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ATLAS OF
Exfoliative Cytology

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to the advancement of
Exfoliative Cytology
in cancer diagnosis

PREFACE

THE STUDY of exfoliated cells found in various secretory and excretory fluids of the body has received added impetus in the past three decades. This has been due primarily to the fact that it proved to be of real value in two broad fields: first, the physiology and endocrinology of the female sex organs, and, later, the diagnosis of certain pathologic conditions, more particularly cancer. A real and urgent need has thus been created for publications providing a detailed description and illustration of the enormous variety of cells desquamating from normal or neoplastic epithelial surfaces. This need has been filled only partly thus far.

The present work is a further contribution toward this end. Its scope is twofold: first, to illustrate in a uniform manner the most significant and representative types of exfoliated cells as a basis for an adequate comparative evaluation; and second, to provide a description and discussion of their general or specific diagnostic significance. It is hoped that such a presentation will not only serve as a helpful manual for those who wish to study the method of cytologic diagnosis but also lay the groundwork for what eventually may emerge as an independent branch of the morphologic sciences, that of "Comparative Exfoliative Cytology."

The essential difference between exfoliative and other branches of cytology is that it deals with desquamated cells which, because of their separation from their site of origin and the ensuing degenerative changes, acquire specific morphologic characteristics. Such cells are free from the pressure of surrounding cells and often assume distinctive forms differing markedly from those of the same type as they appear in tissue sections or those obtained by microdissection or scraping. It is therefore justifiable to deal with them as a separate group.

Should one attempt to describe and illustrate the myriads of normal and abnormal cellular forms found in body fluids, he would be faced with an impossible task. Furthermore, although such an extensive study might have theoretical significance, it would tend to cause confusion rather than to aid those trying to use cytology as a diagnostic procedure. Thus it has been necessary to exercise some selection and to limit description and illustration to forms whose derivation may be considered fairly well established and which appear to be representative of distinct cell types.

It is fully realized that our present knowledge of this new cytologic field is still limited and that an atlas published at the present time could not be anything but incomplete. Indeed, it will require many years of arduous work on the part of many investigators to explore this wide field and its manifold ramifications. By adopting the present plan of loose-leaf publica-

tion we hope to keep abreast of new advances through periodic addition of illustrative and descriptive material.

In this effort the cooperation of other investigators would be most welcome and greatly appreciated. Slides of unusual or rare cytology that might come to their attention could be sent to the author to be illustrated under the standards set for this publication and be included in future plates of this atlas. These contributions, for which proper credit would be given, would make possible the inclusion of many cases of special interest, the cytology of which would be better understood in the light of a comparative evaluation.

The present volume includes thirty-six plates. Of these, twelve (Series A) are devoted to the female genital system; four (Series B) to the urinary and male genital organs; five (Series C) to the respiratory system; five (Series D) to the alimentary tract; two (Series E) to pleural, peritoneal and pericardial exudates; two (Series F) to breast secretions; one (G I) to histiocytic cells; one (G II) to cells related to pregnancy; one (G III) to cells affected by irradiation; one (G IV) to multinucleated cells of various types; and, finally, two (Series H) to the illustration of mitotic figures found in normal and malignant cases.

An effort has been made to segregate the malignant and non-malignant cell types on different plates as far as possible. However, for the sake of close comparison, certain exceptions have been made.

The colored drawings included in twenty-four plates are the work of the well-recognized artist Hashime Murayama, an expert in this type of work. Necessary corrections for cytologic detail were made under the supervision of the author. The original drawings were made at a magnification of 1050 diameters and reduced to 525 in printing. This corresponds to the magnification of the drawings in the two previous monographs,* which were illustrated by the same artist and published in 1943[36] and 1948.[37]

Twelve plates of photomicrographs in color have been included. They were prepared by our photographer, Constantine Railey, who has successfully adapted the Kodak Flexichrome Process to this type of illustrative work. The photomicrographs are reproduced at magnifications of 600 and 1600 diameters.

Obviously, from the point of view of accuracy, drawings cannot be compared with photomicrographs, as it is practically impossible to reproduce with exactness minute details of the structure of nucleus and cytoplasm. However, in dealing with exfoliated cells or cell clusters preserved in toto the drawing presents the advantage of permitting a visualization of overlapping cells and cell structures which cannot be focused in one plane. Photomicrographs assume more importance in demonstrating the general pattern of a smear and the relationship of its various components to one another, particularly in the larger clusters. The drawings and photomicrographs were segregated into separate plates for better reproduction and to permit a ready comparison of cells illustrated in like fashion.

* The magnification of 400 diameters given in these two monographs should be corrected accordingly.

The text is brief. It includes a short historical review of the earliest contributions to exfoliative cytology, a description of the technical procedures used in the preparation and staining of material illustrated in this atlas, a general discussion of cytologic criteria and, finally, a brief description of normal and abnormal cell forms encountered in the various body fluids with special emphasis on neoplastic cell types. A description and explanation of the figures and a special discussion accompany each plate.

It is realized that errors resulting from misinterpretations of cytologic findings are to be expected in a field which is still in its infancy. Detached cells cannot be identified as to their origin and type with the same accuracy as cells in situ, and therefore the general and diagnostic significance of many types of exfoliated cells is still incompletely understood.

The material used for illustrating the atlas has been obtained largely from smears prepared and studied in our laboratory. However, some illustrations have been made from slides which have been generously placed at our disposal by other laboratories. A number of the drawings illustrating cells found in vaginal smears were borrowed from earlier monographs.[26, 27] This was done for the sake of completeness of the present publication and to avoid references to a previous monograph which might not be available to some of the readers.

G. N. P.

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I am particularly grateful to Dr. Doris G. Holmquist for her invaluable cooperation in the writing of the text and the preparation of the plates and for selecting and describing the material in the plates of Series H. I am also indebted to Dr. Xenophon Callas for his helpful cooperation during the early formulation of the text material; to Dr. Nelson D. Holmquist and Constantine Railey for their assistance in the final reading and correction of the manuscript; and to my wife, Mary G. Papanicolaou, a faithful and untiring companion and volunteer assistant throughout the years of my scientific research.

G. N. P.

The following figures also appeared in *Diagnosis of Uterine Cancer by the Vaginal Smear* by George N. Papanicolaou and Herbert F. Traut:

Series	Plate	No.
A	I	3 and 16
	II	1, 2, 5, 6, 10, 11, 12, 15, 17 and 19
	IV	1
	VI	1–18 and 20–23
	VIII	1–8, 16 and 18
	IX	4 and 9
	X	1, 2, 5, 6, 7, 11 and 13
G	I	2, 3, 9 and 13
	II	5–8, 13 and 14

Figures which appeared in *The Epithelia of Woman's Reproductive Organs* by George N. Papanicolaou, Herbert F. Traut and Andrew A. Marchetti are:

Series	Plate	No.
A	III	1, 2, 4, 8, 12 and 16

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CHAPTER

Introduction

The study of exfoliated cells dates back to the middle of the nineteenth century. As early as 1843 Walshe took notice of small tissue fragments expectorated from malignant growths of the respiratory tract.[47] In 1847 Pouchet, in his book concerning ovulation and other related phenomena,[38] reported his observations on the cellular make-up of human vaginal smears. His investigations were limited to the normal cytology, as he set out to ascertain the existence of cyclic morphologic changes which might be correlated with the normal ovulatory process.

One of the earliest references on studies of desquamated cells for the purpose of diagnosing cancer is that of Beale, 1860, who reported the finding of malignant cells in the sputum from a case of cancer of the pharynx.[5] Malignant cells in smears of fresh sputum from cases of carcinoma of the lung were reported by Hampeln in 1876 and 1887,[20, 21] Ménétrier in 1886,[35] Betschardt in 1895[6] and others.

Cytologic smears of other body fluids were also utilized, at a very early date, for the diagnosis of malignancy. Thus Sanders in 1864[43] reported finding fragments of malignant tissue in the urine of a patient with cancer of the bladder. Further observations on cells of the urine were made by Dickinson in 1869.[13] As early as 1892, Ferguson[15] recommended microscopic examination of urine sediment as the best way, exclusive of cystoscopy, of diagnosing tumors of the bladder.

Luecke and Klebs in 1867[28] found malignant cells in smears of ascitic fluid in cases of malignant tumors of the ovary. Later Quincke, in 1875 and 1882,[40, 41] used this principle in the examination of transudates and exudates for cancer cells. In this application, however, the use of smears was soon superseded by the sectioning of sedimented cells as described by Bahrenberg in 1895.[4]

Numerous contributions have since been made by other workers, who have studied the cytology of various fluids of the body, chiefly for establishing criteria for the diagnosis of cancer of different organs. It would be beyond the scope of this short introductory text to give a complete review of the literature in this field. Moreover, this atlas presents chiefly observations and studies which are the outcome of methods and technical procedures developed in our laboratory during the last thirty years.

Originally the vaginal smear technique was used as a means of analyzing the sex cycle in the guinea pig.[46] Its value as a method for determining the morphologic and functional state of the female reproductive organs resulted in its general adoption as a standard method for the study of the sex cycle of female mammals and of problems related to sex physiology and endocrinology. This initiated an era of unprecedented activity which led to significant advances in these fields. Much of the progress which has been made toward an understanding of the mammalian female sex cycle and the role played by the sex hormones can thus be accredited to exfoliative cytology and the unfettering of some of its innate potentialities.

The scope and significance of the vaginal smear were widened considerably by its application to the human.[28] The study of the normal cellular forms characteristic of the various phases of the sex cycle was subsequently expanded to include the many aberrant types found in pathologic conditions and, more particularly, in cancer. The cytologic characteristics of cancer cells are often striking, capturing one's attention immediately by their contrast to those of non-malignant forms. This fact, coupled with the interest which has always been connected with studies related to cancer, has caused a reawakening to the possibilities of using the cytologic method in the diagnosis of malignant lesions.

Its use in recent years has been extended from the study of uterine and vaginal secretions to many other body fluids such as urine; prostatic secretion; semen; sputum; bronchial, gastric, duodenal, rectal and colonic aspirates and washings; pleural, peritoneal and pericardial exudates; breast secretion; and fluid aspirated from other body cavities and cystic growths. Thus far the method, from the point of view of accuracy of diagnosis, has been applied most successfully to cancer of the uterus, lung, bladder, rectum, stomach and pleural and peritoneal linings. Our knowledge of the exfoliative cytology of some of the other organs is less advanced, primarily because of the technical difficulties in obtaining proper material.

It has been recognized that one of the salient features of the cytologic approach is the possibility which it offers for recognition of cancer in its incipiency. This has been demonstrated by the large number of preinvasive carcinomas of the cervix detected in recent years by the use of the smear method in different laboratories. A new interest has thus been created in the study of cytologic and histologic changes in early carcinogenesis.

The recently acquired evidence of the existence of distinctive cytologic patterns in the early developmental stages of cervical carcinoma, in contrast to those of the more advanced stages, has provided more sensitive criteria for the diagnosis of early cancer, and possibly a means of prognosis based on the prevailing cytologic pattern.

Knowledge of the cytology of early cancer is still fragmentary. The only organ in which actual progress has been achieved is the cervix, largely because of its accessibility to biopsy for the confirmation of positive smear findings. The correlation of the cytologic and histologic pictures in the early stages of malignancy is much more difficult in other less accessible organs.

One cannot evaluate by mere analogy findings in smears from one organ on the basis of criteria which have proved valid for another. For example, in a cervical smear, nuclear enlargement, multinucleation, anisokaryosis and hyperchromasia in cells which retain their original type have been definitely linked by biopsy with the presence of an early malignant lesion. However, comparable changes observed in cells exfoliated from other organs, such as the ciliated cells of the bronchial mucosa, have not as yet been proved to be related to malignancy. Because of the limited applicability of the biopsy method in the lung, it is only by prolonged follow-up of cases presenting such structural atypia that its significance and possible correlation with early asymptomatic malignant lesions can be ascertained.

During the past decade much progress has been made in the adaptation of the cytologic method to the diagnosis of cancer. This is attested by the expanding use of the method to cover a greater number of organs and by its rapidly increasing utilization in diagnostic laboratories in this country and abroad.

Despite this noteworthy progress, we are still far from our ultimate goal, the attainment of which will depend not only on the gradual accumulation of observations in the field of applied cytology but to a greater extent on further advances in our fundamental concepts of the morphologic and physiologic properties of the cell.

CHAPTER

II

Technique

THE TECHNIQUES described in this chapter with only few exceptions are those employed in our laboratory at Cornell University Medical College.* Several modifications of these techniques, as well as new methods for the collection of specimens and staining of smears, have been published in the past few years. However, it was felt that chiefly techniques and procedures used in the preparation of material illustrated in this atlas should be given a detailed description here.

PREPARATION AND STAINING OF VAGINAL, ENDOCERVICAL AND ENDOMETRIAL ASPIRATION AND OF CERVICAL SWAB SMEARS

Materials needed

1. Clean slides, labelled beforehand with a piece of index card 1 inch square fastened to one end of the slide by a paper clip. The patient's name and the date and type of smear should be written on the label with pencil. It is advisable to etch the smear type on the slide with a diamond pencil.
2. For vaginal aspiration: glass pipette, 6 inches long, 1/4 inch in diameter, with a rounded tip, slightly curved 2 inches from end and equipped with a strong rubber bulb (Fig. 1).
3. For endocervical or endometrial aspiration: Becton-Dickinson laryngeal cannula, or a similar type as specified in the following descriptions, to be used with a 10 cc. syringe (Fig. 1).
4. For swab smears: an applicator—either a non-absorbent cotton swab or a wooden spatula such as the one devised by Ayre[1] (Fig. 1).
5. Wide-mouthed bottle 1 3/4 x 1 3/4 x 4 1/2 inches, containing a solution of 95% ethyl alcohol and ether, equal parts (Fig. 1).

Preparation of smears

Vaginal smears. The following two paragraphs of the description are reprinted from a previous publication.[24]

The patient is placed upon an examining table in the lithotomy position. The labia are separated and the rubber bulb compressed. The glass pipette is introduced into the posterior fornix of the vagina. The pressure on the bulb is then released and the suction produced serves to aspirate vaginal fluid with its cellular content into the glass tube. While aspiration is in progress, the tip of the tube is moved from one side of the fornix to the other so that all parts are sampled. The pipette is then withdrawn, and the vaginal material is spread upon the surface of a clean microscope slide with a sudden expulsive pressure on the bulb. Further spreading with the convex side of the pipette is advisable when the amount of fluid is copious, as in cases where there is considerable bleeding or abundant mucus. Very thick smears are not well penetrated by the fixing fluids and cannot be uniformly stained.

A few precautions should be observed in the procurement of material for the vaginal smear. The vaginal contents should not have been disturbed by any form of examination or treatment. Douching or bathing will, of

* The author wishes to express his appreciation to Charlotte M. Street, Chief Technologist, Papanicolaou Cytology Laboratory, Cornell University Medical College, for her cooperation in the technical descriptions contained in this chapter.

course, dilute or completely wash away the cellular deposits for a period of several hours. If there is a considerable amount of fluid of either serous or sanguineous character, dilution will occur, in which case it is wise to make several smears to obtain the representative cell constituents which are ordinarily seen in a single smear.

The slide is immersed immediately in the alcohol-ether solution before the smear dries, and left there with the labelled end of the slide at the top. Drying of the smears should be avoided throughout the procedure as it results in flattening and distortion of the cells and their nuclei and a loss of their structural characteristics and affinity for stains.

Endocervical or endometrial smears. Secretion is aspirated from the endocervical canal or the endometrial cavity by means of a laryngeal cannula, using a speculum. Smears are prepared from the aspirated fluid and fixed in alcohol-ether in the same manner as vaginal smears. It is most important to have endometrial smears when carcinoma of the uterine fundus, the tube or the ovary is suspected.

The technique of endometrial aspiration as described by Dr. Sophia J. Kleegman, of New York City, is as follows:*

With the patient in the lithotomy position, the vaginal and cervical specimens are obtained in the usual way for the Papanicolaou cytologic study. The vault of the vagina, cervix and cervical canal are then wiped clean and painted with any disinfectant solution of your preference. The anterior lip of the cervix is grasped with a tenaculum. An antrum cannula attached to a sterile, closed 10 cc. syringe is then inserted through the endocervix into the endometrial cavity, about one-half inch from the fundus. Suction is created by withdrawing the plunger of the syringe almost to the end. The syringe is detached at this point, closed and reattached, and suction is applied in a similar manner a second and then a third time. With each repetition of suction, the cannula is withdrawn a little, but the end of the cannula must be beyond the internal os throughout the test. After the third suction, the cannula must be withdrawn from the uterus, and the contents of the cannula are forcibly expelled on the end of a glass slide by blowing air through the cannula. The secretions are spread thinly over the lower half of the slide, which is dropped promptly into a bottle containing equal parts of 95% alcohol and ether. The slide is then stained according to the Papanicolaou technique. When endometrial tissue is aspirated together with the fluid, this tissue is first quickly picked off the slide, placed in a fixing solution,† and prepared for pathological examination in the usual way. In most cases dilatation of the internal cervical os is not necessary because the cannula is so thin. With a tight nulliparous internal cervical os, a No. 9 Hank's dilator may need to be passed first. In the multiparous cervix, it is often not even necessary to grasp the cervix with a tenaculum. The small caliber of the antrum cannula makes the procedure simple and relatively painless. The antrum cannula chosen should be malleable, with a tip just as narrow as the shank. Usual attention to sterile technique, and gentleness in inserting the cannula are necessary as for all intrauterine work.

Another description of the endometrial aspiration technique is offered by Dr. Mortimer D. Speiser, of New York City, as follows:‡

Endometrial smears should be taken after smears have been obtained from the vagina and cervix and a satisfactory bimanual examination has been completed.

A bivalve speculum is reinserted into the vagina and the cervix properly exposed. The portio vaginalis and the endocervical canal are cleansed with a cotton ball and cotton tipped applicator soaked with an antiseptic solution such as zephiran. If the canal is sufficiently patulous to admit the cotton tipped

* The author wishes to express his appreciation to Sophia J. Kleegman, M.D., Clinical Professor of Obstetrics and Gynecology, New York University College of Medicine, for preparation of the above description of her technique for endometrial aspiration.

† Fixation of tissue in equal parts of 95% alcohol and ether gives very good results. Tissue fixed in this manner can be processed rapidly, as it does not have to be washed, and fixation is comparable to that of the cytologic material.

‡ The author wishes to express his appreciation to Mortimer D. Speiser, M.D., Associate Clinical Professor of Obstetrics and Gynecology, New York University College of Medicine, for preparation of the above description of his technique for endometrial aspiration.

applicator throughout its entirety a tenaculum need not be employed. Where necessary then, a tenaculum is placed upon the anterior lip of the cervix in order to steady it. A Killian cannula is now attached firmly to a 10 cc. Boston Record syringe and the syringe is grasped in the right hand as one grasps a pen. The cannula is now inserted through the cervical canal into the uterine cavity. If this cannot be readily accomplished the small size cervical dilator should be employed to gently dilate the canal. The curved tip of the cannula is then directed upwards and anteriorly in a uterus which is anteverted or anteflexed and posteriorly when the uterus is found to be retroverted or retroflexed. The stem of the cannula is sufficiently flexible that one may adjust the curve as required for flexion or version of the uterine body. The cannula is moved upwards in the cavity of the uterus until the slightest resistance is met as the tip of the cannula reaches the top of the uterine cavity. The barrel of the syringe is now placed in the left hand and held steady while the plunger is withdrawn with the right hand. After suction has been obtained the left hand rotates the barrel from side to side so that the attached cannula will suck up material from the entire cavity. At the same time the syringe with the attached cannula is gently drawn outwards. With an airtight fit between cannula and syringe the plunger is pulled outwards and released three times. Thus suction is more or less continuous as the cannula tip travels from side to side as well as downwards. Finally the cannula is withdrawn from the uterine cavity.

If aspirated material can be seen in the syringe, the cannula is removed from the syringe and the contents of the syringe are now expelled on a glass slide. The cannula is employed to spread the material thinly and evenly over the surface of the slide. It may be necessary to use several slides. As soon as the material has been spread upon the slide and before drying takes place, the slide should be immersed in the alcohol and ether mixture. When large shreds of material are present these particles may be placed in a test tube containing the same alcohol and ether mixture. After the syringe has been emptied, the contents of the cannula should now be spread upon slides. The syringe or a rubber bulb fastened to the cannula can be employed to express the material, which is then spread with the cannula, as suggested previously.

Endometrial smears should be omitted if there is a severe infection or suspicion of pregnancy.

Cervical swab smears. Smears should be taken with a cotton-tipped applicator or a wooden spatula, preferably from the region of the squamo-columnar junction or any area of the cervix which appears to be suspicious. The material obtained is spread on a slide as quickly as possible and immersed immediately in alcohol-ether, as swab smears tend to dry faster than aspiration smears.

Fixation of smears

Fixation does not require more than a few minutes, but a minimum of 15 minutes is advisable for proper adherence of the smear to the slide. If necessary, smears may be kept in the alcohol-ether solution over a long period of time; however, this is not recommended as a general rule, as it may result in an alteration in the staining reaction of the cells. Smears which are to be mailed to a laboratory for staining should be fixed in alcohol-ether for at least 1 hour. If ether is not available, ethyl alcohol (95%) can be used alone. Methyl or even isopropyl though less desirable can be used as substitutes if chemically pure.

For shipping, the following method suggested by Ayre and Dakin[2] may be used. After fixation in alcohol-ether, the smear is covered with two or three drops of glycerine without being allowed to dry. A second clean slide is placed on the smear for protection, and the two are fastened with a rubber band, wrapped in wax paper and packed for mailing. When such preparations are received for staining, they are placed in alcohol-ether until the covering slide becomes loose. The two slides are then separated and both should be stained, since part of the smear often sticks to the covering slide.

A somewhat simpler method for protecting smears from drying during shipping is being

tested in our laboratory. The smears are fixed in a solution of three parts alcohol-ether and two parts diaphane for a minimum of 1 hour and are allowed to dry just prior to shipping. The film of diaphane which forms over the smear protects it from complete drying for a period of a few days. When received, the slides are placed in alcohol-ether in which the diaphane is dissolved.

Another way of protecting a smear is to fix, dehydrate and mount it, omitting the staining. In this manner it can be preserved for an unlimited period of time and mailed as easily as a permanent preparation. When received, the slide is placed in xylol until the coverslip drops off and then stained by the usual procedures.

Staining of vaginal, cervical and endometrial smears

In our laboratory staining of smears for the cytologic diagnosis of cancer is directed toward three chief objectives. (1) Definition of nuclear details. Because of the widespread nuclear abnormalities of cancer cells and their diagnostic significance, good staining of the nucleus is of primary importance. (2) Transparency. This is of particular importance because of the varying thickness and the frequent overlapping of cells. (3) Differentiation of cells. Differences in the staining reaction such as that between acidophilic and basophilic cells help greatly in the identification of certain cell types found in smears.

In the early stages of our work a method was used which consisted of staining the smears first with hematoxylin and then with an aqueous mixture of eosin and water blue.[28] Later it was found that aqueous solutions have the disadvantage of a rather deep cytoplasmic staining. Our present method, introduced in 1942,[29] is based on the use of high alcoholic cytoplasmic stains, which have the advantage of a more transparent staining of the cellular elements and other components of the smear.

The routine staining procedure (No. 265) at present used in our laboratory is as follows:

1. After fixation, transfer slides, without drying, directly from alcohol-ether to 80% alcohol and run down through 70% and 50% alcohols to distilled water.
2. Stain in Harris' hematoxylin* for approximately 6 minutes.
3. Rinse in distilled water. All rinsing should be very gentle to prevent smears from being washed off the slides.
4. Dip in 0.25% aqueous solution of hydrochloric acid about six times.
5. Place in running tap water for 6 minutes.
6. Rinse in distilled water and run through 50%, 70%, 80%, and 95% alcohols.
7. Stain in OG 6† for 1 1/2 minutes.
8. Rinse in 95% alcohol, two changes.
9. Stain in EA 36‡ *or* EA 50§ for 1 1/2 minutes.
10. Rinse in 95% alcohol, three changes. Dehydrate and clear by running through absolute alcohol, a mixture of absolute alcohol and xylol equal parts, and xylol. Permount, gum damar, Canada balsam or any other satisfactory neutral medium may be used for mounting.

* Harris' hematoxylin is prepared from the standard formula, using ammonium aluminum sulfate but omitting the glacial acetic acid, and is filtered into a dark bottle for storage. It is diluted with an equal volume of distilled water before using. When the stain is in use, it should be reinforced often by the addition of a small amount of fresh undiluted stock solution in order to maintain uniform staining results.

† OG 6: Orange G—0.5% solution in 95% alcohol 100 cc.
Phosphotungstic acid 0.015 gm.

‡ EA 36: Light Green SF yellowish—0.1% solution in 95% alcohol 45 cc.
Bismark Brown—0.5% solution in 95% alcohol 10 cc.
Eosin yellowish (water and alcohol soluble)—0.5% solution in 95% alcohol 45 cc.
Phosphotungstic acid 0.2 gm.
Lithium carbonate, saturated aqueous solution 1 drop

All stains used in these preparations are National Aniline and Chemical Company certified stains. The formulae for OG 6 and EA 36, with minor alterations, are taken from a previous publication.[29]

§ EA 50, prepared and marketed by the Ortho Pharmaceutical Corporation, Raritan, N. J., is a stain comparable to EA 36.

COLLECTION, PROCESSING AND STAINING OF OTHER SPECIMENS

Sputum

A deep cough specimen is collected into a wide-mouthed bottle (sputum bottle) containing 70% alcohol. Because of the hardening effect of the alcohol, specimens should be sent to the laboratory and smears made without delay.

Bronchial aspirates or washings

Bronchial secretion is collected at the time of bronchoscopy by aspiration or by lavage with a small amount of physiological saline solution. Aspirated bronchial secretions or bronchial saline washings are mixed immediately with at least an equal quantity of 95% alcohol. The collection tube is rinsed two or three times with 95% alcohol and these washings are added to the specimen. The type of each specimen and side from which it was collected should be accurately recorded on each label.

