Cytology of the Female Genital Tract
CYTOLOGY OF THE FEMALE GENITAL TRACT
INTERNATIONAL HISTOLOGICAL CLASSIFICATION OF TUMOURS
No. 8

CYTOLOGY
OF THE
FEMALE GENITAL TRACT

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ALREADY PUBLISHED IN THIS SERIES:

No. 1. Histological typing of lung tumours, by Leiv Kreyberg in collaboration with A. A. Liebow and E. A. Uehlinger (1967)


No. 3. Histological typing of soft tissue tumours, by F. M. Enzinger in collaboration with R. Lattes and H. Torloni (1969)


No. 7. Histological typing of salivary gland tumours, by A. C. Thackray in collaboration with L. H. Sobin (1972)
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Colour photomicrographs
GENERAL PREFACE TO THE SERIES

Among the prerequisites for comparative studies of cancer are international agreement on histological criteria for the classification of cancer types and a standardized nomenclature. At present, pathologists use different terms for the same pathological entity, and furthermore the same term is sometimes applied to lesions of different types. An internationally agreed classification of tumours, acceptable alike to physicians, surgeons, radiologists, pathologists and statisticians, would enable cancer workers in all parts of the world to compare their findings and would facilitate collaboration among them.

In a report published in 1952,1 a subcommittee of the WHO Expert Committee on Health Statistics discussed the general principles that should govern the statistical classification of tumours and agreed that, to ensure the necessary flexibility and ease in coding, three separate classifications were needed according to (1) anatomical site, (2) histological type, and (3) degree of malignancy. A classification according to anatomical site is available in the International Classification of Diseases, the foundations of which were laid as long ago as 1853 when the first international statistical congress was held in Brussels. Responsibility for the decennial revision of the international lists of causes of disease and death was taken over in 1924 by the Health Organisation of the League of Nations and since 1947 has passed to the World Health Organization. The 1965 revision2 contains a much more detailed classification of neoplasms by anatomical site than did its predecessors.

The question of establishing a universally accepted classification by histological type has received much attention during the last 20 years and a particularly valuable Atlas of Tumor Pathology—already numbering more than 40 volumes—is being published in the USA by the Armed Forces Institute of Pathology under the auspices of the National Research Council. An Illustrated Tumour Nomenclature in English, French, German, Latin, Russian, and Spanish has also been published by the International Union Against Cancer (UICC).

The World Health Organization became involved in 1956 when the WHO Executive Board passed a resolution3 requesting the Director-General to explore the possibility that WHO might organize centres in various parts of the world and arrange for the collection of human tissues and their histological

classification. The main purpose of such centres would be to develop histological
definitions of cancer types and to facilitate the wide adoption of a uniform
omenclature. This resolution was endorsed by the Tenth World Health
Assembly in May 1957 and the following month a Study Group on Histological
Classification of Cancer Types met in Oslo to advise WHO on its implementa-
tion. The Group recommended criteria for selecting tumour sites for study and
suggested a procedure for the drafting of histological classifications and testing
their validity. Briefly, the procedure is as follows:

1. For each tumour site, a tentative histopathological typing and classifica-
tion is drawn up by a group of experts, consisting of up to ten pathologists
working in the field in question.

2. An international reference centre and a number of collaborating labora-
tories are then designated by WHO to evaluate the proposed classification.
These laboratories exchange histological preparations, accompanied by clinical
information. The histological typing is then made in accordance with the
proposed classification. Subsequently, one or more technical meetings are called
by WHO to facilitate an exchange of opinions. If necessary, the classification is
amended to take account of criticisms.

3. The international reference centre then prepares sets of microscope slides
covering all the proposed histological types and sends these with the revised
classification to other pathologists, usually not more than ten, for their
comments and suggestions.

4. When replies have been received from all these reviewers, the classifica-
tion is again revised in accordance with their comments. The international
reference centre then prepares up to 100 sets of microscope slides of the various
histological types and also drafts a text explaining the basis of the classification.
In addition, photomicrographs are taken of the appropriate fields for the
preparation of colour plates and 35-mm transparencies.

Since 1958, WHO has established 23 international reference centres cover-
ing tumours of the lung; breast; soft tissues; oropharynx; bone; ovaries; salivary
glands; thyroid; skin; male urogenital tract; jaws; uterus; stomach and oesopha-
gus; intestines; central nervous system; liver, biliary tract and pancreas;
upper respiratory tract; eye; and endocrine glands; as well as oral pre-
cancerous conditions; the leukaemias and lymphomas; comparative oncology;
and exfoliative cytology. This work has involved more than 200 pathologists
from over 50 countries. The international reference centres for tumours of
the lung; breast; soft tissues; oropharynx; bone; jaws; salivary glands; skin;
and ovaries; and for leukaemias and lymphomas have completed their work,
and some of the classifications prepared by these centres have already been
published (see p. 6).

The World Health Organization is indebted to the many pathologists who have participated and are participating in this large undertaking, especially to the heads of the international reference centres and of the collaborating laboratories. Grateful acknowledgement is also made to the many other international and national organizations whose pioneer work in the field of histological classification of tumours has greatly facilitated the task undertaken by WHO. Finally, WHO wishes to record its appreciation of the co-operation of the International Council of Societies of Pathology (ICSP) which has undertaken to distribute copies of the classifications, with corresponding sets of microscope slides, to national societies of pathology all over the world.
PREFACE TO

CYTOLOGY OF THE FEMALE GENITAL TRACT

The International Academy of Cytology, during the Second International Congress in Paris, May 1965, recommended that, because of the special problems involved, the help of WHO should be sought in developing a standardized nomenclature for cytology that could be used internationally. Accordingly, two temporary advisers met in Geneva in May 1968 to formulate a tentative nomenclature and to develop methods of testing a cytological classification.

