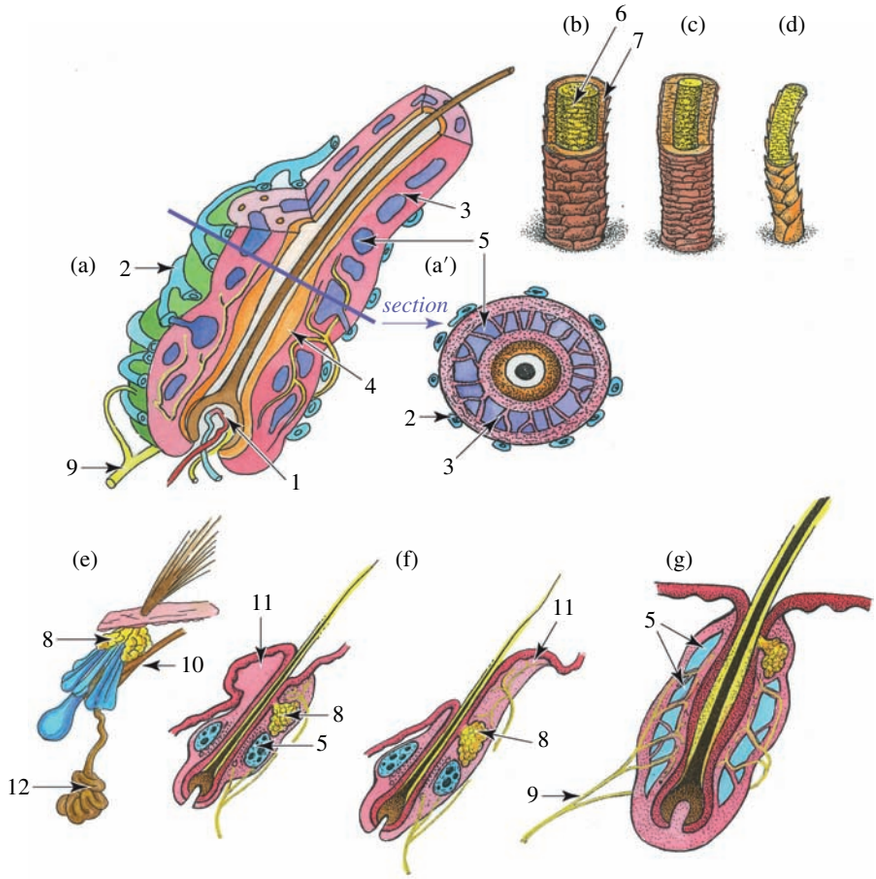

Figure 1.8 Different types of hairs in animals. (a,f,g) Tactile (or sinus) hairs are found on the face. They are deeply rooted into the subcutis or even in the superficial muscles on the face. A few sinus hairs are also found on the medial aspect of the carpus of the cat (**carpal organ**). The hairs are surrounded by venous sinuses. (b,c) Guard hairs with different thicknesses of cortex and medulla; (d) A wool hair. In carnivores, wool hairs are medullated, while in ungulates, they lack a medulla. (e) A complex hair follicle with a single primary hair surrounded by several secondary hairs. (a′) Cross-sectional view of a sinus hair shaft. 1, Dermal papilla with blood vessels and nerve endings; 2, Veins draining blood sinuses; 3, Connective tissue trabeculae surrounding the venous sinuses; 4, Epidermal wall of hair follicle; 5, Blood sinus; 6, Medulla of hair shaft; 7, Cortex of hair shaft; 8, Sebaceous gland; 9. Sensory nerve ending; 10, Arrector pili m; 11, Tactile elevation; 12, An apocrine sweat gland.

lateral aspects of the body exhibit a denser hair coat than ventral aspects of the thorax and abdomen, medial aspects of the thigh, and ventral aspects of the tail. Hairs are sparse on the canine scrotum, but cats exhibit a hairy scrotum. Cats and dogs exhibit compound hair follicles that possess numerous hairs emerging from a common pore [98]. Compound hairs generally consist of one guard hair surrounded by up to nine wool hairs (up to 12 in the cat). The arrector pili muscle bundles from individual hairs join to form a single muscle bundle that inserts into the dermis, while secondary hairs may be associated with sebaceous glands. In addition to these more common hairs, specialized hairs are found in certain regions of the body. For example, **tactile hairs** (or sinus hairs, also called vibrissae, Figure 1.8) are found over the face. These hairs exhibit blood sinuses around the base of the hair follicles designed to amplify the wave motion of the hair and stimulate nerve endings. Walls and trabeculae within the blood sinuses are richly supplied by sensory nerve endings. Scattered over the body surface are specialized sinus guard hairs (**tylotrich hair**) associated with small but visible elevations of the skin called **tactile elevations** [88] (*torus tactiles,* Figure 1.8f). The term is taken from the Greek *tylos*, "a knot or callus." These tylotrich hairs are extremely touch-sensitive. The tactile elevations (or integumentary papules) are small, knob-like structures (0.16–0.42 mm in diameter). The tactile elevations are also touch-sensitive. The tylotrich guard hairs are modified sinus hairs and as such exhibit small venous sinuses.

Hair Replacement: Hairs, except for tactile hairs, are periodically replaced in animals [1, 99]. Outdoor cats and dogs living in colder regions shed hair during the spring and fall, while indoor cats and dogs (especially short-haired breeds) shed throughout the year. Grooming habits of cats serve to remove loose hairs and ectoparasites, stimulate oil glands of the

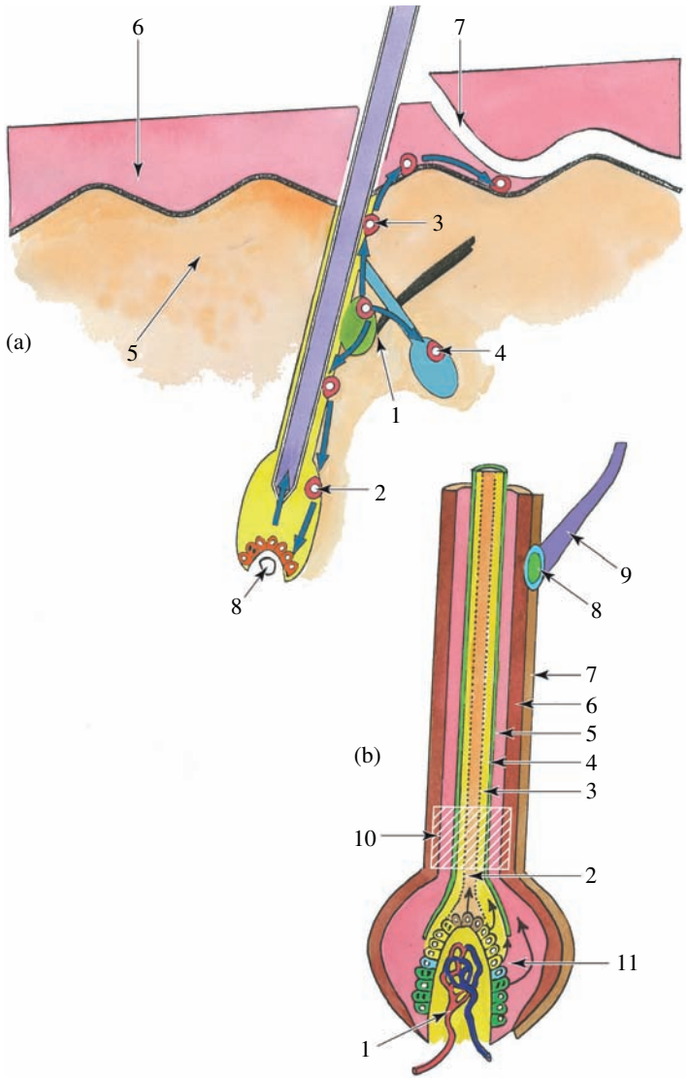

Figure 1.9 Structure of skin, schematic. (a) Keratinocyte stem cells are present in the follicular bulb (1), a part of the external root sheath of the hair follicle where the arrector pili m. attaches; 2, Stem cells can migrate to populate the apex of the dermal papilla to the form the internal root sheath, the cortex and medulla; 3, Stem cells can migrate into the epidermis along the basal lamina and reform the epidermal layer; 4, Stem cells can also differentiate to form sebaceous glands; 5, Dermis; 6, Epidermis; 7, Damaged epidermal layer. Stem cells from hair follicles may eventually restore damaged epidermis; 8, Dermal papilla. (b) Structure of a hair follicle. 1, Dermal papilla; 2, Medulla; 3, Cortex; 4, Cuticle; 5, Internal root sheath; 6, External root sheath; 7, Connective tissue sheath; 8, Follicular bulb region; 9, Arrector pili m.; 10, Cross-hatched region is the keratogenous zone where hard keratin accumulates; 11, Arrows indicate hair bulb cells forming internal and external root sheaths. *Source:* Adapted from Kierszenbaum [12].

skin to waterproof the hair coat, and aid in thermoregulation through the evaporative cooling of saliva. The shedding pattern of hairs depends on several factors, including external temperature and humidity, nutritional status, and health of the animal. The hair cycle can be summarized as follows [98, 100, 101]:

- Anagen, a long period of growth.
- Catagen, the transitional period from growing to resting
- Telogen, a long period of inactivity.

Glands of the Skin: **Sebaceous glands** are associated with hair follicles (Figure 1.4). Their oily secretions help keep the hair coat soft and water-resistant, and contraction of the arrector pili may help to express sebaceous gland secretions. Cats have more sebaceous glands in the face than dogs [65]. **Sweat glands** are classified into two types based on the nature of their secretion. True sweat glands (**merocrine** or **eccrine** glands) are independent of hair follicles (Figure 1.4), and, in carnivores, are predominantly confined to the footpads [1–3] (which are devoid of sebaceous glands). **Apocrine** sweat glands are diffusely distributed over the entire body of the carnivore and are associated with hair follicles [68]. Apocrine glands may release pheromones. Several contractile elements (i.e., myoepithelial cells) surround the sweat glands, which contract to empty the contents of the sweat glands to the body exterior. Sweat glands are supplied by **secretomotor** sympathetic nerve fibers. Circumscribed **tail glands** are modified sebaceous glands found on the dorsal aspect of the tail in an area measuring up to 5 cm in length, caudal to the level of the seventh caudal vertebra of the dog [88] (but located at the

base of the feline tail). The tail gland region appears slightly yellowish and waxy due to secretions of glands in this region. The hairs over this region are coarser than the surrounding region and emerge from single follicular shafts. Tail glands may function to promote sexual behavior through pheromone secretion and conspecific (i.e., belonging to the same species) recognition.