In cases in which secretion is scant, Clerf and Herbut[10] recommend obtaining material from the smaller bronchi by means of a small gauze sponge affixed to the tip of a carrier. Such material is smeared directly on slides which are fixed immediately in alcohol-ether solution.

Aspiration of tracheobronchial secretions by catheter is another method reported by Cahan and Farr.[8] This method is of particular value for cases in which adequate "deep cough" sputum is not produced or bronchoscopy is for any reason undesirable. It has the additional advantage that it is feasible as an office or clinic procedure.

Urine

Voided or catheterized urine may be used for cytologic examination. Female patients should be catheterized to prevent contamination of the urine by vaginal secretion. When a lesion is suspected in the kidney or ureter, a specimen aspirated from the pelvis of the kidney is desirable. The collected urine is mixed immediately with at least an equal amount of 95% alcohol.

It is desirable to have a minimum of 50 cc. of voided or catheterized bladder urine and as much as can be obtained from the ureter or the kidney pelvis. Each specimen should be accurately labelled as to its type and origin.

In cases of suspected carcinoma of the prostate, three specimens are requested: a smear from prostatic secretion obtained by gentle massage and two voided urine specimens, one preceding and the other following the massage.

A fourth specimen of value is semen, collected preferably after the prostatic massage. Examination of semen is of particular importance when there is a suspicion of carcinoma of the testis.

Esophageal specimens

Esophageal washings are best obtained during esophagoscopy. Secretion, if present, or saline solution flushed over the mucosa may be aspirated and collected for study.

Gastric specimens

Gastric fluid may be aspirated with a Rehfuss tube with a bucket tip. The tube is introduced through the mouth in order to avoid contamination by nasopharyngeal cells, the identification of which is sometimes difficult. The patient is kept in an upright position so that the tip of the tube falls to the pyloric region. A small amount of mineral oil may be used as a lubricant for passing the tube. No food should be taken 8 hours prior to the test.

The fluid content of the stomach is aspirated and discarded. The patient is then given 8 ounces of Ringer's solution by mouth while the tube is in position and the solution is withdrawn and reinjected, using a 50 cc. syringe, several times before the final aspiration.[44] Material obtained in this manner is fixed with at least twice its volume of 95% ethyl, methyl or isopropyl alcohol. It is important that it be sent to the laboratory

immediately and centrifuged without delay in order to prevent cell deterioration.

A new improved technique, developed in our laboratory, employs a special apparatus designed to obtain fresh diagnostic material through the use of gentle mechanical irritation.[27]

The apparatus (Fig. 2) consists of a standard 16 French double lumen tube 100 cm. in length with markings at 45, 60 and 75 cm. One lumen, designated the aspirator, is connected proximally to a syringe and distally to a patent silverplated bucket. The other lumen, designated the agitator, is connected proximally to an inflating bulb and distally to an inflatable balloon.

The distal end of the agitator lumen is perforated by eight small holes, approximately 3 mm. in diameter and 1/4 inch apart, through which the balloon can be inflated.

The balloon is made of a condom opened at both ends. To the external surface are tied by single slipped bowknots approximately two hundred and fifty pieces of untreated braided silk, arranged in a regular pattern about 3 mm. apart and cut to leave ends about 2 mm. long.

The construction of the apparatus is greatly simplified by covering the balloon with a wide-meshed, fine net, as suggested by Cooper.[11] This modified apparatus, which gives excellent results, is now in use in our laboratory. Still another modification has recently been suggested by Panico[28] in which the surface of the balloon is made abrasive by attaching with rubber cement 75 to 100 small, round 3 mm. fragments of foamed latex rubber. These are arranged in a regular pattern 1 cm. apart.*

As the success of this method is dependent upon close contact of the abrasive surface of the balloon with the gastric mucosa, it is essential that the stomach of the patient be clear and free of the products of retention. The patient is given no solid food the evening prior to the test and is kept in a fasting state for about 8 hours before collection of the specimen. The balloon is wet in Ringer's solution, and with the patient in the erect position is introduced through the mouth and passed into the stomach. Intubation may be facilitated by allowing the patient to sip Ringer's solution. No ice or mineral oil is used.

In cases of retention additional measures must be taken to cleanse the stomach thoroughly. Two or three days should elapse after a G. I. series before the collection of a gastric balloon specimen.

The total gastric contents are aspirated and discarded, and the stomach is lavaged with Ringer's solution until the return is clear. The balloon is inflated, by about 10 hand compressions of the inflating bulb, and carried by peristalsis toward the pylorus. It is deflated briefly and allowed further passage into the antrum, reinflated and eased back toward the esophagus. When the esophageal sphincter is reached, a gag reflex is elicited. This procedure is repeated five to six times over a 30-minute period, during which time the accumulation of fluid is prevented by frequent gentle aspirations. Representative portions of the aspirates are mixed with at least an equal quantity of 95% alcohol and saved. The balloon is deflated and withdrawn.

Three types of preparation may be made. (1) Any grossly visible fragments of tissue adhering to the balloon net are smeared on slides coated with a thin film of albumin and fixed in alcohol-ether solution. (2) The balloon is rinsed in about 200 cc. of a solution of Ringer's and 80% alcohol, equal parts. About 10 drops of Mayer's albumin are added to the rinse, which is centrifuged at 1500 r.p.m. for 30 minutes. Smears are prepared from the sediment. (3) The aspirates are centrifuged as above and smears are made of the sediment.

* A modification of the balloon technique consisting in the use of an "antral abrasive balloon followed by an enzymatic lavage with chymotrypsin" has been recently reported by Cyrus E. Rubin et al. ("The clinical value of gastrointestinal cytologic diagnosis," *Gastroenterology* Vol. 24, No. 2, October, 1953). A new apparatus for obtaining fresh gastric cells with the aid of a double rotating brush has been devised by J. E. Ayre ("A new rapid method for gastric cancer diagnosis: the stomach brush," *Cancer*, in press).

The specimen most generally used for diagnostic purposes is the rinse from the balloon.

This technique is far superior to the simple gastric aspiration, as the material obtained is freshly exfoliated and therefore better preserved, and may also contain larger clusters of cells or microscopic epithelial fragments.

Another technique has been suggested by Rosenthal and Traut[42] based on the use of the proteolytic enzyme papain. We are indebted to Milton Rosenthal, M.D., of the Culver City Hospital, Culver City, California, for the following description, which includes modifications of the original procedure given by Rosenthal and Traut.

Materials. Buffer solution: This is an isotonic phosphate buffer of approximately pH 7.3, which serves to prevent acid digestion of cells released from their mucous coating and also prevents the inactivation of papain (at pH 4.5). A 5-gallon stock may be made up by adding 750 gm. dibasic sodium acid phosphate ($Na_2HPO_4.12H_2O$) and 0.4 mols of hydrochloric acid to 5 gallons of distilled water (or soft tap water).

Papain powder (Difco, etc.)
Cystein hydrochloride
Waring blendor
Activated charcoal
Large fluted funnel
Filter paper (Whatman No. 2)
Gastric tubes
Asepto syringe (3 oz.)
Tube cultures of Leuconostoc mesenterioides in nutrient broth with sucrose, made up to 10% concentration
Centrifuge and accessories for large centrifuge cups, 250 ml. capacity (e.g., SBV-B Model 1 Intl. or Model 2)

Leuconostoc cultures: After trial of many agents, nutrient broth cultures of Leuconostoc mesenterioides, a mucus-producing saprophyte, was found to be ideal for restoring adhesiveness to the recovered cells which have lost their mucous coating. Tubes of 10 ml. each of broth with 10% sucrose concentration are inoculated and the growth proceeds at room temperature. The tubes may be kept indefinitely and used as needed. It has been found that no material comes off the slides during fixing and staining and the medium does not stain. The organisms themselves do not obscure the cells.

Papain solution: This may be made up fresh or stored for from 24 to 72 hours in the refrigerator. For longer storage it should be sterilized by Seitz filtration. The solution is subject to decomposition by bacterial growth. The activator, cysteine HCl, is not added until just before use.

Three heaping teaspoonfuls of papain powder are placed in the blendor, and buffer solution is added to cover the stirring blades. The mixture is stirred for 3 minutes. Several hundred more milliliters of buffer are then added and stirring is continued for two more minutes. A heaping teaspoonful of activated charcoal is added and wet by intermittent stirring and the mixture is finally stirred for 1 minute. It is then filtered through suitable paper (Whatman No. 2 has proved satisfactory). The first few drops will contain charcoal. These are returned to the funnel. The remaining filtrate should be sparkling clear and amber in color; any sediment in the solution will finally contaminate the cell sediment and obscure and dilute the cells. The concentrated solution is then made up to 1 liter for use. The color is a pale yellow, representing approximately 1.5% papain, but moderate variations in concentration do not matter.

Just before use approximately 1.5 gm. of cysteine HCl are dissolved into the solution. The solution should be warmed before instillation.

Method. After removal of the fasting specimen, or after completion of routine gastric analysis, as much of 1 liter of the papain solution is introduced as will produce a sensation of distension without discomfort. Mucolysis is complete after 10 minutes, and the solution is removed. On rare occasions, most of the solution is lost by duodenal passage. In such a case, new solution may be instilled.

There have been no ill effects in over 3000 patients, except for a mild diarrhea if most of the solution is retained. There have been no more instances of bleeding than might be expected with the intubation of large numbers of patients with gastric ulcer. No massive hemorrhages have occurred, even in patients with recent spontaneous massive hemorrhage.

The turbid suspension is removed and mixed, and as much of it centrifuged as convenient. After the suspension is placed in the large centrifuge cups, several milliliters of mucous Leuconostoc broth are injected below

the papain suspension, to form a separate layer. The cups are carefully balanced to prevent swirling up and mixing of the underlying mucous solution, and the centrifuge brought up to speed (approximately 2000 r.p.m.) quickly to shorten the period of swirling of the solution. After centrifuging, the motor is stopped without braking.

The supernatant fluid is discarded and the tubes inverted for draining for several minutes. A small cotton swab is wet with Leuconostoc broth and the cell sediment smeared onto slides and immediately placed in fixative before drying can take place. Any convenient fixing and staining technique is suitable.

Precautions. It is preferable to allow the fasting patients to drink water as desired in order to reduce rapid duodenal passage of the solution because of dehydration. Any remaining water in the stomach will be discarded.

When there is retention of food due to pyloric obstruction, the stomach must be thoroughly washed out before introduction of the papain solution.

The patient should be cautioned not to swallow sputum and saliva during the lavage, in order to reduce the component of cells derived from those secreta.

Morphine or demerol should be avoided before lavage because of the great dilation of the stomach which they cause.

*Rectal and colonic washings**

Proper cleansing of the intestinal tract is essential for the collection of satisfactory rectal and colonic specimens. Good preparation may be obtained by the following method: the patient is given two ounces of castor oil at 4:00 P.M., the day prior to the examination and permitted only light meals that night and the day of the tests. On the morning of the examination, with the patient lying on the left side, soap suds enemas are given until the returns are clear. Ambulatory patients may be instructed to perform proper preparation.

An effective instrument for rectal washings has been developed at the Kate Depew Strang Clinic, Memorial Hospital, New York (Fig. 3).[22]

This consists of a double lumened brass tube 11 1/2 inches long, made by soldering together two tubes of 1/8 inch inside diameter.

The tip of one tube is set back 1/2 inch and both tips are highly polished to prevent injury to the bowel. The shorter tube carries the saline solution while the longer is used to aspirate the specimen. The distal ends of the tubes are spread apart and to each is attached, by rubber tubing, a 20 cc. test tube with a two-hole rubber stopper in which are inserted glass "elbows." An atomizer bulb is attached to the tube on the short side of the apparatus while the other side is connected to the suction pump usually employed by proctoscopists. About 10 cc. of saline are placed in the first test tube.

The rectal washing is made in conjunction with sigmoidoscopic examination. The area from which the cells are desired is located if possible with the sigmoidoscope with suction pump running. The brass tube of the washing apparatus is then passed through the sigmoidoscope and the forward tip placed on the area. By squeezing the rubber bulb a stream of normal saline is directed over the mucosa. This is immediately aspirated into the second tube. The apparatus is then withdrawn and an equal amount of 95% alcohol added to the washings.

If the suspected lesion is beyond the reach of the sigmoidoscope, a colonic washing may be done.[3] For colonic washing the following materials are needed:

1 liter enema can
Glass Y tube: 2 clamps
Soft rubber catheter, size 18–20
Rubber tubing
Stand (5 foot)
0.9% saline
Water soluble lubricant
Large collection bottle
95% alcohol

An enema of 500 cc. of normal saline at body temperature is given with the patient lying on his left side. If a lesion is suspected above the level of the sigmoid, up to 1200 cc.

* The author wishes to express his appreciation to Genevieve Mary Bader, M.D., Assistant Attending, Strang Prevention Clinic, Memorial Center, New York, for preparation of the descriptions of the techniques for rectal and colonic washing.

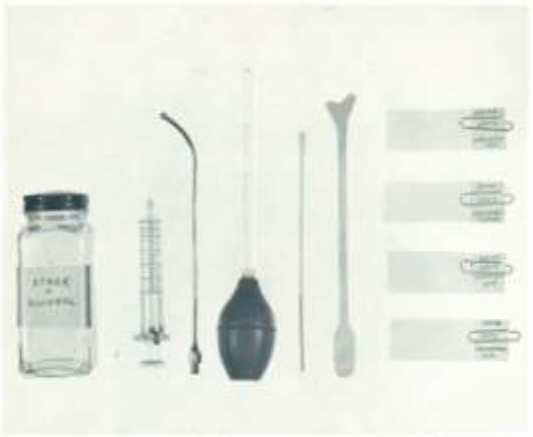


Fig. 1. Apparatus for the preparation of vaginal, cervical and endometrial smears

Fig. 2. Gastric balloon

Fig. 3. Apparatus for rectal washings

of saline may be used. The saline enema is retained for 1–2 minutes. The returns are then collected in a large graduated bottle, moving the catheter carefully up and down to facilitate collection. A return of at least half the quantity of the saline enema may be expected. An equal amount of 95% alcohol is added to the specimen immediately.

The entire specimen is centrifuged at 2500 r.p.m. for 15 minutes and the supernatant fluid decanted. The sediment is smeared on slides previously coated with Mayer's egg albumin and the slides are placed immediately in alcohol-ether fixative. The slides are stained according to procedure No. 267, increasing the wash time in water to 15 minutes.

Pleural, peritoneal or pericardial fluid

Fluid aspirated from the pleural, peritoneal, or pericardial cavities is immediately mixed with at least an equal amount of 50% alcohol. Fifty per cent alcohol is preferable to 95%, as it results in less coagulation of proteins. The sediment smears are thus more cellular.

Specimens centrifuged prior to fixation with alcohol give a larger amount of sediment; however, the immediate addition of alcohol has the advantage of providing a better and more uniform fixation of the cells. Formalin may also be used as a fixative and has an advantage over alcohol as it gives a more richly cellular sediment as well as a good fixation of the cells, but has the disadvantage of causing some shrinkage of the cells and their nuclei. One might profit by the merits of both techniques by dividing the specimen after thorough shaking and fixing one-half with 50% alcohol and the other with 10% aqueous solution of formalin.

Breast secretion

Secretion expressed from the nipple is spread directly on a glass slide coated with albumin and is fixed in alcohol-ether. The difficulty in obtaining secretion from the breast has been a limiting factor in the application of the cytologic method in this organ.

In some cases with no spontaneous secretion, a little fluid can be obtained by weak suction with a breast pump or by gentle massage.

Other fluids

Other fluids, such as nasopharyngeal and antral washings, fluid aspirated from cysts, etc., are mixed immediately with at least an equal volume of 95% alcohol.

It is important to send all specimens to the laboratory as soon as possible and to have them centrifuged promptly. This applies more specifically to certain specimens, such as the gastric, which tend to deteriorate more rapidly. If a delay is unavoidable, more fixative should be added and the specimen should be refrigerated.

Preparation of smears

Sputum. A small amount of sputum is placed on a clean slide previously coated with a thin film of Mayer's egg albumin. With a second clean slide it is crushed gently, using just enough pressure to soften and spread it into a fairly thin, uniform film but not enough to break down the cells. Four to five smears are usually prepared from each specimen. It is advisable to select for the preparation of smears any bloody or other suspicious-looking areas in the sputum. Smears are allowed to dry slightly around the edges for proper adherence to the slides and then fixed in alcohol-ether for 1 hour or longer before staining.

Urine, exudates and bronchial, gastric or other aspirates or washings. The specimens are centrifuged for approximately 30 minutes at medium speed. The supernatant fluid is decanted and one to three drops of Mayer's albumin are mixed with the sediment, depending upon the amount of the sediment. The sediment is removed from the tube with a pipette, a nasal curette or a small spoon (or scoop-shaped instrument) and spread evenly on a slide. Smears are allowed to dry slightly at the edge and then fixed in alcohol-ether. A thin coat of celloidin protects smears from peeling during staining.

Staining of sputum and various sediment smears

Smears other than vaginal, endocervical or endometrial are stained with Procedure No. 267, which is a slight modification of No. 268 (described on page 6).

PROCEDURE No. 267:*

Steps 1–8–Same as in Procedure No. 268, for vaginal smears.

Step 9–Stain in EA 65† for 1 1/2 minutes.

Step 10–Same as in Procedure No. 268.

MODIFIED PROCEDURE No. 267 FOR STAINING URINARY OR GASTRIC SEDIMENT SMEARS: In this method blueing of the nuclei is achieved by the use of ammonium hydroxide rather than running tap water, as the latter tends to loosen cells from the slides. It is particularly useful in the staining of urinary sediment smears which are the least adhesive of the sediments. Other specimen types can be processed in this manner although in thick smears the cytoplasm retains some of the hematoxylin, preventing a clear, transparent cytoplasmic staining.

Steps in Modified Procedure No. 267:

1. Same as standard Procedure No. 267.
2. Stain in Harris' hematoxylin for 2 minutes.
3. Rinse in distilled water, then 50% alcohol.
4. Place in a solution of 1.5% ammonium hydroxide made up in 70% alcohol for 1 minute.
5. Rinse in 70% alcohol, two changes.
6. Run up in 80% and 95% alcohols.

7–10. Same as standard Procedure No. 267.

Contamination of smears. In the process of staining, cells, cell clusters or even small parts of the film of a smear ("floaters") may become detached from a slide and adhere to other slides stained in the same container. This is a rare occurrence yet it may lead to a false positive evaluation if positive "floaters" adhere to a negative smear. Detachment of cells or of cell clusters occurs more often when smears are unusually rich, as is often the case with smears from exudates. One should always be aware of the possibility of contamination when malignant cells are found in a relatively small area of an otherwise negative smear and in only one of the slides prepared from a specimen.

The following are some of the criteria by which one can recognize a "floater":

1. It is usually on a plane of focus higher than that of the remainder of the smear, and often on top of other cells.
2. Its staining often differs from that of the rest of the smear.
3. It may carry with it a substratum which contrasts with that of the host smear.

The following recommendations may help to reduce the chance of contamination.

1. Any movement of slides in and out of solutions should be done slowly and gently and with as little agitation as possible.
2. All solutions used in the staining procedure should be replaced frequently and the jars washed thoroughly.
3. Crowding of the slides within the staining jars should be avoided.
4. Stains should be filtered before re-use.
5. Large and deep staining jars allowing free space beneath the slides are recommended.
6. Since detachment of floaters occurs more frequently in exudates, it is advisable to stain exudate smears in separate jars and renew the solutions more frequently.

* Procedures Nos. 267 and 268 are also very satisfactory for the staining of tissue sections.

† EA 65 differs from EA 36 only in the strength of the Light Green stock solution (0.05% instead of 0.1%). It has the advantage of a lighter and more transparent cytoplasmic staining. However, the differentiation between acidophilic and basophilic cells is better with the EA 36. In our laboratory, vaginal, endocervical and endometrial smears are stained routinely with EA 36 or EA 50 and other smears with EA 65, although any of these stains may be used for all types of smears.

CHAPTER

III

Criteria of Malignancy

THE CRITERIA of malignancy in exfoliative cytology, like those in histopathology, are based on changes in structure of individual cells as well as on their altered interrelationships. In smears a glimpse of the tissue pattern is afforded by cell clusters or small tissue fragments which are frequently found intermixed with the single cells. In exfoliative cytology, however, interest is centered primarily on the morphologic characteristics of the individual cells, whereas in tissue diagnosis attention is shifted in large measure to changes in the architectural pattern. Each method may thus be considered as supplementing the other.

A cell in situ shows its distinctive type and its environmental relationships, but it is like a brick in a wall seen from one side; whereas a fully detached cell which is seen in toto may give a more complete picture of its individual structure. Exfoliated cells differ greatly in appearance from corresponding cells in tissue sections. One may, thus, be fully justified in speaking of cytologic criteria of malignancy as applied to exfoliated cells in contrast to criteria applying to cells in tissues, despite the fact that in both instances one is considering different aspects of the same cell type.

In exfoliative cytology the recognition of malignancy is based on general criteria, which may apply to a great variety of cells, and also on specific criteria, which apply only to characteristic cell types. By the general criteria, malignancy can be recognized as such; but it is by the specific criteria that its distinctive type can be determined. In this early stage of the use of the cytologic method in the diagnosis of cancer, we have to rely chiefly on the general criteria, since the number of specific cell forms which have been conclusively linked with the presence of a particular type of malignancy is still limited.

Accurate identification of exfoliated cells as to their type and origin is complicated by the extreme variation in form in both normal and abnormal cell types, by the presence of many intermediate forms, and by their complete dissociation from their primary site. It is only through experience and persistent and systematic study over a period of years that a more intimate knowledge of a larger number of specific cell types can be acquired.

This atlas is a move in this direction and represents an effort to describe and illustrate as many distinctive and representative normal and abnormal cell forms as can be identified at present in the various body fluids. By further progress, exfoliative cytology may finally reach the status of a more exact science, approaching the accuracy of histopathology in the recognition of various malignant tumors and other pathologic conditions.

The discussion of specific criteria based upon characteristic cell types will be found in the chapters dealing with the various applications and in the discussions of the plates.

OUTLINE OF GENERAL CRITERIA OF MALIGNANCY

The general criteria may be subdivided into: (I) criteria based on structural modifications of cells and their nuclei, (II) those based on changes in the interrelationships of cells as evidenced in cell clusters and tissue fragments and (III) indirect criteria.

I. Structural modifications of cells and their nuclei

A. NUCLEAR CHANGES

1. Disproportionate enlargement of the nucleus, altering appreciably the normal nuclear-cytoplasmic ratio (A IV, 12; A VI, 25; C V, 16, 18, 19; D III, 8).

   Nuclear enlargement beyond normal limits, often upsetting the nuclear-cytoplasmic ratio, may also be seen in non-malignant cells, as in those of the endocervix (A III, 6; A V, 1) or pelvis of the kidney (B II, 3, 9, 10).
2. Increase in the chromatin content, causing hyperchromasia (A V, 6–8; A XI, 6; E II, 12, 14).

   A dark staining of the nuclei in both malignant and non-malignant cells may result from a densification (pyknosis) due to a degenerative change (C I, 28; D I, 4). Overstaining may also give an impression of hyperchromasia and lead to a false evaluation of non-malignant cells (A II, 18).
3. Structural abnormalities such as an aberrant chromatin pattern (A VII, 10, 11; E I, 29), elongation (A VII, 13, 19; C IV, 16; D III, 7; D V, 12), irregularity in outline (A IV, 6; C II, 7; E II, 5), deep indentation and furrowing (A IX, 15; B III, 20), lobulation (A VI, 17; A XI, 10; D III, 5) and budding (F II, 22, 24, 25).
4. Enlarged nucleoli or an increase in their number beyond normal variability (A X, 11, 13; C III, 1, 2; E I, 30, 31).

   The term "nucleolus" is here used to designate both true nucleoli and karyosomes, as their differentiation with our routine technique is not always possible.
5. Multinucleation, when associated with nuclear atypia (A VII, 5; B IV, 20; C III, 11; G IV, 15).

   Multinucleation may result either from mitotic or amitotic nuclear division without cytokinesis, or from a fusion of cells without fusion of the nuclei.
6. Mitotic activity with abnormal mitotic figures (H I, 9, 10, 14, 15, and 17–22; H II, 1, 2, 3).

   Mitotic figures may be seen in non-malignant conditions, such as healing cervical erosions (H I, 4), or in exudates where histiocytes and mesothelial cells find a suitable culture medium (H I, 1–3). Abnormal mitotic figures may also be occasionally seen in non-malignant cells (H I, 16).

7. Marked thickening of the nuclear membrane (A IV, 8; B IV, 2, 16; C V, 14, 24, 32; D IV, 14).

   Thickening of the nuclear membrane by itself should not be stressed as a criterion of malignancy, as it may be seen in chronic infections and other non-malignant pathologic conditions (D IV, 1, 2; D V, 5).

8. Degenerative changes, such as abnormal vacuolation (A VI, 24, 25), fading or complete resorption of the nucleus (B IV, 14; C IV, 10, 11, 12).

   Vacuolation and fading of the nucleus may result from the effects of drying or irradiation (G III, 4, 11, 13). Marked vacuolation may be seen in nuclei of decidual or trophoblastic cells in cases of abortion (G II, 14). Resorption of the nucleus is not infrequent in certain types of non-malignant cells such as the so-called "Pap" cells found in sputum (C I, 29, 30).

B. Cytoplasmic changes

1. Changes reflected in the staining reaction.

   Such changes may be brought to light by special techniques. With the staining procedures used in our laboratory, certain types of malignant cells may exhibit pronounced basophilia or acidophilia. Acidophilia is of special value in the diagnosis of some tumors, such as bronchogenic or other epidermoid carcinomas, where malignant cells may become prominent through their marked orangeophilia (C II, 17; C IV).