At a meeting convened by WHO in Geneva in October 1968, the tentative nomenclature was discussed and modified. The WHO International Reference Centre for Nomenclature in Cytology was subsequently established at the Centre de Cytologie et Dépistage du Cancer [Centre for Cytology and Detection of Cancer], Geneva, with Professor G. Riotton as Head. Representative transparencies and histological sections were selected and circulated to the collaborating centres.

At a second meeting in Geneva in November 1969, the definitions were further modified as a result of the experience of the collaborating centres, and examples were selected from among the transparencies for publication. At this meeting it was recommended that the nomenclature for cytology of the female genital tract should be published first and that a separate publication on extragenital cytology nomenclature should follow later.

The rather special nature of the problems encountered in relation to nomenclature for the cytology of the female genital tract made it sometimes difficult to reach agreement. Nevertheless, it was decided at an early stage to use diagnostic terms rather than Papanicolaou’s numerical classification, so that it was important to follow a standard histological classification. Although the International Reference Centre for Histopathological Definition of Uterine and Placental Tumours has not yet fully tested its classification, it was considered expedient to utilize as far as possible the preliminary classification that the group had devised.¹

It is fully realized that in a new science developing in many parts of the world at the same time it is impossible to achieve complete agreement about terms.

¹ The Terminology Committee of the International Academy of Cytology meeting in June 1970 in Vienna approved the WHO nomenclature for cytology of the female genital tract, with the proviso that it might have to be modified later should any changes be made in the histopathological nomenclature and classification of uterine and placental tumours.
Moreover, the rapidity of the development of cytology is likely to make changes in present definitions and concepts necessary. Nevertheless, international co-operation in the attempt to devise a universal language for cytology will greatly facilitate communication and the comparison of data. Criticisms of the present nomenclature and helpful suggestions will be much appreciated.
Carcinoma of the uterine cervix is the most prevalent form of cancer, other than skin cancer, in the world as a whole, and in developing countries the incidence is much higher than in the more developed ones. The preclinical stages of cancer of the cervix are known to last for several years, and they can be detected by simple, reproducible, and accurate cytological methods. In these pre-invasive stages cure rates approaching 100% can be obtained. Epithelial atypia may even precede carcinoma in situ, and the cytological detection of these lesions allows the destruction of abnormal tissue, so that the development of cancer is prevented.

Although in most countries carcinoma of the endometrium is less frequent than cancer of the cervix, its relative importance is increasing in many areas. It is therefore appropriate to try to develop improved methods of obtaining endometrial cells, so that earlier cytological diagnosis of asymptomatic or at least earlier endometrial lesions can be achieved. Cytology is of little use in the detection of asymptomatic sarcomas, but is sometimes an aid to the diagnosis of carcinoma of the fallopian tubes and ovaries and metastatic carcinoma.

It is not yet generally appreciated that cell identification and interpretation should be considered an integral part of pathology. The basic principle of cytodiagnosis is the accurate identification of cells with the histological entity from which they are derived. The pathologist, because of his training and background, is the obvious person to make cytodiagnostic interpretations. Unfortunately, pathologists with experience and training in cytodiagnosis are limited in number in most countries, and non-existent in others. Since the cytopathologist is the key to success in uterine cancer detection programmes, there is a great need to train additional pathologists in this discipline and also to give special training to clinicians.

Although it is widely accepted that effective uterine cancer control can be achieved by means of mass screening, cytodiagnosis can do more harm than good if not properly performed: existing cancers may be missed, so that the patient is given a false sense of security, and the appearance of cells from non-cancerous lesions may be misinterpreted. It is evident that, unless adequate facilities for histological confirmation, treatment, and follow-up are provided, a cytology programme is ineffective and is doomed to failure.

Since the greatest value of uterine cytology lies in the early detection of uterine cancer, especially asymptomatic cancer of the uterine cervix, its
primary application is in the mass screening of asymptomatic women. This procedure requires cytology technicians specially trained to carry out the routine screening of the large volume of material produced by any realistic detection programme. It is generally agreed that trained cytology technicians can and do perform this important function effectively. There are, however, very few countries in which adequate training standards for cytology technicians exist. The establishment of such standards and the constant review of performance are essential if the maximum benefit is to be derived from the technique.

Without adequate communication, progress is hampered. In the development of teaching material for training purposes a standard nomenclature is essential. Hitherto, methods of communication in cytology have been totally inadequate. The commonly used system of numerical classification to indicate the cytopathologist's opinion as to the degree of probability that a particular lesion is cancer has led to confusion, because the numerical designation varies from one laboratory to another. Now that cytopathologists are better able to recognize the nature of histological lesions on the basis of cell studies, it is both desirable and feasible that cytology reports should be formulated so as to correspond to the anticipated histological diagnosis.

As soon as a standardized cytological nomenclature is accepted, a comprehensive method of formulating cytology reports based on it should be developed so as to express clearly the histological nature of the lesion. While cytology has been developing, a wide variety of terms, some ambiguous, have been used. It is hoped that the nomenclature suggested in this volume will now be adopted in an effort to improve communication.

Throughout the book the descriptions of cells and the illustrations are based on cells derived from specific lesions, including some that are not neoplastic. The definitions relate to well preserved cells derived from the various lesions. Unfortunately the technical quality of routine preparations is often less than perfect. The range of illustrations selected therefore includes some that are encountered routinely as well as exceptionally well preserved cells. There are uterine tumours that do not lend themselves to cytodiagnosis; and there are also tumours of such rarity that their inclusion is not warranted.

