GYNECOLOGIC CYTOPATHOLOGY:

A Color Atlas of Differential Diagnosis

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Translated by VOLKER SCHNEIDER

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Foreword

Diagnostic cytology is an essential part of a gynecological examination. Usually, results are reported by means of a schematic classification, thus excluding differential diagnostic considerations. In this book, Drs. SCHNEIDER and STAEMMLER present a thorough review of all differential diagnostic aspects in gynecological cytopathology and make a major contribution in discussing potentials and limitations of this approach.

In my opinion, this atlas represents a successful attempt to demonstrate the important entities in gynecological cytology. Malignant and premalignant lesions of the squamous epithelium are presented with their inflammatory, degenerative, and reparative changes. The increasingly important alterations of endometrial origin are well covered.

The illustrations are especially noteworthy. Each color plate is supplemented by a schematic drawing making reference to the pertinent cellular elements. Even the beginner in the field can thus gain a clear and visual understanding of the legends.

The presentation of the text is condensed and precise. A differential diagnostic summary closes each chapter. Cytometric data are summarized in the Appendix.

I have no doubt that this book will be a reliable source of information for cytopathologists and cytotechnicians involved in differential diagnostic problems in gynecological cytopathology.

GEORGE L. WIED

Preface and Introduction

Exfoliative cytology plays an important role in early cancer detection and is applied extensively in many hospitals and laboratories. An initial reluctance to accept it no longer exists. The purpose of this presentation is to demonstrate

whether cytology can make further contributions to early cancer detection,
what degree of specification in meaningful diagnostic terms can be achieved in the presence of a premalignant or malignant lesion,
what today's limitations are in the application of gynecological cytology,
what the factors for these limitations are, and finally
what sort of erroneous diagnoses should be expected.

Personal experience and critical evaluation are guidelines in our attempts to expand and refine this inexpensive and technically rather simple method. Practicality represents another essential criterion. Ultimately, our goal is the prevention of unnecessary surgery resulting from lack of expertise in diagnostic cytology and its differential diagnostic aspects as well as prevention of mistakes due to an overestimation of this method.

The atlas is intended to demonstrate typical lesions in gynecological cytology as well as look-alikes and their differential diagnostic aspects. A comparative presentation of characteristic differences for the various differential diagnoses is included. Principles of basic cytology and pathology are not dealt with to a larger extent. We refer the reader to a number of excellent textbooks in this field.

The cytological differentation of lesions shedding well preserved and sufficient material is especially emphasized. Hormonal cytology is covered extensively by a number of texts and therefore is not included in our presentation.

Finally, we are fully aware that differential diagnostic cytology will never be able to replace the histological evaluation of tissue sections despite considerable advances in recent times. However, its value for screening purposes is unique. Therefore, cytology should be regarded by the histopathologist as an outstanding supplementary technique leading to detection of lesions that the histopathologist himself will ultimately confirm, supplement, or correct. This is the way we would wish the histopathologist to look at our attempts for further development of classic gynecological cytology.

Introduction

Each chapter (I to IX) is accompanied by a section with illustrations consisting of color plates, legends, and explanatory schematic drawings. In general, the magnification is 1181.25× (objective 40×, ocular 12.5×, photomicroscope 1.25×, print 1.89×). Under oil immersion, the magnification reaches 2953.13×. The following pictures are exceptions to these general rules: Figs. 147 and 148 (1701×), and Figs. 43, 145, 150, and 151 (1328.91×).

Important cytometric data are summarized in six tables in the Appendix (Chapter X). These cytometric data are referred to on many occasions in the text.

References are limited to important monographs, articles, and papers of the recent literature, in line with the general concept of this outline.

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Dr. K. WEGENER, Professor of Pathology and Chairman of the Department of Pathology, Municipal Hospital, Ludwigshafen, Germany, provided the photographic equipment and enhanced the cooperation of our two departments in every conceivable way.

Mr. S. BIRKER and Mr. H. WADLE, of Ludwigshafen, made the schematic drawings.

