Manual of Gynecological Exfoliative Cytology

United States Naval Medical School
National Naval Medical Center
Bethesda, Maryland

MANUAL

FOR

GYNECOLOGICAL EXFOLIATIVE CYTOLOGY

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PREFACE

Cytology and cytopathology have become important tools of the diagnostic armamentarium. Because of their importance the number of cytologic tests is increasing beyond the temporal capabilities of most laboratories. Additional trained personnel are required to provide this service. In response to this need, training in exfoliative cytology is now included in the curriculum of the course for laboratory technicians at the U. S. Naval Medical School.

This manual is not intended as a substitute for the excellent atlases and texts in the field but rather as a supplement for a specialized course in exfoliative cytology. It was developed to serve as an introductory text and work book for the didactic phase of the program which is followed by a period of approximately seven months of supervised screening of actual slides.

In military laboratories, the predominance of cytology requests arise in examination of the female genitalia. Consequently, the subject matter of the course and of this associated manual is limited primarily to the consideration of gynecologic material.

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CHAPTER I

CELL STRUCTURE AND VARIATION

INTRODUCTION

PROTOPLASM is the general term for the substance found in living forms which performs the functions characteristic of life. In complex animals, such as man, protoplasm is organized into structural and functional units called cells.

CYTOLOGY is the study of the structure and functioning of cells. The study of any aspect of this subject logically may begin with a consideration of the structural organization, the activities and the variations that occur among cells.

STRUCTURAL ORGANIZATION OF PROTOPLASM WITHIN THE CELL

A generalized diagram showing the structural organization of the cell as determined through electron microscopy is shown in Fig. 1 (page 3). In most cells, two regions of the protoplasm are distinguished, namely, the cytoplasm and the nucleoplasm or nucleus. These two regions of the protoplasm have several general components: membranes and a colorless semi-fluid matrix which contains a variety of suspended materials and structures. From the structural viewpoint, the cytoplasm differs from the nucleus mainly in the arrangement of the membranes and in the composition of the suspended materials and structures. These differences in structure are related to the differences in the specialized functions of these two areas of the protoplasm.

THE CYTOPLASM is the outer region of the protoplasm. In mature cells it generally comprises the major portion of the cell. Most of the processes involved in the maintenance of the life of protoplasm are localized in the cytoplasm. In addition, it is the center of the specialized activities of the cell. For example, the secretions produced by gland cells are formed in cytoplasmic structures and the contraction of muscle cells is accomplished by the activity of specialized cytoplasmic fibrils.

THE CELL MEMBRANE is the outer porous layer of the cytoplasm which regulates the movement of materials into and out of the cell. As shown in Fig. 1, the cell membrane may be continuous with an elaborate system of convoluted membranes which form a series of canals through the cytoplasm. This system of membranes is called the endoplasmic reticulum. It is thought that the membranes of this system provide surfaces on which certain biochemical reactions occur and the canals provide pathways along which materials are transported within the cytoplasm.

THE CYTOPLASMIC MATRIX is the colorless fluid-gel complex distributed between the membranes of the endoplasmic reticulum. Through it materials seep or are actively moved. During the process of slide preparation, this material is usually precipitated and, consequently, in fixed and stained cells, it may appear coarsely or finely granular depending upon the nature of the fixation process.

ORGANELLES are tiny variously-shaped structures which are suspended in the cytoplasmic matrix. These organelles are not generally observed in cells fixed and stained by routine laboratory procedures and examined with the compound light microscope. However, knowledge of their presence in the cell is basic to an understanding of the complexity of the structural organization of the cytoplasm. From observations made with the electron microscope, the details of structure of several types of organelles have been described. Furthermore, recent experimental work has shown that each organelle performs a particular function within the cell. The following are three examples of cytoplasmic organelles:

THE CENTRIOLES (usually two) are cylindrically-shaped structures which are located at right angles to each other. They are found just outside the nucleus as shown in Fig. 1. The centrioles become active when the cell begins to undergo division (Fig. 2). At this time they separate and move to opposite poles in the cell. It is thought that they are associated with the formation of the fibrillar structure, called the spindle, which plays an important role in the division of the nucleus.

THE MITOCHRONDRIA (numerous) are distributed throughout the cytoplasmic matrix. In many cells they appear to accumulate at the site of greatest cellular activity. Mitochrondria are membranous sacs. They contain the enzymes which function in the release of energy from food materials. Consequently, they have been called the "power plants" of the cell.

THE GOLGI APPARATUS is another organelle that has been variously described. While there is some doubt concerning the details of its structure as well as its function, there is evidence accumulating that the Golgi apparatus plays a role in the secretory activities of the cell.

INCLUSIONS are a variety of materials that are observed in some cells. They may include substances formed by the cell, e.g., food substances and pigment granules, or they may be microorganisms that have been engulfed by the cell, e.g., bacteria.

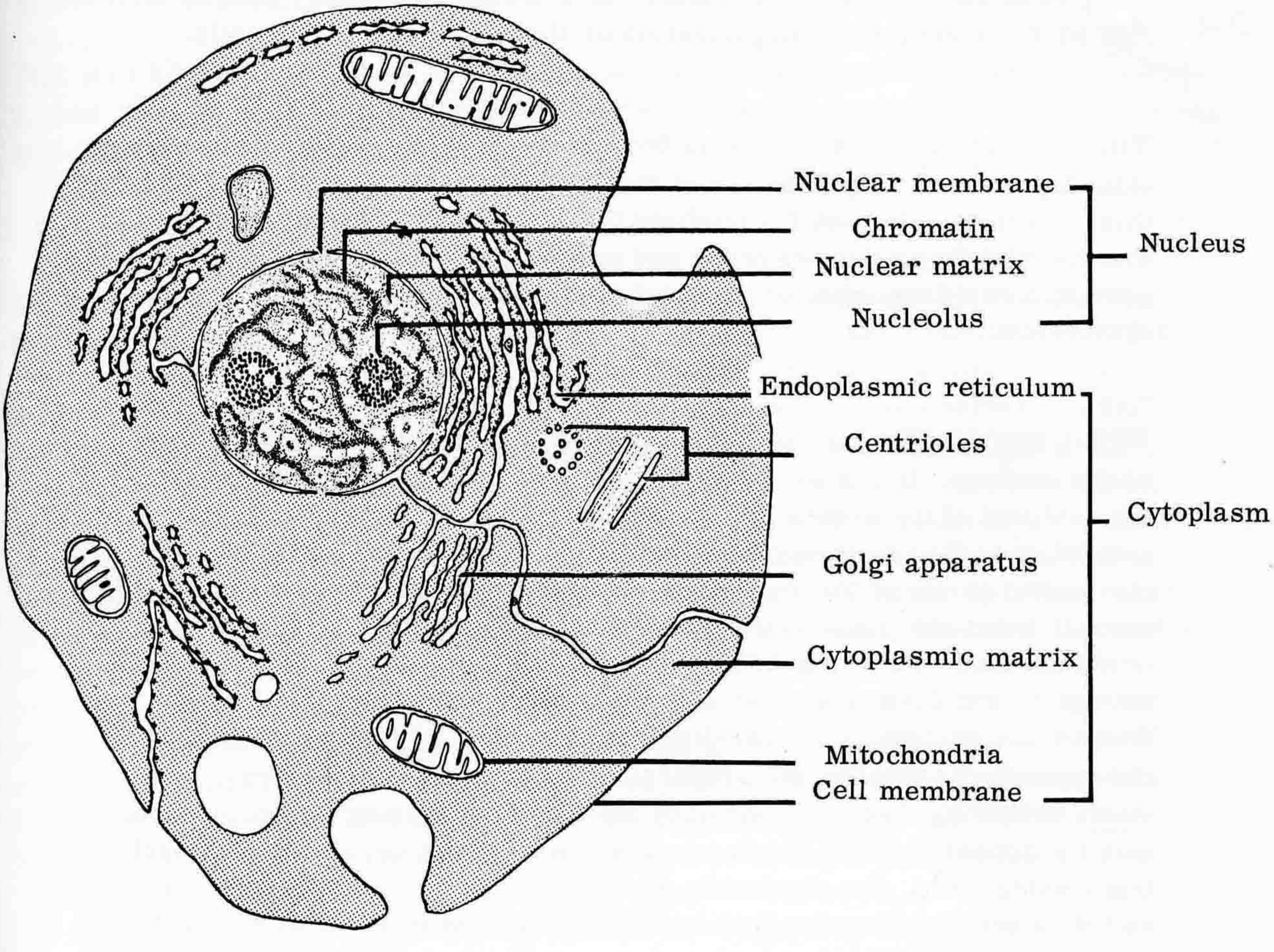

Fig. 1. Diagram showing details of cell structure

THE NUCLEOPLASM (KARYOPLASM) or NUCLEUS is the region of the protoplasm exclusive of the cytoplasm. It contains the genes (hereditary material) that control all of the activities of the cell and that determine the characteristics of the organism. The characteristics of the nucleus are most important in the identification of malignant cells. Consequently, in order to recognize such deviant cells, it is essential that the student of exfoliative cytology become thoroughly familiar with the structural organization of the nuclei of normal cells.

THE NUCLEAR MEMBRANE is the envelope which encloses the nucleus. With high power magnification of the light microscope, it appears as the thin, outer boundary of the nucleus. The electron microscope reveals this membrane as dual-layered and porous. It is thought that the pores permit a rapid transfer of materials between the nucleoplasm and the cytoplasm.

THE CHROMATIN may be considered the most important constituent of the nucleus. It contains most of the hereditary material (genes) of the cell and of the organism. Recent work has shown that the vital activities of the cell are controlled by genes. The appearance of the chromatin is one of the most important criteria used in distinguishing normal from abnormal cells. Consequently, the student is urged to study carefully its normal range of variability so that he can readily recognize the deviations that indicate an abnormality.[1] The appearance of the chromatin in non-dividing cells may vary depending upon the procedures used in the preparation of the slide. In general, however, following routine laboratory fixation and staining techniques, it usually appears bluish-black in color. In growing cells and in actively functioning cells, the chromatin appears finely or coarsely granular and it is usually more or less evenly distributed throughout the nucleus. When the nucleus divides in the process of mitosis, the chromatin substance is equally distributed to the two daughter nuclei in a complicated series of activities involving the formation and division of structures called chromosomes (Page 7).

THE NUCLEOLUS (Pl. NUCLEOLI) is a small, spherical body within the nucleus. The nucleoli are generally more evident in younger than in

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1. Characteristics of abnormal cells will be discussed in detail in Chapters VII and VIII.

older cells. Their function is not definitely known. It is significant, however, to note that there is a size and a number of nucleoli that is characteristic for particular types of cells. Some cells may have a single nucleolus while others may typically have two or three. Increased numbers and/or an enlargement in the size of the nucleoli may be considered as indicative of abnormality in cells.

THE NUCLEAR MATRIX or NUCLEAR SAP is the colorless semi-fluid ground substance of the nucleus in which the chromatin material and the nucleoli are suspended.

ACTIVITIES CHARACTERISTIC OF LIVING CELLS

METABOLISM refers to the numerous biochemical reactions involved in the maintenance of the life of the protoplasm. Essentially, these reactions involve the manipulation of food and many may occur simultaneously within the cell. In general, the cells of higher animals require food, water and a supply of oxygen for the maintenance of life. It is through the metabolic processes that these materials are utilized in sustaining the life of the protoplasm. From the food substances (e.g., carbohydrates, fats and proteins) energy is derived, protoplasmic structures are maintained, repaired or produced and the substances necessary for the normal functioning of the cell (e.g., enzymes and vitamines) are formed. Water, an important component of protoplasm, provides the medium in which the metabolic reactions occur and, in many cases, is a reactant (one of the materials used) in certain processes. Oxygen is necessary for the major reactions involved in the release of energy from the food materials. The energy, in turn, is required for all of these metabolic processes to take place. It is important to note that all of these reactions are collectively called metabolism. They may be considered in two phases.

ANABOLISM may be defined as all the metabolic processes in which complex substances are formed from simpler ones. All food substances enter the cell in a comparatively simple, soluble form. Within the protoplasm, through anabolic processes, some of these simple compounds are converted into complex molecules which may be stored within the cell or may be converted into protoplasm.

CATABOLISM may be defined as all the metabolic processes in which complex molecules are broken down into simpler ones. For example a complex carbohydrate such as glycogen which has been stored within the cell may be broken down into simple sugars such as glucose. Some of these simple sugar molecules may be used as a source of energy for the protoplasm and, in a series of reactions including oxidations, be further broken down into carbon dioxide and water. Accompanying this breakdown of sugar is the release of the energy necessary to sustain the protoplasm. Other simple sugar molecules may be moved out of the cell to other parts of the organism. Still others may immediately become involved in new anabolic processes.

IRRITABILITY refers to the capability of protoplasm to respond to changes in the environment. This characteristic includes the ability to receive stimuli (i.e., "detect" changes in the environment), to convert such stimuli into an "excitation", to transmit this excitation from one part of the cell to another (conductivity) and, within certain limits, to react to the changed environment. In some cases the nature of the response is dependent upon the type of cell. For example, white blood cells which receive a particular type of stimulus respond by migrating to the site of the infection.

CONTRACTILITY refers to the capability of cells to change their protoplasmic dimensions in response to some stimuli. This property is most highly developed in muscle cells.

EXCRETION AND SECRETION are processes involving the removal of the by-products of metabolism. The term excretion refers to the removal of cellular by-products which either have no function or, in some cases, may be injurious to the cell. On the other hand, the term secretion refers to the formation of specialized by-products, such as mucus and digestive juices, that be be important in the functioning of the organism.

GROWTH of a living cell usually includes an increase in size. However, a more significant characteristic of this activity of protoplasm is the process of maturation during which the cells develop those structures that are characteristic of their adult form.

REPRODUCTION is a unique property of protoplasm and refers to the ability of cells to divide and form new cells. Normally, cellular reproduction occurs in the process called mitosis (See Fig. 2).

CELLULAR REPRODUCTION

MITOSIS, sometimes called "equational division," is a type of nuclear division in which the two daughter nuclei formed contain the same kind and amount of chromatin material as the parent cell. It is the type of division that occurs when the somatic (general body) cells reproduce. In fast growing tissues many cells may be seen in different stages of mitotic division. The term mitotic figure is used to refer to the appearance of the chromatin in these cells. In cancerous tissues mitotic figures, some of which may be distorted, are frequently observed. Normally, the cells in mature tissues do not undergo mitosis. Consequently, the appearance of mitotic figures in certain types of cells may be considered to be an indication of an abnormal condition. The changes that occur during mitosis

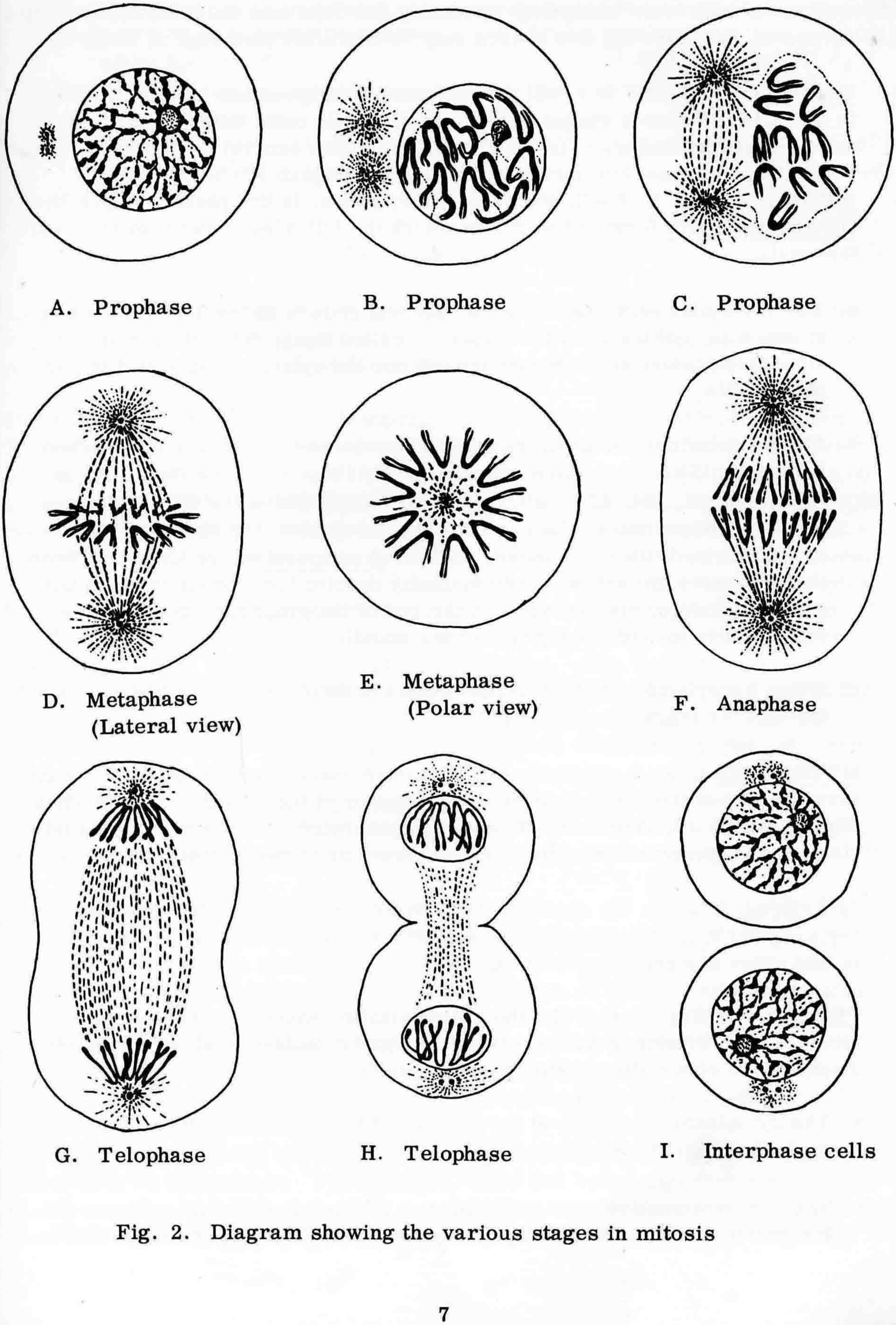


Fig. 2. Diagram showing the various stages in mitosis

are continuous and, while each stage gradually develops into the next, for descriptive purposes, the following five phases may be identified (See Fig. 2, Page 7).

1. INTERPHASE applies to a cell that is metabolically active, usually growing in size and is between stages of division. In these cells the chromatin is usually finely granular.

2. PROPHASE (Fig. 2, A-C), the preparatory phase, is the phase in which the chromosomes are formed and during which the following occur almost simultaneously:

   a. The centrioles separate, move to opposite ends in the cell where each becomes a pole of the fibrillar structure called the spindle. Under ordinary circumstances, neither the centrioles nor the spindle is observed in prophase cells.

   b. The granular chromatin of the nucleus forms into structures called chromosomes. This is an intricate process but its occurrence may be identified. At first, the chromatin appears in deeply-stained strands that have a "beady" appearance. Later, from the chromatin, the short, thick, deeply-stained, clearly visible, rodlike chromosomes are formed. These chromosomes are actually longitudinally double, i.e., each contains two genetically duplicate strands. At the end of the prophase, the chromosomes move toward the equator of the spindle.

   c. While the spindle and the chromosomes are forming, the nucleolus and the nuclear membrane disappear.

3. METAPHASE (Fig. 2., D & E), the alignment phase, is characterized by the arrangement of the chromosomes at the equator of the spindle. It ends when the two genetically duplicate strands have separated. In routinely fixed and stained slide preparations, cells in metaphase are readily observed.

4. ANAPHASE (Fig. 2, F), the migratory phase, is characterized by the movement of the "half chromosomes" to opposite poles of the spindle. Cells in in this stage are readily observed.

5. TELOPHASE (Fig. 2, G & H), the reorganization phase, is characterized by the actual division of the parent nucleus and cytoplasm and during which the following occur almost simultaneously:

   a. The cytoplasm constricts at the equator of the spindle and the parent cell is divided into two daughter cells.

   b. The chromosome lose their identity and the chromatin again assumes the the granular appearance of the interphase cell. (It should be noted that the "half chromosomes" that separated during the anaphase are duplicated prior to the next mitotic division).

c. The spindle disappears.

d. A nucleus is formed within each daughter cell as a nuclear membrane forms around the daughter chromatin material and the nucleolus reappears.

VARIATION AMONG CELLS

Living cells vary considerably in their appearance depending upon the type, location, function, growth stage, metabolic state and a number of other conditions. All of these may be considered within the range of normal variation.

Particular types of cells vary depending upon their location and their function. Epithelial cells which are located on the surface of the body differ in their appearance from those which line its organs. For example, the epithelial cells on the surface of the skin are flattened and tile-shaped with small, rounded to ovoid nuclei that are usually centrally located. On the other hand, the epithelial cells which line the digestive tract are columnar-shaped cells with rounded but somewhat elongated nuclei usually located near the base of the cell. Some leukocytes have a single, rounded nucleus while others are polymorphonuclear and have lobed nuclei. When located in the blood stream or in accumulated body fluids, leukocytes tend to be spherical in shape. However, when located between tissue cells they may be variable in their cell outlines. The muscle fibers found in the intestinal wall are elongated spindle-shaped cells with centrally located nuclei, while those associated with the skeletal system are columnar-shaped cells with peripherally located nuclei. Some specialized cells (e.g., histiocytes) are capable of engulfing micro-organisms and/or foreign materials that may be present. This process is called phagocytosis. These cells vary considerably depending upon the amount of phagocytic activity that has occurred.

The appearance of cells may also vary depending upon the stage of maturation. In general, it may be said that young cells are characterized by the presence of comparatively large nuclei (i.e., a high nuclear to cytoplasmic ratio) and a usually dense cytoplasm which, under magnification of the ordinary light microscope appears mostly homogeneous. In older cells, there is usually a reduction in the nuclear/cytoplasmic ratio and the cytoplasm has generally developed the structures that are associated with the specialized functions of the particular cell. This process of maturation will be discussed in detail in Chapter II.

Any factor which affects the physiological state of a cell may, in turn, have an effect upon its appearance. For example, cells that break away from their parent tissue and remain suspended in body fluids tend to show the effects of their new environment. If the concentration of dissolved substances inside the cells is greater than it is outside, water will flow into the protoplasm and the cell will increase in size. When the situation is reversed there may be a shrinkage in the cells. When the available food supply is limited, metabolic processes may be limited and, in some cases, by-products of metabolism may accumulate in the cell as inclusions.

SUMMARY

The living cell has a highly complex structural organization within which numerous activities take place. Living cells vary in size and shape depending upon the type of cell, its growth stage and its physiological activity. In evaluating the appearance of cells as they are observed in smears, it is important to relate their structure and size to their origin, maturation stage, physiological state as well as to the fixation and staining techniques.

CHAPTER II

THE TISSUES AND THEIR CHARACTERISTICS

INTRODUCTION

In Chapter I it was noted that cells vary in their structure depending upon their particular function within the organism. It may now be pointed out that cells which perform similar functions are grouped into tissues. Furthermore, tissues which cooperatively perform one or a set of functions constitute an organ. Correspondingly, organs are grouped into the systems of the body.

On the basis of their structural composition and organization, the numerous tissues that compose the diverse organs of the body may be divided into four major classes: Epithelial, Connective, Muscular and Nervous Tissues. Most of the work of the cytotechnician involves the examination and evaluation of the cells that slough (exfoliate) from the epithelial tissues into body fluids. Consequently, this chapter contains detailed descriptions of the epithelial tissues. Brief descriptions of the other classes are included for comparison.

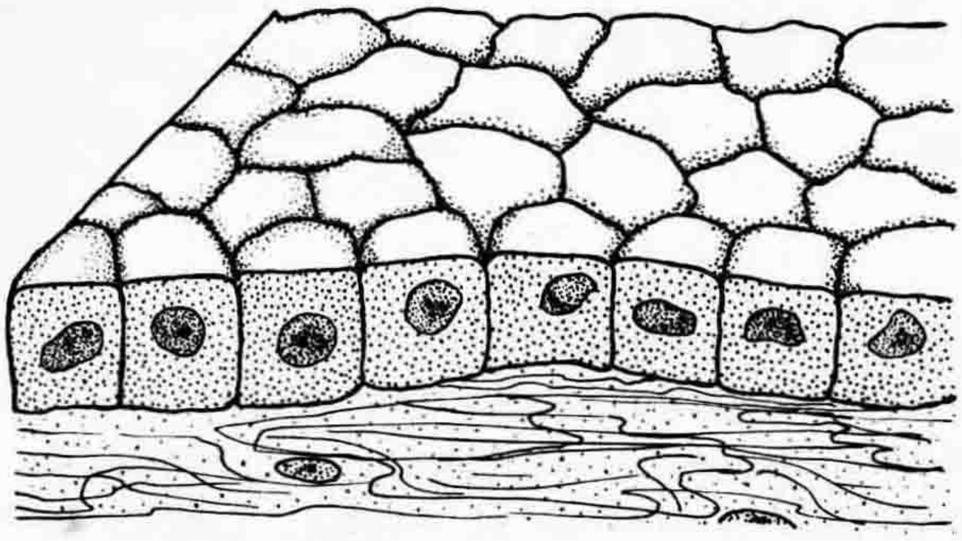
EPITHELIAL TISSUES

EPITHELIAL TISSUES occur as a continuous layer of cells which cover surfaces, line cavities, tubules and ducts in the body. In some cases, epithelial tissue may be considerably modified and compose the major portion of certain organs, e.g., the liver. These tissues are generally subdivided into two broad categories: Simple epithelium, composed of a single layer of cells and stratified epithelium which contains more than one cellular layer (or stratum).

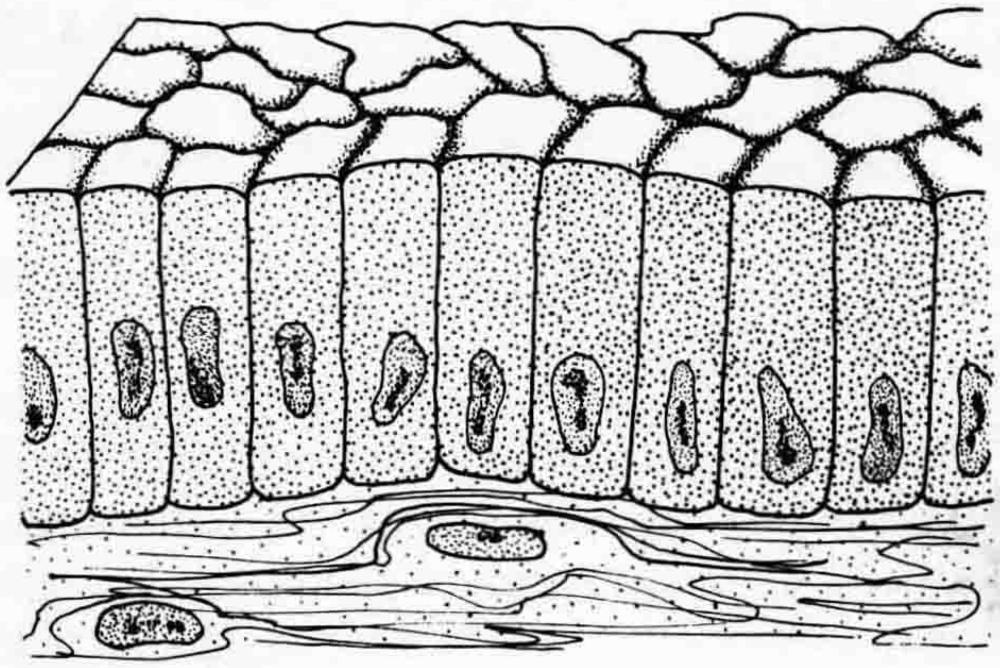
SIMPLE EPITHELIUM consists of a single layer of cells all of which rest on a basement membrane. It occurs in a variety of forms depending upon the particular location and functions. Some examples of simple epithelium are shown in Fig. 3, page 12.

SIMPLE CUBOIDAL EPITHELIUM (Fig. 3A) has been named because of its appearance in vertical section. It is found in the germinal layer of the ovary, in many glands and in portions of their ducts. Usually the cells are short prisms with a top, bottom and six sides. In surface view the cell outlines are generally hexagonal in shape and they are arranged in a rather regular, mosaic pattern (as the blocks in a pavement). In vertical view, the cells appear as a row of squares with centrally located nuclei.

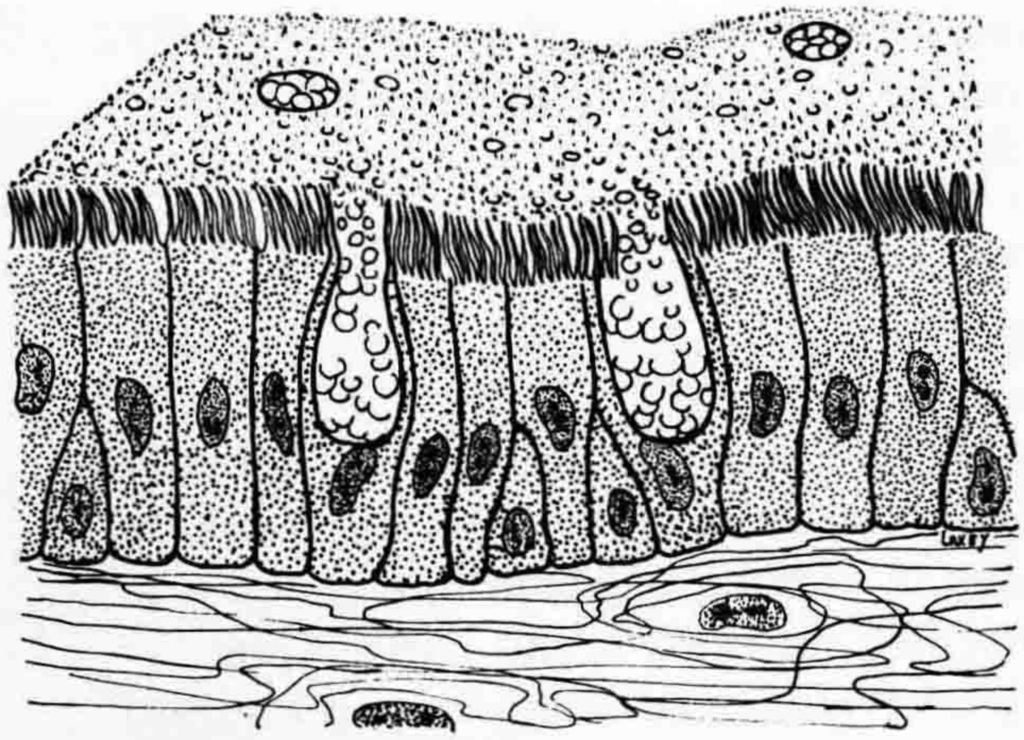
SIMPLE COLUMNAR EPITHELIUM (Fig. 3B), composed of cells that



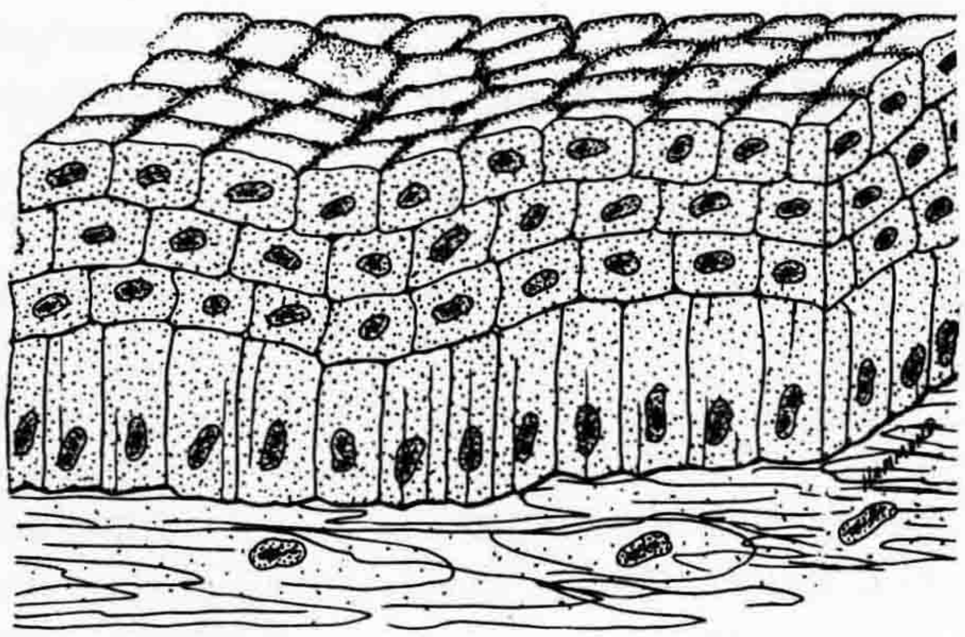
A. Simple cuboidal epithelium



B. Simple columnar epithelium



C. Pseudostratified epithelium



D. Transitional epithelium

Fig. 3. Diagrams of representatives of the epithelial tissues