Artefacts and preparations that are unsatisfactory for a variety of reasons, such as drying and paucity of material, are a recurring problem for the cytopathologist. Since attempts at interpreting such preparations often lead to diagnostic error, they must be rejected as unsatisfactory and repeat specimens requested.

A number of terms used in the past have not been included in the classification. To help readers who might wish to know the corresponding terms in French, Russian, and Spanish, translations of the classification into these languages are also given, immediately following the English
version. The classification reflects the judgement of the members of the International Reference Centre, based on present knowledge, and modifications will undoubtedly be needed in the light of experience.

A note on recommended techniques for cell collection and fixation will be found in Annex 1, page 32. Some terms that are commonly used in clinical cytology of the female genital tract but may be unfamiliar to those new to this field are explained in Annex 2, page 33.
CYTOLOGICAL CLASSIFICATION OF TUMOURS OF THE FEMALE GENITAL TRACT

I. CELLS DERIVED FROM CERVICAL LESIONS

A. Dysplasia
   1. Mild
   2. Moderate
   2. Severe

B. Epidermoid carcinoma in situ

C. Epidermoid carcinoma in situ with minimal stromal invasion

D. Invasive epidermoid microcarcinoma

E. Invasive epidermoid carcinoma
   1. Keratinizing carcinoma
   2. Large cell non-keratinizing carcinoma
   3. Small cell non-keratinizing carcinoma

F. Adenocarcinoma of endocervix

G. Clear cell (mesonephric) carcinoma of cervix

H. Adenosquamous (muco-epidermoid) carcinoma

II. CELLS DERIVED FROM ENDOMETRIAL EPITHELIAL LESIONS

A. Atypical hyperplasia of endometrium

B. Adenocarcinoma of endometrium
III. CELLS DERIVED FROM UTERINE SARCOMAS

A. Leiomyosarcoma

B. Endometrial stromal sarcoma

IV. CELLS DERIVED FROM MÜLLERIAN MIXED TUMOURS

A. Carcinosarcoma

B. Mesodermal mixed tumour

V. CELLS DERIVED FROM TROPHOBLASTIC LESIONS

A. Hydatidiform mole

B. Choriocarcinoma

VI. CELLS DERIVED FROM EXTRAUTERINE MALIGNANT TUMOURS
CLASSIFICATION CYTOLOGIQUE DES TUMEURS GYNÉCOLOGIQUES

I. CELLULES PROVENANT DE LÉSIONS DU COL UTÉRIN

A. Dysplasies
   1. Légères
   2. Modérées
   3. Marquées

B. Carcinome épidermoïde in situ

C. Carcinome épidermoïde in situ avec ébauche d'invasion

D. Carcinome épidermoïde microinvasif

E. Carcinome épidermoïde invasif
   1. Carcinome kératinisant
   2. Carcinome non-kératinisant à grandes cellules
   3. Carcinome non-kératinisant à petites cellules

F. Adénocarcinome de l'endocol

G. Carcinome à cellules claires (mésonephroïde) du col

H. Carcinome adénosquameux (muco-épidermoïde)

II. CELLULES PROVENANT DE LÉSIONS ÉPITHELIALES DE L'ENDOMÈTRE

A. Hyperplasie atypique de l'endomètre

B. Adénocarcinome de l'endomètre
III. CELLULES PROVENANT DE SARCOMES DE L'UTÉRUS

A. LÉIOMYOSARCOME

B. SARCOME DU STROMA ENDOMÉTRIAL

IV. CELLULES PROVENANT DE TUMEURS MIXTES MÜLLÉRIENNES

A. CARCINOSARCOME

B. TUMEURS MIXTES MÉSODERMİQUEs

V. CELLULES PROVENANT DE LÉSIONS DU TROPHOBLASTE

A. MÔLE HYDATIFORME

B. CHORIOCARCINOME

VI. CELLULES PROVENANT DE TUMEURS MALIGNES EXTRA-GÉNITALES
ЦИТОЛОГИЧЕСКАЯ КЛАССИФИКАЦИЯ ОПУХОЛЕЙ ЖЕНСКОЙ ПОЛОВОЙ СФЕРЫ

I. КЛЕТКИ, ПОЛУЧЕННЫЕ ИЗ ПАТОЛОГИЧЕСКОГО ОТДЕЛЯЕМОГО ЦЕРВИКАЛЬНОГО КАНАЛА

А. Дисплазия
   1. Слабая
   2. Умеренная
   3. Резкая

Б. Плоскоклеточный внутриэпителиальный рак

В. Плоскоклеточный внутриэпителиальный рак с начальной инвазией в строму

Г. Инвазивная плоскоклеточная микрокарцинома

Д. Инвазивный плоскоклеточный рак
   1. Ороговевающий
   2. Неороговевающий крупноклеточный рак
   3. Неороговевающий мелкоклеточный рак

Е. Аденокарцинома канала шейки матки

Ж. Светлоклеточный мезонефральный рак канала шейки матки

З. Аденокантона (мукоэпидермоидная карцинома)

II. ЭПИТЕЛИАЛЬНЫЕ КЛЕТКИ, ПОЛУЧЕННЫЕ ИЗ ПОРАЖЕННОГО ЭНДОМЕТРИЯ

А. Атипичная гиперплазия эндометрия

Б. Аденокарцинома эндометрия

— 23 —
III. КЛЕТКИ, ПОЛУЧЕННЫЕ ИЗ САРКОМОЙ МАТКИ

А. Лейомиосаркома

В. Эндометриальная стромальная сарcoma

IV. КЛЕТКИ, ПОЛУЧЕННЫЕ ИЗ МЮЛЛЕРОВСКИХ СМЕШАННЫХ ОПУХОЛЕЙ

А. Карциносаркома

Б. Мезодермальная смешанная опухоль

V. КЛЕТКИ, ПОЛУЧЕННЫЕ ПРИ ТРОФОБЛАСТИЧЕСКОМ ПОРАЖЕНИИ

А. Пузырный занос

Б. Хорионэпителиома (хориокарцинома)

