

## 1 HISTORICAL PERSPECTIVE

The first documented evidence in English literature of cell examination in disease processes appears during the nineteenth century, when malignant cells from a mammary carcinoma were recognized and reported by Professor Johannes Müller of the University of Berlin (Figure 1.1). (Professor Müller trained a number of medical pioneers including Rudolf Virchow, Friedrich Henle, Robert Koch, Paul Ehrlich, and Theodor Schwann.) Alfred Donné is credited with identifying abnormal cells in vaginal smears, and published the first cytology atlas in 1845 containing photomicrographs of cells. In the same year, Lebert published an atlas with 250 cytology figures. He is believed to have laid the foundation of modern cytology. In 1852, Babo developed a centrifuge that he used to study specimens from the body cavity fluids and urinary tract (Figure 1.2).



Figure 1.1 Malignant cells mammary carcinoma, Professor Johannes Muller, nineteenth century.

In 1850, Gottlieb documented cancer cells in detail in an atlas. He wrote:

In cancer ... the cells present peculiarities. The characteristic cancer cells are spherical, ovoid, irregularly polyhedral, and frequently exhibit caudate prolongations. They possess finely granular contents, with a round or oval nucleolated nucleus as large as or larger than a pus-corpuscle. Sometimes cancer cells are double the ordinary size or more and not infrequently contain several nuclei, or even other cells constituting parent or endogenous cells.

Lionel S. Beale – Professor of Pathology, Physiology and Medicine at King's College London – should truly be



Figure 1.2 Urinary bladder cells, centrifuge specimen, Babo (1852).

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Figure 1.3 Laryngeal carcinoma cells. Wood carving, Lionel Beale (1861).

considered the father of cytology. He examined urinary specimens with water and glycerin and reported papillary fragments with cancer cells. In 1861, Beale published a paper on the examination of sputum from a case of laryngeal carcinoma (Figure 1.3). This wood carving is considered among the finest illustrations of tumor cells, nearly a quarter of a century before the birth of George Papanicolaou. Upon his death in 1906 at the age of 76 years, William Osler commented upon Dr. Beale's contributions and influence in medicine in an obituary published in *The Lancet*.

Paul Ehrlich is credited with introducing stains used in cytology and in tissue examinations. He developed aniline and basic dyes used in hematology. The first atlas of urine sediments was published in Germany in 1896. In 1900, Widal and Ravaut published a cytology review of non-neoplastic effusions using Romanowsky stains.

The present era of cytology was started by George Papanicolaou. In association with Charles Stockard in

1917, he published the first paper describing hormonal changes in the vaginal smears of guinea pigs. While at Cornell Medical Center, New York, Dr. Papanicolaou delivered his seminal paper entitled "New cancer diagnosis" at the Third Race Betterment Conference held at Battle Creek, Michigan. This is considered the beginning of modern cytology in the United States. Dr. Papanicolaou introduced the alcohol-based polychromatic stain for use with wet-fixed cellular specimens. One of his earlier colored illustrations (Figure 1.4) – made by Hashime Murayama, a well recognized artist of that era – depicts the accuracy and attention to detail of the great master. Murayama also illustrated the lecture delivered by George Papanicolaou at Battle Creek.

These observations have stood the test of time. Harvard University Press published the *Color Atlas* in 1954, in a ring-binder, facilitating periodic update in the newly emerging field. Students and trainees of Dr. Papanicolaou further promoted the practice of cytology. Mrs. Ruth