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Contents

I. Basic Cytology

Structure of the Normal Cell

Cellular elements in a cytological smear show considerable differences in appearance, if compared with cells in a histological section. After being released from the tissue of origin, they undergo various transformations. The process of dissociation from the parent tissue, exposure to a foreign milieu, and bacterial and biochemical influences are some of the factors responsible for this change in appearance.

In order to detect and recognize these alterations, one should be familiar with the structure of a normal cell under light and electron microscopy (Fig. 1).

The cell consists of nucleus and cytoplasm, the latter containing a variety of cytoplasmic organelles.

The cell membrane serves as the boundary of the cell and consists ultrastructurally of a double layer of lipids with an intervening zone of lower optical density. The width of the entire system is 75 to 100 A (1). The cell membrane has either a smooth surface or is covered by multiple projections (microvilli) that increase its surface.

Intercellular bridges are common. They consist ultrastructurally of attachments (desmosomes) of variable structure and ex-

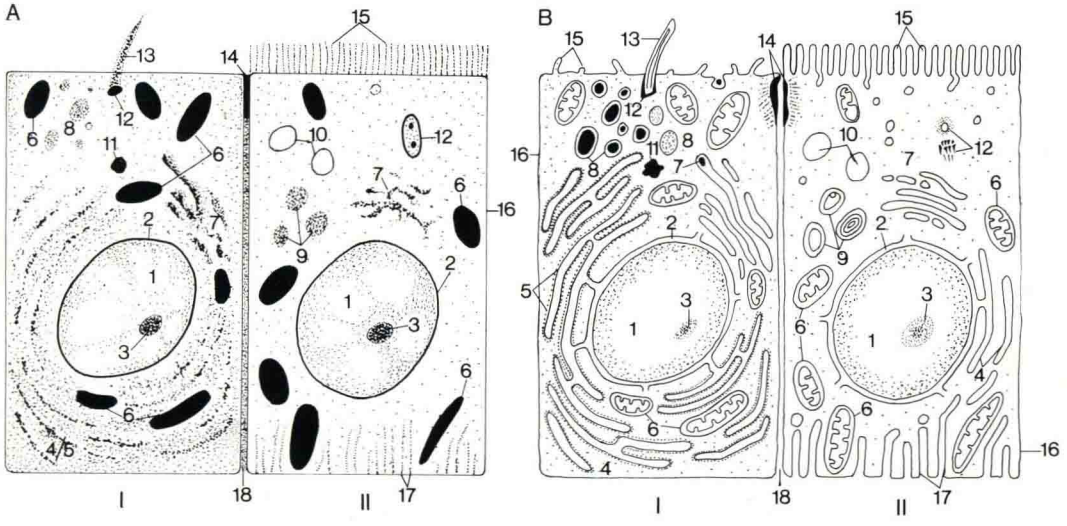

Fig. 1. Comparative illustration of the cellular structure on light microscopy (A I, A II) and electron microscopy (B I, B II) (Stoehr et al., 1969, with modifications).
A I and B I = Cell with predominantly secretory function.
A II and B II = Cell with predominantly resorptive function.

1 Nucleus
2 Nuclear membrane
3 Nucleolus
4 Endoplasmic reticulum
5 Ribosomes
6 Mitochondria
7 Golgi complex
8 Secretory granules
9 Lysosomes
10 Vacuoles
11 Lipid droplets
12 Basal body (in A I)
Centriole (in B I)
Pair of centrioles
(in A II and B II)
13 Cilium
14 Intercellular bridge (in A)
Desmosome (in B)
15 Striated border (in A II)
Microvilli (in B II)
16 Cell membrane
17 Basal striation (in A II)
Invagination of the cell membrane (in B II)
18 Intercellular space

tent. So-called hemi-desmosomes are present between basal cells and the basement membrane. The basement membrane is now considered to be a secretory product of cells, consisting of condensed mucoproteins (1, 12).

The cell is able to take up fluid (pinocytosis) or solid particles (phagocytosis). The material is surrounded by the cell membrane, and a vesicle is formed which subsequently may detach itself from the cell membrane. The resulting vacuoles and inclusion bodies may be enzymatically digested.