VI. КЛЕТКИ, ПОЛУЧЕННЫЕ ИЗ ВНЕМАТОЧНЫХ ЗЛОКАЧЕСТВЕННЫХ ОПУХОЛЕЙ
CLASIFICACIÓN CITOLÓGICA DE LOS TUMORES DEL APARATO GENITAL FEMENINO

I. CÉLULAS DERIVADAS DE LESIONES CERVICALES

A. DISPLASIA
   1. Leve
   2. Moderada
   3. Intensa

B. CARCINOMA EPIDERMOIDE IN SITU

C. CARCINOMA EPIDERMOIDE IN SITU CON INVASIÓN MÍNIMA DEL ESTROMA

D. MICROCARCINOMA EPIDERMOIDE INVASOR

E. CARCINOMA EPIDERMOIDE INVASOR
   1. Carcinoma queratinizante
   2. Carcinoma no queratinizante de células grandes
   3. Carcinoma no queratinizante de células pequeñas

F. ADENOCARCINOMA DEL ENDOCÉRVIX

G. CARCINOMA CERVICAL DE CÉLULAS CLARAS (TIPO MESONÉFRICO)

H. CARCINOMA ADENOESCAMOSO (MUCOEPIDERMOIDE)

II. CÉLULAS DERIVADAS DE LESIONES EPITELIALES DEL ENDOMETRIO

A. HIPERPLASIA ATÍPICA DEL ENDOMETRIO

B. ADENOCARCINOMA DEL ENDOMETRIO
III. CÉLULAS DERIVADAS DE SARCOMAS UTERINOS

A. Leiomiosarcoma

B. Sarcoma estromal endometrial

IV. CÉLULAS DERIVADAS DE TUMORES MIXTOS MÜLLERIANOS

A. Carcinosarcoma

B. Tumor mixto mesodérmico

V. CÉLULAS DERIVADAS DE LESIONES TROFOBLÁSTICAS

A. Mola hidatiforme

B. Coriocarcinoma

VI. CÉLULAS ORIGINADAS DE TUMORES MALIGNOS EXTRAUTERINOS
EXPLANATORY NOTES

I. CELLS DERIVED FROM CERVICAL LESIONS

A. Dysplasia

Dysplastic cells exhibit varying degrees of differentiation. In general, the more differentiated cell types correspond to the milder forms of dysplasia. The nucleus is always enlarged and hyperchromatic.

1. In mild dysplasia the cells are usually of the superficial or intermediate type, rarely of the parabasal type. The chromatin pattern is uniform; there may be numerous small chromocentres.

2. In moderate dysplasia there is a group of cellular patterns intermediate between mild and severe dysplasia.

3. In severe dysplasia the cells are mainly of the parabasal and small intermediate type. The chromatin is dense and uniform, with many darkly stained, slightly enlarged chromocentres and no nucleoli.

B. Epidermoid Carcinoma in Situ

Carcinoma in situ is characterized by the presence of poorly differentiated and undifferentiated squamous epithelial cells that exhibit pronounced nuclear and cytoplasmic abnormalities: an increased nuclear/cytoplasmic ratio, an enlargement of the nucleus, hyperchromasia, and a coarsely granular chromatin network; the nucleoli are not usually identified; binucleation and multinucleation may be present. In addition, the cytoplasm of these cells may show amphophilia and may contain vacuoles. Abnormal cells may occur individually or in loose aggregates. There is often a uniformity of cell type. Dysplastic cells derived from other areas of the cervix are often present.

Such abnormal cells showing a greater degree of differentiation may also be present in those instances of carcinoma in situ in which a certain degree of maturation is apparent towards the surface.

C. Epidermoid Carcinoma in Situ with Minimal Stromal Invasion

At the present time it is rarely possible to distinguish the cells of carcinoma in situ with minimal stromal invasion from those of carcinoma in situ.
D. INVASIVE EPIDERMOID MICROCARCINOMA

The cell pattern is similar to that found in cell preparations from carcinoma in situ, invasive epidermoid carcinoma, or a mixture of the two; however, the characteristic tumour background is usually not present.\(^1\)

E. INVASIVE EPIDERMOID CARCINOMA \(^2\)

1. *Keratinizing carcinoma*

The cell spectrum reflects the histological counterpart. There is a preponderance of relatively large abnormal cells with a high degree of pleomorphism characterized by caudate and elongated forms. The chromatin pattern is coarse and irregular, and there are several dense chromocentres of varying size and shape. Macronucleoli are not prominent. Nuclear degeneration characterized by an opaque nuclear mass is a distinct feature. The cytoplasm may be very abundant and is often eosinophilic or orangeophilic. Pearls or portions of pearls composed of malignant cells are sometimes found.\(^3\) A tumour background may not be present in this form of cancer.\(^1\)

2. *Large-cell non-keratinizing carcinoma*

There are numerous syncytial masses as well as isolated relatively large, cyanophilic cells. The chromatin pattern is coarse and irregular, and there are several dense chromocentres of varying size and shape. The nuclei contain prominent macronucleoli. Although the cell size tends to be uniform, there is a moderate degree of variation.\(^3\) The tumour background is useful for distinguishing these lesions from the large-cell carcinoma in situ.\(^1\)\(^,\)\(^3\)

3. *Small-cell non-keratinizing carcinoma*

Relatively uniform small cyanophilic cells with a high nuclear cytoplasmic ratio predominate. The chromatin pattern is coarse and irregular, and there are several dense chromocentres of varying size and shape. Macronucleoli are prominent.\(^3\) The cells are usually associated with a tumour background.\(^1\)

F. ADENOCARCINOMA OF THE ENDOCERVIX

The cells tend to show some cytoplasmic eosinophilia; cyanophilia or indeterminate staining is less common. The cytoplasm is usually granular

\(^1\) The characteristic background of cell preparations in invasive carcinoma consists of cellular debris, inflammatory exudate, altered and fresh blood, and a loss of normal bacterial flora. In the early phases of invasion these changes are not usually present.