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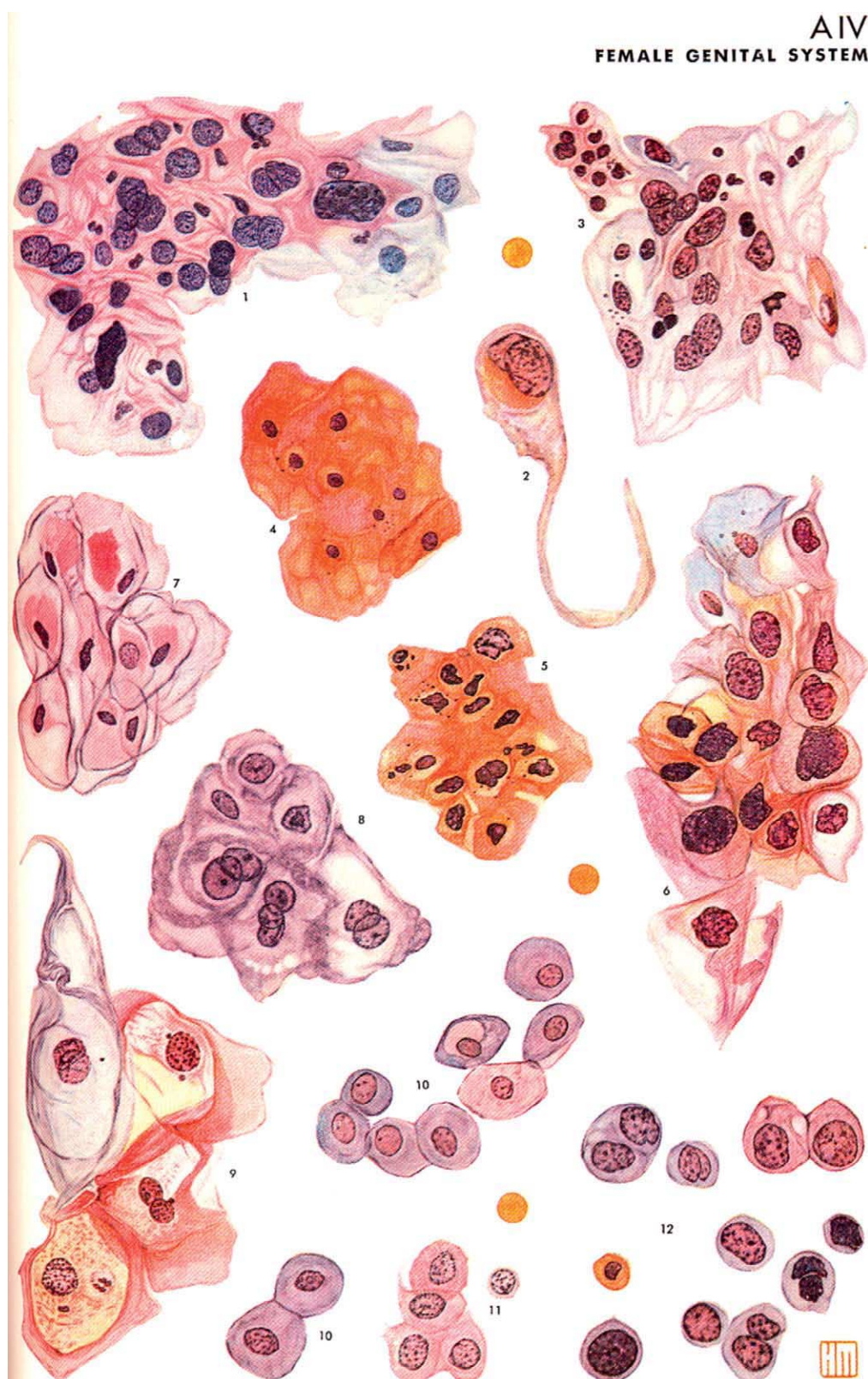
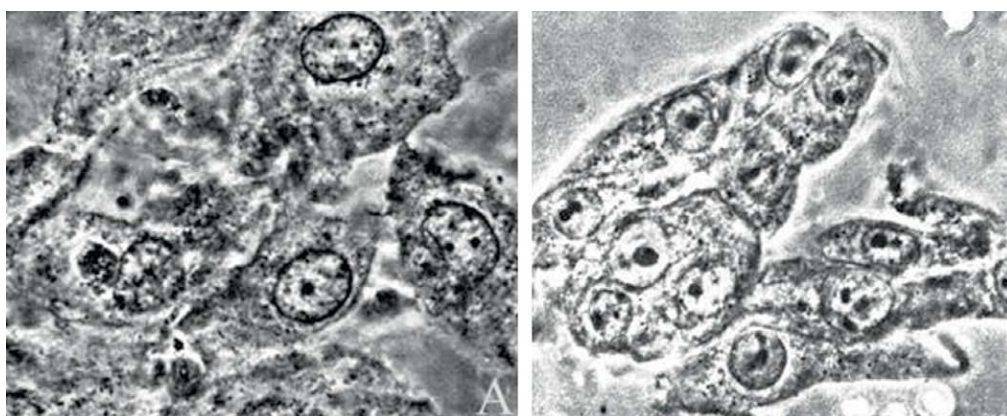


Figure 1.4 Female genital system drawings, George Papanicolaou (1954). (Reproduced with permission: *Atlas of Exfoliative Cytology*, George N Papanicolaou Commonwealth Funds, Harvard University Press, 1954.)

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**Figure 1.5** Concept of developing cervical cancer – angel and devil, Hugh Grady/Emil Novak, early 1940s. (Reproduced with permission: *Gynecological and Obstetrical Pathology, 1st Edition*, Emil Novak, W.B. Saunders, 1940.)



**Figure 1.6** Cervical cells carcinoma, Peter Stoll (1969). (Reproduced with permission: *Gynecological Vital Cytology*, Peter Stoll, Spinger-Verlag, 1969.)