   Orangeophilic cells found in vaginal and cervical smears may be the result of a keratinization of the cervical or vaginal epithelium and are therefore not suggestive of malignancy.

2. Cytoplasmic inclusions such as pigment granules, leucocytes or cellular debris.

   Melanin granules are characteristic constituents of exfoliated melanoma cells (A XI, 14). Leucocytic inclusions are seen most frequently in adenocarcinomas, particularly those of the endometrium (A IX, 8, 10), although they may be present in other types of malignancy.

   Caution must be exercised in evaluating cells with leucocytic inclusions, since such cells may be found in non-malignant conditions such as infections (B IV, 19; D V, 5), after an endometrial curettage (A VIII, 17, 19), or more rarely in hyperplasias and metaplasias of the endometrium (A VIII, 11). In endometritis, leucocytes appear scattered between the cells rather than as intracellular inclusions (A VIII, 16).

   Ingestion of granules, leucocytes and cellular debris by histiocytes is not uncommon (F I, 3; G I, 6, 9, 10). Phagocytosis of lymphocytic cells by histiocytes has been noted in pleural fluid in cases of Hodgkin's disease (E II, 18).

3. Atypical vacuolation.

   Marked vacuolation of the cytoplasm is seen frequently in adenocarcinoma

cells (A IX, 3, 6, 11; A X, 3, 5, 12, 14; F II, 17, 19) although it occurs in other malignant cell forms (A V, 4, 5, 8; E II, 24).

It is also observed in various types of non-malignant cells under normal or pathologic conditions (A II, 10; A V, 3; A VIII, 18; B IV, 10; E I, 15, 16; F I, 7; F II, 5, 6). Marked vacuolation occurs in normal endometrial cells following curettage, giving them a resemblance to adenocarcinoma cells (A VIII, 17, 19). Histiocytes contain vacuoles of varying sizes (G I, 7, 12). When lipoid substances are ingested, the vacuoles are prominent and have a characteristic appearance (G I, 14).

C. Changes of the cell as a whole

1. Enlargement of cells beyond their normal range (A VI, 24; A X, 11, 16; B IV, 16; C V, 13, 14; D III, 5, 7, 8; E II, 21, 24; compare H I, 22 with H I, 1).

   Cellular hypertrophy may occur also in non-malignant cells of the endocervix (A III, 21; A XI, 4), cells of the renal pelvis as seen in ureteral specimens (B II, 9–13), cells of the prostate (B I, 24), cells from the female genital tract during gestation (A III, 22) and others.

2. Aberrance in the form of the cell.

   Cells showing extreme elongation and bizarre shapes are seen frequently in squamous cell carcinomas of the cervix, chiefly in the advanced stages (A IV, 2; A VI, 1–8; A VII, 6, 14, 15, 18), and in bronchogenic or other epidermoid carcinomas (C IV, 1–4, 9–16). Extremely elongated malignant cells of epithelial origin may show great resemblance to fibrocytes. They are frequently referred to as "fiber cells." This term, though descriptive, may give the impression that these are fibrous connective tissue cells. In our laboratory they are designated as "snake" cells (A VII, 6, 14).

   Aberrant or elongated cell forms having a normal nucleus should not be interpreted as malignant (A II, 14, 20).

3. Degenerative or necrotic changes.

   Necrosis is observed frequently in cases of malignant neoplasms (C II, 17; C III, 14; C IV, 10–14; D I, 22) and is of greater diagnostic value in certain types such as uterine adenocarcinoma (A X, 7; A XII, 13).

   As is true for other criteria, degeneration and necrosis alone are not absolute proof of malignancy. Necrotic exfoliated cells may be found also in normal smears, as in rectal and colonic washings. Marked cellular degeneration is evidenced also in smears from certain benign tumors, such as papilloma of the bladder, or in chronic inflammatory conditions (A II, 3, 5, 6, 7, 8; C I, 29, 30; C V, 20) and is particularly conspicuous after irradiation (G III).

II. Criteria based on the interrelationships of cells

1. Irregularity of pattern (A XI, 13; C III, 11; D III, 4, 8; F II, 20).

In exfoliated cell clusters or tissue fragments, irregularity of pattern implies a lack of uniformity in the orientation of the cells and their nuclei.

2. Anisokaryosis and anisocytosis (A X, 11; B III, 23; D II, 5; D III, 8).

The terms "anisokaryosis" and "anisocytosis" refer to marked variations in size of nuclei and cells of the same type within a cluster, not to the variations found in single cells scattered throughout the smear. This is one of the more significant criteria of malignancy.

3. Lack of distinct cell boundaries.

The loss of distinct boundaries within a cluster may be considered an effect of the dedifferentiation of cells, which is not uncommon in malignancy. The diagnostically significant point is the absence of distinct cell borders in clusters of cells of a type in which the boundaries of individual cells are normally distinct (A V, 10; compare with No. 9).

Cell boundaries are sometimes indistinct in clusters of normal cells such as the endometrial (A VIII, 1–7). It should also be taken into consideration that a poor preservation of the specimen may result in the disappearance of cell demarcations.

4. Dense grouping and crowding of cells and nuclei.

Crowding is a significant criterion of malignancy and can be better evaluated in exfoliated cell clusters where cells are preserved in toto (A XII, 10, 11, 12, 14, 15; B IV, 20; D V, 9), than in sections of a tissue or of a centrifuged sediment.

Crowding may be observed in clusters of normal cells, as in the endometrial (A VIII, 6–10).

5. Engulfment of one cell by another.

In many cases this process gives the impression of phagocytosis (C III, 15; D II, 14; F I, 11; F II, 13, 14, 16); in other instances, it appears to result from one cell being pressed into another because of crowding (B III, 1, 3).

6. The grouping of cells into characteristic patterns.

The rosette form of clusters of ovarian cystadenocarcinoma cells (A XII, 9–12; E II, 7, 10; compare A XII, 11, and E II, 7) found in exudates or in endometrial aspirates may be cited as an example.

7. Pronounced stratification.

Stratification in cell clusters is more valid as a criterion of malignancy when associated with hyperchromasia, elongation, or other nuclear abnormalities. Pronounced stratification is usually encountered in the advanced stages of carcinoma of the cervix (A VII, 12, 13).

III. Indirect criteria

1. Presence of blood.

Profuse bleeding and the presence of fresh blood do not suggest cancer as much as old fibrinated blood, with degenerated erythrocytes, as seen in adeno-

carcinoma of the endometrium. Bleeding is practically absent in the early stages of malignancy.

2. Excess of lymphocytes.

Lymphocytes are particularly suggestive of malignancy when found in large aggregations in certain specimens such as sputum and bronchial washings and to a lesser degree in other fluids such as exudates. In some cases of lymphatic leukemia numerous lymphocytes have been noted in various body fluids, such as sputum, bronchial washings, urine and exudates (E I, 19; E II, 17).

Lymphocytes are rarely prominent in normal smears and even in cases of infection are not as numerous as they are in tissue sections. Clusters of normal lymphocytes intermixed with actively proliferating lymphoblasts have been noted in endocervical smears in two cases. In one, a diagnosis of general lymphadenopathy was established. Plasma cells are seen chiefly in cases of chronic inflammation but rarely acquire any prominence in smears.

3. Prominence of histiocytes.

Histiocytes may be found in relatively large numbers in certain malignant conditions, as in adenocarcinomas of the endometrium (G I, 13). In some cases they display high phagocytic and proliferative activity and appear in giant multinucleated forms.

Histiocytes may also be seen in relatively large numbers in normal smears and therefore should not be considered as primarily suggestive of malignancy. They are commonly found in stagnant fluids such as exudates and breast and bronchial secretions and in chronic inflammatory or other conditions when there is cellular debris or foreign matter to be disposed of (E I, 1, 2; F I, 1, 2; F II, 1). They are also prominent in the vaginal and uterine secretions at certain stages of the normal cycle, particularly toward the end of the menstrual exfoliative process (A XII, 2) or following parturition or abortion.

Histiocytes occasionally show atypical features such as hyperchromasia, nuclear enlargement and extensive vacuolation to a degree that may result in their being misinterpreted as malignant cells.

4. Polymorphonuclear leucocytes.

Polymorphonuclear leucocytes are primarily characteristic of infection. In malignancy, they are numerous chiefly in the advanced stages where secondary infections are common.

GENERAL CONSIDERATIONS

In surveying the above criteria it becomes apparent that no single criterion is sufficient to establish conclusively the presence of malignancy. Disproportionate nuclear enlargement, hyperchromasia, multinucleation, abnormal mitosis, vacuolation, anisocytosis and anisokaryosis, stratification, etc., are morphologic aberrations which may be observed singly in apparently normal cells. Sufficient evidence for a positive diagnosis is provided only by the fulfillment of a number of criteria.

A thorough knowledge of normal exfoliated cells and the range of their variability is of paramount importance not only as the logical foundation for an understanding of the abnormal but also as a prerequisite of an adequate diagnostic evaluation.

There are instances in which the consideration of criteria of normalcy and a knowledge of the range of normal variability are essential for the proper interpretation of cytologic findings. The evaluation of smears of urine aspirated from the renal pelvis may be cited as an example (B II, 3, 9–13). In such specimens the exfoliated cellular elements show extreme variations in the size of cells and their nuclei and multinucleation which might easily lead the inexperienced to an erroneous positive diagnosis. Despite this structural atypia* the non-malignant nature of the cells can be recognized by the fact that they retain their transitional type and the nuclei, though enlarged, show a normal chromatin pattern.

Another example is afforded by the ciliated cells, found in bronchial aspirates and washings. They sometimes show abnormal features such as disproportionate nuclear enlargement, multinucleation, and anisokaryosis which might arouse a false suspicion of malignancy (C I, 14, 16, 17, 18; G IV, 3). The non-malignant character of such cells can be established by certain criteria of normalcy, such as the presence of cilia or of a ciliary border. The same remarks apply to ciliated cells appearing in dense clusters which, by virtue of the crowding of the cells, may simulate the grouping of neoplastic cells (C I, 24). The existence of a ciliated cytoplasmic border is an indication of their normal character.

Clusters of atypical cells seen in some cases of bronchiectasis may also simulate the grouping pattern of neoplastic cells (C I, 31–36). Their non-malignant nature is evidenced by the uniform size and normal structure of the nuclei.

One must always be aware of the variability that normally exists in the size of exfoliated cells and their nuclei within a given cell type. As mentioned above, extreme variations may be observed in certain types such as the mucoid cells of the endocervix, cells of the renal pelvis in ureteral specimens, ciliated cells in bronchial washings or cells from the female genital tract in pregnancy and abortive processes. However, the normal nuclear pattern of these cells permits the recognition of their benign character regardless of the variation in size. Exceptions to this rule are the atypical decidual or trophoblastic cells, found in smears following incomplete abortion, in which the nuclear pattern often resembles that of malignant cells (G II, 14, 15, 16).

SOURCES OF ERROR

Aside from errors in interpretation there are others which may result in an incorrect evaluation of cytologic findings. Of these the following may be mentioned:

1. Mislabelling or interchanging of specimens either at collection or in the laboratory.

2. Deterioration of specimens because of delayed processing or poor fixation.

3. Drying of the smears, particularly prior to fixation. This may cause distortion and enlargement of cells and their nuclei and loss of structural detail. Drying is more apt to occur commonly in swab or scraping smears. In endocervical swab smears, it is one of the most frequent causes of false positive evaluations.

4. Improper staining. Overstaining with hematoxylin or other nuclear stains may give to the nucleus or even the whole cell a hyperchromatic appearance. This is another frequent cause of false positive interpretations. Understaining, on the other hand, gives a poor definition of cell structure and does not permit the proper evaluation of the chromatin content of the nucleus.

* The term "atypia" is used in this atlas to designate structural aberrations from the normal type not necessarily suggestive of malignancy.

5. Incomplete or erroneous marking of the specimen type. In urinary specimens for instance, a catheterized urine from the pelvis of the kidney may be labelled only as urine or as bladder or voided urine. This may result in a misinterpretation of the origin and type of certain cells.

6. Contamination. This may occur at the time of or prior to collection of specimens and may result in a serious diagnostic error. This may be illustrated by a few examples. In one instance, cells from a small papillary carcinoma of the bladder situated near a ureteral orifice were carried into the ureter by the catheter and intermixed with the cells of the renal pelvis. A positive cytologic report was given on the ureteral specimen and it was erroneously assumed that the malignancy was in the kidney. In another instance, malignant cells were found in a nasopharyngeal washing, although the nasopharynx was clinically negative. Nine months later malignant cells of the type present in the nasopharyngeal washings were found in a sputum specimen and an unsuspected reticulum cell sarcoma of the lung was discovered. In this case the nasopharyngeal specimen was evidently contaminated by bronchial discharge containing malignant cells.

In a third instance a carcinoma of the stomach was suspected on the basis of malignant cells found in a gastric specimen. Further exploration proved that the positive cells came from a carcinoma of the lung. In reviewing the gastric specimen, the presence of dust cells intermixed with cancer cells was the only clue to the bronchial origin of the malignant cells.

Contamination may also occur during staining. When many slides are processed together, cells or cell clusters may become detached from one smear and adhere to the smear surface of another slide. Cells are detached more frequently from exudate smears; therefore it is advisable to use a separate set of jars for their staining. (See page 12.)

7. Lack of an adequate history. Operative procedures such as endometrial curettage, cauterization of the cervix and bronchoscopy or irradiation may cause marked cytologic changes which in the absence of adequate information may lead to misinterpretation of smear findings.

The above-mentioned possibilities of error should always be taken into consideration in the final evaluation of smear findings.

CLASSIFICATION OF CYTOLOGIC FINDINGS

Smears cannot always be judged as positive or negative. There are cases in which cytologic findings are inconclusive. A classification taking into consideration the relatively large group of questionable smear findings is therefore necessary. In certain laboratories, results are reported as positive, suspicious or negative. However, a subdivision of both positive and negative groups into two subgroups appeared to us desirable and constitutes the basis of our present classification.

One may often experience great difficulty in classifying cells which deviate from the normal type but show no malignant characteristics. Cells with such atypical features as are sometimes seen among the transitional cells of the renal pelvis and the ciliated cells of the lung or in chronic inflammatory conditions and parasitic infestations, as well as cells exfoliating from papillomas and other benign tumors, belong to this category. Their structure is essentially negative for malignancy, yet they cannot properly be included in the group of normal cells. An intermediate class between the entirely normal and the suspicious groups appears thus to be necessary.

A similar need for subdivision exists in the positive group. There are instances in which the results are of an overwhelmingly positive character, leaving no doubt as to their final interpretation. On the other hand, there are cases in which there is strong but not fully convincing evidence of malignancy. To report such cases as definitely positive would not be justified in view of the fact that a positive smear report is often the determining

factor in the decision for major surgery. Therefore it is essential to distinguish between results of a definitely conclusive character and those which carry with them an element of doubt.

These considerations led us to the acceptance of the following system of classification for cytologic findings, consisting of five groups:

Class I—Absence of atypical or abnormal cells.

Class II—Atypical cytology but no evidence of malignancy.

Class III—Cytology suggestive of, but not conclusive for, malignancy.

Class IV—Cytology strongly suggestive of malignancy.

Class V—Cytology conclusive for malignancy.

Regarding the interpretation of the various items of the above classification, it should be emphasized that Class V is actually the only conclusive group. Accuracy of close to 100 per cent can be maintained in this group by competent and conservative utilization of the cytologic method.[8] Of course, a varying degree of error in interpretation is to be expected in each of the other four groups. A Class V report, because of its high accuracy, is the only one which may be considered as justifying a major operative procedure.

CHAPTER

IV

Female Genital System

THE EXFOLIATED CELLS found in vaginal, endocervical and endometrial aspiration smears are shed from the lining epithelium of the vagina, cervix and uterus and appear singly or in clusters.

In vaginal smears, the majority of the cells encountered are of the squamous type and are derived from the epithelium of the vagina and ectocervix. Endocervical cells are not seen frequently. Cells from the endometrial mucosa usually appear in association with uterine bleeding.

The cervical or endocervical aspiration smear contains cells from the lining epithelium of the ectocervix as well as the endocervical mucosa and, more rarely, the endocervical glands. As in the case of the vaginal smear, one does not encounter endometrial cells unless there is exfoliative uterine bleeding.

The direct cervical smear taken with a cotton swab or a wooden spatula contains a varying proportion of ectocervical and endocervical cell types, depending upon the site from which the smear was taken. It has the advantage of procuring a larger number and a greater variety of tumor cells, when the tumor happens to be within the swabbed area. Vaginal and cervical aspiration smears, on the other hand, give a more complete sampling of cells from the entire uterovaginal epithelium.

An endometrial aspiration smear may contain epithelial as well as stroma (tunica propria) cells derived from the uterine mucosa. Endometrial smears, unless taken with great care, may be contaminated with cervical or even vaginal cells.

In order to make a thorough cytologic exploration of the female genital tract it is desirable to have aspiration smears of all three types, vaginal, endocervical and endometrial, as well as a swab or spatula smear from the area of the squamocolumnar junction which is a common site of early cancer of the cervix. The method of preparing these smears has been described in Chapter II.

NORMAL CYTOLOGY OF VAGINA, ENDOCERVIX AND ENDOMETRIUM

The cells found in vaginal, endocervical and endometrial smears may be divided into four groups.

1. Those derived from the squamous epithelial lining of the vagina and ectocervix.
2. Those derived from the endocervical mucosa, including the glands.
3. Those derived from the endometrial mucosa and its glands.
4. Those derived from the fallopian tubes and the ovaries.

Vagina and ectocervix

The squamous epithelium covering the vagina and ectocervix consists of five zones[27] (Fig. 4). The deepest one, the basal zone (C 1 or K 1), is a single layer of small cuboidal or columnar cells with a relatively high nuclear-cytoplasmic ratio. Cells of this zone are not shed normally and are not generally found in the vaginal secretion.

The next, or parabasal zone (C 2 or K 2), is made up of several layers of round, oval or polyhedral cells with a fairly large nucleus and distinct intercellular bridges. The number of the layers and the size of the cells depend upon the stage of the menstrual cycle and the extent of hormonal, chiefly estrogenic, stimulation. The cells of the outer layers are, as a rule, larger than those closer to the basal zone.

Parabasal cells are frequently found in smears and may be subdivided into two types, those poor in glycogen (aglycogenic type) corresponding to the atrophic epithelium of menopause and of primary or low-level amenorrhea (A I, 11–14), and those rich in glycogen (glycogenic type) characteristic of a hypertrophic epithelium containing much glycogen, such as that found in pregnancy or after high estrogenic stimulation (A I, 8). Exfoliated cells of the parabasal type usually lose their intercellular bridges and have a round or oval form with a relatively large round or ovoid nucleus.

Cells with elongated (A II, 18), spindle-like (A II, 14), spinous (A II, 11), tadpole (A II, 20) or other atypical forms are less frequent and appear mainly in pathologic conditions, such as chronic cervicitis, uterine prolapse and cervical polyposis.

The third or intermediate zone (C 3 or K 3) consists of several layers of moderately flattened cells connected by intercellular bridges. In cross sections these cells have a navicular form and may be referred to as navicular cells. The nuclei are relatively large, somewhat flattened, and usually eccentrically placed. The number of layers in this zone, as in the previous one, depends upon the extent of the epithelial growth. The navicular and parabasal zones are more distinctly outlined when the epithelium is high and well developed, as during the peak of the follicular activity or in pregnancy, when they reach their highest degree of development.

Exfoliated cells of the navicular zone are smaller and have a larger nucleus than superficial squamous cells, from which they can be differentiated also by their characteristic form (compare A I, 1 and 5). Navicular cells are quite prominent in pregnancy and differ from those of the normal menstrual cycle in that they have a larger nucleus and a heavier cellular membrane (compare G II, 1, and A I, 5).

The basal, parabasal and navicular zones constitute what has been designated as the "basalis" (Fig. 4, C 1, 2 and 3) in contrast to the "superficialis," which consists of the more superficial zones (C 4 and C 5). Glycogen is found in all layers of the basalis, increasing in quantity from the basal to the navicular zone. Its amount depends upon the growth and secretory activity of the epithelium. It is particularly abundant in pregnancy.

The fourth zone is well defined as an independent zone when the outer layers of the epithelium undergo complete keratinization (K 4). It corresponds to the stratum granulosum of the epidermis. Its cells contain distinct keratohyaline granules. Complete keratinization is rather rare in the cervix and even more rare in the vagina. It is usually associated with pathologic conditions such as uterine prolapse, keratosis or leucoplakia.

In the normal cornified epithelium the stratum granulosum is absent, but its place is often taken by a narrow, dense zone (C 4), the significance of which is not properly understood. This zone is not always well defined and consists of flattened and densely packed cells which are strongly acidophilic and have small pyknotic nuclei. Some investigators consider it homologous to the intraepithelial zone which appears in rodents during the early stage of cornification, while to others it represents the result of densification and collapse of the deeper layers of the superficial zone.

The fourth zone (C 4, K 4) has little practical cytologic significance in exfoliative cytology, since in smears stained by our routine procedures cells from this zone cannot be well distinguished from the cells of the more

superficial layers of the squamous epithelium.

The fifth, or superficial zone (C 5 or K 5), consists of several layers of flattened cells which appear elongated in cross section. Their nuclei are small and pyknotic. The thickness of this zone depends largely upon the functional state of the ovaries and the phase of the menstrual cycle.

Cells exfoliated from this zone are large, flat, sometimes folded and have as a rule an irregular polygonal form and a small, pyknotic nucleus (A I, 1–4, 6, 10). Some of the cells are basophilic (precornified), while those undergoing cornification are acidophilic. A certain amount of glycogen is often retained in the superficial cells, particularly the basophilic (A I, 1).

The ratio of the superficial acidophilic cells to the basophilic depends chiefly upon the estrogenic hormonal level. In the normal menstrual cycle, the number of acidophilic cells increases during the follicular and wanes during the luteal phase.

In the fully keratinized epithelium the cells are scaly and strongly acidophilic or rather orangeophilic (A I, 9). The nuclei have either entirely disappeared or are only faintly outlined. Cells of this zone when exfoliated differ from the cornified cells by the absence of the nucleus and by their greater affinity for the Orange G stain. In contrast to them, cornified cells have a pyknotic nucleus and a predilection for eosin.

Endocervix

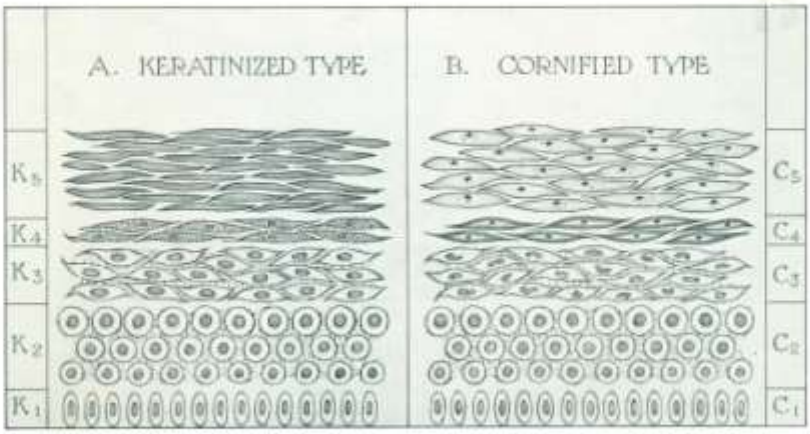
The endocervix, including its glandular components, is covered by simple cuboidal or columnar epithelium consisting of two types of cells, the secretory or mucoid and the ciliated. The mucoid cells are by far the more numerous and their nuclei are basally located. The ciliated cells are scattered singly or in small groups between the mucoid cells and are more superficially situated. The height and form of the secretory cells depend upon their functional state, which is related to various factors such as age, stage of the menstrual cycle, pregnancy, hormonal balance, etc. During the normal cycle the highest secretory activity of the endocervical glands appears to be at the peak of the follicular phase and the transition to the early luteal stage.

Both cell types, secretory and ciliated, are found in endocervical aspiration or swab smears. The ciliated cells are usually sparse in number, although in some instances they are fairly numerous. The ratio of the two cell types depends largely upon the functional state of the epithelium. It should be noted that normally the ciliated cells are more often seen in smears from postmenopausal women. When endocervical exfoliation is marked, relatively large fragments may be detached, containing undifferentiated reserve cells and more rarely subepithelial elements.

Exfoliated mucoid cells may retain their cuboidal or columnar form or may become rounded (A III). Cells derived from the epithelium of the endocervical glands are sometimes recognizable by their taller columnar or goblet cell form. In the cuboidal or columnar cells the nucleus is usually situated at the basal end of the cell, whereas in the rounded cell it tends to be eccentric. The cytoplasm shows the irregular vacuolation characteristic of the mucoid cell type (A III, 3, 8, 11).

When in clusters the cells are usually rounded and have distinct cellular borders. In a cluster viewed from its basal surface (the one adjacent to the stroma), the cellular borders are more distinct and give the cluster the appearance of a honeycomb (A III, 7). This may be interpreted as indicating that the cellular membranes are heavier at the proximal than at the distal end of the cells. The presence of mucin may be demonstrated by special staining (A III, 5). In some instances larger fragments of the endocervical mucosa may be seen, some with glandular stomata or with attached portions of the glands.