\(^2\) Some invasive carcinomas contain a mixture of two or three types, in which case the cellular as well as the tissue diagnosis should reflect the predominant tumour type.

\(^3\) It is important to recognize that abnormal cells from co-existing dysplasia and/or carcinoma in situ may be observed in specimens from patients with invasive carcinoma.
with occasional fine vacuolation, but rarely large discrete vacuoles are present. The large nucleus has a finely granular chromatin pattern with a tendency to marginization. Pyknosis is not seen. Nucleoli are almost always present and are usually multiple. Macronucleoli are commonly observed.¹ Cellular samples may contain malignant glandular cells of endocervical origin, together with malignant squamous cells (or adenosquamous mucoepidermoid carcinoma).

G. CLEAR CELL (MESONEPHRIC) CARCINOMA OF THE CERVIX

These cells can rarely be distinguished cytologically from cells derived from other forms of cervical adenocarcinoma.

H. ADENOSQUAMOUS (MUCOEPIDERMOID) CARCINOMA

There is usually a preponderance of epidermoid carcinoma with occasional cells demonstrating mucin content.

II. CELLS DERIVED FROM ENDOMETRIAL EPITHELIAL LESIONS

A. ATYPICAL HYPERPLASIA OF THE ENDOMETRIUM

The cells display nuclear and cytoplasmic changes of varying intensity. In the less marked cases, the cells retain their typical columnar shape. The nuclei are enlarged, and there is some hyperchromasia. The chromatin pattern is regular. The cytoplasm may be finely or coarsely vacuolated. In the more advanced histological forms the cellular changes are more pronounced, and the cell morphology may resemble closely that of cells derived from well differentiated adenocarcinoma of the endometrium.² In general, there is enlargement of nuclei, with hyperchromasia and some irregularity of the chromatin pattern. The nuclear margin may also show some degree of irregularity. Single nucleoli become predominant in the more severe forms. The cytoplasm is usually vacuolated and the nuclear/cytoplasmic ratio is increased.¹

Cells from atypical endometrial hyperplasia tend to occur in three-dimensional clusters, but isolated elements are also found. Cells from this lesion are usually found on vaginal smears at times when endometrial cells are not normally encountered.

¹ These descriptions refer to well preserved cells obtained by endocervical or endometrial aspiration.
² Cytologically, it is not possible in the present state of knowledge to distinguish cells of "adenocarcinoma in situ" from those of atypical hyperplasia and well differentiated adenocarcinoma.
B. **ADENOCARCINOMA OF THE ENDOMETRIUM**

The cells are found in three-dimensional clusters or singly. They are usually larger than normal columnar endometrial cells. The cyanophilic cytoplasm is generally finely vacuolated, but may be homogeneous. Large vacuoles, which distort the cell, are occasionally found. The nuclei, usually fairly uniform in size, sometimes show pleomorphism. They are round or oval unless distorted by large cytoplasmic vacuoles. The chromatin tends to be finely granular and hyperchromasia is limited. Pyknosis is not found unless there is a squamous component. The majority of cells contain distinct nucleoli. In the less well differentiated tumours the nucleoli tend to be multiple, often large, with irregular outlines.¹

The less differentiated the tumour the larger the cells, the nuclei, and the nucleoli. Multiple nucleoli and/or macronucleoli are not usually found in the well differentiated tumours. Cytoplasmic cyanophilia is more pronounced in the cells derived from undifferentiated endometrial adenocarcinoma. Malignant glandular cells can occasionally be seen together with malignant squamous cells (adenosquamous cells).

### III. CELLS DERIVED FROM UTERINE SARCOMAS

A. **LEIOMYOSARCOMA**

Spindle-shaped, poorly preserved cells with elongated hyperchromatic nuclei are found singly, or rarely in groups, in the presence of a characteristic tumour background.²

B. **ENDOMETRIAL STROMAL SARCOMA (ENDOLYMPHATIC STROMAL MYOSIS)**

This tumour rarely sheds cells that can be detected on vaginal aspiration samples. This is especially true of the endolymphatic stromal myosis variant. The cells from endometrial stromal sarcoma are indistinguishable from the cells derived from leiomyosarcoma.

### IV. CELLS DERIVED FROM MÜLLERIAN MIXED TUMOURS

A. **CARCINOSARCOMA**

Intermingled carcinoma and sarcoma cells.

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¹ See footnote 1 on p. 29.
² The cellular identification of leiomyosarcomas is seldom made unless the tumour is well advanced and symptomatic.
B. MESODERMAL MIXED TUMOUR

The cellular identification is based on the presence of carcinoma cells (usually of glandular origin) together with spindle-shaped cells with indistinct outlines containing elongated deeply hyperchromatic nuclei. The identification of cross-striations in spindle-shaped cells with acidophilic cytoplasm is a distinctive feature, but is not often found.¹

V. CELLS DERIVED FROM TROPHOBLASTIC LESIONS

A. HYDATIDIFORM MOLE

Cytotrophoblastic cells of benign appearance are occasionally found in smears from patients with a hydatidiform mole. They are usually single rounded cells with cyanophilic cytoplasm and rather large hyperchromatic nuclei. The cells are very similar to the cytotrophoblasts found in postabortal smears.