Graham was Papanicolaou's cytotechnologist and she became the head of the cytology laboratory at the Massachusetts General Hospital in Boston. She is credited with describing the "third type" or carcinoma in-situ cell after Dr. Papanicolaou observed the fiber and tadpole forms in squamous cell carcinoma. At nearly the same time, the concept of developing cervical cancer (Figure 1.5) was graphically depicted by Dr. Emil Novak.

Parenthetically, there is documentation that Dr. Aurel Babés of Romania independently recognized cancer cells in cervical smears at practically the same time as Papanicolaou. Drs. Bernard Naylor, George Wied, Leopold G. Koss, James W. Reagan, Stanley R. Patten, and John K. Frost – to name a few – continued to follow and further expand the field of cytopathology. Incidentally, in the late 1950s, John Frost is credited with introducing the word "cytopathology."

Drs. George Wied and Stanley Patten conducted the earlier studies on morphometry and automation. The introduction of computers and the neural network further

advanced this now rapidly evolving field. Work on flow cytometry applicable to cytopathology was initiated in the laboratories of Drs. Koss and Patten. Exfoliated cells in health and disease were studied using phase-contrast microscopy (Figure 1.6).

Drs. Guelfo Sani and colleagues used fluorescent dyes for the study of exfoliated cells (Figure 1.7). Notice the remarkable concepts of developing cervical cancer as seen microscopically.

Although the cytologic examination of gynecological and non-gynecological cellular specimens gained acceptance and much popularity in the United States, evaluation of fine needle aspirations (FNA) failed to gain ground. In the 1930s, the first large series on needle aspiration evaluation using #18 gauge needles was published by Hayes Martin and associates from Memorial Hospital in New York. It appears that Dr. James Ewing, the pathologist at Memorial, was skeptical of FNA utilization in cytology and perhaps impeded its acceptance in the US. Interestingly, Dr. Ewing diagnosed his own bladder cancer by urine



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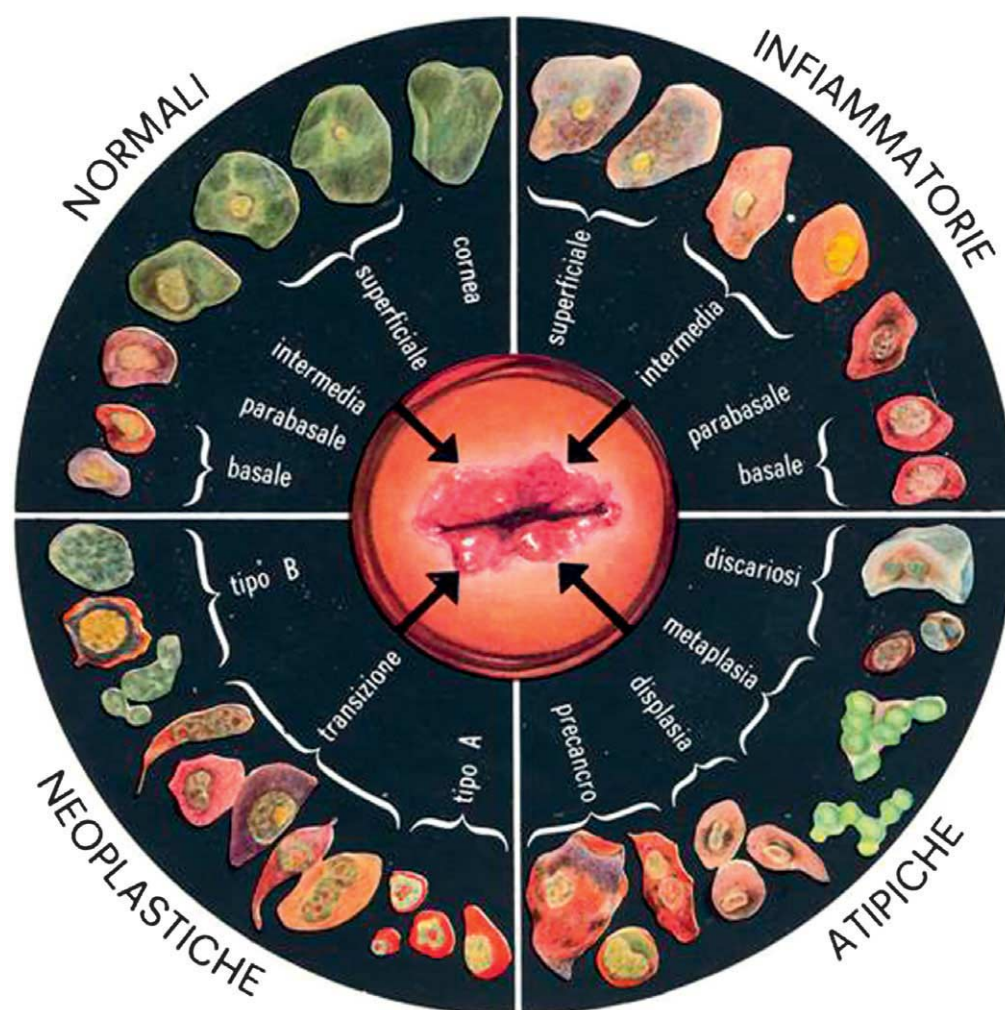