Dermis

The **dermis** (or **corium**) is the layer of the skin below the epidermis (Figure 1.10). It is highly vascular and supports the epidermis. It is separated from the epidermis by a thin basement membrane. The dermis is a connective tissue structure derived from embryonic mesoderm (mesenchyme), while the epidermis is of ectodermal origin. Since the epidermis is avascular, the dermis provides for the needs of the epidermis with several vascular complexes located at different levels.

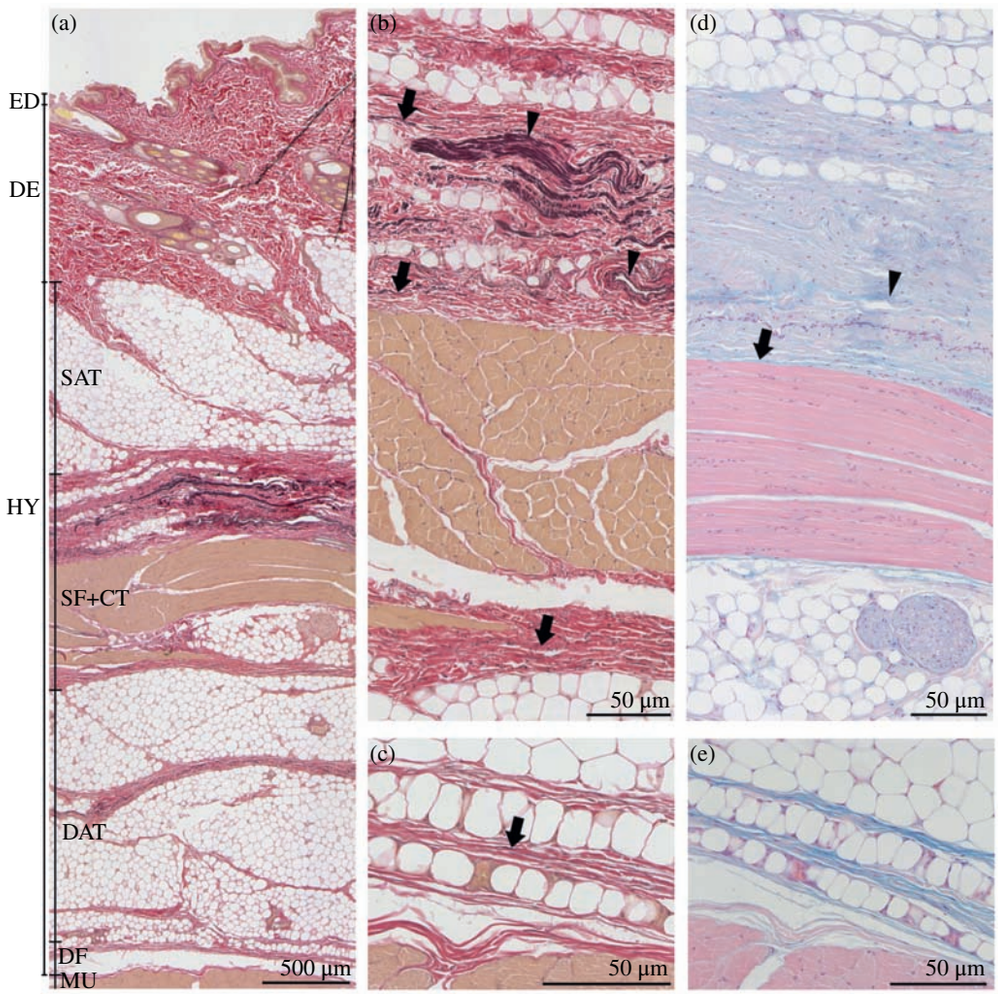

Figure 1.10 Transverse sections of *regio abdominis lateralis* of the dog. (a) Overview micrograph from skin to muscle representing the fascia layers in the dog section. DAT, deep adipose tissue; DE, dermis; DF, deep fascia; ED, epidermis; HY, hypodermis; MU, muscle; SAT, superficial adipose tissue; SF, superficial fascia; SF + CT, superficial fascia inclusive *m. cutaneus trunci*. (b) Elastic fibers are present along the regular collagen fibers in SF (arrows) and along the blood vessels (arrowheads). (c) The DF is loosely attached to the underlying *m. obliquus externus abdominis*. Elastic fibers (arrow) are present in the layers of DF. (d) Alcian blue staining, indicating the presence of hyaluronan, is intense in the layers of SF in relation to muscle (arrow) and blood vessels (arrowhead). (e) Hyaluronan is present within the layers of DF. (a–c) Weigert's Resorcin Fuchsin stain; (d,e) Alcian blue stain. *Source:* Ahmed et al. [102]/Reproduced with permission from John Wiley & Sons, INC.

Dermal thickness varies in different regions of the body [103], being quite thick in the nose and footpads. It is thicker on the dorsal surface than it is on the ventral surface of the body, and the lateral versus the medial surfaces of the limbs. The mean dermal thickness in the dog reported was 0.77 mm (ranging from 0.55 to 1.25 mm). The dermis is thick dorsally (0.95 mm), intermediate laterally on the flank (0.17 mm), and thin ventrally (0.62 mm) [103]. The papillary layer of the cat is relatively low, exhibiting only shallow undulations [1]. The dermis is composed of cellular and extracellular fibrous components of connective tissue (such as fibroblasts, macrophages, plasma cells, and mast cells) and collagenous (most abundant), reticular, and elastic fibers. The extracellular matrix of the dermis is composed of type I, III, and type V collagen fibrils, with type I collagen fibers being the most predominant, microfibrils, and elastic fibers supported by a mucopolysaccharide matrix that is hydrated by an ultrafiltrate of plasma (the source of interstitial fluid and the liquid fraction of lymph) provides the tensile strength and elasticity. Other components of, or found extending within, the dermis include blood and lymph vessels, nerves, glands, hair follicles, and smooth muscle fibers. The hair follicles and glands are epidermal derivatives located at various levels of the dermis but are physically and physiologically separated from it by a basement membrane. Where there are hairs, discrete bundles of smooth muscle called **arrectores pilorum** (arrector pili, *pl.*) extend from near the base of each hair follicle to the superficial papillary layer of the dermis [1–3] (Figure 1.7). The arrector pili insert onto the external basement membrane of the hair follicle and originate in fibers of the superficial dermis.

The dermis has both a superficial and a deeper layer. Where hairs are sparse (as in the digital pads), the superficial layer is called the **papillary layer**, as it forms finger-like projections below the epidermis (Figure 1.7c). Dermal papillae may be wavy and indistinct in regions of the body less prone to friction (for example, heavily-haired skin such as the dorsal aspect of the neck). In regions prone to high friction (such as the footpads), dermal papillae are rather pronounced and interlock with corresponding indentations of the epidermis (epidermal rete ridges) to prevent epidermal sloughing. The papillary layer consists of loose collagenous tissue containing numerous vascular plexuses and nerve endings. The papillary projections are relatively low in cats [1]. The deeper dermis is called the **reticular layer**, based on the lattice-like appearance of collagen fibers in the matrix (Figure 1.10). However, in dogs and cats, the dermis is classically divided into superficial and deep layers rather than papillary and reticular layers [10, 104]. Where the skin has cutaneus muscle (e.g., cutaneus trunci, Figure 1.10), the skeletal muscle fibers are tightly anchored to the superficial layer of the dermis to facilitate movements of the skin.

Cells of the dermis (Figure 1.11): Many cell types populate the dermis, including fibroblasts, myofibroblasts, macrophages (*histiocytes*), and mast cells, with the latter involved in immediate hypersensitivity reactions. Cats have been reported to have more numerous mast cells in the dermis (up to 20/high-power field) than dogs (4–12/high-power field) [10, 54].

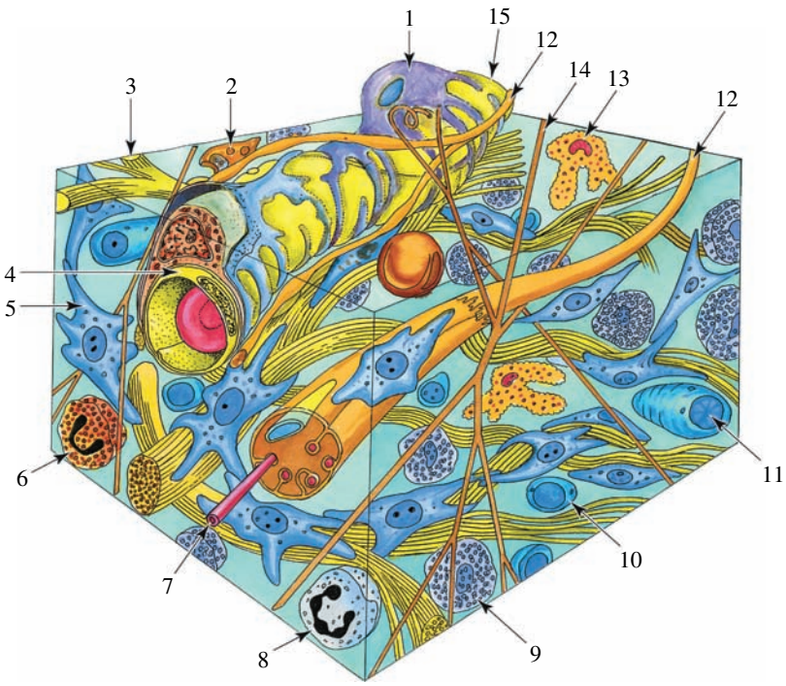

Figure 1.11 Cells and other components of loose connective tissue (superficial fascia). 1, Pericyte; 2, Adipocyte; 3, Collagen fibrils; 4, Capillary endothelium; 5, Fibroblast; 6, Eosinophil; 7, Nonmyelinated axon; 8, Neutrophil; 9, Mast cell; 10, Lymphocyte; 11, Plasma cell; 12, Nonmyelinated axons enclosed by a neurilemmal cell; 13, Macrophage; 14, Elastin fiber; 15, Capillary. Adapted from Williams and Warwick [105].

Fibroblasts and myofibroblasts are important sources of extracellular collagen that is important during wound healing [106]. In cats, activation of fibroblasts and myofibroblasts seems to be slower than in the dog, which may play a role in delayed/or altered open wound contracture [107, 108], perhaps also contributing to a higher risk of wound dehiscence due to pseudo-wound healing [109]. Mast cells of the dermis release vasoactive amines (e.g., serotonin and histamine) as well as pro-inflammatory mediators that can induce immediate hypersensitivity reactions. DCs migrate from the epidermis and are often found in the dermis en route to local lymph nodes.

The Hypodermis (Subdermis or Subcutis or Superficial Fascia)

Beneath the dermis, mesenchymal cells (see fascia) form a layer of loose (or areolar) connective tissue, the hypodermis, consisting of irregular bundles of collagen and elastic fibers and various types of connective tissue cells. The hypodermis is referred to as **subcutaneous connective tissue** by histologists, and as **superficial fascia** by gross anatomists. The hypodermis is relatively thin in carnivores and contains more abundant elastic fibers than that of other domesticated mammals. The hypodermis serves to bind the skin to deeper structures. In some regions, the superficial fascia is directly continuous with the deep fascia (a more organized connective tissue structure, Figures 1.12 and 1.13) or with the periosteum, as on the dorsum of the nose.

The hypodermis is responsible for the following functions: It stores fat as an energy source and serves as a shock absorber, it attaches the dermis to bones and cartilage, it provides support for nerves and blood vessels, and it helps to regulate body temperature. Diseases affecting the dermis can easily spread into the hypodermis and sometimes may also involve muscle bundles as in the case of burns and scars [111]. The adipose tissue in the hypodermis is important for energy homeostasis, and actively secretes adipokines, including adiponectin, interleukin 6, and tumor necrosis factor-alpha (TNFα). Adiponectin has anti-inflammatory and anti-atherosclerotic properties [112, 113] as well as insulin-sensitizing and lipid-lowering effects [114]. Adipocytes of hypodermis appear to secrete higher concentrations of adiponectin and lower concentrations of interleukin 6 and TNFα compared with visceral adipocytes [115].

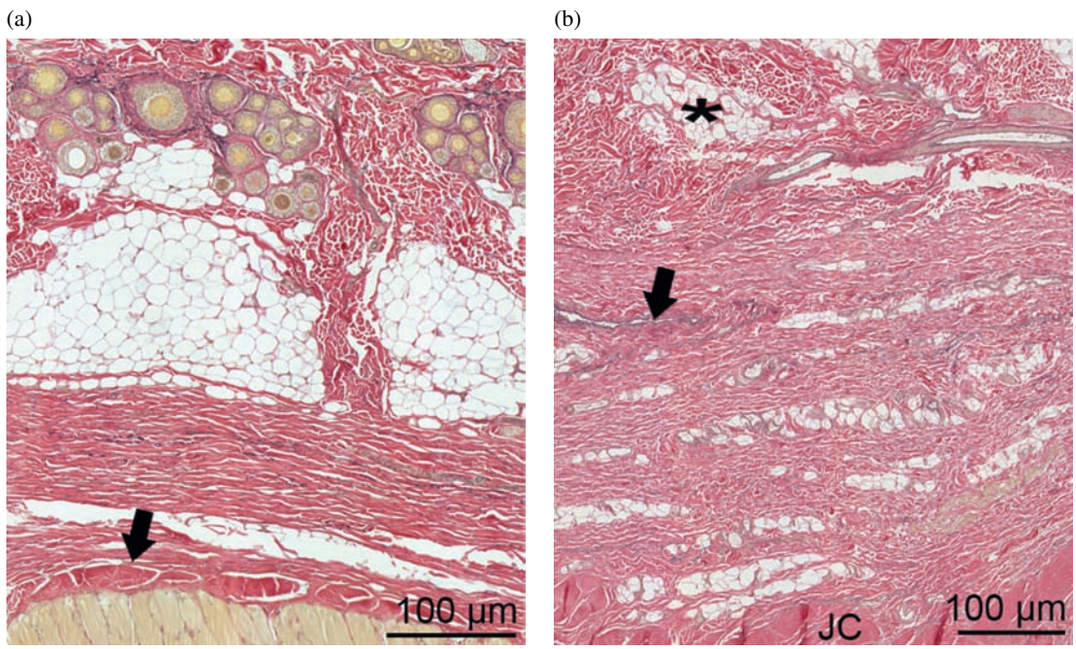

Figure 1.12 Sections of dog limbs. (a) Superficial adipose tissue is present below the dermis and the deep fascia (*fascia lata*; arrow) in this region, which is thick and composed of dense collagen fibers and tightly attached to the epimysium of *m. biceps femoris*. The superficial fascia and deep fascia are fused. (b) Fat deposits (asterisk) are present in the hypodermis of the carpus. Elastic fibers (arrow) are present in the loose areolar tissue in relation to blood vessels. The articular capsule (JC) of the intercarpal joint is present in the section. (a,b) Weigert's Resorcin Fuchsin stain. *Source:* Ahmed et al. [102]/Reproduced with permission from John Wiley & Sons, INC.

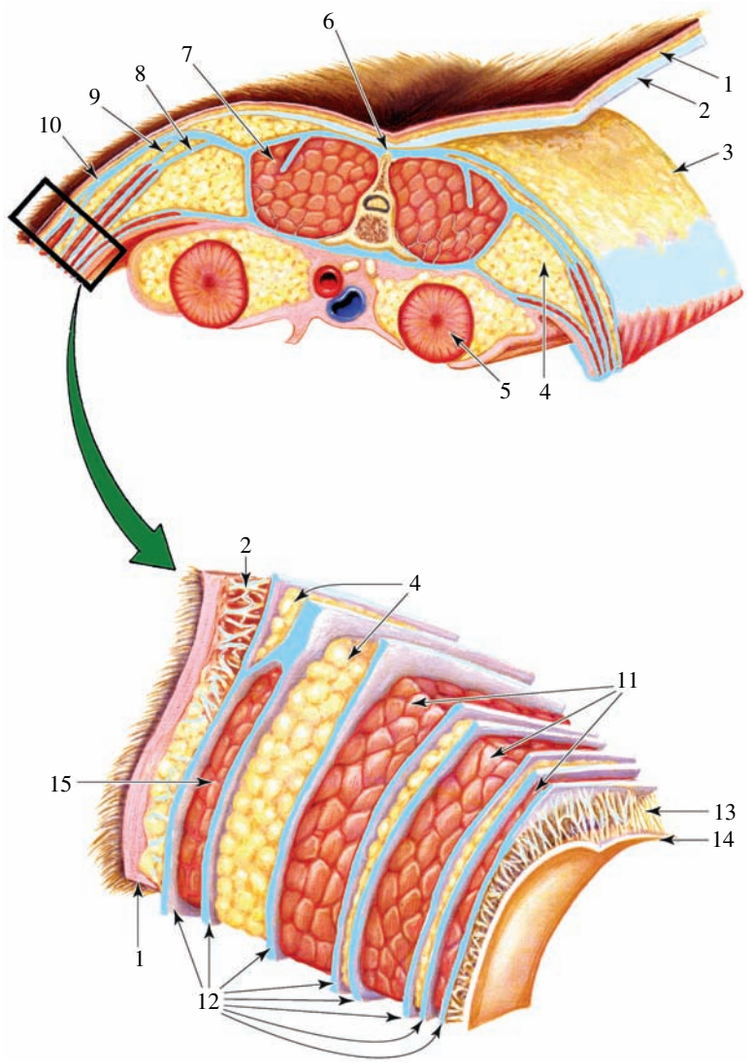

Figure 1.13 Organization of superficial and deep fascia. 1, Skin; 2, Superficial fascia; 3, Fat cells in the superficial fascia, reflected along with deep fascia; 4, Fat; 5, Kidney; 6, Median fibrous raphe (deep fascia attaching to spinous processes of vertebrae); 7, Epaxial muscle; 8–10, 12, Layers of deep fascial laminae; 11, Abdominal muscles; 13, Subserous fascia; 14, Parietal layer of serous membrane; 15, Latissimus dorsi m. *Source:* Adapted from Adams [110].

The hypodermis is a composite of fibrous collagenous tissue (Figure 1.12) in a viscoelastic ground substance of fibroblast origin composed of glycosaminoglycans. It forms an envelope layer beneath the skin. Loss of large amounts of subcutaneus tissue during surgery may impede wound healing [116–118]. This is due to the cellular (e.g., fibroblast that produces collagen fibers required for wound repair) and vascular components of the subcutis that are important for forming granulation tissue. Based on clinical observations of wound healing, cats may have a lower ability to synthesize collagen in the dermis and subdermis [116] than dogs.

The hypodermis connects the skin to underlying structures and permits some degree of skin movement, designed to transmit and dissipate tensional force [119–121]. In certain regions of the body, the hypodermis is characteristically infiltrated by numerous fat cells, thus forming a thick layer of adipose tissue (**panniculus adiposus**). It is divided into superficial adipose tissue exhibiting large fat globules surrounded by perpendicularly oriented fibrous septa (*retinacula cutis superficialis*), and deep adipose tissue with well-defined obliquely oriented fibrous septa (*retinacula cutis profunda*, Figure 1.12) [122]. These adipocytes function as energy stores as well as cushions against concussive forces applied against the skin. Panniculus adiposus is well-defined over the gluteal region. In other regions of the body, a thin layer of striated muscle develops within the hypodermis, giving rise to **panniculus carnosus**. The cutaneus trunk muscle (Figure 1.10) is an example.

Nerve Supply to the Skin: Skin is generously innervated by nerve endings (Figure 1.14). Sensory nerve endings may terminate in the skin as free nerve endings in the epidermis (nonencapsulated receptors), or they may be encapsulated

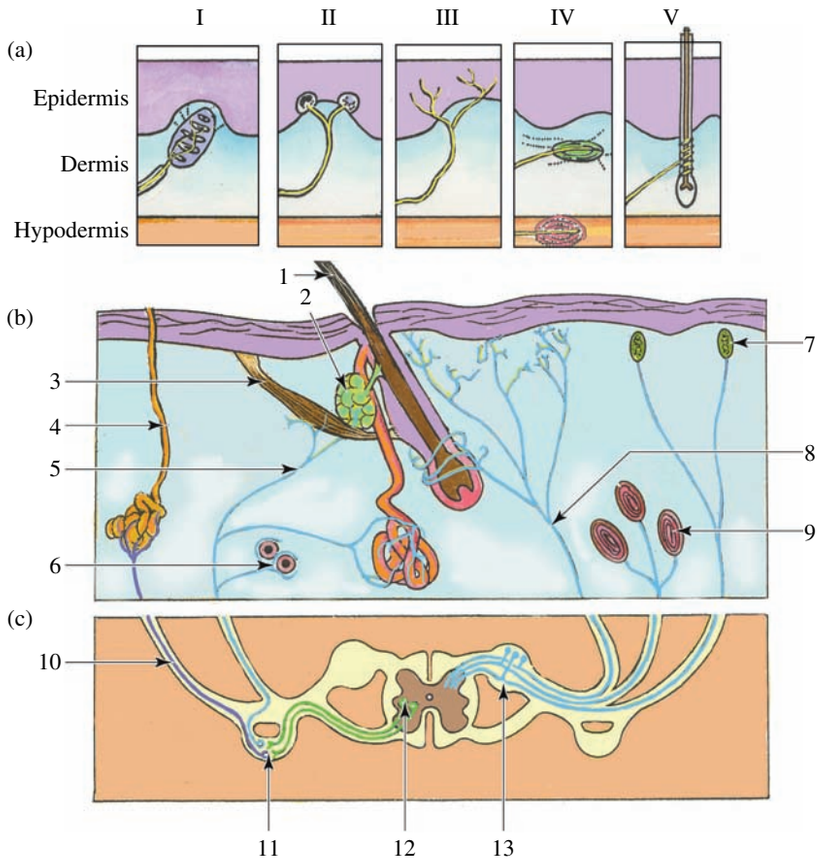

Figure 1.14 (a) Sensory structures of the skin. Various types of receptors are located in the skin. Some are encapsulated; I, *Meissner* corpuscles are in the dermal papillae and detect touch; II, Tactile epithelioid cells (*Merkel* cells) are mechanoreceptors; III, Free nerve endings penetrate the epidermis and respond to pain and temperature differences; IV, *Ruffini* end organs are located in the dermis, and are anchored to the surrounding tissue by fine collagen fibrils and respond to stretch; V, Peritrichial nerve endings wrap around the base and shaft of hair follicles and are responsive to hair movement. (b) Schematic structure of the skin and associated structures. 1, Hair; 2, Sebaceous gland; 3, Arrector pili m.; 4, True (eccrine) sweat gland; 5, Pilomotor (postganglionic sympathetic) nerve to arrector pili m.; 6, Vasomotor (postganglionic sympathetic) nerve to the smooth muscles of blood vessels; 7, *Meissner* corpuscle; 8, Naked sensory nerve endings to hair follicle (peritrichial nerve ending) and to the superficial layers of the dermis and deep layers of the epidermis; 9, *Pacinian* corpuscle and its nerve endings. *Pacinian* corpuscles located in the hypodermis and deep fascial layers respond to pressure. (c) Schematic of the spinal cord level showing nerves to the skin. On the left side of the spinal cord, sympathetic components to the skin are shown, while the right side depicts sensory input from skin structures; 10, Secretomotor (postganglionic sympathetic nerve) fibers to sweat glands; 11, Postganglionic sympathetic neurons located in sympathetic trunk ganglia; 12, Preganglionic sympathetic neurons in the lateral horn; 13, Dorsal root ganglion containing sensory nerve cell bodies. *Source:* Adapted from Kierszenbaum [12] and McGavin and Zachary [123].

by specialized structures within the dermis (encapsulated receptors). **Encapsulated receptors** vary in shape, structure, and function, and are found in the dermis as well as other parts of the body [2]. The simplest is the bulb corpuscle, which is touch-sensitive. Other encapsulated terminals such as the tactile and lamellated corpuscles are mainly pressure-sensitive. **Nonencapsulated nerve endings** are nonmyelinated axons distributed widely in epithelia, hair follicles, connective tissues, periosteum of bones, tendons, joints, and muscles. Cutaneus nerve endings extend into the epidermis from the dermis as nonmyelinated fibers. They are usually confined to the stratum basale and stratum spinosum and perceive pain and temperature variations and are dispersed among keratinocytes, and in the dermis in association with blood vessels and fibroblasts [124]. Other free nerve endings terminate within the epidermis as leaf-like expansions (**nerve plates**, also called the Merkel cell-neurite complexes or the tactile hair discs) in association with specialized epidermal cells called **tactile epithelioid cells**. The cytoplasm of these cells contains abundant granules, presumably neurotransmitters. These cells are thought to serve as mechanoreceptors (or mechanoceptors). Nonencapsulated nerve

endings in the dermis that surround hair follicles form peritrichial nerve endings at the base of these follicles (Figure 1.8) and are sensitive to hair movement.

Sensory and autonomic cutaneus nerve endings in the skin can release several neuropeptides when stimulated. These neuropeptides act as neurotransmitters, paracrine factors, or hormones [125]. Keratinocytes, Merkel cells, and fibroblasts among other cells of the skin have been shown to release neuropeptides [126, 127]. This, in concert with neuropeptides released from nerve endings, may play a role in wound healing. Several of these neuropeptides can act on G protein-coupled receptors [128]. These peptides include Substance P, neurokinin A, calcitonin gene-related peptide (CGRP), and vasoactive intestinal peptide [129]. These molecules, along with the neuropeptides released from the other skin cells may play a crucial role in wound healing [124, 130]. For example, Substance P stimulates vasodilatation [131], upregulates adhesion molecule expression on endothelial cells, monocyte chemotaxis, inflammatory cell activity [132, 133], and synthesis and release of pro-inflammatory cytokines during the inflammatory phase of wound healing [134]. Denervated skin delays wound healing [135, 136]. Diabetic neuropathies have been shown delay wound healing [137, 138].

Blood Supply to the Skin: The epidermis has no direct blood supply of its own; therefore, passive diffusion of fluids from the underlying dermis must provide metabolic support to the deeper, metabolically active epidermal cell layers. The skin is nourished by numerous **cutaneus arteries** (their terminal branches are seen in Figure 1.15). These arteries supply blood to the vascular plexuses that nourish the skin and its appendages [56]. These direct cutaneus vessels can be incorporated into large skin flaps (vascular pedicle grafts), forming **axial pattern flaps** for closing large skin wounds secondary to trauma or resection of diseased skin (Figure 1.16). These are called axial pattern flaps because they include a direct specific artery within the longitudinal axis. A significant amount of blood can be stored in cutaneus vascular plexuses, depending on whether the blood vessels are dilated or not. Dilation or constriction of cutaneus blood vessels (i.e., vasomotion) is controlled by the sympathetic nervous system, whose postganglionic fibers to these vessels form **vasomotor nerve** endings. Increased blood flow to the skin facilitates heat loss by various mechanisms [140], including sweating, and is mediated by

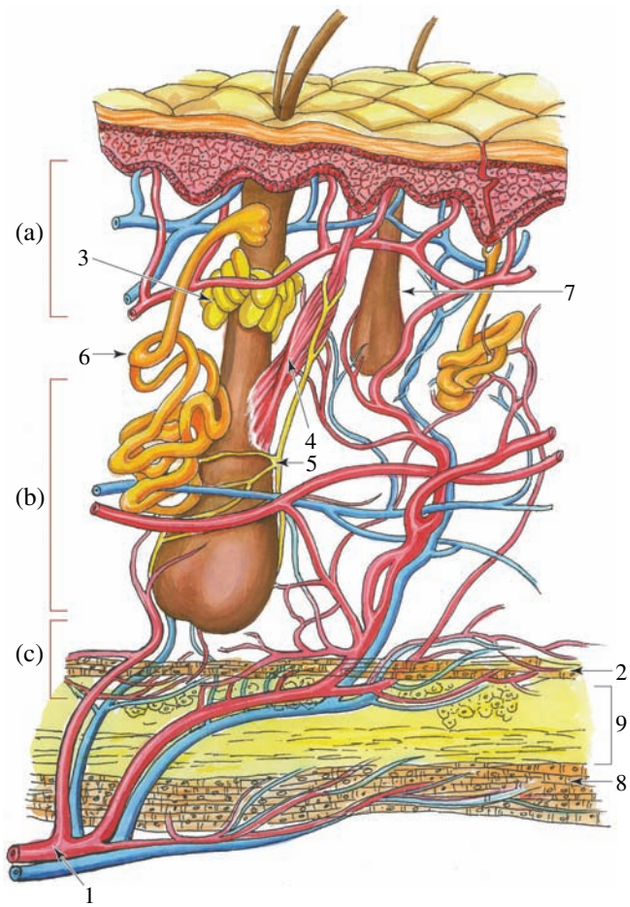

Figure 1.15 Cutaneus circulation in the dog and cat (schematic from neck region). (a) Superficial (papillary) vascular plexus. (b) Middle (or cutaneus) vascular plexus. (c) Deep (or subdermal) vascular plexus. 1, Terminal branches of the direct cutaneus vessels at the level of the cutaneus muscle which supply the subdermal plexus; 2, Subdermal plexus (surrounding a superficial cutaneus muscle); 3, Sebaceous gland; 4. Arrector pili m; 5, Postganglionic sympathetic nerve fibers supplying the arrector pili m; 6, Apocrine sweat gland; 7, Hair follicle; 8, Deeper cutaneus muscle; 9, Panniculus adiposus.

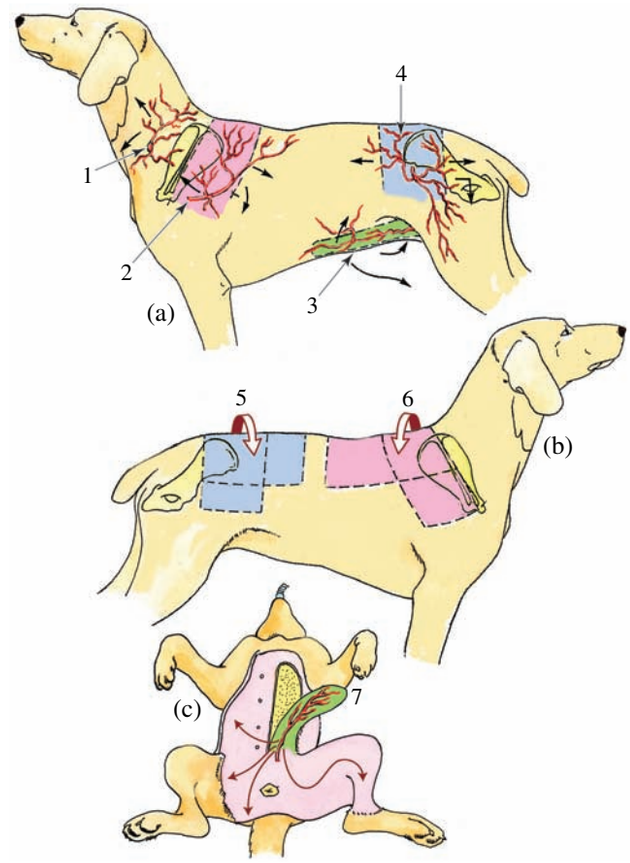

Figure 1.16 Cutaneus blood vessels are important for surgical repositioning of skin flaps. (a) Main cutaneus arteries and the extent of their vascularization: The arrows indicate the direction and the extent to which the skin flap may be moved. (b) The right side of the dog shows the flap extending from the opposite side. (c) Shows the caudal superficial epigastric axial pattern flap. 1, Prescapular branch of superficial cervical artery; 2, Thoracodorsal artery; 3, Caudal superficial epigastric artery; 4, Deep circumflex iliac artery; 5, Moving the skin from the left to right side retaining the deep circumflex iliac arterial supply; 6, Moving the skin supplied by the left thoracodorsal artery to the right side; 7, Caudal superficial epigastric axial pattern flap can be rotated into a variety of positions as indicated by the arrows. *Source:* Adapted from Pavletic [139].

arteriovenous anastomoses (AVAs) under smooth muscle control. Peripheral blood vessels exhibit numerous AVAs between arterioles and venules, which are thus located proximal to terminal capillary plexuses. The AVAs are controlled by sympathetically innervated smooth muscle sphincters. Relaxation of the AVA results in the shunting of large volumes of blood into superficial veins, resulting in radiation heat loss from the skin surface. Thus, the major function of AVAs is to aid in thermoregulation.

Footpad Differences Between the Dog and Cat: Dog footpad exhibits well-defined elongated cornified conical papillae. The epithelium is quite thin underneath these papillae, consisting of three to five cell layers. The dermis exhibits prominent dermal papillae with microvasculature units consisting of several capillaries immediately below the basement membrane, intermeshed with a few venules in the core of the papillae. The Venules form a venous plexus around footpad dermal arteries in the dog, while in the cat, the periarterial venous plexus is not observed in the core of the dermal papillae [141]. AVAs are frequently found in the dermal papillary core. In the cat, the footpad surface is smooth lacking conical papillae. The dermal papillae of the cat are also smaller than those of the dog. The cat also has fewer AVAs in the dermis, with a less complex periarterial network of veins [141]. The periarterial venous network around AVAs serves as countercurrent heat exchangers. The footpads of the cat may be more susceptible to cold injuries such as frostbite.

The cutaneus vasculature is described in terms of arteriosomes and venosomes, each arteriosome consisting of arterial network from a major artery. Similarly, venosomes are described on the venous network draining into a major vein. A three-dimensional block of tissue, consisting of the integument and underlying deep structures, supplied, and drained by specific and dominant named vessels (i.e., matched artriosome and venosome, for example, the thoracodorsal vessels over the lateral flank region) is termed an **angiosome** [142, 143]. Dogs have been shown to have a higher density of tertiary and higher branching of vessels than cats, especially in the trunk region [56, 116, 144]. This may be a reason why the granulation tissue during wound healing appears dark red in dogs while it is of a lighter pink hue in cats [145].

Blood vessels in the skin are organized into three intercommunicating vascular plexuses [88, 104, 146, 147] (Figure 1.15). Some variations in the cutaneus blood supply described herein are encountered in the ears and footpads and at mucocutaneus junctions. The general description given applies as well to the hairy parts of the skin in dogs and cats.

- The deepest **subcutaneus** (or **subdermal**) **plexus** is directly derived from numerous small cutaneus arteries encountered during dissection when the skin is reflected. This is an important vascular plexus of the subcutis that not only provides blood to overlying vascular plexuses, but also to hair bulbs and follicles, sweat glands, and their ducts, and arrector pili. Blood vessels of the subdermal plexus generally run in subcutaneus fatty tissue (panniculus adiposus) and/or the cutaneus muscles (panniculus carnosus), which are associated with the superficial fascia. Where a cutaneus muscle is present, the subdermal plexus is present on either side of the cutaneus muscle. To help preserve skin circulation during surgical manipulation, it is important to undermine the skin along the fascial plane beneath the cutaneus muscle to maintain the integrity of the subdermal plexus and any associated direct cutaneus vessels. In areas lacking a cutaneus muscle layer, undermining is directed to the hypodermal layer below the dermis to ensure the integrity of the subdermal plexus.
- The middle **(or cutaneus) plexus** is derived from secondary arteries running off the subdermal plexus, and it supplies blood to sebaceous glands and hair follicles.
- The most superficial **papillary** (or subpapillary) **plexus** is formed by blood vessels derived from the cutaneus plexus, and it supplies the dermal papillary region. Unlike humans and pigs, carnivores do not exhibit well-defined vascular loops in their dermal papillae, thereby explaining why dog skin does not usually blister in response to superficial burns.

The deep venous plexus of the dermis is located at the interface of the dermis and hypodermis, and the deep subpapillary venous plexus is located between the superficial and deep layers of the dermis. The superficial papillary venous plexus is located beneath the basement membrane [68, 144, 146, 147].

Cutaneus arteries are important for **pedicle flaps**, which are patches of epidermis and dermis that are partially detached from one site, and then grafted to an adjacent site to cover surface defects (Figure 1.16). The pedicle flaps require an intact blood supply for survival. The cutaneus anatomy and its relationship to the blood supply must be considered for safely manipulating skin as in undermining skin to facilitate wound closure or in creating skin flaps [99, 148–151]. The primary course of circulation to the skin is through the deep or subdermal plexus; direct cutaneus arteries, traveling parallel to the overlying skin, supply this vascular network.

The surgical assessment of the skin's laxity or inherent elasticity is important in determining how best to close the wound. Although the skin is elastic, fibrous components (collagen and elastic fibers) within the skin and underlying attachment of the dermis to the hypodermis contribute to pulling the skin in predetermined directions over the body. Cleavage lines of skin tension lines [152–155] (Figure 1.17) represent areas where the skin is pulled in preset tracks over

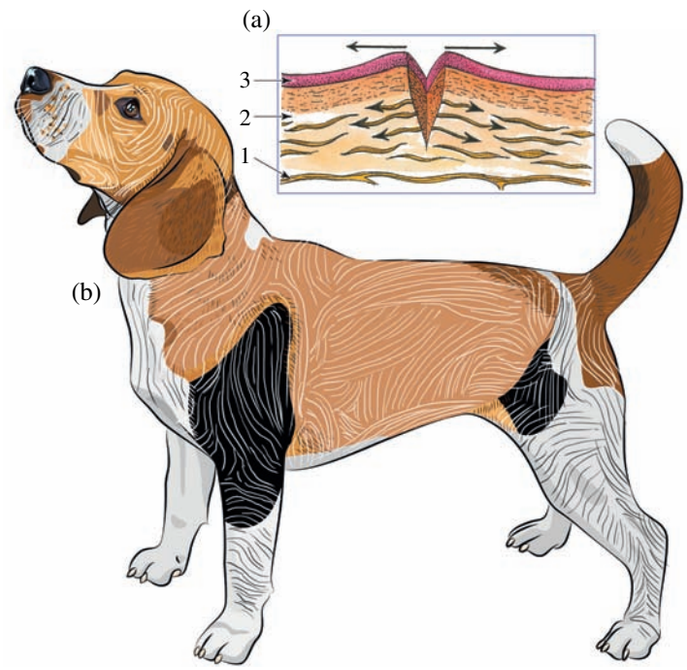

Figure 1.17 Skin tension lines in the dog. (a) Skin has a natural tendency to gape when cut due to the elastic fibers found in the dermis. (b) Tension lines indicate the orientation of the elastic fibers in the skin. 1, an intact elastic fiber; 2, severed and contracted dermal elastic fibers; 3, gaped epidermis. *Source:* Adapted from Oiki et al. [152].

the body surface and can be used as general guides to minimize tension during wound closing. As minimizing skin tension is an important consideration in proper wound closure, it is better to close wounds parallel to tension lines whenever possible. Excessive tension perpendicular to the incision can result in suture failure and wound reopening (dehiscence). Too much tension can also compromise circulation to regional skin and adjacent tissues. Furthermore, this tautness can contribute to the progressive widening of a postoperative surgical scar, thereby minimizing optimal cosmetic results.

Cutaneus Pharmacology: Intramuscular and subcutaneus injections are common means of parenteral drug administration. Subcutaneusly administered drugs may cause pain, tenderness, and local tissue necrosis, especially if the solution is alkaline. The topical application of drugs is also routinely employed in veterinary medicine. Drugs suspended in organic solvents (such as alcohol) or in an oil base tend to be absorbed through the skin, with the drugs exerting both local and systemic effects. The diffusion rate of a drug through the skin is largely determined by the compound's lipid solubility (lipophilicity). The stratum corneum possesses an increased lipid content with densely packed cells, thus creating a formidable barrier against rapid drug penetration. Therefore, the relative thickness of the stratum corneum determines the rate of penetration of even highly lipid-soluble compounds through the skin. Lipid-soluble drugs more readily penetrate the skin by diffusion through hair follicular shafts, sebaceous glands, and sweat glands. The skin of the scrotum and ventral abdomen, for example, is more permeable to lipid-soluble drugs than the skin on the back, thus requiring lesser amounts of a given drug for an equivalent effect.

Transdermal modality of drug delivery: "Transdermal" means across the skin. This method of drug delivery is achieved by the slow release of a drug from a compounded emulsion or from a patch applied on the skin. The drug diffuses across the epidermis to reach the blood vessels of the underlying dermis. The patch mode of drug delivery is popular in human medicine and is gaining acceptance among veterinarians as a preferred route of administration for certain drugs. Fentanyl, for example, is administered transdermally to both humans and animals before and after surgery to reduce pain. The fentanyl patch is applied to the skin, usually on the underside of the earflap. Transdermal drug delivery should be considered only if oral or other means of drug delivery are unavailable. In addition, since a transdermally administered drug requires a longer period to work, it may not be the desired route for drugs that are required immediately. For certain drugs, the effective endpoint may not be quantifiable to determine, whether it is delivered transdermally or not. Other disadvantages of transdermal patches include the patch falling off, getting stuck to another pet or human, or the risk of a pet swallowing it. To prevent these, a transdermal patch should usually be secured by a bandage. The stratum corneum is an excellent barrier to the passage of charged molecules and large molecules. **Iontophoresis** is a technique to facilitate the transport of charged (ionic) molecules into a tissue by passage of a direct electric charge through the electrolyte solution containing the charged therapeutic molecules [156–158]. The epidermis Despite these drawbacks, there are still several advantages to transdermal administration, including the elimination of gastrointestinal side effects, the elimination of daily pill administration, and an effective route for pain control.

Connective Tissue and Fascia

Fascia: Fascia (pl. fasciae) is an important structural component of tissues and organs found beneath the skin [159] (Figures 1.1, 1.10, and 1.12), and this term generally refers to the sheets or layers of predominantly fibrous connective tissue, of varying tensile strength, elasticity, and density, covering those structures. Fascia is the adult remnant of the leftover fetal mesenchyme after the other mesodermal structures were formed during fetal development. It quite literally holds the body together, connecting the dermis and skeletal muscles to the structural core of the skeleton [160], and investing the body cavities and internally suspended visceral organs with a continuous connective tissue covering. The fascial compartments of the skeletal muscles are estimated to account for 10–15% of myofascial force transmission on the skeleton [161]. Thus, nerves, blood vessels, muscles, and internal organs are all covered by fascia. Fascia spans from the dermis toward the skeletal muscles and deeper structures of the body and can be described separately as consisting of superficial fascia, deep fascia, skeletal muscle fascia consisting of epimysium, perimysium, and endomysium, and finally periosteum or visceral fascia covering internal organs [162]. Where the fusion between the superficial and deep fascia is extensive, as in the areas distal to the carpal region, the skin is less mobile and may be more prone to abrasions. Toward the extremities, the superficial fascia is tightly bound to the skin, with similar results, for example, at the tip of the nose. Conversely, where the skin is supple and extensively mobile, the superficial fascia is abundant and without close connections to the deep fascia. For example, in the antebrachium, the cephalic vein is loosely enveloped by superficial fascia with minimal connection to the

deep fascia. Conversely, where the superficial fa the skin is supple and extensively mobile when the superficial fascia is abundant and without close connections to the antebrachium, where the superficial fascia loosely envelops the cephalic vein. In such regions, one must take extra care to avoid hematomas following a venipuncture.

Superficial fascia (***tela subcutanea*** or subcutaneus tissue or the hypodermis) is located immediately beneath the skin. It is loose, containing numerous cellular and fibrous components, and accumulates edematous fluid under certain pathophysiologic states. The fascia consists of abundant collagenous and elastic fibers. Within the fibrous interstices of the hypodermis (Figure 1.11) are found, besides the fibroblasts/fibrocytes and other cells of the hypodermis, migrant white blood cells, mast cells, variable numbers of adipocytes [163] which, in some regions of the body (e.g., over gluteal and flank regions), organize into consistently located structural fat pads. Mast cells are of bone marrow derivation, they respond to allergies and inflammation, by releasing a cascade of biomolecules including histamine, serotonin, heparin, prostaglandins, proteolytic enzymes, and other pro-inflammatory molecules. Mast cell tumors account for 16–21% of skin tumors in dogs; and approximately 20% of skin tumors in cats [164]. The fascia is also an active pool of various progenitor stem cells that possess fibroblastic, chondrogenic [165], osteogenic, and adipogenic differentiation potential [165–168]. Fibroblasts derived from fascia play a major role in wound healing, helping to seal large open wounds [169].

A fat-filled region of superficial fascia is usually referred to as **panniculus adiposus** when it forms a continuous layer of grossly visible fat. These areas are also commonly called fat depots. They perform both a mechanical function as well as serve as nutritional reserves [122, 170]. During starvation, fat is mobilized from all subcutaneus regions, and visceral fat depots (around the heart, kidneys, intestines, and in bone marrow) simultaneously, but subcutaneus fat is depleted first. Thus, one only sees sunken eyes and bony haunches in animals experiencing the extremes of starvation.

Striated muscle fibers (**panniculus carnosus)** develop within superficial fascia in certain regions of the body. These cutaneus skeletal muscles (Figure 1.18) are well-defined, and those present in the trunk region are referred to as cutaneus trunci muscles. The cutaneus trunci muscles are quite extensive in the cat, covering the entire gluteal and femoral

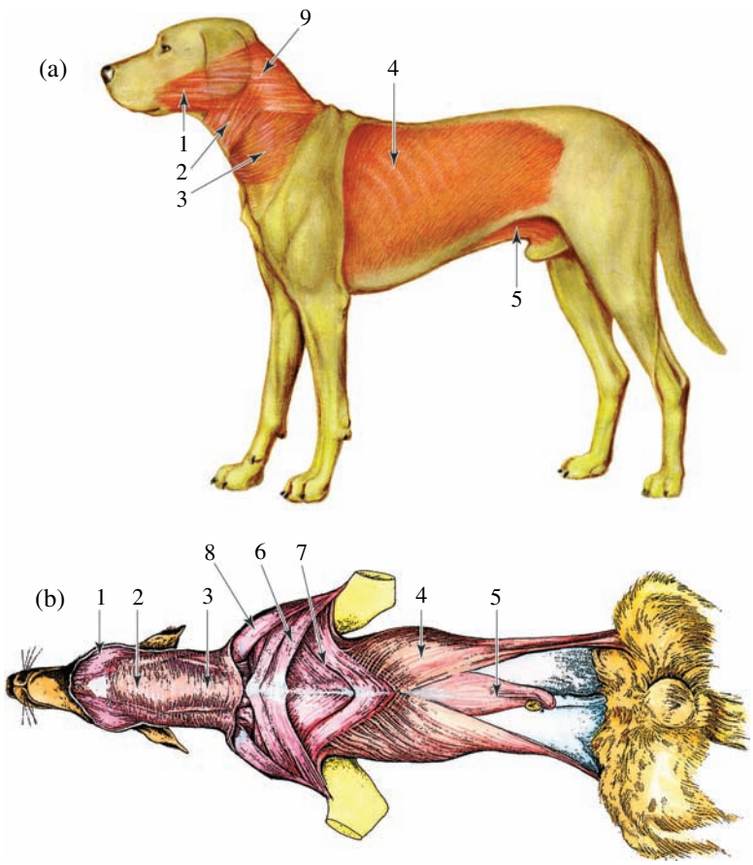

Figure 1.18 Cutaneus muscles of the dog. (a) Lateral view of a dog showing various cutaneus muscles in relation to surface landmarks. (b) Ventral view of a dog with skin removed to show cutaneus as well as some superficial skeletal muscles. 1, Facial part of platysma (cutaneus faciei m.); 2, 3, Sphincter colli superficialis m. (this muscle may not be well defined in some dogs, but better defined in the cat); 4, Cutaneus trunci m.; 5, Cranial preputial m. (in the female this muscle is the supramammarius derived from cutaneus trunci m); 6, Superficial pectoral m.; 7, Deep pectoral m.; 8, Brachiocephalicus m; 9, Platysma. *Source:* Adapted from Nickel et al. [159].

regions [159]. In both the male dog and cat, a muscular slip from the cutaneus trunci continues into the prepuce as the cranial preputial muscle (Figure 1.18). The equivalent muscle in females is the supramammary muscle, located deep within the mammary glands. Other cutaneus muscles include the platysma, and the superficial and deep sphincters of the neck (sphincter colli; not always well-defined). Cutaneus muscles play an important role in ridding the skin of irritants, such as insects, by twitching the skin (e.g., the **panniculus reflex**) [171, 172]. The cutaneus trunci muscle is innervated by the lateral thoracic nerve.

Superficial fascia, which in addition to its cellular components is made up mainly of loose areolar tissue allows the skin to move freely; its bulk, along with its adipocyte content, allows it to act as a thermal insulator. Picking up a fold of skin over the nape is normally an easy task in animals, and when the skin is released, it should readjust to its original location immediately. The skin of dehydrated dogs may take longer to fall back into place due to loss of skin elasticity.

Superficial fascia contains several cell types, and it is usually rich in lymphatics, blood vessels, and nerves (Figure 1.11). The nerves and vessels, which course within the areolar tissue of the hypodermis, can stretch without breaking due to their histologic architectures (the vessels are tortuous). Subcutaneusly injected agents are easily absorbed into the systemic circulation due to the abundant vasculature of superficial fascia. Because there are no compartments in the superficial fascia the initial "buffalo hump" noted when animals are given a large volume of subcutaneus fluid eventually dissipates and the fluid migrates to the most dependent position on the animal's body. Large veins such as the cephalic, saphenous, and external jugular are enclosed by a layer of this superficial fascia. The superficial fascia with its loosely knit fibrous structure is also continuous with the deep fascia. The loosely arrayed fibers of the elastic and mobile superficial fascia blend imperceptibly with the denser and more oriented fibers of the deep fascia. Where two leaves of deep fascia lie side by side (as can be found when one considers the **epimysia** [*pl.*], or the deep fascial envelopes, that surround two neighboring skeletal muscles) there is a transitional zone of loose areolar tissue, which permits gliding movements as muscles contract independently. These planes of areolar tissue constitute the "planes of dissection" one finds during anatomical laboratory dissections or during blunt dissection maneuvers made by a surgeon.

Superficial fascia underlying the dermis of the skin on the trunk of carnivores is not rigidly attached to the **linea alba** (the ventral median fibrous band of the abdominal wall to which the bilaterally symmetrical abdominal muscles attach) nor is it rigidly attached to the mid-dorsal periosteum and ligaments of the spinous processes of the thoracic and lumbar vertebrae, therefore allowing for greater movement of skin from one side to the other. Superficial fascia is scarce over the ears, where the skin is tightly attached to underlying cartilage, and over the muzzle, where the superficial fascia fuses the epimysium of facial muscles to the overlying skin. It is also sparse toward the extremities, yet abundant over ventral regions of the body where the skin is more movable [88].

Deep fascia is composed of densely arranged collagenous fibers that form distinct sheets (Figure 1.13). It is well-developed over the thoracolumbar (also referred to as thoracodorsal), gluteal, femoral, crural, and antebrachial regions; it surrounds all muscles, and it also extends as intermuscular septa between certain essentially fused muscles [159] (e.g., the lumbar portions of the iliocostalis and longissimus muscles). Some of the extrinsic muscles of the limbs (e.g., latissimus dorsi), trunk (e.g., abdominal muscles), and back (epaxial muscles) arise from the internal aspects of the deep fascial envelopes surrounding them (Figure 1.13). The deep fascial layers between the trunk and limb muscles continue inward, fusing with the fibrous layer of the periosteum, thus providing additional resistance to the pull of attached muscles. The deep fascial envelopes of the limb muscles, loosely connected by fibers of the intervening areolar tissue, which blends from one deep fascial leaf to the next, provide for individual muscle separation, which assists in reducing friction when adjacent muscles are contracting/relaxing. These fascial envelopes also increase the surface area available for muscle attachment. In some regions of the body (e.g., thoracolumbar region), the deep fascia is directly continuous with flat tendinous attachments of trunk muscles, such as the latissimus dorsi and the oblique abdominal muscles.

Since deep fascia is often layered into multiple laminae, for ease of description it can, itself, be further subdivided into **superficial** and **deep** layers. Adipose tissue, within the bridging areolar tissue, is usually present between layers of deep fascia [173] (Figure 1.13). For example, the thoracolumbar deep fascia is attached to the supraspinous ligament and the spinous processes of the thoracic and lumbar vertebrae. It divides into a superficial layer that is practically the aponeurosis of the latissimus dorsi muscle and deeper layers that provide attachments to the serratus dorsalis cranialis, serratus dorsalis caudalis and the external and internal abdominal oblique muscles. An even deeper lamina, which is continuous with the fascia covering the epaxial muscles, arises from the transverse processes of the lumbar vertebrae and serves as the origin of the transverse abdominis muscle. In addition to providing attachments to these muscles, a thin layer of areolar tissue is found between layers of trunk muscles where it provides a gliding surface for muscle contraction.

Skeletal Muscle

Organization of Skeletal Muscle: Skeletal muscle normally accounts for about 50% of body weight (57% in greyhounds and 44% in other dogs [174]). These muscle fibers can be considered specialized organs, containing not only muscle tissue, but also nerve endings, blood vessels, and connective tissue. The basic functional unit of skeletal muscle is the muscle fiber (or cell). Each muscle fiber is loosely invested with a random arrangement of collagen fibrils (**endomysium**) to allow for movement during contraction.

Groups of muscle fibers form a **muscle fascicle**, with a thin connective tissue layer, the **perimysium**, surrounding each muscle fascicle [2]. The multisheet-layered perimysium runs transversely to fibers and holds groups of fibers in place. Several muscle fascicles are bundled together by a connective tissue capsule known as the **epimysium**. The epimysium extends inside each muscle and is continuous with the perimysium and endomysium. It is also continuous with the intermuscular connective tissue septa formed by deep fascia. Orthopedic surgeons often make use of intermuscular connective tissue planes to separate adjacent muscles, thus gaining access to bone (Figure 1.1).

Intramuscular connective tissue components account for 1–10% of skeletal muscle, of which the endomysial content can vary between 0.5% and 1.2% of muscle dry weight, and the perimysium can account for between 0.4% and 4.8%. This relatively small variation between muscles in endomysial versus perimysial connective tissue content has been taken to indicate that these differences may dictate their functional differences. Intermuscular connective tissue may play a role in force transmission [175].

Some muscle fibers run the entire length of the muscle fascicle, and these can be up to 20 cm in length. However, most are much shorter with tapered ends that overlap adjacent fibers and are bound together by connective tissue fibers of the endomysium. Tendons extend the endomysium at the ends of individual muscle fibers, forming bundles of parallel fibers, which are composed mainly of collagen, the most abundant protein in the body.

Each skeletal muscle fiber has several nuclei located immediately beneath the cell membrane (**sarcolemma**). Approximately 80% of the cytoplasm (**sarcoplasm**) is occupied by myofibrils and mitochondria. Each myofibril, in turn, is composed of numerous myofilaments [176]. Myofilaments are contractile proteins organized parallel to the long axis of the muscle fiber, and each is made up of numerous actin (thin) and myosin (thick) filaments [1–3].

Nerve Supply to Muscle: Skeletal muscles are associated with both afferent/sensory and efferent/motor nerves. Any nerve terminating inside a muscle cell may carry α and γ motor fibers, which innervate extrafusal and intrafusal fibers, respectively. Postganglionic sympathetic nerve fibers supply unitary smooth muscles of blood vessels, particularly arterioles [177, 178]. Visceral afferent nerves carry sensory information from blood vessels while general somatic afferent nerve fibers provide sensory information from neuromuscular spindles and tendons. Encapsulated and sensory receptors associated with muscle tendons may constitute up to 40–50% of nerve fibers supplying skeletal muscle [179]. These sensory fibers are proprioceptive, and they are concerned with muscle reflex control (such as maintaining muscle tone and coordinated contraction of different muscle groups). Visceral sensory fibers arise from larger blood vessels within skeletal muscles, which travel with the larger nerves that innervate the skeletal muscles. These fibers play a role in local vascular reflexes and pain perception.

The distribution of nerve branches within a muscle follows a fixed pattern, and in general, nerves enter a muscle where it is least mobile. Such an arrangement protects against accidental stretching of nerves when the muscle contracts. Once a nerve enters a muscle, it repeatedly branches along connective tissue planes within. In a fusiform muscle (such as the extensor carpi radialis), major nerve fibers run along the length of the muscle to reach individual muscle fibers. Nerves may run transverse to fibers in a wide muscle [180] (such as the serratus ventralis).

Blood Supply to Muscle: In general, blood vessels and lymphatics accompany the nerve supply to a muscle. The blood supply to a particular muscle may not be as specific as its nerve supply. In addition, the branching pattern of blood vessels varies between muscles; therefore, injury to a given blood vessel may damage one muscle more seriously than the other muscle fibers of the same group. Because of their high oxygen and nutrient demands, muscles depend on a rich blood supply to function properly [181].

Collateral blood supply is a common muscle feature, but due to heavy metabolic demands, major arterial vascular supplies should not be interrupted during surgical procedures since collateral blood supplies will be insufficient in preventing muscle damage. In general, blood and nerve branches are bundled together in **neurovascular pedicles** as they enter a muscle at or close to its attachments. This makes it easy to preserve them during surgical procedures, especially during orthopedic or muscle flap procedures. Complete interruption of blood supply to a muscle will result in **gangrene** (death) of the muscle. Partial blockage of blood supply (**ischemia**) may result in extreme muscle pain due to metabolite build-up (namely H^+ ions) [182].

Each muscle has a characteristic blood supply pattern; the most common is a single set of vessels entering the upper end, with another set at the middle. An additional set of blood vessels is usually seen supplying the muscle at the musculotendinous intersection. Trunk muscles are generally supplied by a series of minor blood vessels, in addition to one or two major vessels. Vascular supplies to these muscles usually tend to be segmental in origin from major blood vessels. Once blood vessels enter a muscle, their branches spread parallel to the long axis of muscle fascicles. These branches pierce the perimysium, forming a capillary network surrounding each muscle fiber that is supported by endomysial fibers (Figure 1.19).

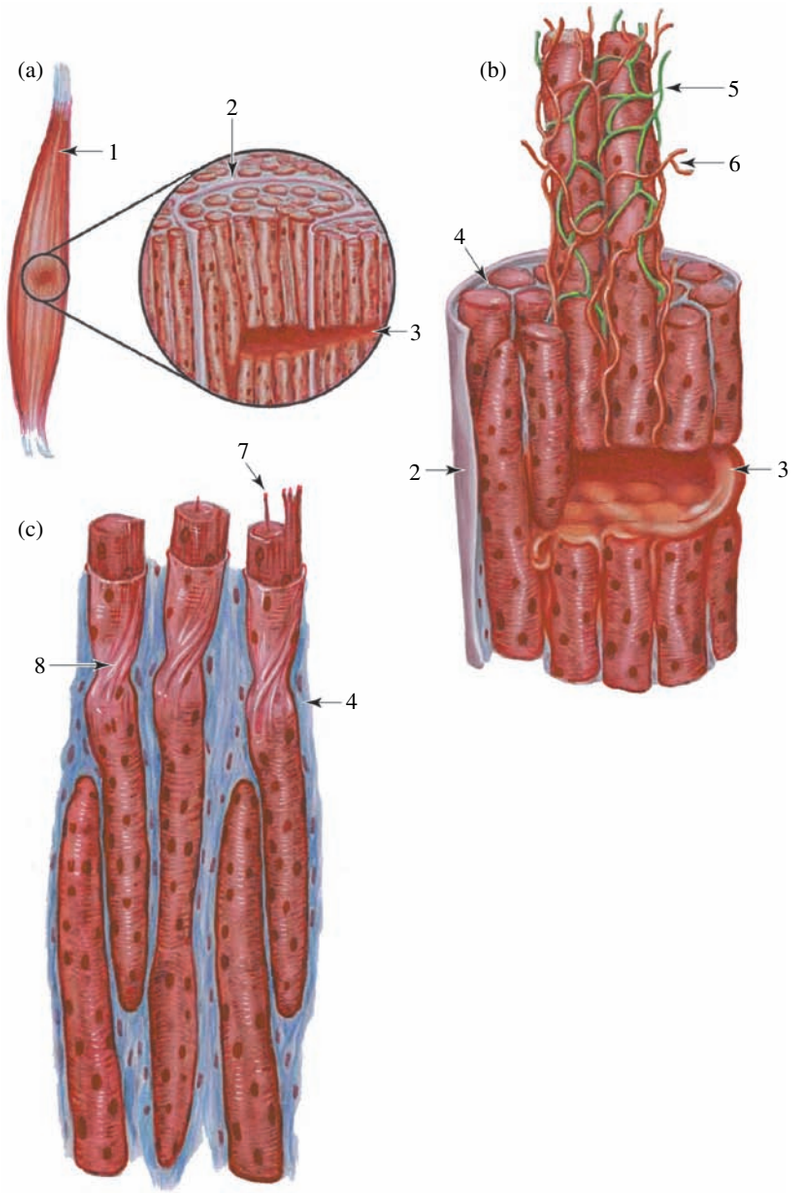

Figure 1.19 Muscle damage and repair. (a) Injury resulting in muscle fiber disruption and interstitial hemorrhage between disrupted muscle fibers. (b) Higher magnification of injury to a muscle. Note the rich blood and lymph capillary plexus surrounding each muscle fiber. The capillaries are sufficiently tortuous to permit their accommodation to changes in length of the fibers. Blood clot formation disrupts blood flow within the muscle fascicle bundled by the perimysium. Resulting inflammation within the fiber bundle contributes to congestion within the perimysium. The fascial compartment overlying the bruised region may appear tight and painful. (c) Muscle may also suffer from crush injuries. In this case, the sarcolemma is not disrupted, and local repair may occur by synthesis of contractile proteins. If the injury disrupts the sarcolemma, regeneration does not occur and the area is invaded by fibroblasts and fibrous scar tissue is formed; 1, Muscle; 2, Perimysium; 3, Hemorrhage; 4, Endomysium; 5, Lymph capillaries; 6, Blood capillaries; 7, Muscle fibril; 8, Crush injury to muscle fibers showing sarcolemma.

Lymphatics originate from lymphatic capillaries surrounding muscle fibers, and they carry away fluid and substances (e.g., proteins) that are too large to enter blood capillary beds. Due to high muscle vascularity, most drugs administered intramuscularly are absorbed more rapidly into the circulation than following subcutaneus injection.

Denervation: When a skeletal muscle loses its motor nerve supply, it also loses its contractile stimulus and fibers gradually degenerate and are replaced by connective tissue. However, for a short period following the loss of motor innervation, muscle exhibits heightened sensitivity to Acetylcholine (**denervation hypersensitivity**), and a period of slight twitching (**fibrillation**) ensues that eventually dissipates. Fibrillation is thought to be due to spontaneous rhythmic discharge within the muscle a few days following denervation. Muscle degeneration can be reversed over a period of months if nerve fibers succeed in reinnervating. Electrical stimulation and muscle massage have been shown to increase blood supply and slow degenerative muscle changes. Once reinnervated, the muscle can gradually regain strength. It is also believed that satellite cells found among muscle fibers can form new muscle cells.

Skeletal Muscle Trauma and Regeneration

Muscle injuries often occur due to over-stretching (**strain**), resulting in the rupture of muscle fibers, and external trauma may also result in the rupture of muscle fibers (**contusion**). These injuries may also result in localized bleeding and edema, due to the rich network of capillaries around individual muscle fibers (Figure 1.19). If the endomysial sheathing and sarcolemma are intact, the muscle fiber regenerates by replenishing myofibrils (Figure 1.19). Bleeding inside muscle is also of clinical importance since blood clots and fluid accumulation may disrupt blood flow (Figure 1.19). Due to the nature of connective tissue sheathing around groups of muscle fibers (perimysial compartments), increased interstitial fluid accumulation may cause pressure occlusion of the incoming blood supply leading to muscle necrosis (compartmental syndrome). If damage to muscle fibers is extensive, dead cells are replaced by connective tissue (**scarring**).

Replacement of muscle tissue with fibrous tissue following injury may result in **muscle contracture**. This type of condition most frequently involves the infraspinatus and quadriceps muscles. Severe strain and irreversible damage of the infraspinatus can occur in hunting dogs, resulting in infraspinatus contracture. Quadriceps muscle injuries following distal femoral fractures or extended limb immobilization following surgery may result in quadriceps contracture, or tie-down, in young dogs. Muscle contracture lesions also occur less commonly in the gracilis and semitendinosus of German shepherd dogs and Belgian shepherds.

Skeletal Muscle Stem Cells: Skeletal muscle fibers are prone to the constant stresses of exercise, weight-bearing, and trauma. Because they are generally considered to be irreversibly post-mitotic, skeletal myofibers require an everlasting source of cells for muscle repair and regeneration (Figure 1.20). Damaged, dysfunctional muscle cells are removed by apoptosis, and new muscle cells may form from satellite cells. Skeletal muscle stem cells, or **satellite cells**, are a pool of undifferentiated reserve cells considered to be the primary source for replacing damaged muscle fibers following injury [184–186]. These satellite cells are set aside underneath the basal lamina of each muscle fiber during fetal development, and function as a major source of myogenic cells crucial to postnatal muscle repair. Only a small population of satellite cells seems to serve as myogenic stem cells [187, 188]. Undifferentiated satellite cells are normally dormant; however, following muscle injury, some satellite cells be stimulated to proliferate, by reentering the cell cycle, then differentiate into myoblasts, and finally join with each other to form multinucleated myotubes. These newly formed myotubes can then fuse with a part of an injured myofiber that survived the initial trauma. Although signals triggering activation and proliferation of satellite cells have not been fully identified, many growth factors and cytokines have been shown to influence the proliferation, differentiation, and fusion of myogenic precursor cells *in vitro*. A variable number of satellite cells are observed within each muscle throughout life, as their number as well as their ability to renew muscle fibers, decreases with age. The number of divisions that satellite cells are capable of appears to be limited [184, 187, 188].

There are believed to be at least two major populations of satellite cells in mature skeletal muscle: (i) committed satellite cells, which differentiate immediately to myoblasts after muscle injury, and (ii) stem satellite cells, which undergo cell division before differentiation [168]. Muscle stem cells remain in a homeostatic or regenerative status regulated by secreted factors and by direct cell–cell contact between stem cells and muscle fibers [189]. Stem satellite cells are capable of replenishing new satellite cells for possible future needs. Additionally, under appropriate conditions, some of these cells are multipotent, exhibiting abilities to differentiate not only into myogenic lineages, but also different mesenchymal cell lineages, and even neural or endothelial cells. Denervation results in muscle atrophy. Satellite cells proliferate within three months

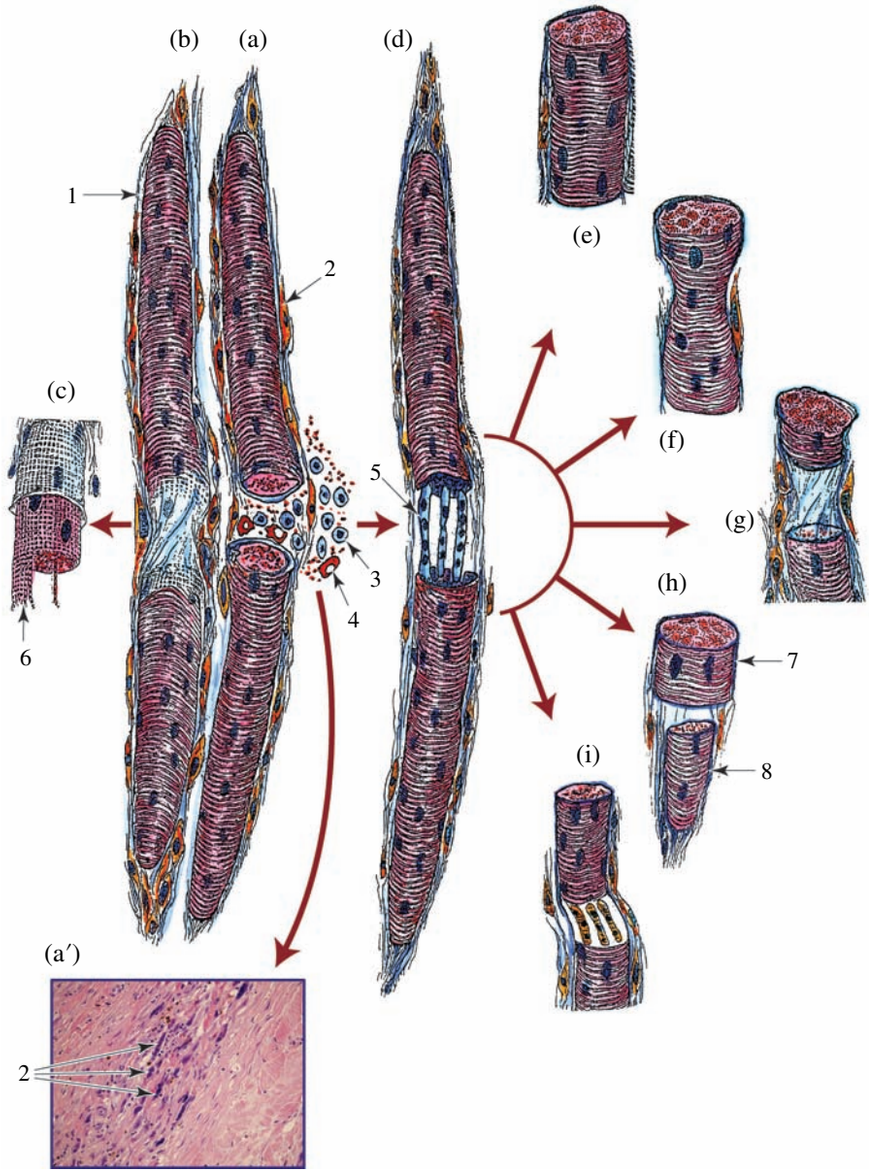

Figure 1.20 Schematic illustration of skeletal muscle fiber regeneration after segmental necrosis and its possible sequelae. (a) Injury to a muscle fiber – during the early phase of regeneration macrophages invade to clean up tissue debris (a′). Satellite cells transform into myoblasts; (b) If the sarcolemma is intact the muscle fiber may regenerate by internal repair – which includes synthesis of myofibrils (c). (d) Later stage of regeneration – myoblasts align themselves end-to-end and fuse to form myotubes. The myotubes now occupy the necrotic segment of the muscle fiber, and a number of possible paths from this stage are schematically indicated; (e) Completely successful restoration of normal fiber caliber. (f) The Regenerated segment is of a smaller caliber than the rest of the fiber. (g) Failure of regeneration results in an empty basement membrane sleeve, which may subsequently scar. (h) Multiple independent fibers due to lack of fusion of the myotubes with the surviving fiber stump. (i) Forked fibers due to incomplete lateral fusion of myotubes. 1, Basal lamina (endomysium); 2, Satellite cell; 3, Macrophage; 4, Myoblast; 5, Myotube; 6, Myofibrils; 7, Surviving stump; 8, Independent regenerated fiber. *Source:* Adapted from Schematically illustrated based on data from Carpenter and Karpati [183].

after denervation [190]. Immune cells infiltrate muscle injury sites and release several cytokines, which play a crucial role in facilitating muscle repair and regeneration [191, 192]. In addition, recent research indicates that injured muscles can release several cytokines that are important in myogenic differentiation [193]. However, muscle stem cell therapy for muscle or tissue repair (to date) is still in its infancy [194].

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