The nuclei of the endocervical mucoid cells (A III, 3) are usually round or oval. They



Drawn by Hashime Murayama

Fig. 4. Diagram showing the various zones of the epithelium of the human vagina and portio vaginalis (ectocervix)

A. Keratinized Type

Stratum corneum	(K5)		
Stratum granulosum	(K4)		
Stratum spinosum superficiale	(K3)	Stratum spinosum	Stratum germinativum or Malpighii
Stratum spinosum profundum	(K2)		
Stratum cylindricum	(K1)		

B. Cornified Type

Cornified or squamous zone	(C5)	Superficialis (Traut, Block and Kuder)
Functionalis of Dierks		
Stratum corneum		
Intraepithelial zone	(C4)	
Verhornungszone of Dierks		
Verdichtungszone of Stemmhorn		
Intermediate or navicular zone	(C3)	
Light zone of Traut, Block and Kuder		
Stratum spinosum superficiale		
Outer basal or parabasal zone	(C2)	Basalis (Dierks)
Dark zone of Traut, Block and Kuder		
Stratum spinosum profundum		
Inner basal zone	(C1)	
Stratum cylindricum		

have a well-outlined membrane and a lightly stained chromatin network dotted with one or more relatively small though distinct karyosomes (A III, 1–3; A XI, 1). In the tall, columnar cells the nucleus is basally located and often concave at its distal pole (A XI, 1).

The nuclei of the endocervical cells exhibit a relatively wide variation in size (A III, 6). Such a variation is often noticeable after a strong or prolonged estrogenic stimulation which may cause some enlargement.

Irregularities in nuclear size are the most frequent cause of false positive evaluation of endocervical cells. Drying of the smear before fixation may also lead to erroneous interpretations because of the ensuing enlargement and distortion of the cells and their nuclei.

It is not uncommon to find stripped nuclei, that is, nuclei without surrounding cytoplasm, in endocervical smears (A III, 16, 17). This is apparently due to an extensive plasmolysis, the etiology of which is not fully understood. In the normal menstrual cycle stripped endocervical nuclei are seen more frequently midmenstrually in association with a high estrogenic index. They may also appear after administration of estrogens.

The ciliated cells generally retain their cuboidal or low columnar form (A III, 2, 12, 13). They can be identified by their cilia and their dense cuticular membrane. A small vacuole, para- or perinuclear, is often present. Ciliated cells appear singly or in small clusters and sometimes in a palisade arrangement. Multinucleation, which is rather infrequent in the mucoid cells, is not uncommon in the ciliated type (A III, 10, 14, 15). The multinucleation of ciliated cells appears to be a general phenomenon, as it is encountered also in ciliated cells of other organs (C I, 13, 14; G IV, 3).

A peculiarity seen chiefly in the ciliated cells is a knob-like protrusion at the distal pole of the nucleus (A III, 1, 2). This is interpreted as a dense concentration of chromatin, since it stains deeply with hematoxylin. In some nuclei one may see strands radiating from the protrusion to the opposite pole of the nucleus (A III, 6, 9). It is likely that this structural peculiarity represents a normal functional state, as it has been linked in some cases with high estrogenic stimulation. It cannot very well be interpreted as a degenerative change, since it is found in cells showing good preservation.

Endometrium

The epithelium lining the endometrium, including its glands, consists of two cell types, the secretory and the ciliated. The secretory cells are of the mucoid variety. They have a cuboidal or low columnar form, with a basally located nucleus. Their secretory activity is limited to the second half of the menstrual cycle.

The ciliated cells are usually inconspicuous and can be better demonstrated with special techniques. They are sometimes numerous but the possible diagnostic significance of this is still not clear. Beneath the surface epithelium and between the glands is the uterine stroma consisting of relatively small cells, which in their undifferentiated state resemble cells of the embryonic mesenchymal type.

Exfoliated endometrial cells are derived from the stroma as well as from the epithelium of the uterus. Clusters of stroma or tunica propria cells consist, as a rule, of closely packed cells of uniform size and appearance (A VIII, 1–3, and 7). The epithelial cells, when they occur in clusters, have a richer supply of cytoplasm than the stroma cells and a somewhat looser texture. They are of the same two varieties found throughout the epithelial lining of the female genital tract: the mucoid or secretory (A VIII, 13–15) and the ciliated (A VIII, 12). The ciliated cells usually retain their original cuboidal or columnar form after exfoliation, in contrast to the secretory, which tend to assume an oval or round shape.

The nuclei of exfoliated endometrial cells are round or oval depending upon the type and form of the cells and have a fine chromatin network. Their nucleoli are sometimes

prominent although they are usually inconspicuous.

The identification of endometrial cells in vaginal and endocervical smears is often difficult, as they may be confused with endocervical cells or histiocytes. The endometrial cells are generally smaller than the endocervical cells and tend to form denser and more compact groups, in which the outlines of the cells are not always clearly defined. However, exceptions to these general rules are not uncommon. A definite overlapping exists between the endometrial and endocervical cell forms, making a differentiation between the two types very difficult.

Ciliated endometrial cells are only rarely seen in smears, and their differentiation from the ciliated endocervical cells, which are much more common, is not always possible. Their identification is easier when they are found in an uncontaminated endometrial smear but their diagnostic significance is still obscure.

Clusters of endometrial cells often exhibit characteristic patterns which facilitate their identification. Such patterns may be seen toward the end of the menstrual phase and during the early proliferative stage, most frequently between the 5th and 9th days of the menstrual cycle (A VIII, 8–10).

A study, conducted over a period of many years, of smears from a large number of normal sex cycles has afforded convincing evidence that the endometrial exfoliative process may extend beyond the 4th or 5th day when the menstrual flow usually stops. During this late exfoliative stage, which is sometimes revealed macroscopically by slight spotting, clusters of undifferentiated stroma cells, desquamated from the still incompletely epithelialized endometrial mucosa, may still be found in the smears.[24, 24a] The cells of these clusters possess the ability to differentiate into phagocytic elements of the histiocytic type. The ones which undergo this differentiation are the peripherally located cells of each cluster, which break away either singly or in small groups and ultimately change into free histiocytes (A XII, 2). The rich supply of histiocytes produced by this process serves to provide phagocytic elements for the cleaning of the female genital tract from the menstrual detritus. A similar process takes place after parturition and abortion. In some clusters the peripheral cells form a distinct zone consisting of larger cells which are often vacuolated, whereas the centrally located cells are more densely grouped and often show signs of degeneration (A VIII, 8–10 and Discussion of A VIII).

Clusters exhibiting high vacuolation of the peripheral cells may be easily misinterpreted as malignant, because of their resemblance to clusters of vacuolated adenocarcinoma cells (A IX, 2–4). This fact should be borne in mind in evaluating smears taken during the postmenstrual phase.

Histiocytes are found commonly in vaginal, endocervical and endometrial smears. They vary greatly in number, form and in size which ranges from that of a small monocyte to that of a large epithelial cell. They are found during all stages of the cycle although the period of their greatest prominence in the normal cycle is toward the end of or shortly after menstruation. It is during this period that they are particularly numerous and display a higher phagocytic activity. They also acquire great prominence during the post-partum and in abortive processes.

Their number as well as their phagocytic activity increase in inflammatory and other pathologic conditions, including malignancy. It is in such atypical states that one may expect to find the large multinucleated forms, although their presence in smears from normal women is not uncommon. In endometrial smears histiocytes are often found in great numbers after menopause. When large and loaded with leucocytic inclusions they exhibit great similarity to adenocarcinoma cells. Their usual prominence in cases of adenocarcinoma of the endometrium and their great variability in size and form make them troublesome

from the diagnostic standpoint, as they may lead to both positive and negative false evaluations.

In pure endometrial smears one may expect to find endometrial cells in larger numbers and greater variety of forms, and in a better state of preservation than in vaginal or endocervical smears. The relative difficulty in obtaining endometrial fluid for a microscopic study is largely responsible for the fact that our knowledge of the normal endometrial exfoliative cytology is still incomplete. Thus the endometrial smear cannot yet be used routinely for evaluating the functional state of the endometrium during the various phases of the normal menstrual cycle. The existence of cyclic modifications of the endometrial cells is, however, apparent and one may safely predict a wider use of the endometrial smear in the future for the determination of the functional changes of the uterine mucosa. In vaginal and endocervical smears exfoliated endometrial cells can be found normally only during the menstrual bleeding and thus give a limited picture of the extent of the endometrial exfoliation during the menstrual cycle.

Fallopian tubes

The epithelial lining of the uterine tubes consists of the same two cell types found in the remainder of the female genital tract: the ciliated and the secretory. The ciliated cells are more numerous and much better developed than in the endometrium or the endocervix. It is likely that cells exfoliated from the tubal epithelium reach the uterine cavity, but their identification is not possible because of the lack of distinctive traits by which they could be differentiated from cells exfoliated from the endometrium. The normal exfoliative cytology of the uterine tubes and the extent of exfoliation are practically unknown.

ATYPICAL (NON-MALIGNANT) CYTOLOGY OF VAGINA, ENDOCERVIX AND ENDOMETRIUM

Atypical cells or smear patterns showing a deviation from the normal type are frequently seen in vaginal, cervical and endometrial smears under abnormal and various non-malignant pathologic conditions. Some of these aberrant cell forms and patterns are illustrated in the plates and described more fully in the plate discussions.

Chronic inflammations of the cervix are probably the most common cause of atypical cytology. In addition to an increased number and variety of inflammatory cells including histiocytes and eosinophils and the occasional presence of blood, various types of atypical epithelial cells are often present in the smears. Their number and form depend upon the site, type and extent of the lesion. In some cases there is a predominance of ectocervical squamous cells while in others the endocervical glandular cells are in the majority.

The changes seen in the ectocervical parabasal and the endocervical cells are probably the most significant (A II). Many cells acquire irregular and sometimes bizarre forms. Parabasal cells of the spinous type with irregular cytoplasmic processes are not uncommon. Both parabasal and endocervical cells tend to become elongated, and their cytoplasm may show pronounced vacuolation. The cells may grow beyond their normal size with a proportional enlargement of their nuclei, but the nuclei retain, as a rule, their normal structure. Both the cytoplasm and the nucleus are often stained more deeply than that of corresponding normal cells, giving an impression of hyperchromasia, which, in association with nuclear enlargement and other structural modifications of the cells, may cause a suspicion of malignancy. The increased cellularity of the smear and the presence of cell clusters exhibiting atypical patterns are additional potential causes of misinterpretation.

In senile atrophic vaginitis, which is one of the common vaginal infections, there is an excess of inflammatory cells including histiocytes and eosinophils and a predominance of parabasal epithelial cells, some of which show a tendency to nuclear enlargement. The nuclear structure, however, remains normal.

In endometritis there is also an increase in the number of inflammatory cells. In this condition, however, the leucocytic infiltration of endometrial cell clusters constitutes a more specific criterion. In contrast to adenocarcinomas in which the ingested leucocytes are enclosed in large intracellular vacuoles (A IX, 8), in endometritis the leucocytes are scattered intercellularly within the clusters (A VIII, 16). In general, lymphocytes and plasma cells are not as prominently represented in smears as they are in sections of infected and inflamed tissue from organs of the female genital system.

Trichomonas vaginalis and Monilia albicans, which are the most common parasites of the female genital organs, are quite frequently associated with chronic infections and tend to increase the cell atypia seen in the smear. In some instances the nuclear modifications exceed normal variability and border on dyskaryosis. Some investigators are inclined to attribute these structural aberrations to a direct action exerted by the parasites. However, the fact that such aberrations appear in a relatively small percentage of parasitic infections indicates that the action of the parasites is probably contributory, merely accentuating an atypia due to some other, probably intrinsic, factor.

Trichomonas and Monilia can be identified in smears stained with our method. However, trichomonads are sometimes poorly preserved and their flagella are only rarely visible. The safest criteria for their identification are their ovoid form and their small elliptic nucleus, which is usually situated near one of the poles (A II, 13). The Monilia spores can be recognized by their elliptic form, the small vacuoles or deeply stained granules which they often contain, their acidophilic reaction and their characteristic budding. The diagnosis of mycotic infections by means of smears is easier when mycelia are present. Trichomonads may be easily confused with stripped nuclei; and Monilia spores, with fragments of nuclei of polymorphonuclears. Trichomonas infestation is generally associated with a bacterial flora of cocci, whereas Monilia is found more frequently in association with a bacillary flora. In addition to the regular bacterial flora a very long bacillary form is often seen in smears from cases of Trichomonas infestation and sometimes of a mycotic infection. These long bacilli have a diagnostic significance, since they are rarely seen in cases in which there is no parasitic infestation. Since Trichomonas and Monilia may result in cell atypia, and since they may occasionally be a causative factor in sterility or miscarriage, their detection is of considerable importance.

Abortions can be recognized cytologically on the basis of the smear pattern rather than by specific cell types, although characteristic cell forms may be seen in certain cases.[28] Some of these forms represent modifications of the navicular cells of pregnancy. A cell of this type, which is acidophilic and has a small pyknotic nucleus, is illustrated in G II, 12. In incomplete abortion the smears usually reveal bleeding with pronounced fibrination, an increase in the number of acidophilic cells and a greater prominence of polymorphonuclears and histiocytes. Similar changes may be noted in ectopic pregnancies. In some cases of missed abortion superficial squamous cells loaded with hemosiderin granules have been observed (G II, 6 and 7).

In endometrial hyperplasias the cytologic criteria are of an indirect nature. In many cases, there is increased cornification associated with atypical bleeding. Specific cytologic criteria, however, are not recognizable in vaginal and cervical smears.

In cases of polypoid hyperplasia of the endocervix or endometrium large clusters of cells or small fragments of tissue exhibiting patterns suggestive of polyposis may be observed in endocervical or endometrial smears. Such fragments may contain stomata of glands or finger-like appendices, which evidently represent portions of detached glands. However, it is difficult to reach a positive diagnosis of a polyp on the strength of such findings,

since almost identical patterns are sometimes observed in apparently normal cases (see Discussion, A XII, 18).

Squamous metaplasia may be recognized in endometrial and endocervical cell clusters when some of the cells exhibit changes suggestive of a transition from the glandular to the squamous type. It is difficult if not impossible to decide whether single cells are actually metaplastic or were derived from the squamous epithelium.

Cauterization of the cervix may cause metaplasia, keratinization and other changes, the specificity of which may be questioned.

More characteristic cytologic changes occur in cervical erosions. There is an increased exfoliation of endocervical cell clusters, some of which assume a rounded form suggestive of a small papillary projection of the endocervical mucosa. Many of the exfoliated columnar cells are somewhat larger and stouter than their normal counterparts (A III, 21; A XI, 1) and often exhibit a palisade arrangement (A XI, 2). Nuclear enlargement with some variation in size within a single cluster and multinucleation may also occur in some cases (A XI, 4). Mitotic figures are more likely to be observed in healing erosions.

Epithelial pearls (A I, 7) are a rather common occurrence in vaginal smears, particularly in cases in which there is a relatively large number of cornified cells suggestive of a high estrogenic level. They indicate a certain hypertrophy of the epithelium but not a malignant change. They are sometimes seen in cases of superficial cell dyskaryosis but this is probably because of the pronounced cornification which often prevails in such cases.

Cell clusters or small tissue fragments showing stratification are often present in vaginal, cervical and endocervical smears in nonmalignant conditions such as chronic cervical infections. Sometimes stratification is very pronounced in cell clusters exfoliated from cervical stumps (A II, 18) and may normally be seen in cervical and endocervical smears from senile women. Stratification is commonly observed in malignancies of the epidermoid type (A VII, 15). The diagnostic significance of clusters or tissue fragments showing stratification depends largely upon the type and structure of their cellular components. The presence of nuclear abnormalities is one of the criteria by which their malignant nature can be recognized.

Stratification is often combined with or may even be considered as the result of keratinization of the cervical epithelium. When the keratinization process is incomplete the cells still retain their nuclei, which are totally resorbed when the keratinization is complete, as in leucoplakia. It is likely that keratinization, though an inherent quality of the squamous cells, does not express itself unless brought out by an external factor causing local irritation. Uterine prolapse may be considered one of the most frequent causes of keratinization of the cervical epithelium. The massive exfoliation of keratinized cells in the form of small squamae showing pronounced orangeophilia and the complete loss of nuclei permits a fairly accurate diagnosis of leucoplakia. The presence of a few keratinized cells may cause a suspicion of leucoplakia, but it is not sufficient to establish a positive diagnosis of this condition.

Another peculiarity which may be observed in endocervical smears from senile women is the frequent presence of groups of stripped nuclei. The nuclei have an apparently normal structure but are usually hypochromatic and tend to appear in small clumps or aggregates.

Marked cellular and nuclear abnormalities may be noted in smears taken after irradiation therapy (G III). These changes are extreme during the first two weeks following irradiation, particularly in the cells of the squamous type, both normal and abnormal. Later on they tend to subside and in many cases are hardly noticeable after a period of a few months. In other cases, however, the cell modification persists for a much longer period of time. In the latter cases it is often difficult to decide whether the atypia indicates the per-

sistence or reappearance of an active lesion or is the result of drastic and permanent structural derangements of the normal epithelium caused by irradiation. Our difficulty in interpreting postradiation changes is that we still lack specific criteria for an objective and accurate differentiation between the abnormal forms of irradiated non-malignant and malignant cells. The studies of Ruth Graham[18, 19] mark a great progress toward a better understanding of the subtle differences in the changes in non-malignant and malignant cells and give promise of a more competent future use of the cytologic method in the evaluation of the adequacy of irradiation therapy in individual cases.

MALIGNANT CYTOLOGY OF VAGINA, ENDOCERVIX AND ENDOMETRIUM

Cells desquamated from malignant neoplasms of the uterus may be subdivided into two types, the squamous or epidermoid found in the epidermoid carcinomas of the cervix, vagina or vulva or in adenoacanthomas, and the adenomatous found in the adenocarcinomas of the cervix, endometrium and tubes, or in cystadenocarcinomas of the ovaries.

Epidermoid carcinomas

From the cytologic standpoint the epidermoid carcinomas of the cervix may be divided into two distinct groups, one of which includes the early and the other the more advanced stages of malignancy.

Early malignancy. In the early stages the morphologic changes of the exfoliated malignant cells are limited almost entirely to the nucleus. The most characteristic of these are disproportionate enlargement, irregularity in form and outline, hyperchromasia and multinucleation (A IV and A V). The cytoplasm may exhibit some structural modifications such as vacuolation, either perinuclear ("cavitation") or generalized (A IV, 5, 8, 9; A V, 4, 5, 8) and acidophilia or orangeophilia (A V, 6 and A VI, 10, 13, 14); but the cells as a whole retain their original type in contrast to the highly aberrant forms characteristic of advanced malignancy (A XI, 13).

The term "dyskaryosis" has been introduced[32] to designate the cytologic patterns of early cervical malignancy characterized chiefly by an abnormal nuclear morphology. Depending upon the predominating type of the cells which exhibit nuclear abnormalities, it is possible to recognize four distinct types of dyskaryosis: the superficial (A IV, 1, 3, 5, 6), navicular or intermediate (A IV, 8, 9), parabasal (A IV, 12; A V, 4, 6, 7, 8) and endocervical (A V, 10, 12, 13; A XI, 6).

These patterns may be quite frequently intermixed in the individual cases. This remark applies particularly to the three squamous cell varieties—superficial, intermediate and parabasal—which are often found together in various combinations. Coexistence of a dyskaryotic pattern with an advanced malignant cytology may also be occasionally observed. One might be tempted to interpret this as indicating a possible transition from an early to an advanced malignant state.

Dyskaryotic patterns are usually found in smears from cases in which the lesion is still in an intraepithelial, preinvasive stage (stage 0) or, more rarely, in cases of early invasion (stage 1). The percentage of cases in which dyskaryotic changes have been proved by biopsy to correspond to a malignant lesion varies with the type of the pattern, being much higher in the endocervical and parabasal than in the superficial and intermediate types. In many cases of the latter two varieties, the histopathologic findings revealed the presence of borderline changes corresponding to the condition which some pathologists designate as dysplasia.

Evidence is steadily accumulating in favor of the view that the course and rate of development of intraepithelial carcinomas of the cervix are not the same in all cases. Some of these early preinvasive lesions may progress rapidly to an advanced invasive stage; others may remain dormant over a period of many years; while still others may undergo spontaneous

regression. A long-range study and analysis of the cytologic patterns prevailing in cases of early malignant lesions of the cervix and their correlation with histopathologic material and clinical data may eventually lead to the development of dependable cytologic criteria which would permit not only a diagnostic but also a prognostic evaluation of individual cases.

Thus far a follow-up by repeat smears of a number of cases representing the various types of dyskaryosis indicates that "reversibility"—that is, a spontaneous disappearance of abnormal dyskaryotic cells—occurs more frequently in the superficial and intermediate than in the parabasal and endocervical cell types. The parabasal and endocervical cell varieties are much more likely to advance to an invasive stage. The endocervical variety is probably the one which carries with it the most unfavorable prognosis. A long postponement of action on the part of the clinician appears thus to be highly undesirable in cases in which the cytologic specimens show a predominance of abnormal cells of the endocervical and the parabasal cell types.

Cytologic patterns characteristic of dyskaryosis apparently occur with somewhat higher frequency in pregnant women. In a number of such cases observed in our laboratory the predominating patterns were of the superficial and intermediate cell varieties which, as stated above, have a greater tendency to undergo complete regression. This may be one of the reasons why a relatively large percentage of the abnormal cytologic patterns of pregnancy disappear entirely after the end of gestation. Such a post-partum regression of carcinomas in situ of the cervix appearing during pregnancy has been previously reported on the basis of studies of tissue sections of biopsy specimens.

The nature and malignant potentialities of these abnormal histologic and cytologic patterns and the interpretation of the hormonal and other factors involved in their transitory appearance during pregnancy are still matters of controversy. Opinions are divided as to whether they represent true malignant lesions or deviations from the normal morphologic structure caused by hormonal or other physiologic factors related to gestation. The presence of similar lesions, both non-reversible and reversible, in non-pregnant women indicates that other, more general, biologic factors are involved in this phenomenon and that the gestational factor has only a contributory significance.

The mechanism of the process of total disappearance of early malignant or potentially malignant lesions is not fully understood. It is still debatable whether this process is actually one of true reversibility, that is, a spontaneous normalization of a distinctly abnormal epithelial area, or rather a process of elimination by total exfoliation. It is, of course, conceivable that the continual shedding of cells from the squamous epithelium of the cervix and vagina, and the periodic exfoliation of the endometrial mucosa might result in the complete disappearance of some of these intraepithelial lesions. The massive epithelial desquamation occurring during the post-partum period may explain the more frequent disappearance of intraepithelial lesions appearing during pregnancy as compared with the disappearance of similar lesions in the non-pregnant state.

Advanced malignancy. In the more advanced stages of squamous cell carcinoma of the cervix the cytologic changes are more pronounced and are reflected not only in the modification of the nuclear structure, but also in that of the cytoplasm and the cell as a whole. An elongation of the cells is one of the most characteristic features. Cells may become spindle-shaped or filiform, resembling fibrocytes or smooth muscle fibers (A VI, 1, 2, 4, 5, 6; A VII, 6, 14, 15). Others may acquire a tadpole appearance (A VI, 7) or other unusual and bizarre forms (A VI, 3). Many of these cells are acidophilic and show an affinity for eosin or Orange G.

Although these forms are quite character-

istic, the diagnosis of malignancy should be based primarily on coexisting nuclear abnormalities. A spindle-like, filiform, or tadpole cell with a normal nucleus should not be interpreted as malignant. Such atypical cells may be found in chronic inflammatory or other conditions (A II, 14, 18, 20).

Some of the aberrant epidermoid cells observed in advanced malignancy of the cervix are often referred to as "differentiated." This term appears to be more fitting for designating the dyskaryotic cell types of early malignancy which still retain the identifying characteristics of the normal cell type. The unqualified use of the term "differentiated" to specify also aberrant epidermoid forms as seen in advanced malignancy is apt to be misleading.

The term "undifferentiated" is often used in describing malignant cells which, because of cytolysis and other degenerative and necrotic changes, can no longer be identified with any distinctive normal or abnormal cytologic type. The term "dedifferentiated" appears to be more appropriate for designating these cells; whereas the term "undifferentiated" should be retained for cells which have not as yet become differentiated and are closer to the embryonic type.

The grouping of cells into large clusters showing anisokaryosis and crowding is observed more frequently in advanced malignancy (A VII, 19; A XI, 13). Groups of malignant cells found in the early stages tend to be smaller, and the individual cells do not exhibit the extreme deviations from the normal cell type which are met with in later invasive stages.

Cytologically it is difficult to differentiate between epidermoid carcinomas of the cervix and those of the vagina or the vulva. In general, it may be stated that the exfoliated malignant cells in carcinoma of the vulva tend to be larger than those in carcinoma of the cervix (A VI, 20-22).

Cell clusters showing pronounced stratification are seen mainly in the advanced stages of cervical carcinomas (A VII, 12, 13, 19). Stratification may also be observed normally in senile smears or in certain conditions such as chronic infections or hypertrophic cervical stumps (A II, 18). Although stratification is more common in malignant cell clusters, the diagnosis of malignancy should be based largely on the presence of nuclear abnormalities.

Mitosis is occasionally observed in cells exfoliated from epidermoid carcinomas, but is rather rare (A V, 12; A VI, 28). Mitoses are also seen in non-malignant conditions (H I, 4) such as regenerative processes in healing cervical erosions.

Adenocarcinomas

In adenocarcinomas of the endometrium, the exfoliated malignant cells are usually smaller and more densely grouped than those of the squamous type (A XII, 6, 7, 8, 14). The cytoplasm may show vacuolation, which is often extreme. The vacuoles are not infrequently filled with leucocytes in various stages of degeneration (A IX, 6, 8, 10). The nuclei are ovoid or round or may show irregularity in form. In some cases the nucleoli are greatly enlarged and very prominent (A IX, 13). Although there is an apparent increase in the chromatin content, hyperchromasia is not as marked as in the nuclei of the malignant epidermoid cells. The nuclei may assume an eccentric position, particularly in cells having large vacuoles.

Exfoliated adenocarcinoma cells tend to become rounded or oval, losing their original columnar or cuboidal form. Their glandular origin can still be ascertained by certain criteria such as the vacuolated appearance of the cytoplasm and the eccentricity of the nucleus. However, in some cases there is a marked elongation of the cells and their nuclei causing them to resemble malignant cells of the epidermoid type.