Figure 1.7 Normal and pathologic cervical cells as seen by fluorescence microscopy, Guelfo Sani (1963). (Reproduced with permission: *Fluorescence Microscopy in the Diagnosis of Cancer*, Guelfo Sani, Ugo Citti, Giuliano Caramazza, and Pietro Quinto, Charles C. Thomas Publisher Ltd., 1964.)

cytology. In Europe, hematologists were quite familiar with bone marrow specimens and utilization of Romanowsky stains. They were able to recognize tumor cells in the bone marrow aspirates and expanded their studies to include other organs such as thyroid, breast, and lung. Dr. Paul Lopes-Cardozo published a book on clinical cytology in 1954 and his *Atlas on Clinical Cytology* in 1976. In 1952, Nils Söderström published his experience with thyroid aspirations. Drs. Franzen Sixten, Joseph Zajicek, and Trossten Löwhagen – all from the Karolinska Institute in Stockholm, Sweden – should be attributed with popularizing the present use of FNAs.

Methylene Blue was used as a quick stain for FNA specimens over a number of years, in preference to the Romanowsky procedure which required a number of staining steps. A major improvement occurred in 1971 with the introduction of the Diff-Quik stain, permitting

FNA to truly become a bedside diagnostic procedure. The Ultrafast Papanicolaou stain introduced a few years ago combines the properties of Papanicolaou and the convenience of Diff-Quik stains.

## FURTHER READING

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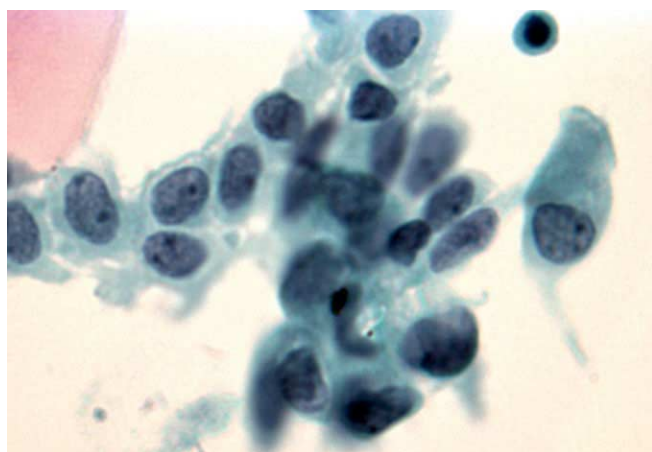
## 2 NORMAL CELL MORPHOLOGY – EUPLASIA (CELLS IN NORMAL HEALTH AND PHYSIOLOGIC STATE)

### THE CELL AS A WHOLE

#### (a) General features

The cell is an independently functioning, structural unit of an organism. It almost always contains a nucleus and cytoplasm; some cells (mature erythrocytes) do not have a recognizable nucleus. *One or more than one type of cells when organized as part of a purposeful, working arrangement in the body form a tissue.* Clonal derivation of the *euplastic* (healthy, normal, and physiologically responsive) cells and tissues imparts certain predictable morphologic and physiologic characteristics to the cells and tissues. These characteristics are diagnostically important. Familiarity and recognition of the cellular and tissue attributes is essential for proper evaluation and diagnosis of “normal” cells and tissues.

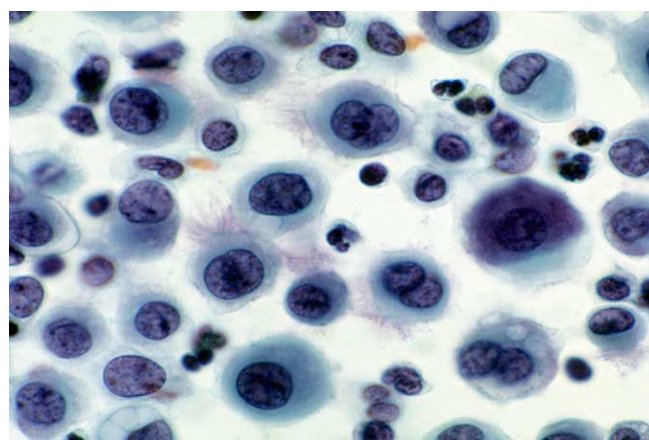
Cellular reactions to the body can be conveniently divided into the *growth* and the *functional* activities. These include: physiologically healthy or resting (*euplastic*), dying or dead or physiologically regressive (*retroplastic*), stimulated or reactive (*proplastic*), and stimulated with uncertain or autonomous growth (*neoplastic*) activities.



