EXFOLIATIVE CYTOPATHOLOGY

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PREFACE

TO serve best the patient, the physician who obtains the cellular specimen and the pathologist who interprets it must know all the values and limitations of this relatively new discipline in laboratory medicine.

Exfoliative Cytopathology is designed to familiarize medical technicians and graduate students with the morphologic alteration of the exfoliated cells. I have made special effort to present, in the simplest and most concise manner, the basic systematic knowledge needed for interpretation. Cytology is useful in almost all areas of medicine. No pretension to new discoveries is made; rather I have tried to present the current knowledge in a schematic but comprehensive manner. To retain this practical approach when controversies exist, only my personal experience is presented. Wherever possible, the morphologic details are illustrated in simple line drawings.

Because of the limited scope of this work, very little discussion of the anatomy, histology, physiology, and histopathology of the different organs studied is given. The reader is referred to various excellent existing textbooks.

Unless indicated, all the photomicrographs are taken from the specimens of patients seen at Grady Memorial Hospital in Atlanta.

The references with each chapter are not intended to be an exhaustive review of the literature, but rather a personal selection.

I wish to thank the staff of the Cytopathology Laboratory at Grady Memorial Hospital, especially Alan S. Clepper, M.D., John W. Ross, M.D., Miss Susan Elliott, C.T. (ASCP), Miss Cecelia Denman, C.T. (ASCP), Mrs. Jean Colwell, C.T. (ASCP), Mrs. Brenda Forbes, C.T. (ASCP), and Miss Cheryl Ray for their help in the preparation of this book.

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Z. M. N.

CONTENTS

INTRODUCTION

EXFOLIATIVE cytopathology is the study of the normal and the disease-altered desquamated cells. Because of the need for continuous renewal of the body tissues, cellular exfoliation is an unceasing process. The rate of desquamation varies with each tissue, its function, and metabolic capacities. Some of these desquamated cells accumulate in natural cavities and in recesses. The majority are lost from the surface or through the gastrointestinal tract. There are two types of cellular exfoliation from which samples are studied.

NATURAL SPONTANEOUS EXFOLIATION

The physiologically desquamated cells will often show, besides the pathologic changes and the normal changes of natural aging, the results of their separation from confinement in the organized structures. The samples of cells to be studied are usually suspended in fluid and removed by bulb or syringe aspiration. Good examples are the accumulation of cervicovaginal cells in the vaginal pool secretion in the posterior fornix and of mesothelial cells in the effusions of the pleural and abdominal cavities.

ARTIFICIAL EXFOLIATION (SURFACE BIOPSY)

Artificial exfoliation occurs when the surface of the mucosa is scraped and viable cells are traumatically exfoliated before their natural time of shedding. According to the type of information desired, the scraping will be energetic, as in the case of cervical carcinoma where the deep basal cells are needed for examination, or very gentle, as in the case of hormonal studies where the cells desired must come from the surface layer of the vaginal mucosa. The artificially exfoliated cells often appear in sheets and are smaller and less mature than the spontaneously desquamated ones.

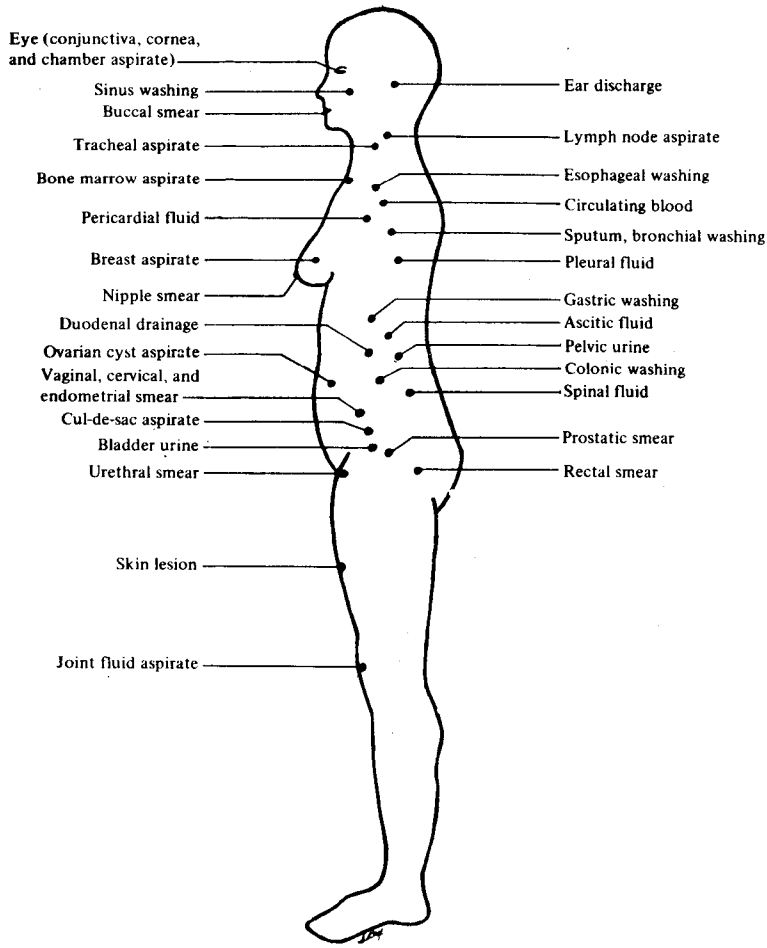

FIG. 1. The most common sources of cytologic specimens.

CYTOPATHOLOGY IN RELATION TO HISTOPATHOLOGY

Advantages

Cytopathologic methods provide a rapid and simple means of diagnosis that can, on occasion, supplement or replace a frozen section or a biopsy.

The cytopathologic process produces no injury to tissue and allows frequent repetition of cellular sampling, important in the evaluation of the progression or post-treatment regression of a lesion.

The smears cover a wider surface than that involved in a biopsy.

The cells can be obtained from areas inaccessible to a biopsy, for example, from the bottom of a crypt, the renal pelvis, or the bottom of a diverticulum.

The intimate cellular structures are often more clearly seen in an isolated cell of a smear because of the minimum shrinkage and distortion in such cells. Furthermore, the entire cell is studied in a three-dimensional view, not merely a section from it.

The vaginal smears permit an easier evaluation of the hormonal status of a patient and the type of genital inflammation.

Limitations

The interpretation of the morphologic cellular changes is based mainly on individual observation and often cannot be forced into rigid criteria.

The cytologic diagnosis is not final; it must be confirmed by histology.

The screening of a smear can be time consuming. Often the nature of the lesion is not as obvious as in a histologic section.

The interrelation and arrangement of the cells cannot be established. Neighboring cells in a smear often originate from different parts of an organ.

The relation of the cells to the supporting stroma cannot be determined by cytology, which is important in the diagnosis of an invasive carcinoma as compared with an in situ one.

The size of the lesion cannot be approximated by cytology, since the number of exfoliated cells often has no relation to the size of the lesion.

The type of lesion, in situ as compared with early invasion, adenocarcinoma, or sarcoma, is more difficult to determine by a smear.

The sample of the cells studied may originate from an unwanted site (liver cells in fluid aspiration, bronchial carcinoma cells in a gastric washing, or rectal cells in the vaginal smear).

GENERALITIES AND TECHNIQUE

No amount of skill and experience will enable the cytopathologist to render an accurate interpretation from a poorly obtained or fixed cellular sample. He does a disservice to the patient if he accepts such a smear for interpretation. Furthermore, a direct and close relationship and understanding should always exist between the clinician and the cytopathologist if the full benefit of this method is to be obtained. The majority of the recommended methods for obtaining satisfactory specimens are described at the beginning of the different chapters, but based on the most common mistakes in technique seen in our laboratory the following recommendations are emphasized:

Do not use a lubricant on the instruments or gloves. If moisture is necessary, use warm saline solution.

Do not use talcum powder on the gloves. Eliminate all dust before touching the patient, instruments, or slides.

Do not permit the cells to dry while trying to spread them perfectly on the slide. A poorly distributed thick smear is only a slight handicap for the diagnosis and is preferable to a smear that has been well spread but dried and then fixed.

Do not biopsy before taking the smear. The presence of excess blood will render interpretation of the smear difficult by masking or diluting the epithelial cells.

Do not allow the patient to douche or use an intravaginal medication or contraceptive substance for at least 24 hours before the examination. If she has done so, postpone the cellular sampling unless a visible, scrapable lesion is present.

A history of previous biopsy or conization, with an indication of the time lapse, should always be given because of the eventual diagnostic pitfalls presented by the regenerating cells.

The smears should be taken before swabbing the cervix with acetic acid as used during colposcopy.

Slide Examination

The examination of a slide should be systematic from one end to the other, in a series of overlapping sweeps, preferably with the use of a mechanical stage holder of the microscope. One should not attempt to follow the cellular streaks trapped in the mucus. Too many important clues needed for the diagnosis can be missed in this way.

To begin with, the observer should, with a low-power (X50) magnification, get a general impression of the quality of the smear, the possible presence of tissue structures, and the location of the best cells to be examined. Under higher magnification (X150) the actual screening of the smear can begin by moving the slide horizontally or vertically from one end to the other. The oil immersion examination of a cell is permitted only when an answer to a specific inquiry concerning a definite cellular structure is demanded; for example, does the nuclear membrane touch the cytoplasmic border? The continuous use of high magnification for screening will produce an increased number of false positive interpretations. (Nothing looks more malignant than a lymphocyte seen through an oil immersion objective (X1250).

The significant cells found in the slides should be marked with an ink dot, preferably semitransparent, so that the dot will not completely obscure any ink-covered cells. To call the attention of the pathologist to a specially diagnostic structure, a red line drawn with a wax pencil can be added on the cover slip. If more characteristic cells are found later, the screener should make an effort to remove from the slide, if need be, the earlier markings placed on the first but less diagnostic cells encountered. It is as bad for a screener to place too many dots on a slide as not enough.

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1
TISSUE AND BASIC CELLULAR STRUCTURES

GENERAL CELLULAR MORPHOLOGY

CELLS are the essential units of the structure of all living organisms. Their structure varies with their function, but a large number of their components are common to all. The nucleus, needed for reproduction, is surrounded by a cytoplasm supplying most of the life-sustaining functions. A cell can survive without a nucleus (red blood cell) but not without a cytoplasm (stripped nucleus). The cells contain multiple structures, some living (organelles), others without life (inclusions), each having different specific functions to perform. If we are to examine these different components with the light microscope, the cells must be stained. As illustrated in the diagram of a ciliated cell (Fig. 2), the most important components are as follows.

Cell Membrane (Plasmalemma)

The cytoplasmic membrane is a semipermeable membrane that allows a selective two-way traffic of substances needed by the cell for growth and multiplication or rejected as metabolic waste.

Cytoplasm

The consistency of the cytoplasm varies from liquid to a firm jellylike viscosity. Usually finely granular, it is composed mainly of water that contains (in solution or suspension) inorganic ions, anabolic and catabolic compounds, nuclear proteins (RNA = ribonucleic acid), glycogen (contained in large vacuoles), lipids (fat droplets), and different enzymes and catalysts needed for the breakdown and absorption of its nourishment (oxidizing and reduction phenomenon). The centrosomes containing the dark-staining centrioles are important for the cell division.

Organelles

Of the different organelles contained in the cytoplasm, the most common are:

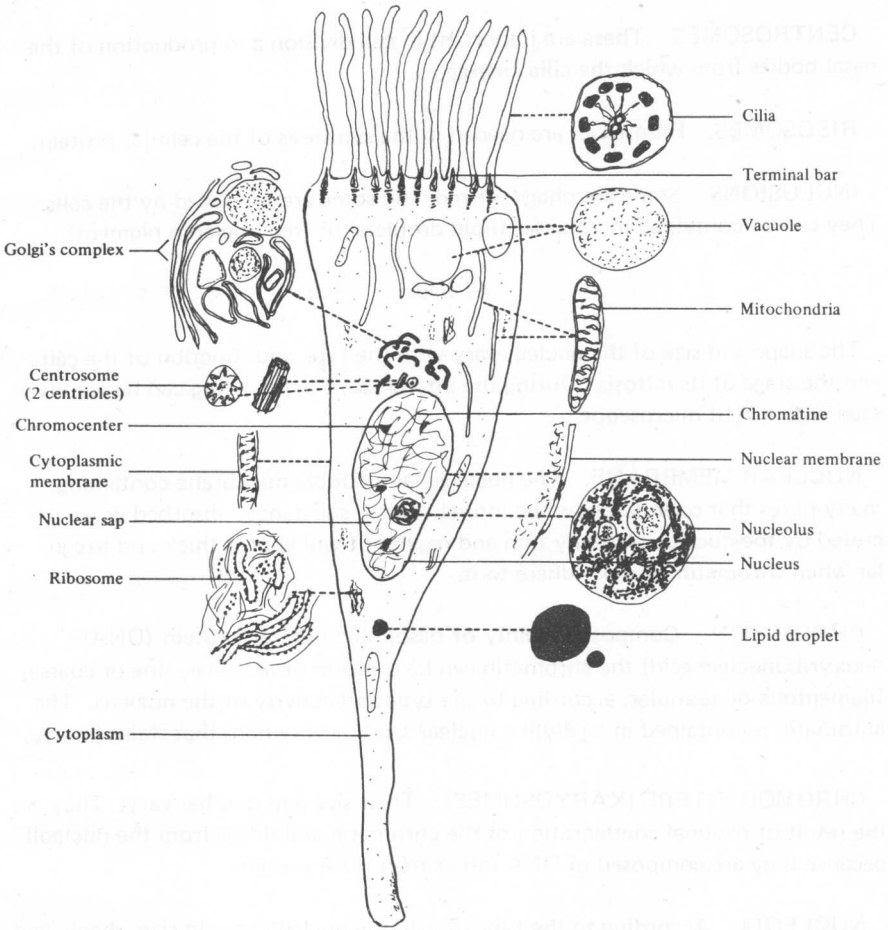

FIG. 2. A ciliated columnar cell. Some of its components are enlarged to show the structural details seen only with the electron microscope.

MITOCHONDRIA. These produce some of the enzymes needed for metabolism. Their injury may result in a degenerative cloudy swelling of the cells and an eventual necrosis.

GOLGI COMPLEX. The function is not well known, but it has a possible action in the production of cellular secretion. It is best seen when the cells are silver stained.

CENTROSOMES. These are important in cell division and production of the basal bodies from which the cilia emerge.

RIBOSOMES. Ribosomes are needed in the synthesis of the cellular protein.

INCLUSIONS. Some are phagocytized, and some are produced by the cells. They can be contained in vacuoles (lipid droplets) or free (melanin pigment).

Nucleus

The shape and size of the nucleus vary with the type and function of the cell and the stage of its mitosis. During the interphase, the following can be readily seen with a light microscope.

NUCLEAR MEMBRANE. The nucleus has a double membrane containing many pores that open to allow the interchange of substances absorbed or excreted by the nucleus. Usually thin and regular, it will appear thick and irregular when chromatin clumps adhere to it.

CHROMATIN. Composed mainly of basophilic nuclear protein (DNA = deoxyribonucleic acid) the chromatin can be uniform or irregular, fine or coarse, filamentous or granular, according to the type and activity of the nucleus. The chromatin is contained in a jellylike nuclear sap (karyolymph) that stains poorly.

CHROMOCENTERS (KARYOSOMES). Their size and number vary. They are the result of regional condensation of the chromatin and differ from the nucleoli because they are composed of DNA rather than RNA protein.

NUCLEOLI. According to the type of cell, the nucleoli vary in size, shape, and number. They are composed mainly of ribonucleic protein (RNA) and give an acidophilic reaction with the Papanicolaou stain.

LIFE OF A CELL

The life of a cell can be summarized as follows:

(1) Birth (mitotic division from a basal or reserve cell).
(2) Maturation and differentiation (for instance, increase in size, formation of cilia, formation of secretory vacuoles, or pigmentation).
(3) Functioning period, when the full-grown cell secretes, filters, absorbs, etc.
(4) Degeneration, slow or rapid decrease of its functions, followed by its death and desquamation.

CELLULAR DIVISION (FIG. 3)

The growth, regeneration, and healing of wounds of the tissues depend on the capacity of all cells to divide and form new cells where needed. These processes are called mitosis in somatic cells and meiosis in sex cells.

Mitosis

This cellular division, as illustrated in Fig. 3, is a complex process in which the chromatin arranges itself into a long chain that breaks up into 46 pairs of rods called chromosomes (prophase). Each chromosome divides lengthwise into two

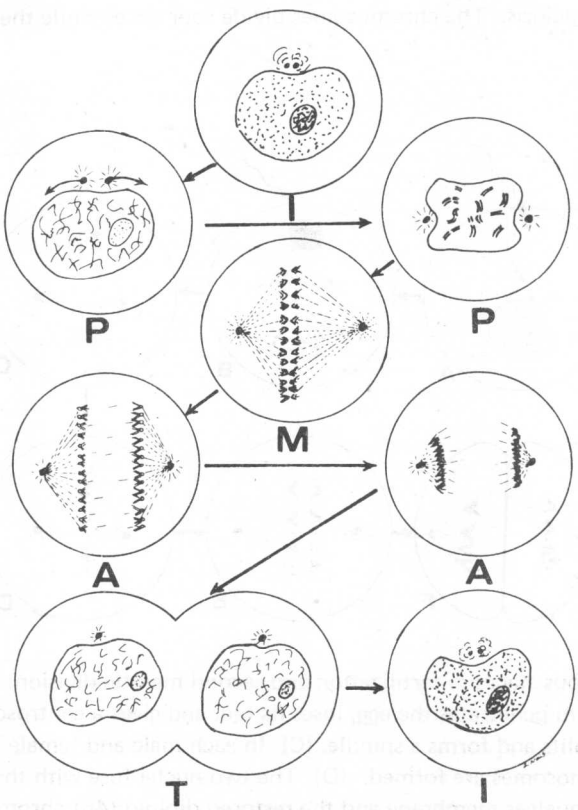

FIG. 3. Various stages in cellular mitosis: I, interphase; P, prophase; M, metaphase; A, anaphase; and T, telophase.

and they align themselves into an *equatorial plate* dividing the mitotic spindle, while the nuclear membrane disintegrates (metaphase). These divided chromosomes continue to separate into two groups (anaphase). Each new group contains the same number of chromosomes as the original cell. The new chromosomes mass at each end of the spindle, and nuclear membranes form again around each of them. The cytoplasm begins to divide (telophase) to form two new separate daughter cells, each with its new nucleus in a resting phase (interphase).

Meiosis and Fertilization

As illustrated in Fig. 4, the fertilization produces a mixture of the chromosomes from the male and the female germ cells. Before their union, these cells must have only half the number of chromosomes (haploid state) to preserve, when combined, the normal total number of 46 chromosomes per cell (diploid state). This division and reduction to half the number of chromosomes occurs during gametogenesis and is called meiosis. The chromosomes divide four times while the cells divide only twice.

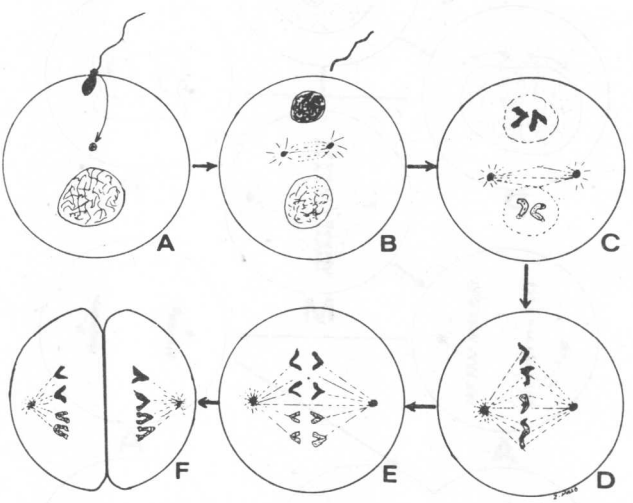

FIG. 4. Various stages in fertilization and second meiotic division: (A) The head of a sperm penetrates the egg, loses its tail, and gives a centrosome. (B) The centrosome splits and forms a spindle. (C) In each male and female nucleus, 23 pairs of chromosomes are formed. (D) The two nuclei fuse with the disappearance of their nuclear membrane and the restored diploid (46) chromosomes line up. (E) The mixed chromosomes (of the male and female cells) divide. (F) Two separate daughter cells are formed.

EPITHELIAL TISSUES (FIG. 5)

The majority of the exfoliated cells to be considered in this work arise chiefly from the epithelium, which covers the entire external surface of the human body and the inner surface of the hollow organs. The different epithelial tissues that are significant for cytodiagnosis are divided into:

(1) Simple epithelium, where every cell is directly attached to the basement membrane, sometimes by a very narrow and elongated cytoplasmic process (cells from the pseudostratified epithelium, for example).
(2) Stratified epithelium, where only the basal cells are in direct contact with the basement membrane and the remaining cells overlap each other in a variable number of layers.

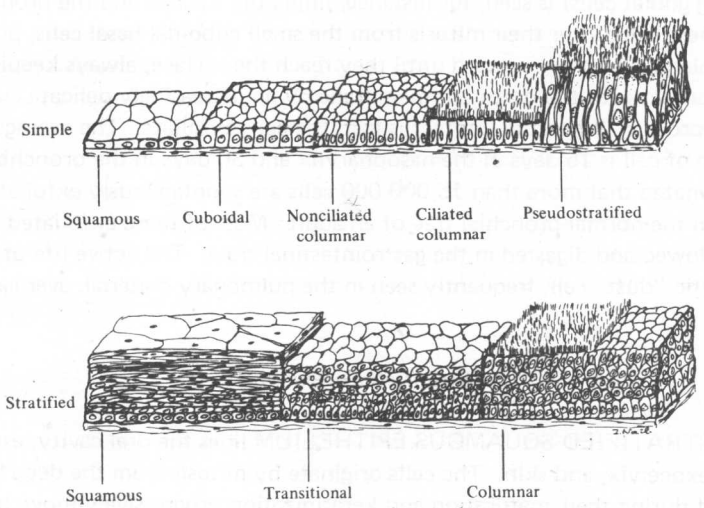

FIG. 5. Various types of epithelium.

Simple Epithelium

THE SIMPLE SQUAMOUS EPITHELIUM is found, for instance, in the mesothelial tissue lining the pleural, pericardial, and abdominal cavities and as a part of the serosa on the surface of the organs that they contain. The life length and the amount of exfoliation of its component cells vary enormously and depend upon multiple external factors. The average life span of these cells is 18 days.