The nucleus is surrounded by a double-layered membrane. Nuclear pores allow communication between nuclear sap (karyolymph) and cytoplasm. A direct connection exists between the outer membrane of the nucleus and the tubular system of the endoplasmic reticulum.

The nucleus consists of a basic substance (karyolymph), which contains the nucleoproteins. The nucleoproteins form a fine network of threads condensed in some areas into cyanophilic granules, the chromocenters. They represent those portions of the genetic material that remain visible in the interphase. Deoxyribonucleic acid (DNA) is the main component of the nucleoproteins and is the chemical basis for the blue staining reaction of the nucleus with basic dyes (hematoxylin). One or multiple eosinophilic nucleoli are normal constituents of the nucleus. They are round or oval and consist of ribonucleic acid (RNA). They are surrounded by a cyanophilic (basophilic) layer of heterochromatin (18). The nucleolus plays an important role in RNA synthesis, which is induced and controlled by chromosomal DNA. Messenger RNA carries genetic information into the cytoplasm, where protein synthesis takes place.

The endoplasmic reticulum is one of the most important cytoplasmic organelles and consists of a tubular system that is present in variable amounts. It may form cisternae and is in direct continuity with the cell membrane and the nuclear membrane. The outer surface of the "rough" endoplasmic reticulum is covered by small RNA particles (ribosomes). The "smooth" endoplasmic reticulum is free of ribosomes. The endoplasmic reticulum plays an important role in protein synthesis. Ribosomes attached to the rough endoplasmic reticulum synthesize protein for extracellular use, while free ribosomes seem to produce protein for intracellular use.

The Golgi complex appears as an irregular network of three components:

- a group of large vacuoles,
- flat, saccular formations in the vicinity of the vacuoles, and
- numerous small vesicles.

The membranes of the Golgi complex are free of ribosomes. In actively secreting cells, the Golgi complex is usually well developed; secretory granules may be discernible.

Mitochondria consist ultrastructurally of a double membrane. The inner membrane is folded and projects into the lumen, forming so-called cristae and tubuli mitochondriales, thus enlarging the available surface considerably. Round particles (complex macromolecules) cover these surfaces and contain the enzymes of cell respiration (1, 12, 13, 22). Mitochondria play a key role in the cellular enzyme system, containing the enzymes of the Krebs cycle, fatty acid cycle, and oxidative phosphorylation, and therefore represent important centers for energy production in the cell.

Lysosomes contain hydrolytic enzymes that are responsible for the digestion and removal of phagocytized foreign material.

Centrioles are paired cylindrical structures in the cell center, forming the spindle with its fibrillary apparatus in mitosis.

Tonofibrils consist of bundles of filaments that are present in all cells. They are particularly well developed in stratified squamous epithelium, increasing in number with the degree of maturation. Therefore, they seem to be connected with keratin formation. Myofibrils and neurofibrils are other fibrillary formations that may be observed.

General Considerations in the Assessment of a Cytological Specimen

Diagnostic interpretation of a cytological smear is a step-by-step procedure. The environment of the cells present in the slide background should first be evaluated. Features of the cellular elements, their arrangement, and their numerical presentation are then considered, so that the examiner can reach a diagnostic conclusion.

Background (Diathesis)

The slide background represents an important general criterion in the evaluation of the specimen. Cells originating in an invasive tumor are usually associated with a dirty background consisting of tumor cell necrosis (tumor diathesis; Fig. 104). Atypical cells of a noninvasive lesion are usually found in a clean environment. However, it should be kept in mind that inflammatory reactions of the uterus (e. g., pyometra and severe endocervicitis) or vagina (e. g., Trichomonas infection) produce a dirty background in the absence of malignancy.

Number of Atypical Cells

Anatomical location, type, and degree of the presenting lesion and the sampling technique determine the number of cells present in the specimen:

- ▶ Malignant cells dissociate more readily and therefore shed in larger numbers than normal cells.
- ▶ Undifferentiated carcinoma possesses a higher degree of dissociation than well differentiated carcinoma.
- ▶ Atypical glandular cells occurring in large numbers usually derive from an endocervical lesion.
- ▶ Few atypical cells of glandular differentiation are more likely to originate from an endometrial carcinoma.

Arrangement of Cells

Sampling technique and type of lesion present are determining factors for the cellular arrangement. In the vast majority of cases, isolated cells as well as cell groupings are present. The latter, so-called cell aggregates or microbiopsies, provide information about the tissue of origin. Cell aggregates occur as sheets, syncytia, or clusters.

A sheet (Fig. 62) is represented by a flat group of cells with regular arrangement, preserved nuclear polarity, and well defined cell borders (16, 17).
Occurrence: normal tissue, dysplasia.

A syncytium (Fig. 99) consists of irregularly arranged cells with loss of nuclear polarity and ill defined cell borders.
Occurrence: carcinoma in situ, invasive squamous cell carcinoma.

A cluster (Fig. 20), or cell ball, is a three-dimensionally arranged group of cells with ill defined cell borders. It is of glandular origin and typical for benign or malignant glandular tissue.
Occurrence: Small clusters are common in endometrial carcinoma. Larger, more loosely arranged clusters, rosettes, or side-by-side groupings are more characteristic of endocervical adenocarcinoma.

Cell Size and Cell Shape

Cellular size remains constant for a specific tissue. However, sampling technique, biochemical or bacterial influences, biological factors (e. g., sex hormones), and therapeutic measures (e. g., ionizing radiation) may alter the size.

Cellular shape indicates origin as well as functional status. However, specific features are usually lost after exfoliation: All desquamated cells tend to take a spherical shape after losing contact with the natural en-

vironment and its prevailing tissue pressure. Forcibly removed cells mimic more closely the specific configuration of the parent tissue. A classification into isodiametric cell shapes (polygonal and round cells) and anisodiametric forms (oval, elongated, cylindrical, spindly, or tadpole cells) may be made.

Cell Borders and Cell Aggregates

Well differentiated cells possess distinct cellular borders. When occurring in aggregates, they form sheets.

Poorly differentiated cells are characterized by ill defined cell borders. Their aggregates are called syncytia.

Cell Function

The cytoplasm undergoes a specific functional adaptation. Morphological examples of functional adaptation are tonofibrils, neurofibrils, myofibrils, and cilia. Fibrillary structures in spindle cells may indicate early keratinization (Fig. 84).

Cytoplasmic inclusions appear as granules (pigments) or droplets (vacuoles). The vacuoles contain protein, lipids, or secretions, they indicate a secretory or resorptive function of the cell.

Glandular cells are characterized by large vacuoles displacing the nucleus to an excentric position (Fig. 138).

The Nucleus

Whereas the cytoplasm is a reflection of origin, functional status, and degree of cellular differentiation, the nucleus represents biological activity and malignant potential.

Nuclear size is variable but constant for a specific cell type. The nuclear-cytoplasmic ratio is of great diagnostic value. It decreases with maturation and differentiation, being 1:3 for basal cells (23) but 1:100 for mature superficial cells (24) (see Tables 1 to 6 in the Appendix). Nuclear configuration corresponds usually to cell shape. Isodiametric cells contain normally round or spherical nuclei, whereas anisodiametric cells possess oval, ellipsoid, or irregular nuclei.

The number of nuclei varies very little. Most cells contain a single nucleus. Inflammatory or hyperplastic conditions may induce multinucleation. Histiocytes (Fig. 33), cells of herpes simplex genitalis (Fig. 41), and trophoblast cells (Fig. 54) may be multinucleate. Multinucleation does not therefore represent a criterion for malignancy.

Nuclear displacement to marginal positions may occur with cellular differentiation. The nuclei of immature cells are usually centrally located.

The nuclear membrane may appear thickened owing to deposition of chromatinic material beneath its inner surface (Fig. 90).

The chromatin pattern is of great importance in diagnostic cytology. It is directly related to the amount of deoxyribonucleic acid. The various premalignant and malignant lesions of the cervix show characteristic distribution patterns (16, 17). In the interphase, the chromatin pattern of a normal nucleus is uniformly finely granular.

In atypical cells, the chromatin tends to clump together, leaving free zones of clearing in between.

The chromatin pattern of the nucleus is classified as:

- ▶ uniformly finely granular chromatin,
- ▶ irregularly finely granular chromatin,
- ▶ uniformly coarsely granular chromatin,
- ▶ irregularly coarsely granular chromatin with chromatin clumping and chromatin clearing,
- ▶ transparent, opaque chromatin (ground-glass appearance), and
- ▶ pyknotic chromatin (karyopyknosis).

In dysplastic cells, the chromatin pattern is finely granular and hyperchromatic (see page 79), while in squamous cell carcinoma its distribution is coarsely granular (see page 114). These alterations in the chromatin pattern

are caused by an increase of deoxyribonucleic acid (16, 47, 51). Nuclei with uniformly coarsely granular chromatin pattern may represent an early mitotic stage. This pattern is a common finding in carcinoma in situ (see page 95).

Nucleoli are composed of ribonucleic acid (RNA) and stain eosinophilic with the Papanicolaou technique. They are normally round or oval. Irregular shape is usually a sign of a pathological condition.

For practical purposes, it is important to remember that the presence of macronucleoli is indicative of an infiltrating lesion. Their occurrence in carcinoma in situ is exceptional.

Cells in the Cytological Smear

The technically satisfactory gynecological sample contains epithelial cells derived from ectocervix, endocervix, and endometrium, as well as the cellular elements of the bloodstream. The cells of the ectocervix usually predominate.

Squamous Cells of the Ectocervix

The ectocervix of the adult woman in the sexually active period of life is covered by a stratified squamous epithelium, which is composed of

basal cell layer,
parabasal cell layer,
intermediate cell layer (superficial and deep zone),
cornified zone of DIERKS (not always present), and
superficial cell layer.

The nonkeratinizing, stratified squamous epithelium of the ectocervix borders abruptly on the simple columnar epithelium of the endocervical canal, forming the squamocolumnar junction. The location of the squamocolumnar junction varies with age. In the reproductive age group, the squamocolumnar junction is usually located on the ectocervix. After menopause, it slowly recedes into the endocervical canal and is, therefore, no longer visible (44).

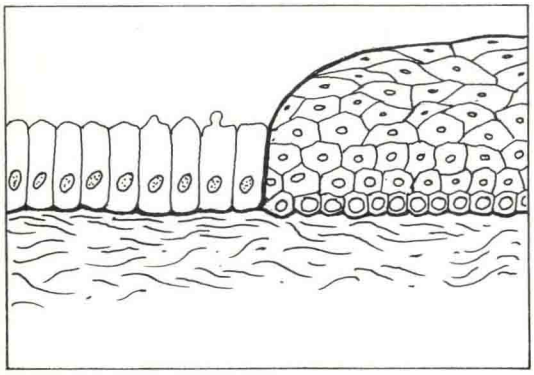

Fig. 2. Squamocolumnar junction of the uterine cervix.

The internal os marks the transition between endocervical and endometrial columnar epithelium. As in the ectocervix, the location varies considerably with age.

The zone of transformation between squamous epithelium of the ectocervix and columnar epithelium of the endocervix (Fig. 2) is of special significance, because the majority of cervical carcinomas and their precursors arise in this location. The importance of the transformation zone in carcinogenesis at the uterine cervix is well documented (4, 7, 12, 16, 35, 44).

The primary function of the squamous epithelium of the ectocervix is protection against chemical, mechanical, and bacterial injury. In the sexually active period of life, the squamous epithelium consists of multiple layers and thus is particularly well suited to fulfilling its function.

Special conditions, for example, mechanical trauma in cases of descensus or uterine prolapse, lead to keratinization of the epithelium (hyperkeratosis) and thus to the occurrence of keratinizing superficial cells and anucleate squames in the smear. It is only in these instances that the previously mentioned zone of DIERKS may be observed (20).

After menopause the epithelial lining decreases to varying degrees. In some women, a totally atrophic pattern develops, with concurrent loss of protection. These women are, therefore, highly susceptible to senile vaginitis.

A brief description of the typical features of the different cell types follows. Their cytometric characteristics are summarized in the tables in the Appendix.

Basal Cells

These cells are rarely found in the smear, for example, after disruption of the upper layers of the epithelium. Basal cells are the