**Figure 2.1** Cervical specimen depicting uniform nuclear features among columnar and cuboidal endocervical cells. LBP, Pap stain.

Cellular activity level is primarily reflected in nuclear morphology. An evaluation and familiarity with the various nuclear constituents evident in a well-prepared cellular specimen including nuclear envelope (membrane), chromatin, parachromatin distribution and clearing, and nucleoli are the most critical components of clinical cytopathology. Morphological changes in one or more of these nuclear components often mirror the underlying disease process.

While the growth cycle is reflected in the nuclear features, functional differentiation of the cell is observed primarily within the cytoplasm. In most instances, this cytoplasmic mantle surrounding the nucleus determines the type or the anatomic nature of the site and the tissue of the cell origin. The nuclei of an intermediate cell from the cervix and an astrocyte from the central nervous system are morphologically similar, as are the nuclei of parabasal and intermediate cells from the cervix. Similarly, various types of bronchial epithelial cells can only be characterized by the cytoplasmic appearance, nuclei of both the ciliated and non-ciliated cells being morphologically identical (Figures 2.1 and 2.2).



**Figure 2.2** Bronchial cells. Ciliated and non-ciliated forms can be distinguished by the cytoplasmic features. Bronchial wash, Pap stain.



## CHAPTER 2

Proper evaluation, interpretation, and recognition of normal (euplastic) cells are fundamental to the diagnostic cytopathology; in this context, the value of quality of cytopreparations cannot be minimized. “Normal,” baseline cells represent resting phase and a reflection of the various physiological transient changes. Depending upon the tissue (nerve cells and lymphocytes vs. adipocytes and myocytes), various cells may reveal morphologic alterations that can be overlapping and mimicking “atypical” or “neoplastic” features. There is both a morphological and functional overlap between the physiological adaptive (i.e. pregnancy, obesity) and “early” reactive changes (oral contraceptives, environmental, physical, and biochemical irritants) observed in cellular specimens. For example, fat cells (adipocytes) can vary considerably in size depending upon the lipids accumulation and physical effects. Smooth muscle cells of the uterus during pregnancy similarly can demonstrate considerable hypertrophy and hyperplasia. It must be appreciated that there is a continuum in the tissue changes, and even a clonal group of cells may reflect different cellular changes simultaneously.

The classic features of “normal” cells are valuable when evaluating biologic behavior among cells derived from the same clone, such as in a tissue fragment or micro-biopsy often seen in cytologic preparations. Quite commonly, cells obtained from a clonal “normal” area such as a cervix, mesothelium, bronchial mucosa or the urothelium, although occurring singly and unattached, preserve and display similar and morphological characteristics. It must be recognized that these cells are in the same growth phase and are preserved identically.

*Normal (euplastic, resting) cell.* The normal resting or healthy (euplastic) cell exhibits certain cytomorphologic characteristics, including roundness, uniformity, predictability, and symmetry (Figure 2.3A,B).

Recognition of these fundamental cytological features of the euplastic or healthy cell is critical in the evaluation and diagnosis of the cellular samples in health and disease processes. In small tissue fragments, micro-biopsies and histopathology samples, surprisingly these crucial features are evaluated subconsciously, and one is able to separate “normal” from “abnormal” almost instantaneously. Such quick decisions are a common occurrence in interpretations of routine histopathology sections such as cervical and bronchial biopsies and other tissues (Tables 2.1 and 2.2). It must be recognized that this assessment is helpful when the cells are derived from a single clone and occur together, situated on a single basement membrane and interconnected by cytoplasmic processes (Figure 2.4). Certain physical,