Differences in the size of the cells and the nuclei, though often marked, are in general less pronounced in adenocarcinoma than in the cells of epidermoid carcinomas. Mitoses,

though rare, are seen occasionally (H II, 13).

Degeneration and necrosis of adenocarcinoma cells is a common occurrence. Some of the necrotic cells show karyorrhexis and assume a characteristic appearance as the nucleus breaks down into irregular chromatin granules scattered throughout the cytoplasm (A X, 7; A XII, 13). A thorough screening of a smear under high power for abnormal or necrotic adenocarcinoma cells is very essential, particularly in cases in which clusters are absent.

Cells that have a large vacuole filled with leucocytes and an eccentrically placed nucleus are also characteristic of adenocarcinoma. However, such cells may be confused with large histiocytes phagocytosing inflammatory cells.

In smears taken within a few days after a curettage, clusters of endometrial or endocervical cells infiltrated by leucocytes may be found (A VIII, 17, 19). Because of their great similarity to clusters of adenocarcinoma cells containing ingested leucocytes, they may lead to an erroneous diagnosis of malignancy, although their non-malignant nature can usually be recognized on closer examination.

The cytology of early adenocarcinomas of the endometrium has not thus far been as thoroughly explored as that of the carcinomas of the cervix. As already stated, exfoliated endometrial cells cannot be expected to be found in smears of fluid aspirated from the vagina or cervix, except in the presence of uterine bleeding. The chances of finding endometrial cells in such smears are therefore very slight in the early stages of endometrial malignancy when bleeding is either slight or absent. This is why one has to rely on an endometrial rather than on a vaginal or cervical smear for the detection of early uterine cancer.

Another point to be considered is that the spotting and identification of early adenocarcinoma cells or cell clusters is much easier in a pure endometrial aspiration smear than in a vaginal or cervical smear. The criteria by which adenocarcinoma cells can be identified, such as distinct nuclear abnormalities, vacuolation and leucocytic infiltration, may be absent in early adenocarcinoma cells, making difficult their differentiation from the normal cell type (A XII, 7). Clusters of such cells found in a vaginal or cervical smear could not be identified as malignant endometrial cells as confidently as they could be in an endometrial smear.

This actually happened in the case of the cluster illustrated in A XII, 7, which was observed in an endocervical smear. Though impressed by its atypical features, the author was unable to recognize definitely its origin and malignant nature because of its similarity to clusters of endocervical cells present in the same smear. This cluster would have stood out in an endometrial smear, and its abnormal features would probably not have been overlooked. In view of the fact that the presence of an adenocarcinoma was later proved, the true origin and nature of this cluster are now apparent.

Adenocarcinomas of the cervix have a smear pattern comparable to that of the adenocarcinomas of the endometrium but their cells are somewhat larger and closer to the endocervical glandular cell type (A X, 3–8, 10, 11, 14). Since there is an overlap in the range in size and form of the cells of adenocarcinomas of the endometrium and those of the cervix, the differentiation between these two types is difficult.

Adenoacanthomas present a mixed cytologic picture in smears. Some exfoliated malignant cells show squamous metaplasia (A X, 9), whereas others retain the glandular type characteristic of adenocarcinoma. When metaplasia is marked, the malignant cells resemble closely those of epidermoid carcinomas of the cervix (A VI, 24).

In adenocarcinomas of the tubes, malignant cells are expected to be found more often in endometrial than in endocervical or vaginal smears (A XII, 15). The cells may be identified as malignant, but the site of their origin

cannot be determined by cytologic criteria.

Cells from cystadenocarcinomas of the ovary are not infrequently recovered in endometrial smears, even in the absence of metastasis to the endometrium (A XII, 10–12). Their occurrence in vaginal or endocervical smears is rather rare (A XII, 9). Clusters of ovarian cystadenocarcinoma cells often appear in the form of rosettes or other distinctive patterns that make possible their differentiation from cells of adenocarcinoma of the endometrium. The exact route through which malignant cells migrate from the ovaries to the uterus is still unknown.

Cells from hydatidiform moles (A XII, 16, 17) and chorioepitheliomas of the endometrium (A X, 15, 16) are only rarely encountered in vaginal smears. Their cytologic type has not yet been well established. Chorioepitheliomas, as a rule, show extreme cellular and nuclear abnormalities and degenerative changes. The chance of recovering cells from tumors of this type is better in endometrial than in vaginal or cervical smears.

CHAPTER

V

Urinary and Male Genital Systems

CELLS FROM the urinary organs, that is, the kidneys, ureters, urinary bladder and urethra, and from some of the male reproductive organs are constantly being shed from their epithelial surfaces and carried out of the body by the urine.

Although the number of the exfoliated cells reaching the excretory ducts is very large, there is no appreciable accumulation as in the case of the female genital tract because of their continual evacuation. Centrifugation of the urine is thus necessary for obtaining a cellular sediment from which slide spreads may be prepared.

Techniques of handling the urine specimens and preparing and staining the smears have already been described (see pages 11, 12).

The exfoliative cytology of the urinary and male genital organs differs in the various types of specimens, which may be classified into five groups according to the method used for their collection.

1. Urine aspirated from the ureter and the pelvis of the kidney.
2. Catheterized bladder urine.
3. Voided urine.
4. Prostatic secretion.
5. Semen.

CYTOLOGY OF URINE ASPIRATED FROM THE URETER AND THE PELVIS OF THE KIDNEY

Non-malignant cytology

Urine aspirated from the ureter and the pelvis of the kidney is usually rich in exfoliated cells. Most of these are derived from the transitional epithelium lining these organs, although cells shed from the tubules of the kidney may also be found in ureteral specimens. The renal origin of cells may be revealed by their relatively smaller size and their frequent arrangement into clusters of cylindrical form. Cells derived from the epithelium of the ureter and the renal pelvis exhibit the characteristics of the transitional cell type, which makes possible their recognition within certain limits. However, many cells are poorly differentiated or show degeneration and cannot be easily identified as to their origin and type. Ureteral specimens are sometimes contaminated by cells dislodged from the epithelium of the urethra and bladder during the introduction of the catheter.

Cells found in ureteral specimens display a great variability in size, form and structure. They appear singly or in clusters in which the lines of demarcation between the individual cells are usually well preserved. The cells show vacuolation of the cytoplasm, which is often differentiated into a central, lighter staining endoplasmic area and a deeper staining ectoplasmic zone (B II, 1).

The nuclei, when well preserved, are round or oval and have a fine chromatin network with one or more small karyosomes. The arrangement of the chromatin may sometimes give the impression of proliferative activity, but mitotic figures are very rarely seen. The nuclei vary greatly in size, often to a point far surpassing the range of variation seen in other types of exfoliated cells (B II, 9–13). Nuclear enlargement per se is not, therefore, as valid a criterion of malignancy in ureteral urine as

it is in cells observed in other body fluids.

Another characteristic of ureteral specimens is the frequent presence of bi- or multinucleated cells (B II, 6–13). In some instances, as many as 80 nuclei have been counted in one cell. These cells bear a certain resemblance to the giant histiocytes, from which, however, they can be distinguished by differences in the form, structure and distribution of the nuclei and the vacuolated pattern of the cytoplasm. (Compare B II, 6–8 with G I, 11, 13 and 15.) It is possible that these giant multinucleated cells are syncytia formed by a fusion of many cells probably after exfoliation, since such giant forms cannot be seen in sections of the ureter or pelvis; although smaller multinucleated cells may be found occasionally in sections of the renal tubules (B II, 17). Other possibilities are that the exfoliated giant forms found in ureteral smears are artefacts resulting from the trauma of catheterization or the result of amitotic or mitotic division of the nucleus without a corresponding division of the cytoplasm. Cells of this type have been reported as occurring more frequently in smears of post-partum women.[16]

Cells with cuboidal or columnar form are often seen in ureteral smears in cases of benign papillary growths. However, their occasional presence in cases in which there is no evidence of such a growth limits their diagnostic significance.

Renal calculi may cause the appearance of atypical transitional cells. These are mostly small or medium-sized cells with a deeply stained nucleus and are usually intermixed with many inflammatory cells and erythrocytes (B II, 4). Because of the apparent nuclear hyperchromasia their differentiation from malignant cells is at times difficult.

Malignant cytology

In the presence of malignant tumors arising in the transitional epithelium of the pelvis or ureter, there is usually a copious exfoliation of cells exhibiting distinct criteria of malignancy, nuclear as well as cytoplasmic (B III, 21). In order to evaluate these cells accurately one must be well acquainted with the extreme cytologic variability of the normal ureteral specimen (B II, 9–13).

There is evidence that in neoplasms originating in the transitional epithelium of the renal pelvis and the ureter, the exfoliation of malignant cells appears early. In two cases of early carcinomas of the pelvis of the kidney observed in our laboratory, an abundant shedding of distinctly malignant cells was noted. In both instances the diagnosis was made originally by the cytologic examination of ureteral urine and later confirmed by a nephrectomy performed on the basis of repeated positive cytologic findings.

In the first case, the lesion was invisible macroscopically and the surgeon left the operating room with the sad feeling that a normal kidney had been removed. The microscopic examination of samples from the epithelium of the pelvis revealed the presence of a typical carcinoma in situ.[17] A cluster of exfoliated malignant cells found in a ureteral specimen from this case is illustrated in B III, 15 (compare with B III, 21).

The second case is also of particular significance from the diagnostic standpoint. The patient had intermittent hematuria, and physical examination revealed an irregular, hard prostate. Clinical findings, including an intravenous pyelogram, were negative. The urine sediment smears were evaluated as positive for malignancy, Class V. A transurethral resection of the prostate was performed and a pathologic diagnosis of adenomatous hyperplasia of the prostate was made. A cytologic diagnosis of voided urine 2 months later was again positive for malignancy, Class V, and as a result the patient was rehospitalized. At this time a retrograde pyelogram was essentially normal, revealing only a slight irregularity in the upper calyceal group on the right. As the clinical suspicion was carcinoma of the prostate, a second transurethral resection was performed and the pathologic diagnosis was adenomatous hyperplasia of the

prostate. Cytologic examination of voided urine as well as of urine from the right ureter was positive, Class V. Urine from the left ureter was negative. On the strength of the cytologic findings and the slightly suspicious retrograde pyelogram a right nephrectomy was performed. A tumor 3 mm. in diameter covering the tip of one of the minor calyces was found and was diagnosed pathologically as a papillary carcinoma of the right kidney. The time elapsed from the first positive cytologic findings to the nephrectomy was 5½ months.

Malignant tumors of the kidney parenchyma cannot be detected through the recovery and identification of exfoliated cells in urine specimens unless they erode into the excretory tubules. Cells exfoliated from parenchymal carcinomas are found most frequently in specimens of ureteral urine, but even then they do not appear in the urine in as large numbers as cells from neoplasms developing in the transitional epithelium of the pelvis or ureter.

Exfoliated cells from clear cell carcinomas or hypernephromas of the kidney are usually well differentiated and show no obvious structural abnormalities by which their malignant character could be readily recognized (B III, 7; B IV, 1 and 3). Cells of this type can be identified in tissue section by their rich glycogenic content; however, attempts to demonstrate the presence of glycogen in exfoliated cells from these tumors by specific staining procedures have thus far been unsuccessful. Furthermore, in specimens of ureteral urine, the normal exfoliated cells from the ureter and the renal pelvis show great structural variability which constitutes a further handicap in the recognition and evaluation of the tumor cells from the parenchyma of the kidney. (Compare B III, 7, with B II, 3 and 9–13.)

Wilm's tumor is a more rare neoplastic disease and, like other parenchymal tumors of the kidney, cannot be diagnosed cytologically unless it has already invaded the excretory ducts. Cells exfoliated from tumors of this type are smaller than those of the clear cell carcinoma and have the appearance of undifferentiated embryonic cells. Their recognition is based on a knowledge of their specific type rather than on general criteria of malignancy.

These adverse factors reduce considerably the chances of diagnosing by the cytologic method parenchymal tumors of the kidney, particularly in their early developmental stages.

CYTOLOGY OF CATHETERIZED BLADDER URINE

Non-malignant cytology

The urinary bladder acts as a receptacle for cells exfoliated not only from its own epithelium but also, to a lesser extent, from the epithelium of the kidney pelves and the ureters. However, most of the cells found in catheterized specimens are cells desquamated from the epithelium of the bladder. Cells with strikingly atypical forms like those found in ureteral specimens are only rarely seen in normal bladder urine, as, for instance, after irritation caused by the passage of a renal calculus. Cells of prostatic or urethral origin are also a rare occurrence in catheterized bladder specimens. Urethral cells may sometimes be introduced into the bladder by the catheter.

The urinary vesicle is lined with epithelium of the transitional type which in histologic sections shows a uniform structural pattern. Exfoliated bladder cells, however, exhibit a variety of types and forms (B I, 5, 6, 12, 13, and 15–17). Some (B I, 5 and 6) are similar to cells found in ureteral specimens, while others (B I, 12, 13, 16 and 17) exhibit forms resembling the superficial squamous cells seen in vaginal smears. Vacuolation may often be noted but is only rarely pronounced (B I, 17).

The nuclei, when well preserved (B I, 5, 6, 15 and 16), are of the vesicular type and have a lightly stained chromatin network and one or more small though distinct karyosomes. Their structure shows some resemblance to

that of the nuclei of endocervical cells. (Compare B I, 5 and 6, with A III, 2, 6 and 16, and A XI, 1.) In the larger, more superficial cells, the nuclei may show shrinking or pyknosis (B I, 12, 13 and 17).

Hormonal influences cause morphologic changes in the epithelium of the urinary bladder which are reflected in the cytologic picture. Such changes may occur normally during pregnancy and result in the appearance of characteristic cells of the navicular glycogenic type (B I, 13) showing great similarity to those observed in the vaginal fluid during gestation.[35] Following treatment with an estrogenic hormone, a variety of cell forms comparable to those found in the vaginal secretion during the peak of the ovarian follicular activity may be present in the urine of both sexes.

Malignant cytology

Neoplasms of the urinary bladder exfoliate, as a rule, copiously. The cytologic pattern prevailing in each individual case depends upon the type of the tumor. Papillary growths may often be recognized as such by the presence of cuboidal or columnar cells (B II, 21 and 22). Malignant papillomas usually exhibit marked nuclear abnormalities (B II, 22) and throw off many degenerate and necrotic cells. In cases of early or low-grade malignancy, as in B II, 21, malignant and non-malignant cells may be intermixed. The benign, borderline or malignant nature of a papilloma can often be established on the basis of cytologic findings.

In some instances malignant cells may appear in a urine sediment smear long before the malignant nature of the tumor is corroborated by biopsy. The malignant cells illustrated in B II, 22 were found in the smear of a case in which a biopsy performed at the time of the preparation of the smear was diagnosed as benign papilloma. The malignant character of this growth was proved by a second biopsy 13 months later. This case gives further evidence to the well-recognized fact that a negative biopsy does not necessarily invalidate a positive smear.

Carcinomas of the urinary bladder show a high rate of exfoliation and marked nuclear changes even in their early developmental stages. In a case of a small, unsuspected carcinoma of a bladder diverticulum, detected by the cytologic examination of urine, as well as in several other early cases, the number of malignant cells and clusters was impressively high.

In advanced cases of carcinoma of the bladder, one usually finds many clusters of malignant cells showing practically all the important criteria of malignancy, such as marked nuclear enlargement, hyperchromasia, anisocytosis, anisokaryosis, multinucleation, crowding and engulfment (B III, 1-6). Malignant cells of the squamous type may be seen in cases of carcinoma showing epidermoid changes (B III, 10 and 13).

The rich desquamation and the pronounced polymorphism of carcinoma cells of the urinary vesicle, as well as its greater accessibility for the procurement of adequate specimens, account for the higher accuracy and better results in the cytologic diagnosis of carcinoma of the bladder as compared with other areas of the urinary tract.

CYTOLOGY OF VOIDED URINE

Smears prepared from voided urine contain cells derived primarily from the bladder and, to a lesser extent, from the ureter, pelvis and kidney, as well as from the urethral canal. In the male, cells from the prostate may also be found, whereas in the female the voided urine is generally contaminated by vaginal cells. For this reason a catheterized specimen is necessary for a competent cytologic study of the female urinary tract.

The male urethra is lined by three different types of epithelium, the transitional, the pseudostratified columnar, and the stratified squamous, whereas the female urethra is lined by only two types, the pseudostratified columnar and stratified squamous.

The normal exfoliative cytology of the male and female urethra and their glandular components and the extent of exfoliation taking place normally within these organs have not as yet been fully explored. It is thus difficult to recognize the various types of desquamated urethral cells with the exception of those derived from the outer squamous portion of the urethra (B I, 2–4). Cells from carcinomas developing in the squamous or penile urethra may also be identified cytologically (B II, 18).

CYTOLOGY OF PROSTATIC SECRETIONS

Non-malignant cytology

The lining of the tubulo-alveolar glands of the prostate consists of cuboidal or columnar secretory cells. That of the ducts is also of the cuboidal or columnar type, becoming transitional near the opening into the urethra. The prostate is a secretory organ showing wide variations in size, functional activity and even structure, due to the action of various factors, such as hormonal influences and age. It is an organ in which benign hypertrophy, metaplasia and chronic infections are quite frequently encountered.

The morphologic changes associated with these altered conditions are reflected in its exfoliative cytology, which embraces a great variety of representative cell forms. Exfoliated prostatic cells exhibit extreme variations in size ranging from very small undifferentiated epithelial cells of apparently glandular origin with a small nucleus and a minimal amount of cytoplasm, to very large cells with one or more vesicular nuclei, resembling the transitional or squamous cell types. Intermediate cell types are also seen, some with a cuboidal or columnar form, reminding one of cells exfoliated from benign papillary growths. The origin, nature and diagnostic significance of these diverse cell types still need further clarification.

One of the special difficulties connected with the study of the prostate is that its secretion normally is not evacuated into the urethra except during ejaculation. It is thus only by massage that one can obtain an adequate specimen for a cytologic study. Voided or catheterized urine collected after massage may also contain a number of prostatic cells. For this reason no fewer than three specimens are considered essential for a competent cytologic exploration of the prostate: one smear prepared from the secretion obtained by massage, and two voided urine specimens, one taken before and the other after massage. A comparison of the cytologic pictures in the two urine specimens permits a more accurate evaluation of the origin of the exfoliated elements.

Certain changes appearing in the prostatic cytology following the use of estrogens are striking.[21] Many of the exfoliated cells are full of glycogen, and show great resemblance to cervical parabasal glycogenic cells found in vaginal or endocervical fluid at the height of the ovarian follicular activity or after the administration of estrogens (B I, 7; compare with A I, 8).

Malignant cytology

Cells exfoliated from different types of malignant neoplasms of the prostate show considerable variability in size and form. In certain types the cells are small and have a relatively uniform appearance. The activation and hyperchromasia of the nuclei and the irregular pattern and crowding of the cells in the clusters are practically the only criteria by which their malignant character can be recognized (B III, 22; B IV, 12). In other types the cells and the nuclei show marked structural aberrations which readily reveal their malignant character (B IV, 7, 8). Epidermoid carcinomas may be recognized as such by their distinctive cytology (B IV, 14, 15). After prolonged estrogenic therapy exfoliated malignant cells may often show hypertrophy and a tendency toward squamous metaplasia[21] (B III, 23; compare with the cells in No. 22).

In benign hypertrophy or adenomatous hyperplasia of the prostate, various forms of

atypical cells may be encountered. Some of these are unusually large and have large vesicular nuclei (B I, 24). Cells of this type, however, may be found also in malignant cases. The differentiation between the benign and malignant forms is based largely on nuclear structure (compare B I, 24, with 22).

Other cells appear in clusters which exhibit a distinctly adenomatous pattern (B IV, 5, 6, 9, 10 and 18) and are consistent with a diagnosis of adenomatous hyperplasia. The clusters illustrated in B IV 4 to 6 are from a case of particular diagnostic interest. The smears were interpreted as suggestive of adenomatous hyperplasia with some clusters (Nos. 4 and 5) indicating a benign condition, while others (No. 6) showed enlarged and hyperchromatic nuclei and an atypical pattern suggesting the possibility of a malignant change (Class III). At that time a biopsy was diagnosed as adenomatous hyperplasia, but a second biopsy performed six years later revealed the existence of a carcinoma of the prostate. This is one of the cases which illustrate that cytologic smears may sometimes present certain advantages over biopsy in uncovering early or hidden carcinomas. The case also gives valuable information regarding the slow rate of development of some of the prostatic carcinomas and at the same time shows the prognostic potentialities of the cytologic method.

Spermatozoa may be seen frequently in prostatic secretion or in urine following prostatic massage, particularly if the seminal vesicle has also been massaged. The spermatozoa are often degenerating and are phagocytosed by histiocytes. In cases of chronic infections the smears contain a large number of polymorphonuclear leucocytes.

SEMEN

Examination of semen may be of value when malignant tumors of the testis are suspected. In a case of teratoma of the testis studied by us, cells displaying malignant characteristics were found in smears prepared from an ejaculate. The relatively simple and uniform cytology of the normal semen facilitates the recognition of any abnormal or atypical cells. Atypical cells with pyknotic nuclei, interpreted as degenerating spermatids, are sometimes seen in semen specimens.

One can approximate the source of malignant cells in the ejaculate by collecting the semen in two separate containers at the time of ejaculation. Since the fluid and cellular contributions of the prostate and ampulla of the ductus deferens appear in the first portion and that of the seminal vesicles in the second portion of the ejaculate,[23a] the portion in which the preponderance of the malignant cells appear may act as an indicator of the site of the malignancy.

The exfoliative cytology of the seminal vesicle as well as that of the epididymis and the seminal ducts and the extent of exfoliation occurring in these organs are practically unknown. It is, therefore, very difficult to identify cells of such a derivation in either the urine or the ejaculate. In direct smears from the epithelium of the seminal vesicle, some bizarre and markedly atypical cells have been noted, which have been related to certain atypical cells found in prostatic secretion smears.[24]

CHAPTER

VI

Respiratory System

THE RESPIRATORY ORGANS and their passages are lined with pseudostratified columnar epithelium, consisting of two types of cells, the ciliated and the secretory or goblet cells.

Cells exfoliated from the lining epithelium of the respiratory tract and its passages can be recovered in sputum produced by a deep cough, or in bronchial or tracheal aspirates and washings. The techniques of obtaining specimens and the preparation and staining of the smears are described in Chapter II.

NON-MALIGNANT CYTOLOGY OF THE RESPIRATORY ORGANS

Bronchial aspirates or washings

The epithelial elements found in a bronchial aspirate or washing are differentiated cells of the ciliated and goblet type and undifferentiated reserve cells from the basal area of the epithelium.

The ciliated cells have a columnar form and may be identified by their cilia or, in their absence, by the heavy cuticular membrane covering their distal surface (C I, 1, 2, 4–10 and 13). They are, as a rule, found in profusion in bronchial aspiration or washing smears. The cytoplasm is often vacuolated, particularly in the distal portion of the cells. Larger and more distinct vacuoles, when present, are usually in close proximity to the nucleus, most frequently at its distal pole (C I, 7 and 10). The nuclei of the ciliated cells have an ovoid or rounded form and when well preserved show a fairly characteristic granular arrangement of the chromatin (C I, 1, 4 and 7).

Ciliated cells exfoliated from various regions of the respiratory tract show marked variations in size and form. Those found in nasopharyngeal washings tend to be taller (C I, 20), whereas those from the laryngeal mucosa (C I, 3) are larger and stouter, than those of the bronchial mucosa.

Multinucleation is encountered quite frequently in ciliated cells found in bronchial aspirates and washings (C I, 2, 13 and 14). This feature is also observed, though to a lesser extent, in ciliated cells exfoliated from other organs, such as the cervix (A III, 10, 14). When multinucleation is associated with anisokaryosis it might easily be misinterpreted as a sign of malignancy (C I, 17). The presence of cilia is a criterion by which the benign nature of a cell can be fairly accurately ascertained. Exfoliated cells, however, often lose their cilia and, if there is multinucleation and the nuclei show marked variation in size or are fused into a single mass (C I, 16), the cells may be interpreted erroneously as malignant.

The significance of multinucleation in ciliated cells is not clear. Does it represent a morphologic variation corresponding to a normal functional state or a pathologic aberration? If pathologic it cannot be considered an indication of malignancy, since it is seen in benign as well as malignant cases. It is, however, possible that it may indicate the presence of malignant potentiality, although evidence in support of this view is lacking at present.

The secretory or goblet cells have a columnar form and a basally located nucleus and may be recognized by their mucoid con-

tent. When the secretion is abundant, the nucleus is caved in and assumes a conical form (C I, 19). The secretory cells appear singly or in small aggregations. In bronchial smears they are much less numerous than the ciliated cells. A relatively large increase in their number has been observed in certain pathologic conditions involving a more abundant secretion of mucus, as in asthma.

The undifferentiated cells are probably reserve cells derived from the deeper layers of the epithelium (C I, 27, and C V, 9, 10). They are small and usually appear in compact groups. Their nuclei are round and show a granular arrangement of the chromatin. When such undifferentiated cells are overstained with hematoxylin, they may be misinterpreted as malignant cells of the anaplastic or oat cell type, particularly when anisokaryosis is present (C V, 9, 10; compare with 11).

Bronchial aspirate or washing smears from normal cases contain, as a rule, a small or moderate number of leucocytes and histiocytes. The presence of a large number of polymorphonuclears, including eosinophils, or of plasma cells is suggestive of an inflammatory condition or an infection. Evidence of suppuration arouses a suspicion of an abscess. Lymphocytes in large aggregations are more frequently seen in cases of malignant neoplasms or in leukemia than in non-malignant conditions and, though not pathognomonic, should not be ignored even in the absence of other indications of malignancy.

The number of histiocytes varies greatly but is usually small or moderate in the normal cases. Those of the dust cell variety are a familiar constituent of the normal smear and can be readily identified by their foamy cytoplasm and their content of dust or carbon particles (G I, 5, 6). In patients with lipoid pneumonia or in users of mineral or other oils, the histiocytes contain many large characteristic vacuoles formed by the ingestion of fat or oil droplets and are known as lipophages (G I, 14). Large syncytial histiocytes (foreign body giant cells) are only occasionally seen in normal cases. Their presence in large numbers is suggestive of some pathologic process causing an increased phagocytic activity. In cases of chronic inflammatory conditions and infections, the histiocytes are usually numerous and display a great diversity in size and form. Their nuclei may show enlargement and some hyperchromasia which may give them a certain resemblance to malignant cells.

Erythrocytes are present in bronchial aspirates and washings almost without exception, but their diagnostic value is rather limited since they are frequently the result of trauma caused by bronchoscopy. Bronchial specimens containing much blood are unsatisfactory, as the exfoliated cellular elements are diluted and there is a decreased cellularity of the smear.

Sputum

The cytology of sputum differs greatly from that of the bronchial aspirate. Ciliated, goblet and undifferentiated bronchial epithelial cells are not commonly seen except soon after bronchoscopy. This seems to indicate that exfoliation of the bronchial epithelium is normally rather limited. However, the sputum contains a great variety of other cells. Most of these are of the squamous type and are derived from the epithelium of the upper part of the respiratory tract, such as the anterior and the upper half of the posterior surfaces of the epiglottis, the aryepiglottic folds, the vocal cords, the oral portion of the pharynx, and the oral cavity and tongue.

The majority of squamous cells found in the sputum are of the superficial type. Most of these are acidophilic and display great variation in size and form. Smaller cells of the parabasal type, both basophilic and acidophilic, are also present. Those which are strongly acidophilic or orangeophilic sometimes resemble the orangeophilic cells characteristic of bronchogenic epidermoid carcinoma. Their identification as benign is based chiefly on the normal structure of the nucleus. The predominance of squamous cells in a sputum

smear in association with an absence of histiocytes of the "dust cell" type indicates that the specimen was not produced by deep cough.

Cells with atypical features, which in some instances may cause a suspicion of malignancy, are often seen in cases of chronic inflammatory conditions including pneumonia, tuberculosis and bronchiectasis. Some of these cells have a distinctive form and therefore a diagnostic value, such as the cell known in our laboratory as the "Pap" cell because it was first noted 7 years ago in the author's sputum during an exacerbation of a chronic inflammatory condition of the upper respiratory tract. It is a relatively small acidophilic cell with an elliptic form and an ovoid pyknotic nucleus (C I, 28). Dense clusters of cells of this type have been seen in many cases of chronic respiratory infections. In such cases a characteristic fading of the nucleus is often noted (C I, 29 and 30), particularly in the later resolving stages of the inflammatory process.

The exact nature, origin and diagnostic and prognostic significance of the "Pap" cells has not as yet been established. According to one view they are small squamous cells from the upper portion of the respiratory tract. Another view supported by good evidence is that they represent a squamous metaplastic change of epithelial cells of the ciliated type. "Pap" cells have been seen intermixed with malignant cells in smears from a few cases of malignancy. In these cases there was no definite evidence of a transition of the "Pap" cells to those of the malignant type, and their presence might be attributed to the co-existence of a malignant with a chronic inflammatory condition. In other instances they have been found intermixed with larger squamous cells of the parabasal type, giving the impression of an extensive metaplastic process. A few of these cases were diagnosed as malignant.

Marked nuclear atypia—chiefly enlargement, irregularity in form and hyperchromasia—in squamous cells of the superficial and parabasal type has been noted in some sputum smears from cases in which clinical and other examinations failed to prove the presence of malignancy. Squamous cells of this type show great resemblance to cells found in vaginal and cervical smears in cases of superficial cell dyskaryosis. Their origin and diagnostic significance need to be further clarified by a study and long follow-up of a larger number of cases.

Epithelial pearls are occasionally found in sputum smears from both non-malignant (C I, 25 and 26) and malignant (C II, 15 and 16) cases. Their diagnostic value is thus limited, since there are no clean-cut and objective criteria that would permit an accurate differentiation between the malignant and non-malignant types. The general configuration of a pearl and the appearance of the central cell often have a suggestive but only rarely a diagnostic value. Epithelial pearls are seen even more frequently in esophageal washing specimens. This may sometimes be a source of error in determining the origin of the pearls, since a sputum specimen may occasionally contain esophageal cells. Curschmann's spirals may also be seen in sputum and bronchial specimens. Their specific diagnostic significance has yet to be clarified.

Bronchiectasis is occasionally revealed by characteristic compact clusters of medium-sized cells which probably represent fragments exfoliated from small papillary projections developing within the bronchi of the diseased area. The cells appear to be of the mucoid type with nuclei having a normal structure. In some clusters (C V, 1) the form and grouping of the cells is typical of a benign papillary growth, while in others they simulate a neoplastic pattern. In a case of bronchiectasis in a male child, 8 years of age, some of the cell clusters found in the sputum and bronchial washings showed pronounced squamous metaplasia (C I, 35 and 36).

Clusters of densely grouped ciliated cells (C I, 24) may also be found occasionally in sputum and bronchial washings in various

non-malignant cases. Such groups of cells like those of bronchiectasis may sometimes be misinterpreted as neoplastic.

Leucocytes and histiocytes are commonly seen in sputum and bronchial aspirates, and their appearance, interpretation and significance are similar to those of cells of this type observed in other fluids. Red blood cells have a greater diagnostic significance in the sputum than in the bronchial aspirates and washings, as in the latter the presence of blood may be the result of trauma.

MALIGNANT CYTOLOGY OF THE RESPIRATORY ORGANS

The use of the cytologic method in the diagnosis of malignant lesions of the respiratory tract has been generally acclaimed as one of its most successful applications. Statistics show that its diagnostic accuracy is higher than that of bronchoscopy. The cytologic method may indeed provide not only an accurate diagnosis of a malignant neoplasm[8] but often a recognition of its type.[16]

The bronchogenic epidermoid carcinoma, which is the most frequently encountered tumor of the lung, is characterized by the presence of malignant epidermoid cells some of which resemble greatly those found in the epidermoid carcinomas of the cervix and other organs. Their highly aberrant and bizarre, yet very distinctive, forms and their strong acidophilia and orangeophilia make their detection and identification relatively easy. The recognition of such cells is facilitated by the enlargement, hyperchromasia and characteristic degenerative changes of the nucleus (C III, 8 and 9, and C IV). The degeneration of the nucleus may result in its complete resorption and the appearance of anucleated, strongly orangeophilic cells, the so-called "ghost" cells (C IV, 11–14). The malignant epidermoid cells have a tendency to appear singly or in small groups in the smears, whereas the cells of the undifferentiated or pleomorphic bronchogenic carcinomas usually form larger and more impressive clusters of cells showing an irregular pattern and marked nuclear abnormalities (C III, 11).

Carcinomas of the oat cell or anaplastic type can be identified by the relatively small size of the cells, the marked nuclear hyperchromasia and anisokaryosis, and the scantiness of the cytoplasm which is partly the result of extensive plasmolysis (C II, 4 and 5, and C V, 11 and 12).

Adenocarcinomas may be recognized as such when the cells are well preserved and their glandular type is discernible (C II, 7 and C V, 23, 25 and 33–35). The vacuolation of the cytoplasm and the eccentricity of the nucleus are traits characteristic of adenocarcinoma of the lung as well as of other organs.

Alveolar cell or terminal bronchiolar carcinomas of the lung exfoliate copiously, and their presence—sometimes even their type—can be diagnosed by the examination of sputum or bronchial washings (C III, 4, and C V, 24). Their general cytology is that of an adenocarcinoma. Cells exfoliated from tumors of this type often show multinucleation, but this should not be considered a specific criterion for alveolar cell carcinoma, since multinucleated cells are also found in other types of pulmonary malignancy.

Cells exfoliated from malignant neoplasms of the lymphoid type, such as Hodgkin's disease and reticulum-cell sarcoma, usually exhibit distinctive cytologic features permitting the identification of their type. Malignant cells from such tumors appear mostly single or in relatively small clusters (C II, 9, and C V, 27).

Metastatic neoplasms of the lung do not, as a rule, exfoliate as freely as primary carcinomas. Furthermore, a metastatic growth may lie within the parenchyma without ever reaching the lumina of the bronchial tree. The detection of metastatic tumors by the cytologic method is therefore less likely than that of a primary lesion. Some of the metastatic carcinomas retain their original cytologic character and their type and origin may sometimes be recognized by distinctive cell forms.

With regard to the relative value of sputum and bronchial aspiration or washing specimens, it is felt that each one presents certain advantages. The sputum examination implies a simpler technique, and can be repeated as frequently as desired. However, bronchial specimens are better in other respects. Cells from oat cell or anaplastic carcinomas are easier to detect in bronchial aspirates or washings, though their identification is often rendered difficult by the frequent presence in such specimens of clusters of undifferentiated reserve cells which greatly resemble cells of the anaplastic type. (See C V, comparing 9 and 10 with 11 and 12.) Malignant epidermoid cells can be recognized more safely in bronchial aspirates, because of the virtual absence from such specimens of non-malignant squamous cells which, when present in large numbers as in sputum smears, render the detection and identification of the malignant cells more difficult. Another advantage of the bronchial specimen is that it may contribute evidence as to the site of the lesion. As there are cases of carcinoma in which the bronchial washing is positive and the sputum negative, and vice versa, one might expect to be able to apply this diagnostic method more advantageously by using specimens of both kinds.

In the case of negative cytologic findings, a minimum of three specimens taken at different times should be examined.

MALIGNANT CYTOLOGY OF THE LARYNX

Carcinomas of the larynx may be diagnosed by the cytologic method through the examination of sputum specimens, which may contain malignant cells exfoliated from tumors of this organ. However, when a lesion is visible, it is preferable to take a direct swab or scraping smear. As a rule, in carcinomas of the larynx, the malignant cells are of the squamous type and show marked acidophilia or orangeophilia and pronounced cellular and nuclear abnormalities (C IV, 5–8) which resemble closely those of the epidermoid carcinomas of the lung.

Carcinomas of the nasopharynx and the cranial sinuses may be diagnosed by nasal or sinusoidal (antral) washings. Cells from such carcinomas are shown in C V, 28–31. The normal cytology of nasopharyngeal washings consists of cells of both the ciliated and mucoid type (C I, 20–22). Inflammatory cells are often numerous because of the frequent occurrence of local infections. Lymphocytes are a relatively common constituent of the nasopharyngeal smears and should not therefore cause as much suspicion of malignancy as when found in smears of other regions of the respiratory tract such as the bronchi.

In examining nasopharyngeal washings one should always consider the possibility of contamination by bronchial discharge. Histiocytes of the dust cell type are indeed fairly frequent contaminants of smears of this type. When malignant cells and dust cells are found intermixed in nasopharyngeal washings, the possibility of contamination by bronchial discharge must be borne in mind, particularly when the nasopharynx and the larynx are clinically negative. The case discussed on page 20 may be referred to as an example of such a contamination.

CHAPTER

VII

Digestive System

THE CYTOLOGIC METHOD in the diagnosis of cancer and other pathologic conditions is discussed in this chapter in its application to four regions of the gastrointestinal tract: the esophagus, the stomach, the duodenum and the sigmoid colon and rectum.

CYTOLOGY OF THE ESOPHAGUS

The epithelium lining the esophagus is of the stratified squamous type. Exfoliated esophageal cells are carried into the stomach and are present in large numbers in aspirated gastric fluid intermixed with cells exfoliated from the gastric mucosa. For a competent study of the esophageal cytology, it is advisable to have an esophageal washing or a direct smear if there is a lesion present which can be visualized by esophagoscopy.

The techniques of obtaining and processing esophageal specimens are given on page 7.

Non-malignant cytology

Most of the cells found in smears of esophageal washings are of the squamous type and may be classified into two groups corresponding to those found in vaginal and cervical smears, the superficial cells and the cells derived from the deeper epithelial layers (parabasal). The superficial cells are large and have an irregular or polygonal form and a pyknotic nucleus. The deeper cells are smaller and have a round or ovoid form and a larger and better-preserved nucleus (D I, 10). Some of the superficial cells found in esophageal specimens are the result of contamination by cells of the oral and pharyngeal mucosae.

Epithelial pearls are occasionally seen in esophageal specimens (D II, 16). Their presence in larger numbers is rare and may be attributed to a hypertrophic epithelium. The scarcity of normal cells of the mucoid type indicates that there is no appreciable exfoliation from the epithelium of the esophageal glands. When glandular cells of the mucoid type are found, the possibility of contamination by cells from the nasopharyngeal and gastric mucosae or from a gastric papilloma (D IV, 4) should be considered.

Malignant cytology

The excellent results obtained by the use of the cytologic method in the diagnosis of malignant lesions of the esophagus is proof of an active exfoliation of cells from tumors developing in this organ. The relative simplicity of the normal exfoliative cytology of the esophagus greatly facilitates the detection and identification of desquamating malignant cells. Depending upon the origin and type of the tumor one may recognize two cell types, the epidermoid and the glandular. In both types, the malignant character of the cells is usually indicated by marked nuclear abnormalities.

The majority of the malignant neoplasms of the esophagus are of the epidermoid type and are characterized by exfoliated cells similar to those desquamating from epidermoid carcinomas of other organs (D IV, 14, 16, 17). A distinctive cell type often seen in smears of esophageal carcinomas exhibits a disproportionately large nucleus and a scanty rim of cytoplasm as illustrated in D IV, 18. Epithelial pearls with abnormal features,

when present in esophageal smears, constitute a fairly good criterion for malignant neoplasms of this organ (D IV, 11 and 12). It is of interest to note that in a case of an esophageal carcinoma, malignant epidermoid cells, including an epithelial pearl, were present in a sputum smear. These cells reached the respiratory tract through an existing tracheoesophageal fistula.

Adenocarcinomas of the esophagus are rather rare and often represent extensions from primary tumors of the cardia of the stomach. Cells exfoliating from an adenocarcinoma can usually be identified by their glandular type. However, the site of the lesion cannot be safely established by the finding of adenocarcinoma cells in an esophageal specimen because of the possibility—though remote—that gastric fluid containing malignant cells from an adenocarcinoma of the stomach may be regurgitated into the esophagus during the collection of the specimen.

CYTOLOGY OF THE STOMACH

Cells exfoliated from the gastric mucosa are studied in smears prepared from the sediment of centrifugated gastric fluid. The techniques for obtaining and processing this fluid are discussed on pages 7–10.

Normal cytology

The gastric mucosa is lined with a simple columnar epithelium composed of mucus-secreting cells. Well-preserved exfoliated cells of this type usually retain their columnar form, which can be clearly seen in single cells and small clusters (D III, 2). In the larger clusters the cells are viewed from their distal or proximal ends, which exhibit a rounded or polygonal form with fairly distinct intercellular lines of demarcation (D III, 1). The intercellular lines are particularly sharp and often give a honeycomb appearance to a cluster seen from its proximal (basal) surface (D III, 2). When cells become partially detached from the periphery of such a cluster, their columnar type, which is presumably that of the other constituent cells, can be visualized (D III, 2). Cells with a goblet form are seen occasionally (D I, 2 and D IV, 9). Normally, gastric cells are well differentiated and show a limited variation in size and form.

In the ordinary gastric aspirate, well-preserved exfoliated cells are relatively scarce. This may be attributed to two factors. One is that the gastric epithelium is protected by a mucoid coat which tends to limit exfoliation. A second factor is the action of the gastric secretions which causes cytolysis and results in the presence of many stripped nuclei.

Cells of the parietal and zymogenic types of the gastric glands are not seen normally in the fluid of the stomach. Perhaps such cells are at times present but cannot be identified because of lack of adequate criteria. The only instance in which cells resembling the parietal type have been noted by the author was in a case of benign gastric ulcer. The recognition of mucous neck cells is very difficult because of their structural similarity to the mucous cells of the surface epithelium.

The majority of cells encountered in smears from gastric fluid are cells carried into the stomach from the esophagus and the oral mucosa. These are cells of the squamous type showing considerable variability in size and form. One finds many large superficial cells of the cornified type as well as smaller cells closer to the parabasal type. Other extraneous cells often found in the gastric fluid are derived from the respiratory epithelium. Their site of origin is the tracheobronchial epithelium or the nasopharynx. The recognition of these foreign cells is not always easy, particularly in the case of nasopharyngeal cells which may be carried into the stomach during intubation through the nose. When clustered into a somewhat irregular pattern, such cells may simulate the grouping of neoplastic cells. Ciliation, when present, is a criterion by which the derivation of the cells from the respiratory epithelium can be ascertained (D I, 14, 16). Cells from the duodenum and even the pancreatic and common bile ducts may occa-

sionally reach the stomach by reflux. It is likely that some of the goblet cells found in gastric smears reach the stomach in this manner. Lung histiocytes (dust cells) are not infrequent constituents of gastric specimens and can usually be identified by their characteristic cytoplasmic inclusions.

Cells from various foodstuffs are sometimes found, particularly in impure specimens. These may be identified by their disintegrated appearance and by their morphologic characteristics.

Since the introduction of the balloon technique, which specifies a thorough preliminary cleansing of the stomach, the gastric specimens have been practically free from extraneous cells, thus giving a clearer picture of gastric exfoliative cytology. Of even greater importance is the fact that the cells in such specimens are better preserved and appear in larger epithelial fragments.

Polymorphonuclear leucocytes are found in gastric smears in varying numbers and are particularly prominent in chronic irritations. Lymphocytes are more rarely seen and acquire diagnostic significance only when present in large aggregations. Erythrocytes may be noted occasionally in normal smears, but are of little importance unless found in large numbers indicating actual bleeding or unless they are in a state of degeneration revealing the presence of chronic bleeding. Histiocytes—excluding the extraneous dust cells—vary greatly in number and size but are not so prominent a constituent of the normal gastric smear as of smears of other fluids. Their activity increases in cases of inflammatory processes.

Atypical (non-malignant) cytology

Atypical smear patterns or cells deviating from the normal type yet devoid of malignant characteristics are found under certain conditions in the stomach as in other organs.

In gastric or duodenal ulcers no distinctive cytologic type can be singled out which would permit a definite diagnosis. In rare instances and when the mucosa is eroded to the muscularis one may find well-preserved smooth muscle fibers which permit a diagnosis of the lesion as well as of its extent. In many cases the presence of an ulcer is suggested by an increased number of inflammatory cells. In specimens obtained by the gastric balloon technique a marked increase in the number of exfoliated epithelial cells and cell clusters is not infrequently noticed. These changes, however, cannot be considered diagnostic of this condition.

In some cases of chronic hypertrophic gastritis, a somewhat distinctive cytology has been observed, characterized by medium-sized, rounded and often vacuolated cells with a relatively large and sometimes eccentric nucleus, as shown in D IV, 1 and 2. Such cells may appear either singly or in clusters.

Papillary growths of the stomach may be revealed by clusters of columnar cells arranged in a characteristic pattern suggestive of detached papillary tufts or by cell clusters exhibiting a palisade arrangement (D IV, 3 and 4). Polypoid growths may also show desquamated fragments consisting of columnar cells with an arrangement resembling that observed in papillomas (D IV, 5). However, a definite diagnosis of a polypoid growth cannot be based on evidence provided by the mere presence of columnar cells in the smear, since various forms of columnar cells derived from the normal epithelium of the stomach may frequently be seen in gastric smears.

Malignant cytology

In carcinomas of the stomach the cytologic picture in individual cases presents great differences depending upon the type and site of the tumor, the extent of exfoliation and the state of preservation of the cells.

In some instances, one finds large, irregular clusters of cells exhibiting pronounced abnormalities, both nuclear and cytoplasmic, revealing the malignant nature of the cells (D II, 17, 18, 21 and D III, 7 and 8). In other instances the cells are well differentiated and, though less striking in their manifestation of

the general criteria of malignancy, conform more closely to the adenocarcinomatous pattern (D II, 3, 4, 5, 6, 19 and D IV, 10). In adenocarcinomas of the mucous type as in D II, 1 and 19, the mucoid character of the cells can be demonstrated by special staining.

In some instances the exfoliated malignant cells have a columnar form (D II, 1 and D III, 7), while in others a papillary arrangement of the cells is discernible (D III, 4 and D IV, 7 and 8).

In a case of ulcerated carcinoma a large desquamated fragment showed an unusual and very irregular pattern (D IV, 6) which at low magnification gave the impression of irregularly arranged smooth muscle fibers. By examination under high power and by comparison with sections of the tumor, it was identified as a part of the tumor itself.

In anaplastic carcinomas (D II, 7) the cells are smaller and their identification is often difficult. Hyperchromasia and irregularity of the nuclei are some of the distinctive general criteria. Malignant tumors of the lymphoid type, though consisting of smaller cells, can be diagnosed more readily by their characteristic morphology. The presence as well as the lymphoid character of such neoplasms have been recognized in several cases studied in our laboratory by the cytologic examination of gastric specimens.

The great variability of the cytologic pattern of malignant cells and the good preservation of the material obtained by the balloon technique can be seen in the cells illustrated in the five gastric plates. D III, 9 is an example of a well-preserved adenocarcinoma fragment which is almost as informative as a tissue section. Mitotic figures are occasionally seen in smears from malignant cases, particularly in specimens obtained by the gastric balloon technique (H II, 2).

It appears that exfoliation begins early in the malignant tumors of the stomach, as has been proved by several cases in which carcinomas have been detected at a very early stage by the examination of smears.[11] The extent of exfoliation in carcinomas of the stomach as well as in those of other organs does not apparently depend on the developmental stage of the tumor as much as on its type and site and the size of its free surface. Tumors developing in the pyloric end of the stomach are more likely to be missed because of the frequent difficulty in obtaining cells from this region by ordinary aspiration or even by the balloon technique. Scirrhous carcinomas or sarcomas and metastatic tumors can be diagnosed cytologically only when the tumor reaches the lumen of the stomach. Superficial malignant lesions covered by a necrotic membrane may also fail to shed cells that would reveal their presence.

CYTOLOGY OF FLUID ASPIRATED FROM THE DUODENUM

In fluid aspirated from the upper portion of the duodenum, one may find not only cells exfoliated from the duodenum, but also cells reaching the duodenum through the common duct. The latter include cells exfoliated from the ampulla, the bile and pancreatic duct systems and the pancreas. Fresh cells from these sources may often be obtained in a good state of preservation despite the action of the digestive enzymes which cause a rapid disintegration of cells entering the intestine from the stomach. Duodenal specimens are often contaminated by gastric or even squamous cells. Our knowledge of the exfoliative cytology of this region is still incomplete.

Non-malignant cytology

The exact site of origin of normal cells found in duodenal smears is obscure in most instances. Cells, such as those illustrated in D I, 11, are interpreted as derived from the epithelium of the duct systems. They have a somewhat distinctive appearance and a characteristic nuclear structure, probably due to early necrosis. Cells of this type are often numerous in duodenal smears. Their occasional presence in gastric smears may be attributed to a reflux action. The cells illus-

trated in D I, 18 are of the glandular type (see group on right), and are probably of pancreatic origin. In the group to the left there is evidence of secretion of a substance which is probably of mucoid nature as indicated by its staining reaction.

In chronic infections the smears usually contain a large number of inflammatory cells, mainly polymorphonuclear leucocytes which, judging from their good preservation, appear to withstand well the action of the digestive enzymes. Excessive bile secretion is sometimes indicated by the amount of bile pigment present in the smear.

Malignant cytology

The possibility of diagnosing malignant neoplasms of the duodenum, the pancreas and the pancreatic and biliary duct systems by the cytologic examination of duodenal drainage specimens has been demonstrated in a number of cases studied in our laboratory in which well-preserved malignant cells were recovered in the smears.

Some cases of carcinoma of the pancreas are illustrated in D II, 8, 14, 15 and D V, 9, 10 and 11. In all these cases, the cells show distinct general criteria of malignancy and reveal to some extent the glandular type of the tumor. When cells with such pronounced abnormal features are found, a diagnosis of malignancy is relatively safe because of the limited variability of the non-malignant cytology of duodenal specimens.

Fluid from the duodenum is obtained by drainage through a Rehfuss tube. Mention should be made of the necessity of processing these specimens immediately because of the rapid deterioration of the cellular elements.

CYTOLOGY OF THE GALL BLADDER AND THE DUCTAL SYSTEM

In several instances, an opportunity was afforded for the study of fluid aspirated from the gall bladder, biliary ducts and common duct at the time of operation. Non-malignant and malignant cells aspirated from the common duct are illustrated in D V, 1–6. The cells illustrated in D V, 1 are interpreted as non-malignant, as are the columnar cells in No. 2, although they were found in a case of adenocarcinoma of the common duct. Cells with obvious malignant characteristics from the latter case are shown in D V, 3. The cells illustrated in D V, 4, 5 and 6 are more difficult to interpret. They are relatively large and were at first considered suspicious for malignancy. However, no such evidence was obtained at operation, and a lymph node biopsy was diagnosed as chronic lymphadenitis. The extensive intercellular leucocytic infiltration of the larger cluster is consistent with the diagnosis of a chronic inflammatory condition.

D V, 7 and 8, show malignant cells found in bile aspirated from the gall bladder in a case diagnosed as carcinoma of the gall bladder with metastases. Cells from another case exhibiting similar cytologic characteristics are shown in D II, 20. They were recovered in a gall bladder aspirate from a case diagnosed as adenocarcinoma presumably arising in the bile ducts. The adenomatous type of the tumor in both cases is apparent in some of the illustrated clusters. Cells found in fluid aspirated from the biliary duct of a patient with cholangiocarcinoma are depicted in D IV, 19. In this case there is a possibility that the tumor was metastatic from a carcinoma of the rectum resected 6 1/2 years earlier.

CYTOLOGY OF THE RECTUM AND SIGMOID COLON

The use of the cytologic method in the diagnosis of malignant neoplasms of the rectum and sigmoid colon has received great impetus in the past few years through the development of improved technical procedures for obtaining adequate material.[9, 22] The techniques now in use are described in Chapter II, pages 10 and 11.

Non-malignant cytology

The large intestine is lined with a simple epithelium consisting of columnar cells with a striated cuticular border and interspersed goblet cells. The surface epithelium is continuous with that of the crypts of Lieberkühn,

where the columnar cells are lower and the goblet cells much more numerous.

The surface epithelium undergoes extensive desquamation. However, only a few of the cells found in rectal and colonic washings are well preserved and retain their columnar form and their normal appearance. The majority show marked degeneration and necrotic changes.

The cytology of rectal and colonic washings is relatively simple because of the structural uniformity of the epithelial lining of the large intestine and the absence of contaminating cells from other organs. Undigested foodstuffs and other fecal matter are generally eliminated by the proper preparation of the patient. Normal exfoliated cells show a limited variability in size and form, a fact which facilitates the recognition of cells derived from pathologic lesions. A cytologic feature which helps to distinguish between rectal and colonic washings is the more frequent presence of taller columnar cells in smears prepared from colonic specimens.

Since the large majority of the desquamated epithelial cells of the large intestine show degenerative changes, the finding of well-preserved clusters and epithelial fragments consisting of columnar cells may justly arouse a suspicion of a benign polypoid growth (D I, 17). There is good evidence that benign polyps exfoliate copiously, as proved by the large fragments of polypoid tissue, including the stomata of glands, which are often seen in smears from such cases. However, the differentiation of these polypoid fragments from large sheets of epithelium occasionally found in other benign pathologic conditions requires much experience.

Cells found in a colonic washing from a case of adenoma of the sigmoid are illustrated in D V, 13. Their type shows a close resemblance to that of the goblet cells.

Malignant cytology

Malignant cells from adenocarcinomas of the colon or rectum can be detected and identified with a high degree of accuracy because of their striking contrast to the relatively uniform exfoliated cells of the normal intestinal mucosa and those of the benign polyps. The malignant cells are as a rule larger and show marked nuclear abnormalities characteristic of malignancy. The clusters are well defined and exhibit considerable crowding, irregularity of pattern and loss of polarity. The cytoplasm is usually poorly preserved and the intercellular boundaries are indistinct (D I, 20, 22, 23, and D V, 14).

In some cases the malignant cells retain their columnar form (D I, 21 and D V, 12). In D I, 21, from a case of carcinoma of the sigmoid, the cells are relatively small and the nuclear changes are not very pronounced, leaving some doubt as to whether these cell groups are actually malignant or simply atypical. These cells were found in swab smears taken from the area of the lesion. The cells in D V, 12 show unmistakably malignant features.

One of the changes characteristic of the adenocarcinomas of the large intestine is the elongation of the nuclei, which appears at a relatively early stage and constitutes one of the best criteria of early malignancy of this region. Enlargement, elongation and some hyperchromasia of the nuclei are among the first changes by which the presence of a malignant neoplasm can be recognized, as seen in D V, 15, in which non-malignant and malignant cells from a very early adenocarcinoma of the descending colon are shown side by side.

The elongation of malignant cells and of their hyperchromatic nuclei is often extreme. Some of these cells acquire forms resembling the form of the "snake" cells of the advanced epidermoid carcinomas of the cervix. Being somewhat smaller and more slender, they may be appropriately designated as "needle" cells. When in clusters, they usually display an irregular pattern.

Carcinomas of the anus are better detected by direct smears, although malignant cells may also be recovered in rectal washings. The cells are of the epidermoid type.

CHAPTER

VIII

Pleural, Peritoneal and Pericardial Exudates

THE EXAMINATION of fluid aspirated from the pleural, peritoneal and pericardial cavities has long been practiced by pathologists, though less by means of smear preparations than by sections of the cellular sediment. The descriptions given in this chapter are based exclusively on the examination of sediment smears prepared and stained in accordance with the principles used in the other applications. The techniques are given on page 11.

NON-MALIGNANT CYTOLOGY

The pleural, peritoneal and pericardial cavities are lined with a simple squamous epithelium known as mesothelium. Fluid aspirated from these cavities contains chiefly mesothelial cells and a varying number of histiocytes, leucocytes and erythrocytes. It should be borne in mind that since an accumulation of fluid in these cavities is a reflection of some pathologic process, there is a lack of adequate material for the study of the strictly normal exfoliative cytology and the extent of the exfoliative process in these cavities.

Desquamated mesothelial cells tend to lose their polyhedral form and become rounded (E I, 4, 6, 8–9, 10–12; and E II, 1 and 2). Their cytoplasm is rather compact and stains light pink with our standard Procedure No. 267; although with other staining procedures it appears to be predominantly basophilic. It is finely vacuolated and often shows a light ectoplasmic rim which may be an artifact due to shrinking (E I, 4 and 9). The nucleus is comparatively large and vesicular with a fine chromatin network and one or more small, relatively inconspicuous nucleoli. It is usually centrally located, although an eccentric position is not infrequent. Binucleation may be seen occasionally but multinucleation is a rare occurrence. Mitoses are more frequently noted in exudate smears (E I, 7 and 11) than in other smear types.

Normally, there is a relatively limited variation in the size and form of the mesothelial cells and of their nuclei. However, in cases of chronic infections and other non-malignant conditions, such as cirrhosis of the liver, enlargement of the cells and the nuclei beyond normal limits, nuclear hyperchromasia and, in clusters, anisocytosis, anisokaryosis, engulfment and other atypical features may be observed (E I, 7, 9, 12 and 17).

The histiocytes show a much greater variability in size and form than the mesothelial cells, ranging from small cells of approximately the size of a monocyte to giant multinucleated forms (E I, 1–3). Their foamy and lighter-staining cytoplasm and its less distinct outline, their looser grouping pattern and the type and form of their nuclei aid in their differentiation from the mesothelial cells. However, these distinctive traits are sometimes absent, particularly in the more atypical forms, the identification of which may be very difficult or even impossible. Histiocytes in mitosis are found in varying numbers in smears, indicating that they actively proliferate within exudates (E I, 3; H I, 1–3).

A cell form which is rather frequently seen in exudates is the so-called signet ring cell, the nucleus and cytoplasm of which are arranged in the form of a ring around a large central vacuole (E I, 5, 13 and 15). Cells of this type

are seen quite frequently in cases of cirrhosis of the liver. There is good evidence that histiocytes may assume this form, but the possibility that mesothelial cells may also show this extreme type of vacuolation cannot be ruled out. A signet ring cell in mitosis is shown in H II, 12.

Histiocytes and leucocytes, particularly polymorphonuclears, are usually numerous in cases of inflammatory processes. The relative predominance of lymphocytes carries some suspicion of a malignant neoplasm (E I, 18), although an excess of lymphocytes may also be present in tuberculosis and in other infections. Active phagocytosis of leucocytes by histiocytes may be observed during the resolving stage of an inflammatory process.

Erythrocytes are generally present in exudate specimens as the result of trauma and, therefore, are of little diagnostic significance. The presence of old fibrinated blood or of histiocytes which contain phagocytosed erythrocytes or blood pigment (heart failure cells) is suggestive of a pathologic condition accompanied by extravasation of blood.

MALIGNANT CYTOLOGY

All malignant tumors of pleural, peritoneal or pericardial lining membranes with the exception of mesothelioma are of metastatic origin. The fact that exfoliated malignant cells remain viable for a long time and proliferate actively within the fluid of these cavities may account for their presence in unusually large numbers as well as for the relative frequency of mitotic figures in exudate smears from some malignant cases. The finding of distinctly abnormal mitotic figures in addition to other criteria helps in establishing a diagnosis of malignancy (H I, 5-8, 10, 12, 14, 19-22; and H II, 1, 3-8, 15).

In most instances the diagnosis of primary and metastatic malignant tumors of the pleural, peritoneal and pericardial membranes is based on general criteria which are discussed in Chapter III. Only in cases in which the original cell type of the tumor is retained may one have a clue as to its primary site. Tumors metastatic from bronchogenic carcinomas of the lung, cystadenocarcinomas of the ovary and adenocarcinomas of the breast and gastrointestinal tract are more likely to reveal their cytologic type. Special stains for mucin may help in the diagnosis by demonstrating the mucoid type of the cells.[24] Melanotic melanomas can be recognized by their characteristic melanin inclusions. Neoplasms of lymphoid origin also have a distinctive cytology. The differentiation of mesotheliomas (E I, 20) from other malignant neoplasms, particularly bronchogenic carcinomas, is not always possible, although their malignant character is usually apparent.

A more detailed discussion of the various types of metastatic tumors found in exudates and a comparison of their cytologic features are given in the Discussions of plates E I and E II.

Aside from its role in the diagnosis of malignancy, the cytologic examination of exudate specimens is of particular value in the study of the morphologic effects of various therapeutic agents or modes of treatment. The occurrence of degenerative and necrotic cytologic changes and the ensuing numerical decrease or even ultimate disappearance of the malignant cells can be followed by means of repeated smear examinations and give a fairly good indication of the effectiveness of the treatment. The reaction of other cells, such as histiocytes and leucocytes, may also offer additional information.

CHAPTER

IX

Breast

THE STUDY of the exfoliative cytology of the breast in both normal and pathologic conditions has been rather limited, mainly because of the relatively infrequent occurrence of spontaneous secretion. Our knowledge is therefore incomplete on a number of important points such as the extent of normal exfoliation, the morphologic characteristics and range of variability of the exfoliated elements and the possible presence of cyclic changes. Secretion occurs more frequently in chronic infections, duct papillomas or carcinomas and other pathologic conditions, but even in these conditions the cases with spontaneous discharge are not sufficiently numerous to permit an extensive use of this diagnostic method.

Various techniques for inducing discharge have been tried and have met with partial success but have not as yet been developed to a point to be considered practical and generally acceptable. A description of the techniques for obtaining specimens and preparing and staining the smears is given on page 11.

NORMAL CYTOLOGY

The mammary gland is a compound gland and consists of a number of independent units each having its separate duct system. The larger excretory ducts become dilated under the areola, forming what is known as the sinus lactiferous, and terminate at the summit of the nipple by independent openings.

The gland reaches its highest degree of development during pregnancy and lactation. In the resting stage its alveolar components are not fully developed and it is the duct system which constitutes the bulk of the gland.

The alveoli are lined with a simple epithelium consisting of cuboidal or low columnar cells, the size and form of which depend upon the degree of secretory activity. The ducts also are lined with simple cuboidal or low columnar epithelium which becomes pseudostratified in the main lactiferous duct, and stratified squamous toward the nipple.

The normal exfoliative cytology of the breast includes a limited number of cell types and is thus relatively simple. Most of the desquamated cells are of ductal origin and are derived chiefly from the epithelium of the larger ducts.

Exfoliation appears to be very scant in normal cases, and in many instances the smears are practically acellular or contain only a few squamous cells from the region of the nipple.

The cells found in normal breast secretion smears vary in size and form. Some are small and appear singly or in dense and compact clusters. Their nuclei are relatively large and often wrinkled, possibly because of shrinkage at fixation, although cells with well-preserved nuclei are also seen. The cytoplasm usually shows distinct vacuolation.

In the larger cells vacuolation is more pronounced, giving to the cytoplasm a foamy appearance. The nuclei are often eccentrically located and are only slightly larger than those of the smaller cells. Bi- or multinucleation is not infrequent. The large cells also appear in clusters, some of which exhibit marked varia-

tion in the size of the cells (F I, 1; F II, 1).

The origin and nature of both large and small cells are still debated. The smaller cells appearing in compact clusters are apparently desquamated ductal cells, but most of the larger cells have a striking similarity to histiocytes found in fluids from other organs. The foamy cytoplasm, the eccentricity and occasional kidney-shape of the nucleus, the ingestion of blood (F I, 3), the loose grouping pattern, and the diversity in size of the cells within a group are characteristics which these cells have in common with histiocytes (compare F II, 1, and G I, 2). Furthermore the fact that they are much more numerous in cases of chronic inflammatory conditions, as in mastitis, constitutes additional evidence in favor of their histiocytic nature.

On the other hand the demonstration of a lipoid substance within the vacuoles of the large cells has led certain investigators to attribute to them a secretory function.[12] It has also been claimed that this secretory activity follows a cyclic pattern. It is, of course, possible that here we are dealing with two types of cells which, though morphologically indistinguishable, perform different functions, one secretory and the other phagocytic. If this proves to be true, then there is the possibility that the small undifferentiated cells have the potentiality to differentiate into two functionally different types, one secretory and the other phagocytic, as is apparently the case in the endometrium (see page 26). Factual support of this interpretation is not yet available. However, there are good reasons to consider the large foamy cells as potentially phagocytic elements which are structurally similar to the histiocytes found in other body fluids.

ATYPICAL (NON-MALIGNANT) CYTOLOGY

Certain benign pathologic conditions such as papillomas, cysts and chronic mastitis may be recognized by clean-cut cytologic smear patterns. In papillomas there is usually an exfoliation of well-organized papillary fragments in addition to the single cells and small clusters. The individual cells show enlargement, metaplasia, engulfment, and vacuolation which is often extreme. The nuclei do not display, as a rule, marked deviations from their normal size and structure (F I, 6–7). However, there are cases in which structural atypia and nuclear enlargement and hyperchromasia are noted, arousing a suspicion of a malignant transformation (F II, 3–6). In other instances the cells are small and less differentiated, as in F II, 2.

In benign breast cysts an adequate specimen for cytologic study can be obtained by needle aspiration. Such specimens may contain clusters of cells (F I, 4; F II, 7–10) which may be cyst lining cells or fragments of papillary projections, as are often seen in ductal cysts. The larger fragments have a lobulated pattern and the cell borders are rounded, and some have a cuticular rim (F II, 7–9). The cytoplasm shows a fine, uniform vacuolation. Many of the cells of this type are acidophilic, though basophilic cells are often intermixed with the acidophilic ones (F II, 10). Some of the cells, particularly those which are strongly acidophilic, appear to contain secretory granules. The nuclei of well-preserved cells are spherical (F II, 7 and 9) with some variation in size. Occasionally binucleation may be observed (F II, 9). In some clusters exhibiting signs of degeneration the nuclei are pyknotic or faded (F II, 10).

In chronic mastitis there is generally a marked increase in the histiocytic elements. Leucocytes are present in varying numbers. In some cases there is extravasation of blood which is phagocytosed by the histiocytic elements. In acute infections there is a profusion of polymorphonuclears, while the histiocytes are less numerous. Lymphocytes are frequently seen scattered sparsely throughout the smear but rarely acquire much prominence.

MALIGNANT CYTOLOGY

The malignant neoplasms of the breast which are most likely to be detected by the

cytologic method are primary adenocarcinomas developing within the larger ducts.

Exfoliation apparently begins very early in the carcinomas of the breast, as proved by several cases in which numerous malignant cells were found in smears prior to the appearance of clinical signs other than nipple discharge. None of these cases had a palpable mass, and subsequent operations revealed the presence of early infiltrating carcinoma without evidence of lymph node involvement. The preoperative diagnosis of malignancy in these cases was based exclusively on the cytologic findings. Cells recovered in breast secretion smears from five cases of this category are illustrated in F I, 8, 9, 10 and 11; F II, 11–16 and 28. The clusters in F I, 8, 9 and 10, and F II, 28 exhibit a pattern resembling that seen in papillomas. In the group of malignant cells shown in F II, 11–16, the nuclear changes are more pronounced and similar to those seen in the advanced cases.

The recognition of malignant cells in breast smears is based chiefly on nuclear criteria such as enlargement, hyperchromasia, irregularity in outline, and prominence of nucleoli. These changes are more distinctly expressed in the advanced cases. Additional criteria such as irregularity of pattern, crowding and loss of individuality of the cells are afforded by the large clusters (F I, 12–16, and F II, 20–25).

A relatively frequent feature in the carcinomas of the breast is the cupping of one cell around another, giving the impression of phagocytic activity, although it is probable that the engulfment is due to pressure (F I, 11, 16 and F II, 14, 16, 26 and 27). The same picture may be seen in pleural or peritoneal exudates in cases of metastatic carcinoma of the breast (E II, 13). This feature should not be considered as pathognomonic for carcinoma of the breast, as it may also be seen in other carcinomas as well as in non-malignant cell types.

Of the cytoplasmic changes, one may mention vacuolation which is often extreme (F I, 10 and F II, 17, 19). This also occurs frequently in papillomas.

In most cases malignant cells appear in clusters, although in some instances only single cells may be found. The single cells may be well preserved (F II, 12, 15 and 24), or they may be cytolized, with only scattered, stripped nuclei appearing in the smear (F II, 25). The abnormal structure and form, larger size and hyperchromasia of these nuclei permit their identification as malignant. The usual absence of stripped nuclei from normal smears facilitates their detection and correct evaluation.

The presence of necrotic cells in breast secretion smears is another manifestation of malignancy. Such cells may be numerous in some cases, whereas in others they are entirely absent.

Mitotic figures may be seen in breast smears from malignant cases but not so frequently as in clusters of metastatic adenocarcinomas of the breast observed in smears prepared from exudates.

Cells found in a scraping of an ulcerated lesion diagnosed as carcinoma of the breast associated with Paget's disease are shown in F II, 26 and 27. They are cells from the deeper layers characteristic of carcinoma, but no cells were present which would make possible a cytologic diagnosis of Paget's disease. This latter condition may be diagnosed cytologically through the finding of superficial keratinized cells with abnormal nuclei. The presence of keratinization without nuclear abnormality is seen in leucoplakia.

Malignant changes in breast cysts may be detected by the cytologic examination of fluid aspirated from the cyst. Smears from needle biopsies of solid tumors prepared and stained by our techniques also provide adequate material for a cytologic examination, but it is felt that special training and experience in pathology are necessary for the interpretation of such material.

Plates

To assist the reader in making a more profitable use of this atlas, some comment on the selection of the illustrations and their organization into plates is in order. The plan of arrangement as originally conceived provided for groups or series of plates based on various smear types, with separate plates for non-malignant and malignant cell forms within each series.

Such an arrangement was deemed desirable and would have been possible if all the material from which representative cells and cell clusters were to be chosen for illustration had been at hand at the initial stage of the work. This, however, was not the case. The selection and illustration of representative cytologic types were conducted gradually over a period of many years, during which time exfoliative cytology continued to advance steadily and to expand into new applications, made possible through the introduction of improved technical procedures.

In order to cover the new material which was being accumulated, the original plan for twenty-four plates of drawings was expanded to include twelve plates of photomicrographs. This called for an addition of new plates in each of the old series. In the digestive system (Series D), for example, the number of plates was increased from two, as originally planned, to five. This was considered necessary because of new and important advances, chiefly the gastric balloon technique and newer procedures for obtaining rectal and colonic washings, which made possible the procurement of more satisfactory and better-preserved material. As the new photomicrographic plates were added after many of the original drawing plates had been engraved, some repetition was unavoidable if the better material available was to be illustrated.

On the other hand, the segregation of the non-malignant from the malignant cells in the new plates was found to be impracticable because of space limitations. Exceptions to the segregation plan were also made for the sake of a close comparison of normal and malignant cell types, as in the plates concerning dyskaryosis (A IV and A V), in which normal and corresponding malignant cells were illustrated side by side.

Series A through F deal with the exfoliative cytology of various body systems. Series G (Miscellaneous) includes four plates, each of which is composed either of groups of cells found in various smears under many different forms, such as histiocytes (G I) and multinucleated cells (G IV), or of specific cell types found in particular conditions such as pregnancy (G II) or after irradiation (G III). Series H is devoted to the illustration of mitosis as seen in smears prepared from various body fluids. These special subjects were treated on separate plates in order to provide a more comparative picture of their cytology.

SERIES PLATE

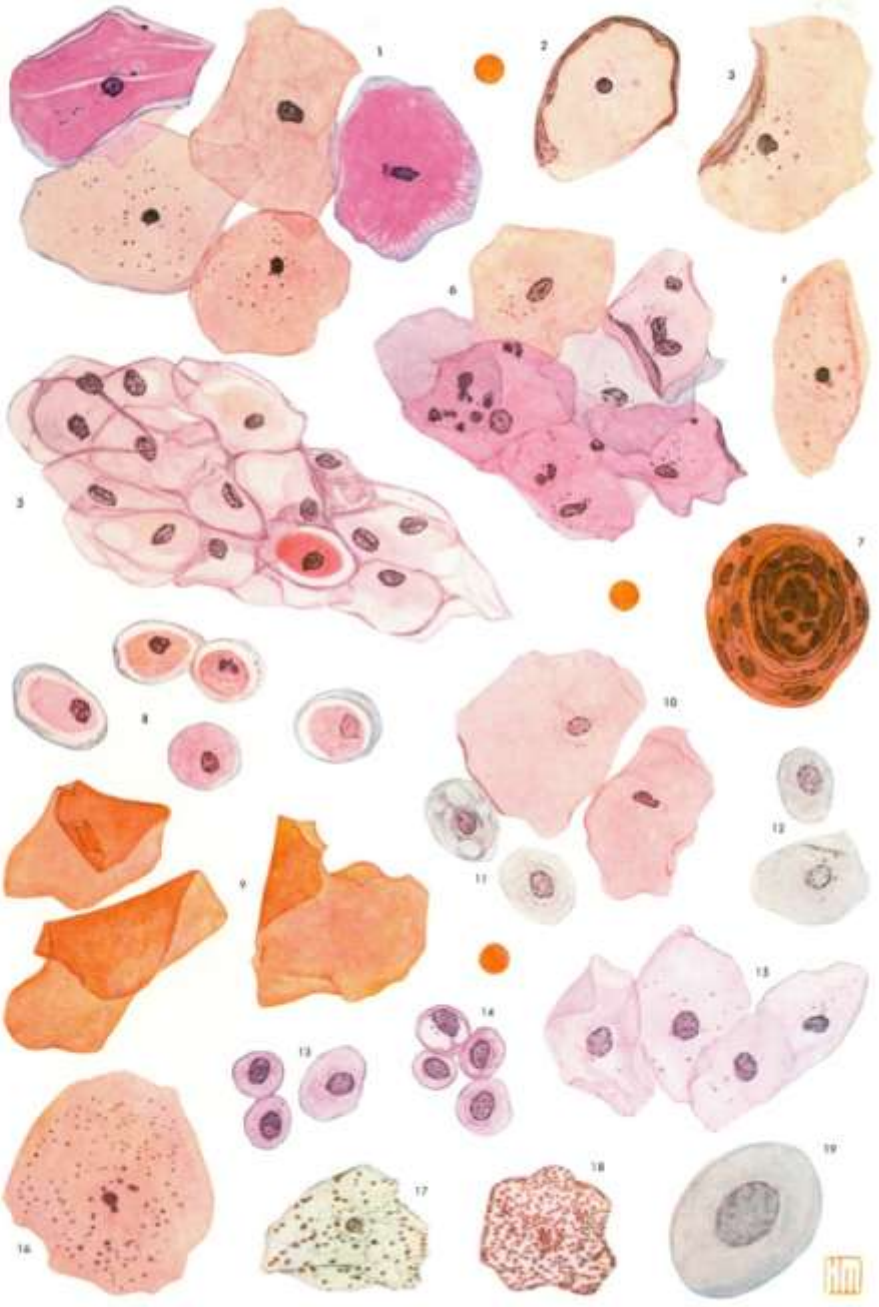
A I

Female Genital System

Non-malignant squamous epithelial cells found in vaginal and cervical aspiration or swab smears from normal women

A1

FEMALE GENITAL SYSTEM



A I
DESCRIPTION

Non-malignant squamous epithelial cells found in vaginal and cervical aspiration or swab smears from normal women. Drawings x 525 except No. 19, which is x 1050.

1 and 2. Superficial squamous cells, late follicular (preovulatory) stage. Vaginal smears. Glycogen series.* Ages 36 and 23 respectively.

3 and 4. Superficial squamous cells, early luteal (postovulatory) stage. Vaginal smear. OG–EA series. Age 45.

5. Cells of the intermediate or navicular type. Vaginal smear. Glycogen series. Age 50.

6. Superficial squamous cells, early luteal stage. Vaginal smear. Glycogen series. Age 43.

7. Epithelial pearl. Case negative for malignancy. Vaginal smear. OG–EA series. Age 29.

8. Cervical parabasal cells filled with glycogen. Postmenopausal patient receiving estrogen therapy. Cervical smear. Glycogen series. Age 52.

9. Superficial squamous cells showing complete keratinization. Vaginal smear. Glycogen series. Age 48.

10–12. Parabasal and cornified superficial squamous cells from a woman in early menopause. Vaginal smear. Glycogen series. Age 43.

13–15. Parabasal (Nos. 13 and 14) and superficial squamous (No. 15) cells. Three years after menopause. Note relatively large nuclei of the superficial cells. Vaginal smear. Glycogen series. Age 49.

16. Acidophilic superficial squamous cell showing numerous chromatin granules. Early menopausal changes with irregular bleeding. Vaginal smear. Eosin–Water blue series. Age 49.

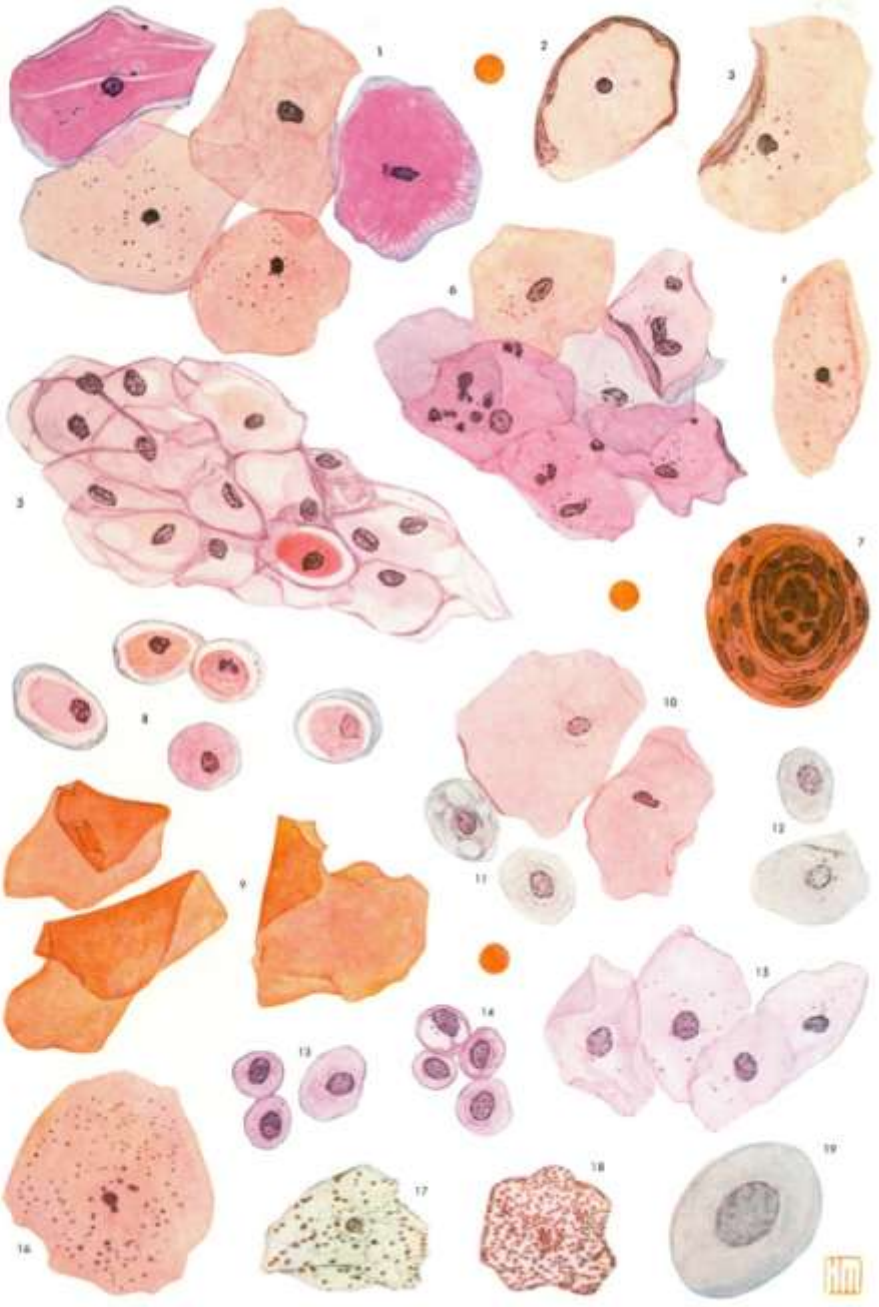
17 and 18. Superficial squamous cells, basophilic (No. 17) and acidophilic (No. 18), containing blood pigment granules. Seventh day of the menstrual period. Vaginal smear. Glycogen series. Age 43. (Compare with A I, 16 and G II, 6 and 7.)

19. Cervical parabasal cell (x 1050). Vaginal smear. OG–EA series.

*The stains referred to as "glycogen series" are still in the experimental stage and will be published at a later date.

A1

FEMALE GENITAL SYSTEM



A I
DISCUSSION

Non-malignant squamous epithelial cells found in vaginal and cervical aspiration or swab smears from normal women

Four types of cells may be recognized in the squamous epithelium of the vagina and the ectocervix: the basal, parabasal, intermediate or navicular and superficial.[37] Only the last three varieties are shown in this plate. The basal cells may be seen in smears only when fragments of epithelium including the basal zone are exfoliated.

No. 1 illustrates a group of cells characteristic of the late follicular stage of the normal menstrual cycle. Three cells show varying degrees of cornification, whereas two others are filled with glycogen, as indicated by their fuchsin color. All cells have pyknotic nuclei, and three contain chromatin granules which apparently emanate from the nucleus. The cells of the follicular phase tend to be flat and discrete.

The characteristic curling which is often seen near the peak of the late follicular stage, prior to or shortly after ovulation, is shown in Nos. 2 and 3. Following ovulation the cytoplasmic granules often show acidophilia and fading (No. 4).

No. 5 illustrates a cluster of navicular cells. Cells of this type are as a rule filled with glycogen, as shown in one cell of this group. Their nuclei have an oval or elongated form and are often folded and/or eccentrically located.

A cluster of cells representative of the luteal phase is shown in No. 6. The cells are less discrete and more densely grouped and have larger nuclei than those of the follicular phase.

Epithelial pearls are not infrequent in vaginal smears, more particularly in smears showing high cornification. The pearl illustrated in No. 7 consists of normal cells and does not indicate a malignant condition. Pearls with abnormal cells and nuclei may also be seen in certain pathologic conditions, as in superficial cell dyskaryosis associated with a high estrogenic level.

The cells illustrated in No. 8 are large parabasal cells of ectocervical origin that are loaded with glycogen. They are characteristic of a high cervical epithelium of the glycogenic type. Hormones stimulating epithelial growth in the cervix, such as estrogens, may cause the appearance of such cells in the smears. It is of interest that similar cells may also be seen in prostatic secretion, most frequently following hormonal therapy (see B I, 7).

Complete keratinization of the superficial squamous cells, as indicated by orangeophilia and total resorption of the nucleus, is often seen in vaginal smears (No. 9). Such keratinized cells are generally of ectocervical origin. When they occur in large numbers, they may be interpreted as suggestive of keratosis or leukoplakia.

After menopause the cytologic pattern varies greatly. Its distinctive type depends largely upon the prevailing hormonal status. The most characteristic cells found in postmenopausal smears, particularly in those of the atrophic type, are round or oval parabasal cells with a relatively large nucleus (Nos. 11–14). They are often vacuolated (Nos. 11 and 14) and are as a rule poor in glycogen (compare with No. 8), a fact which indicates a rather low estrogenic level. In general, postmenopausal cells are basophilic and are smaller and stain lighter than cells from younger women. Cytoplasmic granules may be seen (No. 15) but are rarely prominent. When the estrogenic level is high, one may find in the smears cornified cells intermixed with the parabasal (No. 10). However, these cornified cells differ somewhat in size and appearance from those found during the follicular phase of the normal menstrual cycle (compare with No. 1).

Superficial squamous cells sometimes contain an unusually large number of chromatin granules, some of which are in direct contact with the nucleus (No. 16). The granules may be seen in a variety of cases, and their signifi-

cance for diagnostic purposes is not clear.

Cells with hemosiderin granules (Nos. 17 and 18) may be seen occasionally in missed or incomplete abortions, in atypical bleeding, or even in the late stage of the normal menstrual period. The high refractiveness, yellowish tint and characteristic grouping of the granules help toward their identification.

A high magnification of a postmenopausal parabasal cell (No. 19) shows more clearly the pale and structureless appearance of cells of this type.

SERIES PLATE

A II

Female Genital System

Non-malignant epithelial cells found in vaginal and cervical aspiration or swab smears in normal and pathologic conditions

AII
DESCRIPTION

Non-malignant epithelial cells found in vaginal and cervical aspiration or swab smears in normal and pathologic conditions. Drawings x 525.

1. Cells with large, hypochromatic nuclei characteristic of a postmenopausal, senile state. Vaginal smear. OG–EA series. Age 68, 23 years after menopause.
2. Atypical cervical cells, showing nuclear hypertrophy and para- or perinuclear vacuolation. Vaginal smear. OG–EA series. Age 28. Chronic cervicitis.

3 and 4. Atypical cervical cells. Vaginal smear. OG–EA series. Age 54, 7 years after menopause. Cervical polyp, chronic cervicitis, Trichomonas infestation.

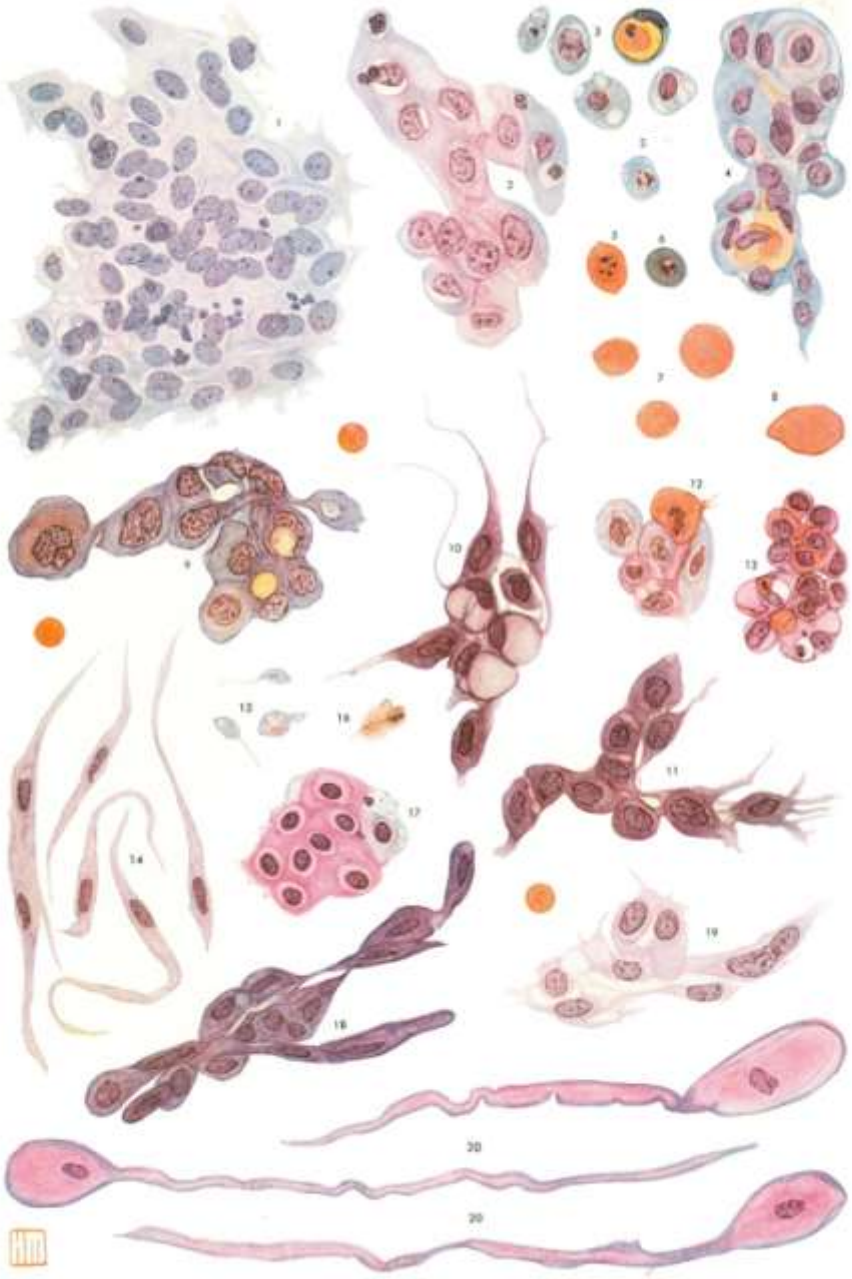
5 and 6. Cervical cells with nuclei disintegrating in the form of chromatin clumps (karyolysis). Vaginal smears. Glycogen series. No. 5 was found in a case of carcinoma of the cervix, age 50; No. 6, in a normal case, age 30.

7 and 8. Orangeophilic parabasal cells showing complete nuclear resorption. Vaginal smears. Glycogen series. Ages 59 and 41 respectively.

9. Cervical cells with atypical features such as vacuolation of the cytoplasm and hypertrophy of the nucleus. Vaginal smear. OG–EA series. Age 43. Chronic cervicitis, cervical erosion. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

10 and 11. Atypical non-malignant cervical cells of the spinous type showing hyperchromasia and large vacuoles. Vaginal smear. Eosin–Water blue series. Age 67. Pathologic diagnosis: adenoacanthoma of the uterus.

12. Atypical cervical cells. One cell is orangeophilic. Vaginal smear. Eosin–Water blue series. Age 28. Chronic cervicitis.
13. Cervical cells showing vacuolation and multinucleation. Cervical aspiration smear. OG–EA series. Age 62, menopause. Prolapsed uterus.
14. Cervical epithelial cells, with spindle-like form. Endocervical swab smear. Glycogen series. Age 38. Thirteenth day of the period. History of estrogenic therapy.
15. Trichomonads, one in the process of budding. Note nuclei and flagella. Vaginal smear. Eosin–Water blue series.
16. Trichomonad stained by the Bodian technique. Vaginal smear. Age 26.
17. Cells with distinct perinuclear vacuolation as often seen in Trichomonas infestation. Vaginal smear. Eosin–Water blue series. Age 44.
18. Cervical cells exhibiting stratification and some hyperchromasia. Vaginal smear. OG–EA series. Age 60. Prolapsed cervical stump after supravaginal hysterectomy.
19. Cervical cells of the spinous type showing variation in nuclear size. Vaginal smear. Eosin–Water blue series. Age 51. Cervical polyp.
20. Unusually large tadpole cells, apparently of ectocervical origin, containing glycogen. Cervical aspiration smear. Glycogen series. Age 41. Chronic cervicitis, Monilia, Nabothian cyst of cervix.



A II
DISCUSSION

Non-malignant epithelial cells found in vaginal and cervical aspiration or swab smears in normal and pathologic conditions

A large cluster of cells from a vaginal smear taken 23 years after menopause is shown in No. 1. The cellular borders are indistinct. The nuclei are relatively large and hypochromatic. They have an ovoid form and are grouped in an irregular, though characteristic, fashion. The cytolysis of such clusters results in the appearance of stripped nuclei, some of which tend to form aggregates as indicated in the center of No. 1. Aggregates of such stripped nuclei are found more frequently in endocervical smears of the advanced menopausal (senile) type.

Nos. 2–4, 9, 12, 13, 17 and 19 show clusters of atypical cells found in vaginal and cervical smears in non-malignant conditions such as chronic cervicitis, erosion, cervical or endocervical polyps, uterine prolapse and Trichomonas infestation. These clusters present various atypical features such as cellular and nuclear hypertrophy, vacuolation, elongation of cells and their nuclei, an irregular pattern, a lack of sharply defined cellular borders, multinucleation and engulfment. Such structural deviations have been found to be within the range of variability observed in chronic inflammatory and other non-malignant lesions.

Nos. 5 and 6 show karyolysis in two parabasal cells, the former from a case of cervical malignancy, the latter from a non-malignant case. Karyolysis occurs not only in malignancy but also in chronic inflammatory and other conditions and may be observed normally in smears from senile women.

Nos. 7 and 8 show orangeophilic parabasal cells in which the nuclei have been totally resorbed. Such cells found in vaginal smears do not indicate the presence of malignancy, whereas cells with a similar appearance in sputum or in bronchial washings ("ghost" cells; see C III, 14) are suggestive of a bronchogenic epidermoid carcinoma.

Atypical ectocervical cells of the spinous type showing elongation and pronounced vacuolation are illustrated in Nos. 10 and 11. They were observed in a vaginal smear of a woman with adenoacanthoma of the uterus associated with chronic cervicitis. In a previous monograph they were interpreted as adenoacanthoma cells,[38] but we are now inclined to interpret them as atypical cervical cells from the stratum spinosum of the cervix.

Squamous cells with a spindle-like form are illustrated in No. 14. The normal structure of their nuclei precludes their interpretation as malignant epidermoid cells (compare with A VII, 6, 14, 15 and 18). In this case there is a history of estrogenic therapy which might have been responsible for this atypia.

Nos. 15 and 16 illustrate four trichomonads. One is in the process of budding, whereas the other three show some of the flagella, particularly No. 16, which has been stained with the Bodian technique. The small elliptic nucleus is very characteristic and constitutes the best criterion for the identification of trichomonads in smears stained with our standard procedures.

The perinuclear vacuolation which is frequently observed in exfoliated vaginal and cervical cells in cases of Trichomonas infestation is shown in No. 17.

The cluster of atypical cervical cells shown in No. 18 was found in the vaginal smear of a woman with a prolapsed cervical stump. The smear was overstained with hematoxylin; this may account for the apparent hyperchromasia. The cells are interpreted as normal on the basis of their nuclear structure.

No. 20 illustrates three unusually long glycogenic cervical cells of the tadpole type (compare with A I, 8). Despite their atypical form the benign character of these cells is evident (compare with A IV, 2).

SERIES PLATE

A III

Female Genital System

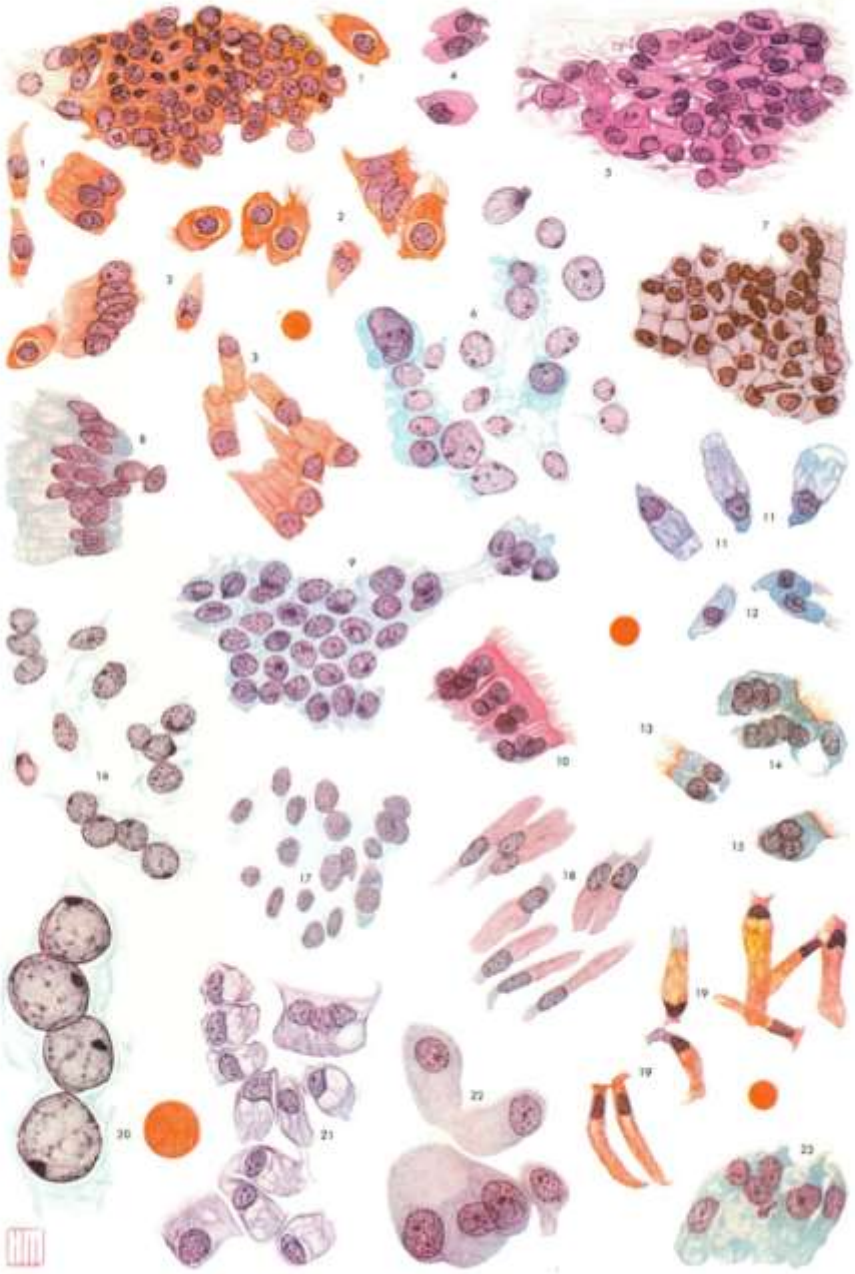
Non-malignant endocervical cells found in cervical and endocervical smears

AIII
DESCRIPTION

Non-malignant endocervical cells found in cervical and endocervical smears. Drawings x 525 with the exception of No. 20, which is x 1050.

1. Endocervical ciliated and mucoid cells illustrated as they appear singly or in cluster formation. Cervical aspiration smear. OG–EA series. The detached cells show their columnar form.
2. Endocervical ciliated and mucoid cells. Cervical aspiration smear. OG–EA series. Age 41.
3. Endocervical mucoid cells. Cervical aspiration smear. OG–EA series. Age 41.
4 and 5. Endocervical mucoid cells, stained for mucin. Cervical aspiration smear. Special mucin stain.
6. Endocervical cells showing anisokaryosis and stripped nuclei resulting from cytolysis. Cervical aspiration smear. Glycogen series. Age 46. One of the nuclei shows polarization and protrusion of chromatin.
7. Endocervical mucoid cells viewed from the basal surface of the epithelium. Cervical aspiration smear. Eosin–Water blue series.
8. Side view of endocervical mucoid cells showing their columnar form. Cervical aspiration smear. Glycogen series.
9. Endocervical cells viewed from the distal (superficial) surface, showing many nuclei with chromatin protrusions. Cervical aspiration smear. Glycogen series. Peak of follicular activity.
10. Fragment of ciliated epithelium showing multinucleation. Cervical aspiration smear. Glycogen series. Age 51. Menopause.
11. Mucoid cells. Cervical aspiration smear. Glycogen series. Age 46.
12. Ciliated cells. (Same case as No. 11.)
13–15. Ciliated cells, some of which are multinucleated. Endocervical smear. OG–EA series. Age 34.
16. Stripped endocervical nuclei. Cervical aspiration smear. Glycogen series. Age 38. Advanced follicular stage.
17. Stripped endocervical nuclei and one ciliated cell. Cervical aspiration smear. OG–EA series. Age 52. Menopause. Note faded hypochromatic appearance.
18. Group of tall, columnar mucoid cells. Cervical aspiration smear. Glycogen series. Age 55. Cervical polyp.
19. Tall, columnar mucoid cells. Endocervical smear. OG–EA series. Age 34. Nabothian cyst of the cervix.
20. Four nuclei from No. 16. Magnification 1050 diameters to show detailed nuclear structure.
21. Atypical endocervical columnar cells, "stout" type, showing some hypertrophy and vacuolation. Cervical aspiration smear. Glycogen series. Age 49. Cervical erosion.
22. Hypertrophic endocervical cells. Endocervical smear. Glycogen series. Age 27. Normal pregnancy. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
23. Endocervical cells showing some hypertrophy. Cervical aspiration smear. OG–EA series. Age 19. Incomplete abortion.

A III
FEMALE GENITAL SYSTEM



A III
DISCUSSION

Non-malignant endocervical cells found in cervical and endocervical smears

Normal endocervical cells of the secretory (mucoid) and ciliated types are illustrated in Nos. 1–4, 5, 7, 8, 10–12 and 13–15. Cells in side view reveal their columnar form, which cannot be visualized in clusters viewed from their basal (proximal) or superficial (distal) surfaces. It has been observed that the cellular borders are thicker at the proximal than the distal end of the cells. This may be the reason that clusters presenting their basal surface to view show a honeycomb pattern as illustrated in Nos. 5 and 7. A few cells on the upper right free surface of No. 7 show the columnar type. The mucoid content of the secretory cells may be demonstrated by special staining as in Nos. 4 and 5.

Ciliated cells in endocervical smears are often seen intermixed with secretory cells. Ciliated cells may also be noted in sections of the endocervix[37] in both the lining epithelium and the glands, but not so frequently as in endocervical smears. This is probably because their demonstration is easier in smears where cells are preserved in toto than in sections.

Ciliated cells can be recognized as such not only by their cilia, when present, but also by the cuticular membrane, the basal corpuscles and the characteristic perinuclear vacuolation they often exhibit (No. 2). They have round or slightly oval nuclei contrasting with the more pronounced ovoid shape of the nuclei of the secretory cells. They are often multinucleated (Nos. 10, 14 and 15), a trait which cannot be well discerned in sections. It appears that multinucleation in exfoliated endocervical cells is more common after menopause (No. 10).

Multinucleation is not limited to the ciliated endocervical cells. It is frequently seen in ciliated cells exfoliated from the bronchial mucosa (C I). Multinucleation in itself, observed in well-differentiated cells with normal nuclei, is not considered a criterion of malignancy.

No. 6 illustrates an aggregation of endocervical cells some of which have undergone cytolysis resulting in the appearance of many stripped nuclei (see also Nos. 16, 17 and 20). Stripped endocervical nuclei are not uncommon and sometimes show marked variation in size (No. 6). This is frequently seen in association with a high estrogenic index near the peak of the follicular phase,[37] in hyperestrin states, in hyperplasias, or during estrogenic therapy. The structure of stripped nuclei is, as a rule, normal. The nucleoli are generally distinct and stand out in contrast to the relatively pale chromatin net (No. 20). It should be noted that in endocervical cells encountered in smears of advanced postmenopausal or senile women, the nucleus is often distinctly hypochromatic (No. 17).

A rather unusual feature seen in endocervical nuclei, chiefly of the ciliated cell type, is a protruding knob of chromatin in the distal pole of the nucleus. Such nuclei are shown in No. 9 and also in a few ciliated cells of Nos. 1 and 2 and in a few stripped nuclei of Nos. 6 and 16. They are most frequently observed in conditions in which there is some nuclear activation, as in regeneration following an erosion, or after hormone stimulation, particularly estrogenic; but their full significance is not clear. The presence of this change in well-preserved cells precludes its interpretation as a degenerative process. In some nuclei the strands of chromatin appear to converge and are continuous with the protruding knob (Nos. 6 and 9). A similar chromatin knob may be seen also in ciliated cells of other organs, such as those of the bronchial mucosa (C I, 5, 9 and 20) or of the nasopharynx, and more rarely in cells of the mucoid type.

In No. 18 a group of tall columnar cells containing mucin is illustrated. Though found in a case of cervical polyp, these cells are not necessarily diagnostic of this condition. The same remark applies to the very tall cells shown in No. 19, found in the case of a woman with a large Nabothian cyst which was ruptured during the examination. