Principles of Histology

(14)

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A. Introduction

In order to understand the interactions between living and non-living systems, we must know something about cell, tissues, and organs - their properties, structures, and how they are studied. The science of examining the gross structures of living systems is called anatomy. The examination of tissues on the microscopic level is termed histology; histology is the microscopic analog of anatomy. It is the metallography of living systems.

The basic unit of structure in crystalline materials is the grain or crystallite - in living systems it is the cell. Though grains and cells are roughly the same size, they are obviously greatly different in complexity, structure, and methods of examination. The cell can be a complete living organism, such as a protozoa; a single crystal can be a complete functioning unit. For our purposes, the cells will be part of a different level of organization, called a tissue. A tissue is generally considered to consist of a group of similar cells working together to perform some role or function in the living system. Tissues, then, consist of cells with a fluid or gel-like material between the cells, often called extracellular or intercellular (outside or between the cells) fluid.

Tissues are generally classified into four fundamental categories: epithelial, connective, muscular, and nervous. These will be considered in more detailllater. These tissues and their subtypes can often be traced to their embryologic origins.

Roughly two to four weeks (in the human case) after fertilization of the ovum (female egg cell), after it is firmly lodged in and attached to the wall of the uterus, a disc consisting of three distinct cell layers becomes evident. It is called the germ disc. The cell layess are the entoderm (endoderm), the mesoderm, and the ectoderm. These embryologic tissues are the origin of all body tissues. Figure 1 is a diagram showing how the final tissue types are related to the germ tissues. The terms presented in Figure 1 are often used in histology and physiology texts. How the final tissues are derived from the embryologic structures is the subject of courses in embryology and development and cannot be considered here.*

The techniques and procedures used in preparing tissues for microscopic examination are discussed in all histology texts. With the exception of direct microscopic examination of living tissues or cultures, histology is usually done on dead, dried, fixed (crosslinked or otherwise treated to minimize changes in shape or structure), stained and static specimens. The living cell is none of these. Living cells can be examined directly in a suitable chamber with an optical microscope. Time lapse studies can be readily done, which leads to a totally different view of the cell than one sees with conventional histological specimens.

The following quote is from Paul Weiss, one of the real experts on cells:

*See, for example, J. Langman, Medical Embryology, 2nd ed., Williams and Wilkins, 1969.

"To one brought up in the classical tradition of static microscopic anatomy, the first exposure to the living cell is a shattering experience, as all his solid groundings in morphological definition and structural fixity melt and are swept away. Instead of a clearly delineated portrait of cellular architecture, grooved in his memory by the stereotypes of fixed dead specimens and their textbook effigies, he is confronted with an object in constant change, never the same from moment to moment, unceasingly stirring and churning, every momentary configuration but a fleeting event. Particles inside the cell keep milling around like crowds of people in a market place. The contour of the free cell likewise changes continually with the buffetings, thrusts, and recessions of the interior."

The slides illustrated some of the various stains commonly used and the features observed. One of the most common general stains is hematoxylin and eosin (H & E). Eosin is an acidic dye and stains acidophilic structures. Hematoxylin is a basic dye and stains basophilic structures. A wide variety of staining solutions are available to detect different tissue features.

The scanning electron microscope (SEM) is now commonly used to study cells and tissues. Generally the samples are fixed, dehydrated, and critically point dried to minimize sample distortion. Frozen samples can be examined directly and freeze-etch experiments performed. There has even been some work on examining fully wet samples (unfrozen) directly in the SEM using a modified electron detector.

B. The Cell

The cell consists of an aqueous, gel-like fluid, called protoplasm enclosed by a structure called the cell membrane. Protoplasm is roughly 75% water, containing dissolved macromolecules, lipids, salts, and many other solutes. Most cells contain a nucleus, partly membrane-separated from the rest of the cell interior. A wide variety of subcellular structures or organelles are also present, performing a large number of specialized jobs. The endoplasmic reticulum and ribosomes are assiciated with protein synthesis. The mitochondria are involved with cell metabolic activity and energy generation and storage. The Golgi apparatus is involved with cell secretory processes. Filamentary or fibrilar structures are present which are apparently involved in cell motion and shape. Lysosomes function as digestive systems for the cell.

The properties of the cell membrane are very important in understanding the interactions between cells and between a cell and its extracellular environment, including implants. Unfortunately we do not have time to discuss cell membrane properties in this course. They are discussed in detail in MSE 753 - Surface Properties of Biological Systems.

Some of the general functions of cells are irritability - the capacity to respond to stimuli; electrical conductivity and membrane potentials; ability to change shape; respiration; absorption and secretion; and growth and reproduction.

We must be primarily concerned with the general functions and properties of the cell rather than its detailed makeup. The various cell types will be discussed as we discuss the fundamental tissue types.

*See R. G. Kessel and C. Y. Shih, Scanning Electron Microscopy in Biology: A Student's Atlas, Springer-Verlag, 1974.

C. The Cellular Environment

The cell undergoes a continual dynamic interchange with its environment. The intercellular material consists of a fluid matrix often containing fibrous structures. The intercellular material often provides the major strength of a tissue, it is the medium by which cells communicate, it is the diffusion medium. The intercellular material may be a fluid, such as blood, or it may be a somewhat structured fibrous and gelatinous material - often called ground substance. The ground substance may consist of fibers in a gel-like matrix or may be relatively fiber-free.

The fibrous material consists of one or more of three types of fibers - collagenous, reticular, or elastic - in a gel-like matrix. The amorphous material consists of little or no fiber component. The gel-like material contains a relatively high polysaccharide concentration as well as proteins, ions and other species.

The collagenous fibers (and probably the reticular) consist of 1-12 micron diameter bundles of smaller fibrils. The fibrils in turn are bundles of 0.3 - 0.5 micron diameter microfibrils with diameters of 200 - 1000 A. The microfibrils are made up of a staggered array of tropocollagen protein molecules, each molecule made up of a triple chainccoiled helix (See Figure 2). The association of tropocollagen molecules is called collagen. The structure of elastic fibers is dependent on the protein elastin.

It is believed that the fibers are formed extracellularly in close assiciation with cells called fibroblasts. Fibroblasts will be discussed more later.

The intercellular material is the transport vehicle between the cell and the nearby blood vessels.

D. Fundamental Tissue Types (Figure 3).

1. Epithelium - Epithelium (Figure 4) is one of the four basic types of tissue. It is the tissue generally covering all free body surfaces, including the skin, organ linings, and the linings of most body cavities. It is essentially the interfacial transition between tissue and non-tissue mediums. The cells in epithelial tissue are called epithelial cells. These cells are usually contiguous with little intercellular material. The cells usually rest on an extracellular layer called the basement membrane. The basement membrane is in essence the substrate on which epithelium exists. The basement membrane is always associated with connective tissue and depends on the connective tissue for its survival. Epithelial tissue is usually non-vascular - it contains no blood vessels or lymphatics. Thus it must depend entirely on diffusion for nutrition and communication with the rest of the organism. Epithelial tissue then must be relatively thin, or the diffusion barrier would be too great for a viable tissue to exist. The tissue consists of single or multiple cell layers, but is rarely more than a few cell layers thick.

The major functions of epithelial tissue are protection, absorption, and secretion. Some of the slides illustrated several of these functions.

We usually consider three types of epithelial tissue, largely based on cell shape:

1. Squamous or flat epithelium - examples are the inner surfaces (intimas) of vessels, the air sacs of the lung.

2. Cuboidal - many of the epithelia involved in absorption or secretion.

3. Columnar - intestinal epithelium.

If the tissue consists of only one of the above types as a single cell layer, it is called simple epithelium. Epithelia consisting of multiple cell layers are usually called stratified (See Figure 4).

Epithelial tissue is thin and relatively non-porous and is thus membranous in nature. It is greatly involved in wound healing processes and is very important in some aspects of foreign body reaction. The free surfaces of epithelium are often highly specialized and may contain structures such as cilia (hair-like structures which aid in mass movement) or villi (structures which increase epithelial surface area for absorptive or secretive functions).

2. Ordinary Connective Tissue (Figure 5)
The major functions of connective tissue are support and binding - other functions are transport, storage, protection, and repair.

Connective tissue is usually highly fibrilar, consisting of one or more of the three types of fibers already discussed in ground substance. It is usually a very strong tissue, particularly when the fibers are aligned and the tissue is highly structured.

Some of the more specialized types of connective tissue include cartilage, tendon and bone.

The most common cell type in general connective tissue is the fibroblast - a very versatile and diversified cell. Its extensive endoplasmic reticulum attests to its major function - the synthesis and deposition of collagen. It can orient along lines of stress thus providing for an oriented tissue. Its fibril production is thought to be regulated by the local oxygen concentration. The fibroblast is usually relatively fixed or immobile but is capable of slow, gliding movements.

Figure 3 shows the various sub-classes of connective tissue, all usually derived from the mesodermal mesenchyme.

Ordinary loose or areolar (from areola, meaning a small space) connective tissue (Figure 5) is perhaps closest in structure to the embryonic mesenchymal tissue from which it came. It is a relatively loose, open tissue, consisting of a large amount of intercellular material containing relatively few cells and, generally, unoriented fibers.

Loose connective tissue is the chief packing or filling tissue in the adult and the prototype of all connective tissue. It is a loosely organized, fibroelastic material encountered in nearly every histological section of the body. Collagen fibers are the most prominent. Elastic fibers tend to form a continuous network but are often difficult to see. Regions of ground substance are present wherein no structure is evident. The loose, open structure permits rapid diffusion and even allows cell movement through the tissue. This is very important in wound healing.

Connective tissue is vascular. Capillaries are generally present, especially near the basement membranes.

The most common cell (Figure 6) is the fibroblast - the second most common cell in connective tissue is the macrophage (also called monocyte or histiocyte). The macrophage is nearly as abundant as fibroblasts and can exist as a fixed or a mobile cell. Immobile macrophages look very similar to fibroblasts. When an immobile macrophage is stimulated, it can become detached and is then free to wander. Wandering macrophages may vary in size and shape. Their major role is to ingest unneeded particulate matter by phagocytosis, which distinguishes them from fibroblasts. Macrophages are scavengers for extravascular blood, dead cell, bacteria, and foreign bodies. The ingested material is attached and destroyed, where possible. If confronted with an object too large to be ingested, many macrophages (up to 80-90) may actually coalesce to form a multinucleated cell, often called a foreign body giant cell.

Fat or adipose cells (Figure 6) are often present. These are large spherical cells consisting of a large vacuole of fatty material surrounded by a thin film of cytoplasm. Its primary function is storage of food.

Mast cells (Figure 6) are also widely distributed in connective tissue. They tend to concentrate near small blood vessels. Their exact role is unknown, though they do produce heparin and other mucopolysaccharides, serotonin, and histamine - these compounds are important in inflammatory reactions and wound healing.

A number of other cell types are present in relatively low numbers. These are generally leucocyte (white cell)-related (Figure 7). Neutrophils can pass between the epithelial cells of the capillary walls to phagocytize extravascular bacteria and other foreign material. Eosinophils are also present in connective tissue and are apparently involved in the ingestion and destruction of foreign matter.

Lymphocytes are formed in the lymph nodes and are found in small amounts in blood. Lymphocytes are small, free cells in connective tissue, usually 7 - 8 microns in diameter. They tend to concentrate near inflamed areas.

A relatively uncommon connective tissue cell is the plasma cell, though it is found in large numbers in areas of inflammation, usually with lymphocytes. Plasma cells may just be mature lymphocytes. These cells are probably involved in immunologic processes.

If fat cells are the principle cell type, the tissue is called adipose or fat tissue.

Ordinary connective tissue can also exist in a denser form, wherein the fibers are somewhat closely packed. Dense connective tissue has fewer cells and more fibers than the loose type. If the fibers are highly uniaxial and the morphology is quite regular, the tissue is dense regular connective tissue. Examples are tendons and ligaments. Dense irregular connective tissue is found in organ capsules, bone sheaths, the dermis of the skin, and other areas. The irregular tissue is usually biaxially oriented and thus sheetlike in nature.

3. Muscle Tissue

Muscle tissue is specialized for producing movement. Muscle cells have a contractile character and are very long and thin; muscle cells are often called muscle fiber. The muscle cells (fibers) are arranged in bundles, which are bound to each other by connective tissue. The connective tissue also provides the blood vessels which provide the high food and oxygen requirements of the muscle.

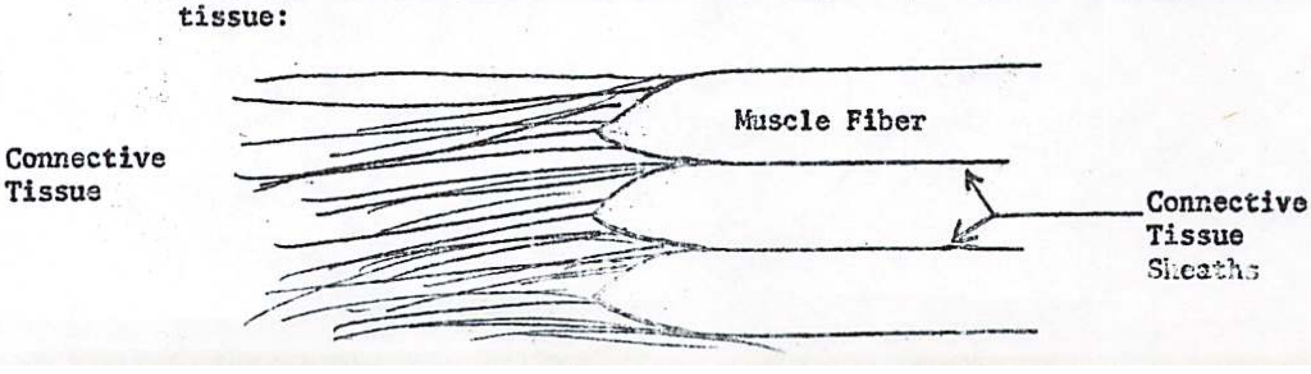
Muscle tissue can be divided into three types, based on their structural and functional characteristics:

Smooth muscle contains no stripes or striations. It is an involuntary muscle, meaning it is not under the organism's conscious voluntary control. Smooth muscle forms the contractile portion of the wall of the digestive tract. It is also found in vessels, in the dermis, and in many other areas. The cell (fiber) length may vary from 20 to 500 microns; 200 x 6 microns is common for the intestinal tract and large blood vessels. Smooth muscle cells in many regions are closely associated with connective tissue. It is often difficult to distinguish between smooth muscle and connective tissue.

Striated Muscle is what we normally call muscle - the meat of animals. It is a richly vascular tissue. Each individual muscle cell is long, cylindrical, and multi-nucleated - about 35 nuclei per millimeter of cell (fiber) length. The cytoplasm contains alternating dark and light bands or striations, hence the name striated muscle. The cytoplasm of the muscle cell contains myofibrils, which are bundles of fine filaments. It is banding in these fibers that give striated muscle its striated appearance. The multi-nucleated cells (fibers) of striated muscle is in contrast to smooth muscles, where the cells have only one nucleus. The general structure of striated skeletal muscle is given in Figures 8 and 9. The cells (fibers) are packed into bundles, which are the anatomical structural unit of skeletal muscle (the fascicles). The individual cells or fibers, the fascicles (muscle cell bundles), and the entire muscle are enveloped in connective tissue sheaths called endomysium, perimysium, and epimysium, respectively. Figure 8 clearly shows the perimysium and epimysium; the endomysium is invisible. The connective tissue serves to bind the muscle fibers and bundles (fascicles) together and to provide the rich vascular supply of the muscle. Each muscle cell is surrounded by fine capillaries which are readily accomodated to changes in length due to muscle contractions and expansion. The more connective tissue between the fibers, generally the tougher themmeat. Filet,mignon, for example, has very little connective tissue.

The muscle fibers may vary from 10 to greater than 100 microns in diameter. They may run the entire length of the muscle or may terminate in connective tissue somewhere within the muscle.

At the ends of the muscle, the connective tissue surrounding each muscle fiber blends into the connective tissue of the tendon, whose connective tissue in turn blends into the collagen fibers of bone. There are no sharp interfaces at the bone-tendon-muscle junctions. It is continuous. The muscle fibers fit like fingers in a glove of connective tissue:



Car Cardiac (heart) muscle is a specialized type of striated muscle. Unlike skeletal muscle, it is involuntary. The cardiac muscle fibers are composed of separate cells joined end-to-end at special junctions called intercalated disks, which are transverse to the longitutinal axis of the fiber. The fibers are not simple cylinders, but branch and connect to form a 3-D network. The disks may be continuous across the entire fiber, but more often it is divided into offset segments. The myofilaments are probably identical to those in skeletal muscle - they do not cross the intercalated discs. Specialized cardiac muscle exists in the electrical conducting systems of the heart - the nodes and Purkinje fibers. Connective tissue is not prominent in cardiac muscle. it does extend between all fibers, binding them together and forming a find capillary network.

4. Specialized Connective Tissue - Cartilage

A. Introduction. Cartilage and bone are the high strength tissues of the body whose major functions are support and bearing. Both have high fiber (collagen) contents in the intercellular substance. Cartilage also contains sufficient sulfated mucopolysaccharides to produce a firm rubber-like intercellular substance. Thus the surface of cartilage can take a high polish and has excellent lubricity. Nature uses cartilage as the wear and lubrication surface of articulating (moveable) joints. It provides an interface of low friction. Only in a few areas such as joints, however, does cartilage remain as cartilage. More generally cartilage is the precursor to bone. Cartilage generally tends to calcify or mineralize, thus serving as a model for the formation of bone.

B. Hyaline Cartilage. There are 3 major types of cartilage: Hyaline, elastic, and fiber. We will discuss only the hyaline type.

Hyaline is the most common type of cartilage, forming joint linings and being the major cartilage type in the nose, trachea, etc. The fetal skeleton is largely hyaline cartilage, which is later replaced by bone. Several slides illustrated the cellularity of hyaline cartilage. It appears translucent and pearl white and glossy-like to the eye. Hyaline is from the Greek hyalos, meaning glass. Cartilage cells are called chondrocytes, present in spaces in a relatively rigid intercellular matrix.- the spaces are called lacunae. Hyaline cartilage is surrounded by a connective tissue membrane called the perichondrium. The outer part of the perichondrium is dense connective tissue while the inner part merges into cartilage. The perichondrium is thus the transitcon surface zone. At surfaces where cartilage is interfaces with cartilage, with bone, and with other connective tissue, the perichondrium may not be present.

Cartilage develops from embryonic cells which differentiate to become chondroblasts. Mature chondroblasts are chondrocytes. The chondrocytes can divide and multiply, thus cartilage can grow. Cartilage can expand from within - this is called interstitial growth, and is limited to young, non-rigid cartilage. The perichondrial fibroblasts can also multiply and differentiate to chondroblasts, which then surround themselves with an intercellular substance. Thus a new cartilage layer is formed directly under the perichondrium. This type of growth is from the surface out - and is called appositional growth.

More on cartilage later when we discuss bone.

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7. J.A. Schilling, "Wound Healing," Physiological Reviews, 48 (1968) 374.

8. The chapter on wound healing and inflammation in any surgery or pathology textbook.

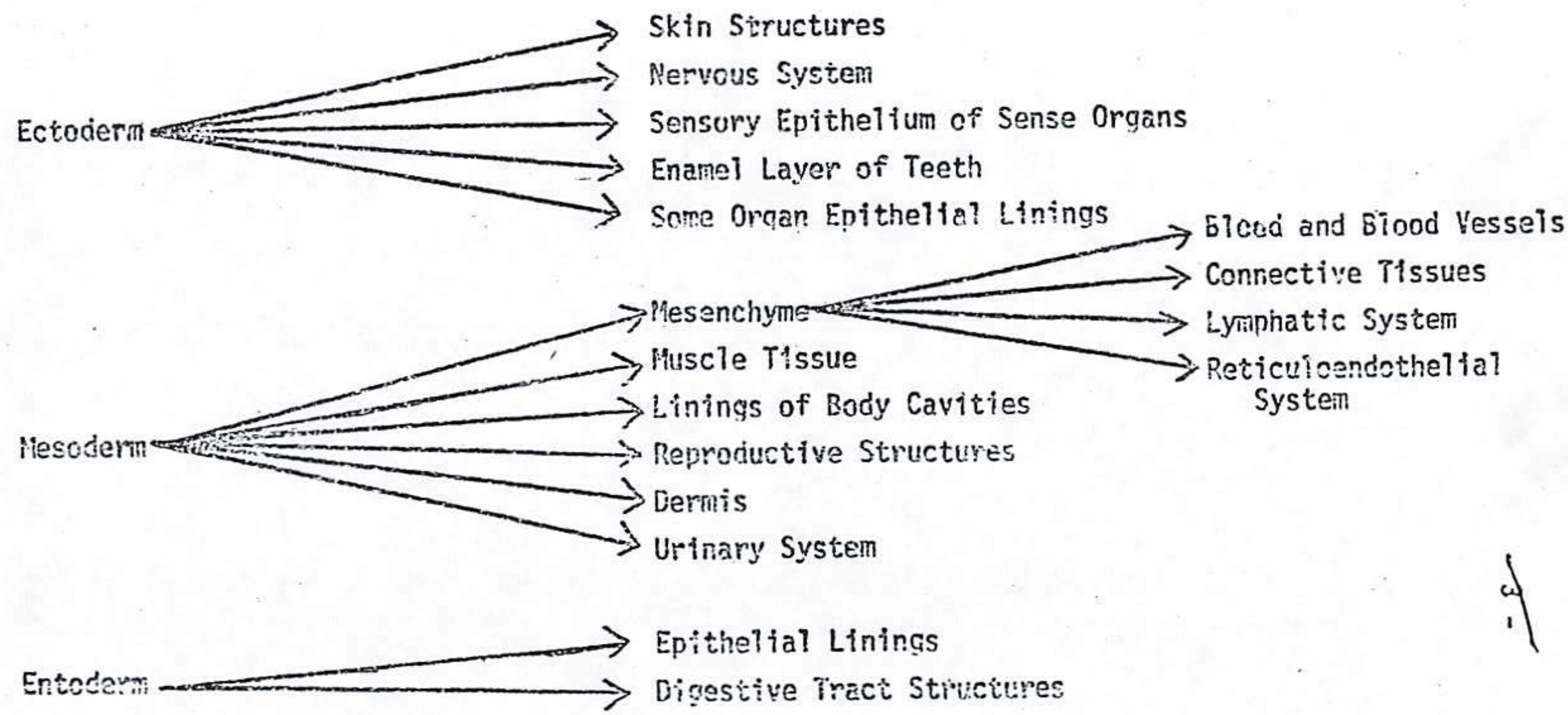


Figure 1. Relationship of Body Tissues to Their Embryologic Origins

from A. W. Ham, Histology, 5th ed, Lippincott, 1965, p. 166

A Morphologic Classification of the Tissues

1. Epithelial tissue
   - covering and lining membrane division
     - simple
       - squamous
       - cuboidal
       - columnar
         - ciliated
         - nonciliated
     - pseudostratified-columnar
       - ciliated
       - nonciliated
     - stratified
       - squamous
         - keratinizing
         - nonkeratinizing
       - cuboidal
       - columnar
       - transitional
   - glandular division
     - endocrine
       - cord and clump type
       - follicular type
     - exocrine
       - simple
         - tubular
         - alveolar
       - compound
         - tubular
         - alveolar
         - tubulo-alveolar
2. Connective tissue
   - Ordinary
     - loose—adipose
     - dense
       - regularly arranged
       - irregularly arranged
   - Special
     - hemopoietic (blood cell forming)
       - lymphatic
         - nonencapsulated
         - encapsulated
           - lymph nodes
           - spleen
           - thymus
       - myeloid
         - red
         - yellow
     - supporting
       - cartilage
         - hyaline
         - fibro
         - elastic
       - bone
         - immature
         - mature
           - cancellous
           - compact
3. Muscular tissue
   - smooth (involuntary)
   - striated (voluntary)
   - striated involuntary (cardiac)
4. Nervous tissue
   - of central nervous system
     - gray matter
     - white matter
   - of peripheral nervous system
     - nerves
     - ganglia
     - nerve endings

Figure 3: Fundamental Tissue Types

Connective Tissues

Tissue	Reticular fibers	Collagenous fibers	Elastic fibers	Ground substance	Fixed cells	Free cells
Loose areolar	++	+++	++	+++	++	+++
Dense collagenous	(±)	+++++	(±)	+	+	(±)
Dense elastic	++	+	+++++	++	+	(±)
Mucous	(±)	+++	(±)	+++++	++	(±)
Reticular	++++	+	+	++	+++	+++++

R. O. Greep, ed., Histology, 2nd ed, McGraw-Hill,

Fig. 5

Figure 5. Diagram of a section through subcutaneous, loose areolar connective tissue. (1) Collagenous fiber. (2) Elastic fiber. (3) Lymphocyte. (4) Monocyte. (5) Macrophage. (6) Fibroblasts. (7) Mast cell. (8) Undifferentiated mesenchymal cell. (9) Plasma cell. (10) Capillary. (11) Fat cell.

Fig. 4

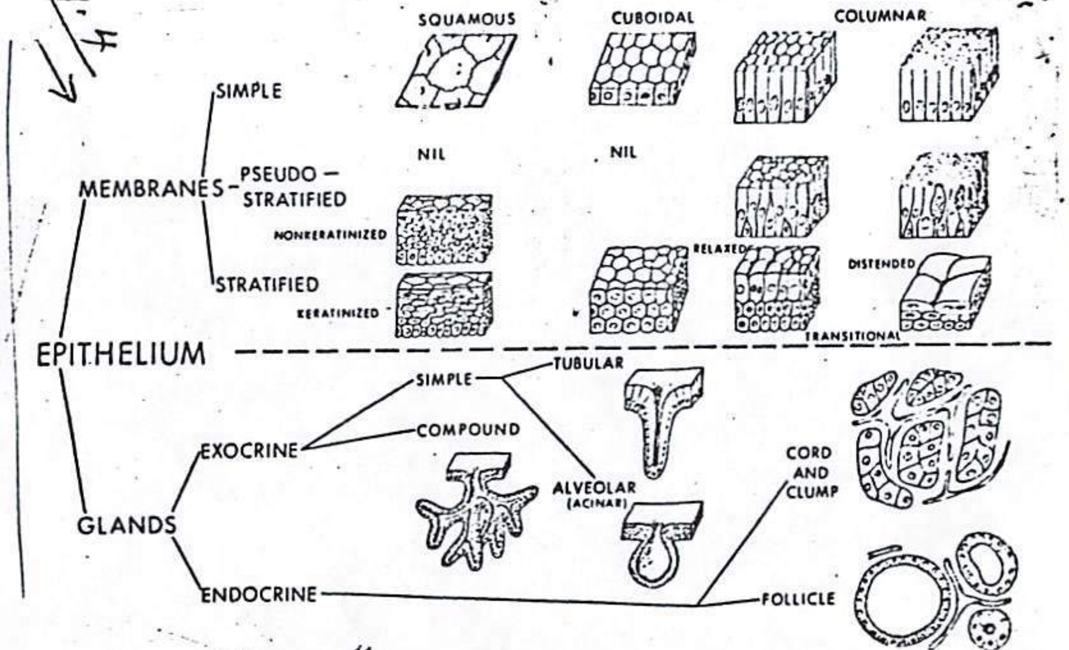


Figure 4. Diagram of the classification of epithelia.

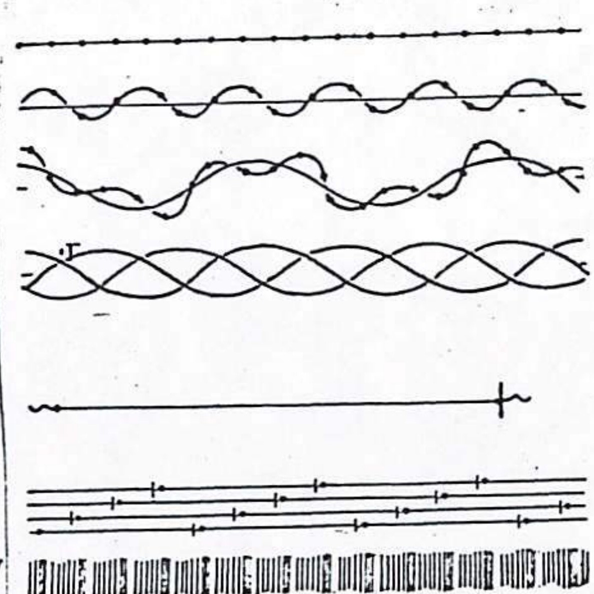
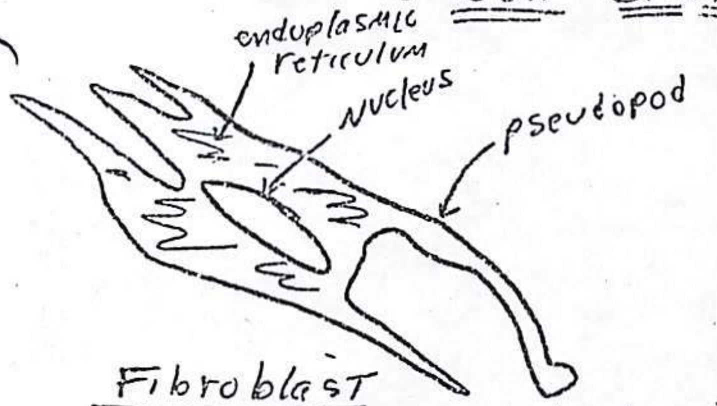


Fig. 2

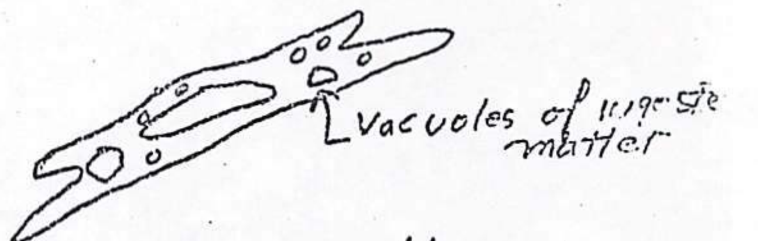
Figure 2.

FIGURE 6

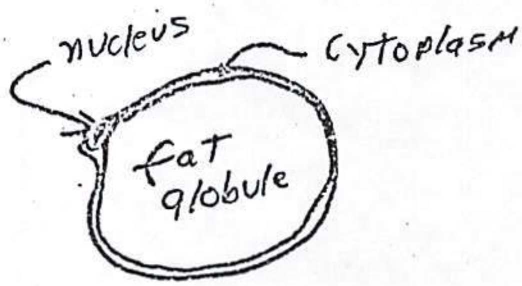
Cell Sketches (not to scale)



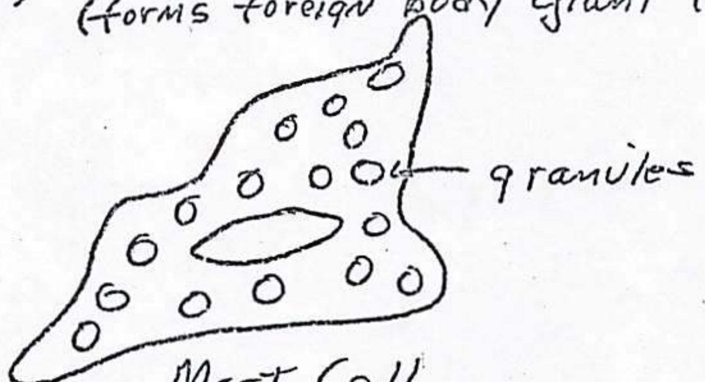
Fibroblast
(synthesis & deposition of collagen)



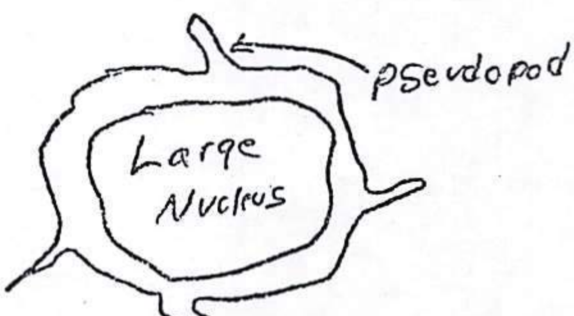
Macrophage or Monocyte
(phagocytosis — scavenging)
(forms foreign body giant cell



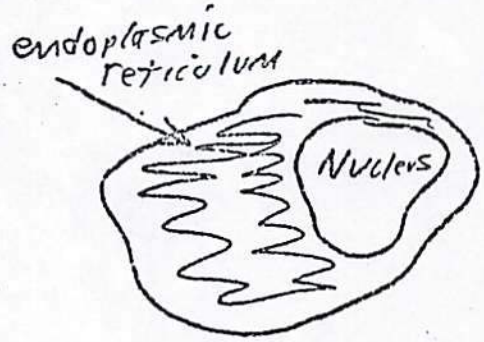
Adipose (fat) Cell
(lipid storage)



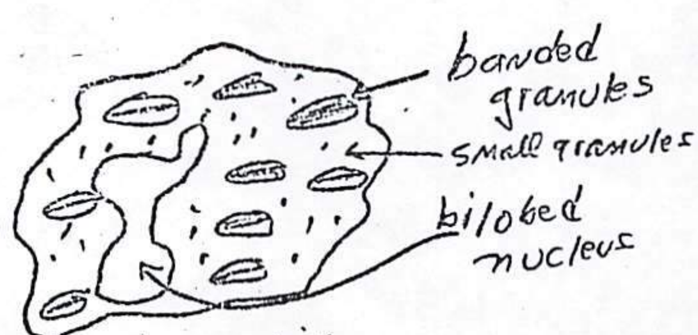
Mast Cell
(produces heparin & histamine)



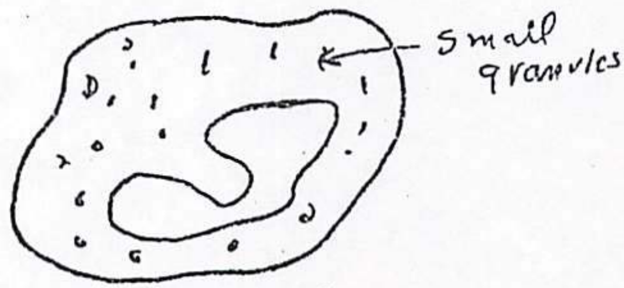
Lymphocyte
(immunological activity and/or precursor)



Plasma Cell
(immunologically active)



Eosinophil
(allergic effects and ?)



Neutrophil
(phagocytosis)

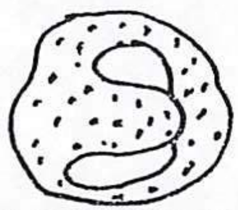
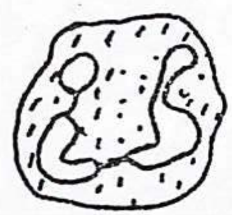
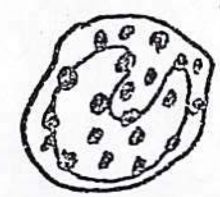
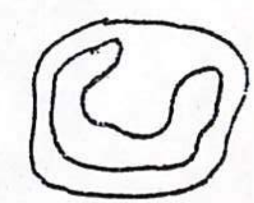
FIGURE 7

White Blood Cells — Leukocytes

Leukocytes → Granular; Nongranular

Granular → Eosinophils, Neutrophils, Basophils

Nongranular → Lymphocytes, Monocytes

	Eosinophils	Neutrophils	Basophils	Lymphocytes	Monocytes
Percentage of all White cells in blood	1-3 %	60-70 %	0.5 %	20-30 %	3-8 %
Diameter (microns)	10-15	10-12	10-12	Most about 5 some " 7 a few up to 12	12-15 (up to 20 in blood smear)
FUNCTION	Not well known. May liberate histamine — some phagocytosis	Phagocytosis	? Granules contain histamine & heparin	Some immunologic activity — may be precursor to plasma cells	Phagocytosis. Known as Macrophage in tissues
Sketch					
Remarks	2 lobed nucleus	2-5 lobed nucleus	Most of cell is nucleus — which is irregular in shape and often obscured by dark granules	Most of cell is nucleus	Kidney-shaped nucleus. A young cell which reaches full development after leaving blood & enters tissues.

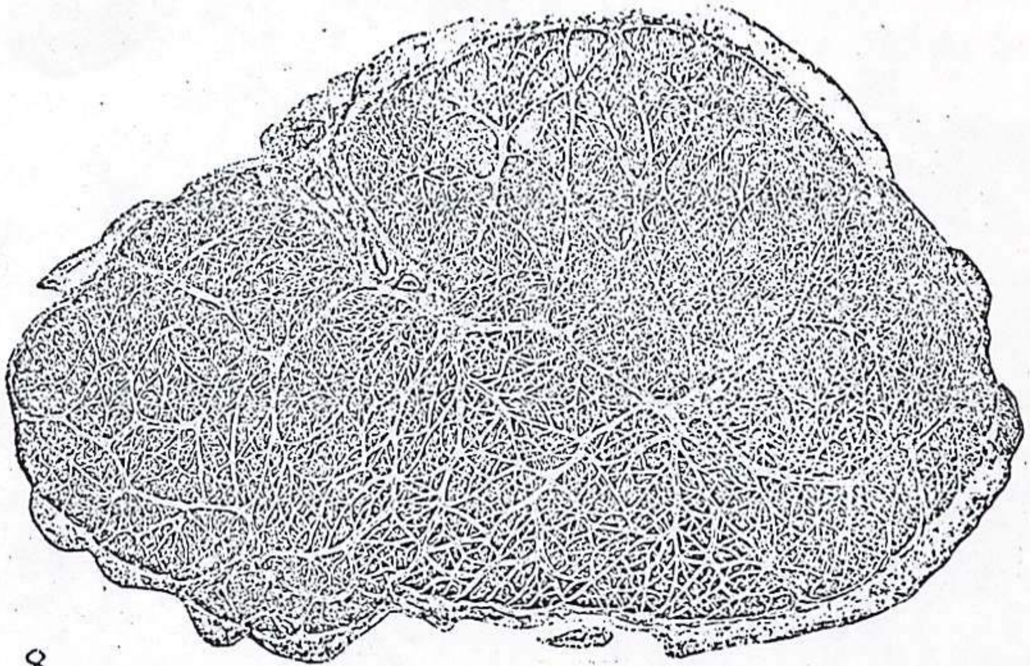


Figure 8. Cross section through human sartorius muscle, showing the subdivision into bundles of various sizes by connective tissue. × 4. (Photograph by Müller, from Heidenhain.)

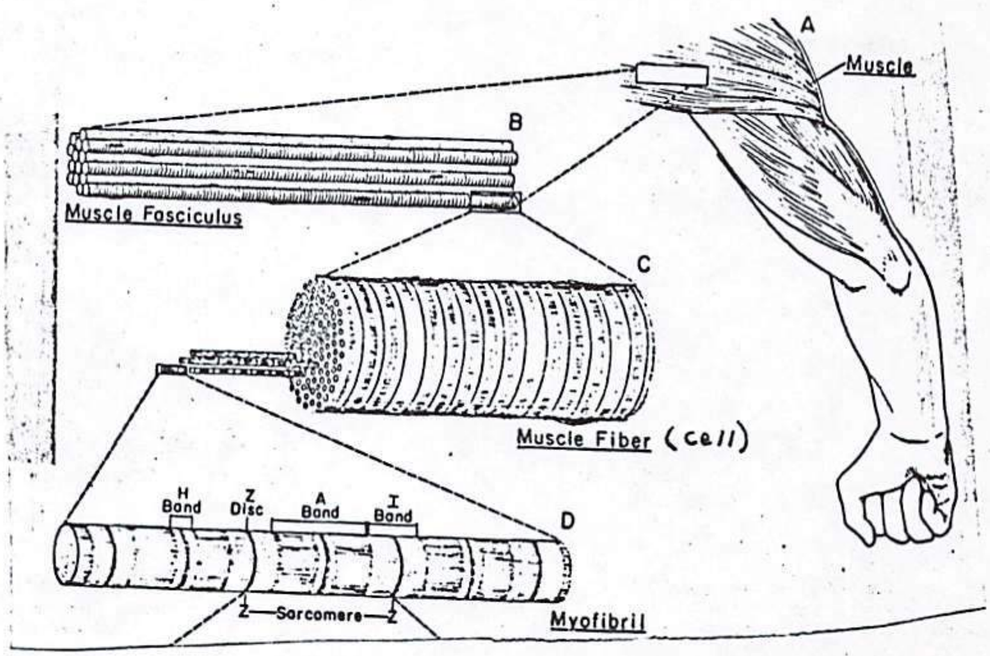


Figure 9.