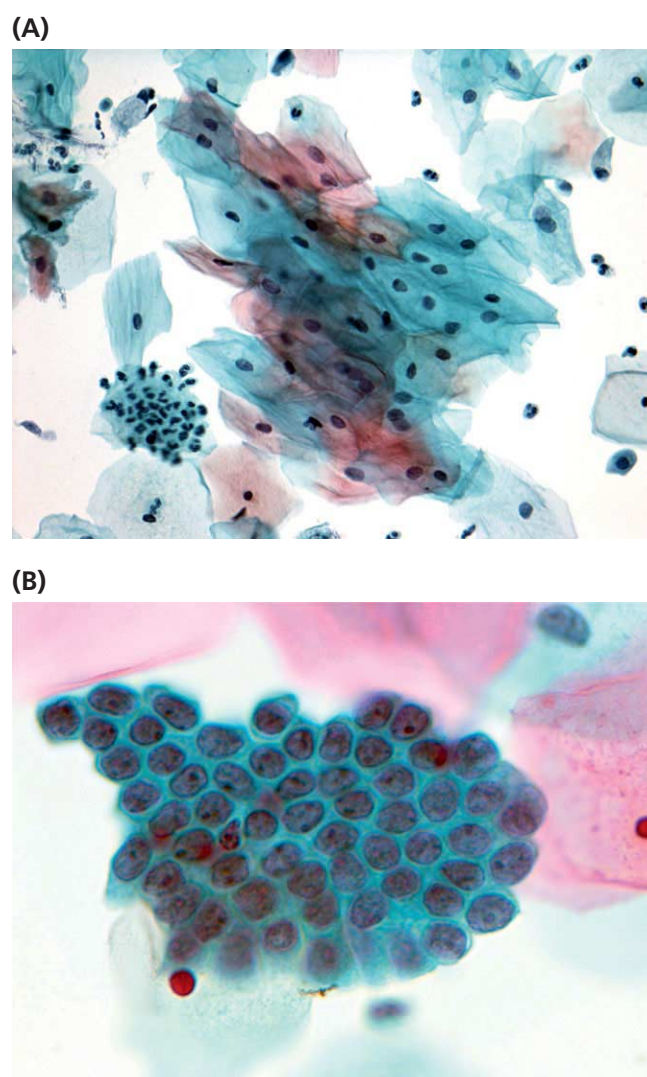


Figure 2.3 (A) and (B) show the morphologic features of euplastic cells. Notice the chromatin, parachromatin, and nuclear membrane features. Vaginal cervical smear, Pap stain.

metabolic or physiological processes can alter this attribute of the “normal” tissues. For example, the normal squamous cells of the epidermis, although polyhedral in shape, associated with tone filaments and intercellular bridges can often be seen to mold against each other, becoming flattened and wafer-like as a result of mechanical pressures. Similarly, the fat cells in the subcutaneous region as well as the osteocytes immediately adjacent to the periosteal layer of the bone can be reshaped by physical forces.

### **Roundedness**

Healthy cells naturally tend to acquire a round or rounded shape, especially when devoid of any convergent physical forces. As an example, the mesothelial cells exfoliated in the effusion fluids acquire a rounded appearance (Figure 2.5).



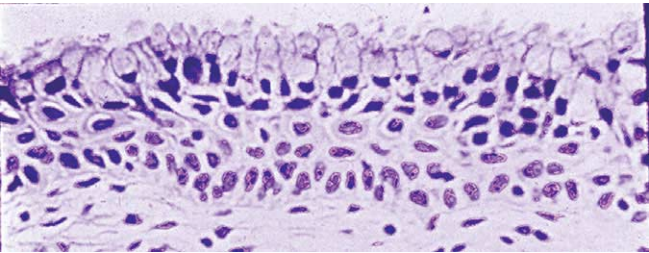
NORMAL CELL MORPHOLOGY – EUPLASIA

**Table 2.1** Diagnostic cytomorphologic features helpful in differentiating between true tissue fragments and non-tissue fragments

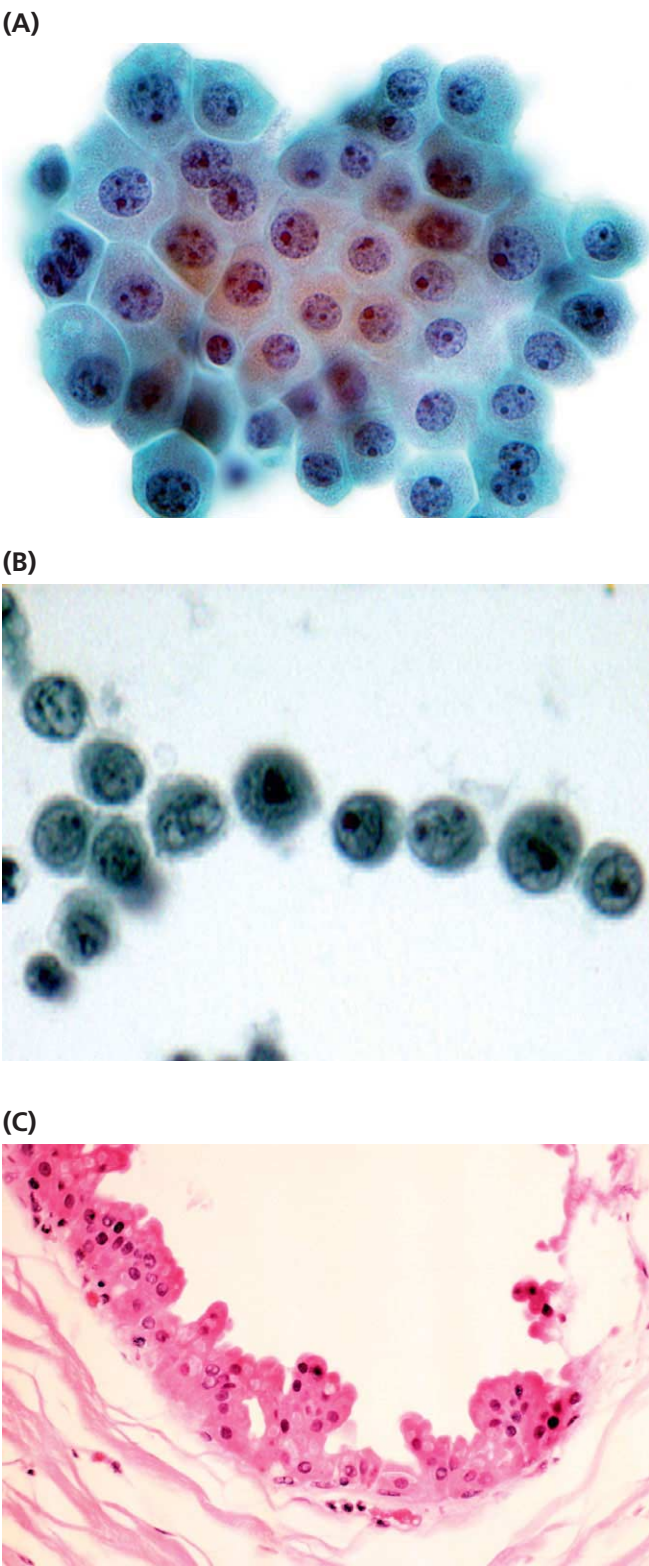
Diagnostic true tissue fragment (DTTF)	Similar structures
	Clumps, aggregates, clusters, conglomerates, morula, pseudo-acini, pseudo-papillae. It must be appreciated that these represent three planes of focus and not necessarily three superimposed sheets
	Diagnostic features
No windows	Intercellular spaces – windows
Fence-like outer border	Generally loop-to-loop pattern
Obtuse angle between cells	Acute angle between cells
Tight intercellular junctions	Loose junctions
True acini (three-dimensional structures with top ( <i>en-face</i> viewing) and inner layer with true luminal border)	No true acini No distinct epithelial sheet No true inner border No true bottom layer
True polypoid or papillary fragment epithelial center sheet – lumen with inner stroma and core	No true sheet No luminal border No stroma

**Table 2.2** Salient cytomorphologic features helpful in differentiating between endocervical and endometrial cells

Features	Endocervical cell	Endometrial cell
Cytoplasm	Variable	Scant
Cytoplasmic border	Distinct	Indistinct
Nuclei	Oval or round	Irregular
Multiple	Frequently	Usually single
Chromatin	Granular	Clumped
Size variation	Variable	Little
Preservation	Good	Poor/variable



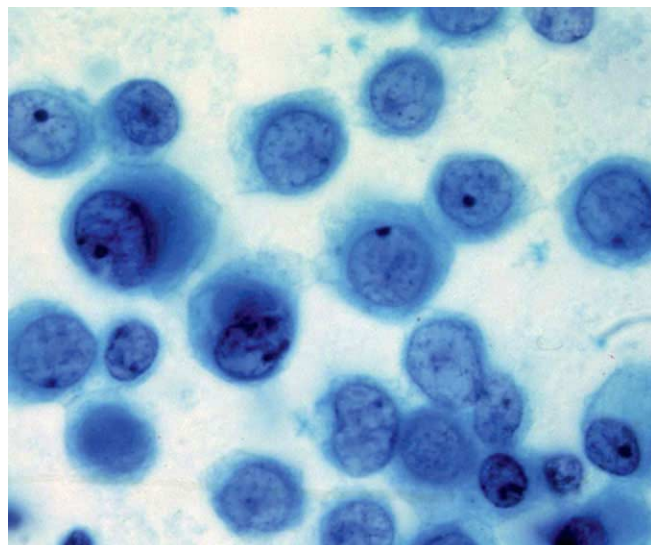
**Figure 2.4** Endocervical and reserve cells showing cellular changes resulting from hormonal and physiological effects. These cells are resting on a basement membrane and reveal features of benign cells. Cervical biopsy, H/E stain.



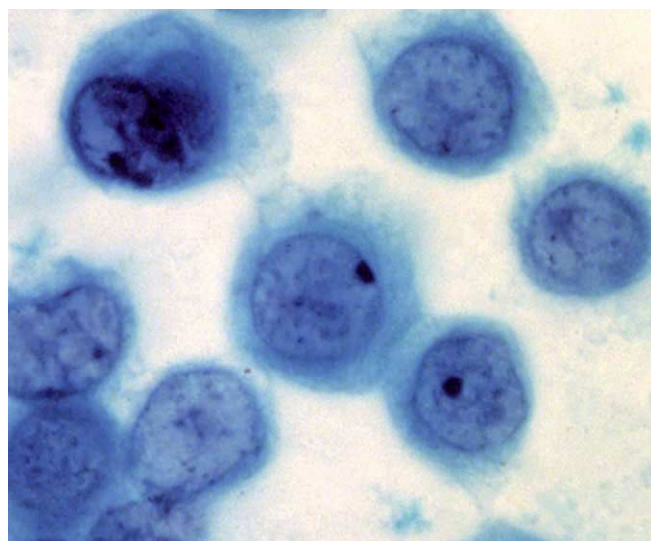
**Figure 2.5** (A–C) Roundedness in the cell and nuclear organelles. Metaplastic cells (apocrine cells) in breast cyst, fine needle aspiration. (A) cytospin slide. (B) mesothelial cells abdominal fluid, cytospin, Pap stain. (C) histologic section of a breast cyst lined by apocrine cells, H/E.

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(A)



(B)

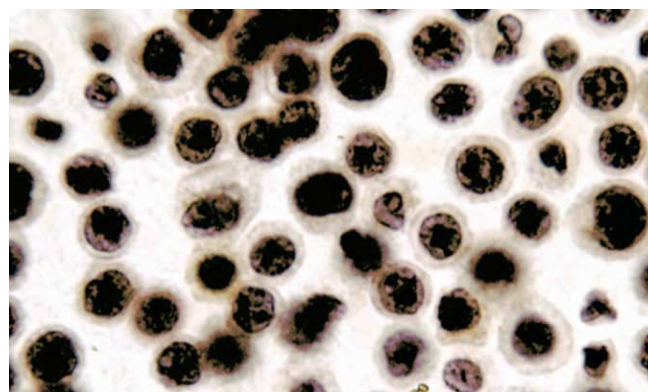


**Figure 2.6** Pleural fluid, refrigerated for 3 days. Notice the well-preserved round nuclei of mesothelial cells on low power (A) and preservation of their nuclear details on high power (B). Millipore filter preparation, Pap stain.

Similarly, urothelial cells suspended in the fluid within the urinary bladder for some time also acquire blunted edges and a rounded appearance. It must be appreciated that this feature is determined by the cytokeratin make up of the cell to a certain degree, i.e. cells with low molecular weight cytokeratin are more often affected by physical forces. They are often retained to variable degrees in other benign and malignant cells (Figures 2.6 and 2.7).

### **Uniformity**

This refers to the cell size, shape, texture, and nuclear structure. Cells within the same micro-fragment exhibit identical



**Figure 2.7** Pleural fluid with gastric adenocarcinoma cells after 3 days of refrigeration. Notice the hyperchromasia and lack of distinction between malignant and benign mesothelial cells. Millipore filter, Pap stain.

morphometric and textural characteristics. Nuclear chromatin, irrespective of its basic morphological appearance (granular, meshy, or fibrillary) tends to be uniform, i.e. minimal variation in shape and size, as well as its distribution within the nuclear sap. Similarly, the shape of the nuclear membrane (outer rim of the nuclear envelope) is uniformly round or oval (Figure 2.8). However, this can be altered by physiological and physiochemical forces, such as secretion within the cytoplasm or by the neighboring cells and structures (best studied in living cells by phase contrast microscopy). Morphological changes can occur due to specimen fixation and preservative used; commonly utilized 95% ethanol provides the least amount of morphologic alterations, thus making cellular interpretation meaningful.

### **Predictability**

Euplastic cells derived from the same site and assembled as a tissue fragment demonstrate certain morphological features which are extremely reliable in assessing their biologic nature. These include the size, shape, polarity, and location of the nuclei; orientation of the cytoplasmic vacuoles; size and shape of the cells; size of the nuclei; shape of the nuclear envelope; the nucleocytoplasmic (N:C) ratios; the thickness of the nuclear membrane; the size, location, and distribution of chromatin material as well as pattern; the texture of parachromatin shape; and the number of nucleoli (Figure 2.9). Among euplastic cells, all cellular components are predictable (as discussed below). Under certain physiological conditions the cells can show some deviation in their predictable behavior; however, this is minimal (i.e. less than one to four or six times variation in the size of nuclei and number of nucleoli).