B. CHORIOCARCINOMA

The syncytial trophoblastic cells show anisokaryosis, and the nuclei have features similar to those of the young cytotrophoblastic nuclei. The nucleus of the cytotrophoblast has an irregular membrane and contains coarse, irregularly dispersed chromatin. The nucleoli are distinct and may be multiple. There is a tumour background with a predominance of altered blood. The hormonal pattern often reflects high estrogenic activity.

VI. CELLS DERIVED FROM EXTRAUTERINE MALIGNANT TUMOURS

In most instances it is not possible to distinguish between cells derived from primary uterine and extrauterine cancer, particularly when associated with a tumour background. The possibility of extrauterine cancer must be considered where there are groups of malignant tumour cells without a tumour background.

¹ The cellular identification of mesodermal mixed tumours is seldom made unless the tumour is well advanced and symptomatic.
Cytological preparations can be obtained from different areas of the female genital tract. Specially recommended sites are:

(a) the lateral vaginal wall  
(b) the posterior fornix  
(c) the exocervix  
(d) the endocervix  
(e) the endometrium

Cell collection should always be carried out under direct visual control of the exocervix after the introduction of a non-lubricated speculum. Material from the lateral vaginal wall is obtained by scraping with a wooden spatula. This type of sample is particularly suitable for cytohormonal evaluation.

Cell preparations from the posterior fornix can be collected either by aspiration through a glass pipette or by scraping with a wooden spatula. The wooden spatula is the instrument of choice for obtaining cell samples from the exocervix. The entire periorificial area should be scraped.

Cell samples from the endocervix can be obtained by introducing a cotton applicator into the endocervical canal and rotating it one full circle, or by aspiration. Special endocervical scrapers are also commercially available.

Material from the endometrium is aspirated through a tube attached to a syringe. The samples must be quickly spread on a glass slide and immediately fixed (before any drying occurs).

The recommended staining technique is the one described by Papanicolaou.
ANNEX 2

NOTES ON TERMS COMMONLY USED IN CLINICAL CYTOLOGY OF THE FEMALE GENITAL TRACT

AGGREGATES OF EPITHELIAL CELLS

*Sheet:* a monolayer in which the component cells are regularly arranged in relation to one another and distinct cell boundaries.

*Syncytium:* cells that are irregularly arranged with respect to one another and have indistinct cell boundaries.

*Glandular grouping:* a three-dimensional group of cells with poorly defined cell boundaries. They may occur as loose or tight clusters.

ANUCLEATE SQUAME

An orangeophilic large flat squamous cell without a nucleus (indicating keratinization of squamous epithelium).

ATROPHIC SMEAR

A smear characterized by the predominance of parabasal cells.

CANDIDA

A variety of yeast seen in the form of hyphae and conidia.

CYANOPHILIA

The property of staining blue or purplish with basic stains.

CYTOLYSIS (CYTOPLASMOLYSIS)

The dissolution of cytoplasm (frequently associated with the presence of *Lactobacillus vaginalis*).

CYTOSTATIC EFFECT

The effect of cytostatic drugs on cells. It is not qualitatively different from the irradiation effect but is less marked.
DECIDUAL CELLS

Endometrial stromal cells modified by progesterone, occurring singly or in sheets. They are larger than ordinary stromal cells, and have a dense, amphophilic cytoplasm, sometimes diffusely vacuolated, and a centrally located vesicular nucleus.

DEGENERATIVE CHANGES

These may be cytoplasmic and/or nuclear in type. The cytoplasm may show an altered staining reaction, coarse granularity and/or vacuolation, frayed borders, and sometimes lysis. The nucleus may show pyknosis, karyorrhexis, simple enlargement with reduced staining affinity, or ultimately karyolysis.

DIFFERENTIATION

The process of morphological and functional specialization of cells.

ENDOCERVICAL COLUMNAR CELLS

Columnar cells may be non-ciliated or ciliated and they occur singly, in strips, in sheets, and in glandular groupings. When viewed end-on, the cell is small and polygonal in form, and when seen in profile the cell is of columnar form. Such configurations are more common in cells removed by abrasion; when they desquamate spontaneously some of the cells tend to assume a rounded form. The cell size is variable, usually 1½ to 2 times the size of an endometrial cell. The amphophilic or faintly eosinophilic cytoplasm is finely granular and, depending on the metabolic state of the cell, may be diffusely vacuolated or dominated by a single vacuole. The round or oval nucleus, which is approximately 1½ times the size of an endometrial cell nucleus, has a thin, well defined nuclear membrane. A protrusion of the nuclear content may occur at one nuclear pole during the ovulatory phase. The vesicular nucleus usually contains two to three chromocentres and sometimes a small nucleolus. Binucleation and rarely multinucleation are observed.

ENDOCERVICAL RESERVE CELLS

Multipotential subcolumnar cells of the endocervix, identified only in cases of reserve cell hyperplasia. They are usually observed in sheets and have poorly defined cell borders. They are small, oval, or polygonal, and have scanty, finely vacuolated, cyanophilic cytoplasm. The nuclei are centrally located. They are round or oval, or occasionally have a reniform shape with longitudinal grooves or folds. The chromatin is fine and has numerous chromocentres. Reserve cells when occurring singly cannot readily be distinguished from small histiocytes or endometrial stromal cells.
The characteristics of endometrial cells depend on the site of origin, the stage of the menstrual cycle, menopausal status, the angle from which some of the cells are viewed, and the mode of cell collection. Two main types are recognized—epithelial and stromal cells. The endometrial cell nucleus is approximately the size of the intermediate cell nucleus.

**Endometrial epithelial cells.** Three cell types are observed:

1. the secretory (non-ciliated) cell;
2. the ciliated cell;
3. the intercalated cell.

Epithelial cells present in cellular samples are usually non-ciliated. Although isolated cells may be observed, they are usually shed in aggregates. The aggregates may be composed of epithelial cells only, or there may be an admixture of stromal cells.

The epithelial cell is small and columnar to cuboidal, with a scanty, finely vacuolated, cyanophilic cytoplasm and an eccentrically placed nucleus displaying a finely uniform chromatin pattern. One or occasionally two chromocentres may be identified in the nucleus. Nucleoli are not identified.

The intercalated cells are probably compressed secretory cells. The cells are long, thin, prismatic forms, some of which have a bulbous dilatation in the vicinity of the centrally located nucleus.

**Endometrial stromal cells.** These cells can be derived from the spongiosa layer (deep stromal cells) or the compact layer (superficial stromal cells) of the functional endometrium. They may be shed singly or in groups. The superficial stromal cell is small, round, or irregular, and has indistinct cell borders. There is considerable variation in size, particularly when there is evidence of phagocytosis by these cells. The cytoplasm is finely vacuolated and faintly cyanophilic. The nucleus, which is often eccentric, is round, oval, or reniform, and has a prominent granular chromatin pattern. The deep stromal cell is smaller and contains less cytoplasm than its superficial counterpart. Vacuolation of the cytoplasm is less evident. The nucleus is oval or spindle-shaped.

**Eosinophilia**

The property of staining various shades of red or pink with eosin dye.

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1 Endometrial cells are usually observed in cell samples obtained during and for some days after menstruation. The presence of endometrial cells in smears obtained during the second half of the menstrual cycle in women of reproductive age and in post-menopausal women must be considered as abnormal shedding.
EPITHELIAL PEARL

A concentric structure, usually containing keratinized cells in the centre and squamous cells at the periphery with nuclei still visible.

ESTROGENIC EFFECT

The action of estrogens on a target tissue, commonly understood as producing proliferation and differentiation of cells in the squamous epithelium of the vagina. The proportion of superficial cells reflects the estrogenic effect on the vaginal epithelium.

FOLIC ACID DEFICIENCY: CHANGES IN SQUAMOUS CELLS

Changes characterized mainly by the enlargement of the whole cell, nuclear enlargement, and binucleation. Cytoplasmic staining and nuclear structure remain normal.

FOLLICULAR CERVICITIS

Cell studies show heavy concentrations of lymphoid cells and reticulum cells. The latter often show phagocytosis. Histologically, there is either a superficial band-like lymphoid infiltrate or well-formed lymphoid follicles.

HAEMOPHILUS VAGINALIS

When this organism is present, very small rods and "clue cells" are found in the cell studies.

HERPESVIRUS-INFECTED CELLS

These are usually parabasal or endocervical cells, often occurring in clusters. There is generally cellular and nuclear enlargement and often multinucleation. Two main characteristic changes can be identified in the nucleus: (1) a ground-glass appearance with margination of chromatinic material (most frequently observed in primary infections); and (2) prominent, large, intranuclear eosinophilic inclusions with a peri-inclusional halo (most frequently observed in recurrent infections).

HISTIOCYTE

A cell characterized by an ill-defined border and a finely vacuolated cytoplasm occasionally containing engulfed particles. The nucleus is round or reniform and often eccentric.
INCLUSION

Contents of extraneous or abnormal intrinsic particles within a cell nucleus or cytoplasm.

INFLAMMATION: CHANGES IN EPITHELIAL CELLS

Changes may be seen in both cytoplasm and nucleus. In the cytoplasm there may be an altered staining reaction, increased granularity, and possibly vacuolation. The nucleus may be either pyknotic or large with a persisting regular though somewhat blurred chromatin pattern. There may be an apparent thickening of the nuclear membrane due to accumulation of chromatin.

IRRADIATION EFFECT

The effect of irradiation on cells. The changes seen include cytomegaly, karyomegaly, vacuolation of the cytoplasm, polychromasia, multinucleation, and varying degrees of nuclear degeneration. There is often an increase in leucocytes around the margins of the epithelial cells.

KARYOLYSIS

A form of degeneration in which the nucleus of the cell swells, gradually loses its chromatin, and finally disappears.

KARYORRHEXIS

Nuclear fragmentation.

KERATINIZATION

The formation of anucleate squames.

KERATINIZED CARCINOMA CELL

A malignant squamous epithelial cell that stains orange with the Papanicolaou stain.

LACTOBACILLUS VAGINALIS

The *Lactobacillus vaginalis* (formerly known as Döderlein's bacillus) is a deeply staining, rod-shaped saprophytic micro-organism, which thrives in the presence of a low vaginal pH and cells with a high glycogen content.
LEPTOTHRIX

A filamentous micro-organism frequently associated with *Trichomonas vaginalis*.

METAPLASTIC CELLS

Cells of squamous type that have replaced endocervical columnar cells, and to less extent endometrial glandular cells. They occur singly, in loose sheets, or in tight groups. In the early stages of development (immature metaplasia) the cells, in addition to having the features of parabasal cells, are larger than reserve cells but smaller than parabasal cells. The cell borders are sharply defined and have a dense, homogeneous, cyanophilic cytoplasm. The nucleus is large and vesicular and contains chromocentres.

In the later stage (mature metaplasia) the cells, in addition to having the features and size of parabasal and intermediate cells, have more sharply defined cell borders, denser staining cytoplasm, and a larger vesicular nucleus containing chromocentres.

In both types of cell the cytoplasm often appears to be divided into an outer dense zone and a relatively pale perinuclear zone.

Atypical metaplastic cells with no apparent evidence of abnormal differentiation are sometimes seen. They exhibit slight enlargement and hyperchromasia of the vesicular nuclei.

MULTINUCLEATED GIANT CELLS

Four types can be identified: epithelial, histiocytic (including the Langhans type), trophoblastic, and malignant.

NAVICULAR CELL

A boat-shaped variant of the intermediate cell. It has condensed peripheral cytoplasm and a high glycogen content. The nucleus is vesicular, often elongated, and located eccentrically.

OVULATORY CYCLE PATTERNS

1. **Follicular phase**

From menstruation to ovulation the smears show large, flat, superficial cells and intermediate squamous cells. Under normal conditions the number of polymorphonuclear leucocytes decreases and the percentage of superficial cells increases throughout this phase.
2. **Ovulatory phase**

The mid-cycle days during which ovulation occurs. The smear is “clean”, with little, if any, mucus or polymorphonuclear leucocytes. The squamous cells are flat, mostly isolated, and much less numerous than at any other time of the cycle. Owing to the estrogenic effect the percentage of superficial cells is at its peak.

The post-ovulatory reaction is expressed by the folding of cells and the appearance of small clusters, sometimes accompanied by mucus and polymorphonuclear leucocytes.

3. **Luteal phase**

Owing to progesterone activity exfoliation is abundant. The squamous cells are folded and are found in large dense clusters. The percentage of intermediate cells is increased during this phase with the exception of the premenstrual period. (An increase of superficial cells may occur 2-4 days before menstruation.) At the end of the premenstrual period abundant polymorphonuclear leucocytes and granular mucus are present.

4. **Menstrual phase**

During menstruation the smears may show a late luteal phase pattern or an early follicular phase picture. Numerous red blood cells and groups of endometrial cells are present.

**Parakeratotic cells**

These appear as miniature counterparts of superficial squamous cells. They may be isolated or occur in small sheets; in contrast to the normal superficial cells they display anisocytosis and pleomorphism. They often appear as elongated forms. The cytoplasm is eosinophilic and rarely cyanophilic. The nuclei are usually small and pyknotic.

**Perinuclear halo**

A vacuole around the nucleus.

**Phagocytosis or engulfment**

The occurrence of cells, cell fragments, or other particles within a cell. The appearance of whole cells within another cell is usually referred to as engulfment, while the presence of particles or fragments within a cell is usually referred to as phagocytosis.
PLASMA CELL

An inflammatory cell slightly larger than a lymphocyte with a characteristic, eccentrically placed, round nucleus containing radially arranged chromatin bars. The cytoplasm is ample and distinct and has sharp boundaries.

PYKNOSIS

Nuclear shrinkage resulting in the formation of condensed, structureless, hyperchromatic nuclei.

REGENERATION (OF SQUAMOUS OR ENDOCERVICAL CELLS)

Regenerating squamous cells have features of parabasal cells: large nuclei and frequently prominent nucleoli, which may be multiple. They are readily recognized when they occur in sheets.

Regenerating endocervical cells resemble non-ciliated endocervical cells but have a larger nucleus with prominent single or multiple nucleoli. They occur in sheets. More marked changes may be seen in chronic endocervicitis or in IUD users. (Regenerating cells that are forcibly removed often have cytoplasmic processes.)

RESERVE CELLS

See Endocervical reserve cells.

SEX CHROMATIN BODY

One of the X chromosomes identified when contiguous with the nuclear membrane as a planoconvex body.

SQUAMOUS CELL

_Basal:_ a small, rounded, cyanophilic cell derived from the basal layer. It has a centrally placed vesicular nucleus. The nuclear/cytoplasmic ratio is between 1:2 and 1:3. (This cell is not found in cytological specimens under normal conditions.)

_Parabasal:_ a small, round, or oval, cyanophilic cell with thick dense cytoplasm and a vesicular nucleus. The nuclear/cytoplasmic ratio varies from 1:3 to 1:6.

_Intermediate:_ a polygonal cell with cyanophilic cytoplasm. The nucleus is vesicular and has a diameter larger than 6 μm. The nuclear/cytoplasmic ratio is less than 1:6.
Superficial: a large, thin, polygonal cell with a pyknotic nucleus (6 \( \mu m \) or less). The cytoplasm may be either eosinophilic or cyanophilic.

Syncytium

See Aggregates of epithelial cells.

Trichomonas Vaginalis

An ovoid protozoan with weakly staining amphophilic cytoplasm and an indistinct, small, eccentric nucleus. Sometimes it is possible to recognize the axostyle, one or more of the four flagella, or the undulating membrane. Deeply staining and sometimes eosinophilic intracytoplasmic granules may be present.

Trophoblastic Cells

These are of two types:

The syncytial trophoblast is a multinucleated giant cell that can contain up to 20 or more nuclei. The cytoplasm is amphophilic or eosinophilic. The nuclei have coarse, uniformly distributed chromatin and are sometimes irregular. Nucleoli are rarely observed.

Cytotrophoblastic cells are large cells with uniformly stained amphophilic cytoplasm. The nucleus is large, irregular, usually lobulated, sometimes vacuolated, and often hyperchromatic.

Undifferentiated Cells

Cells lacking normal differentiation, organization, and specialized function.

Vesicular

Usually applied to the description of nuclei with uniform, delicate chromatin network, as opposed to heavily stained, pyknotic nuclei.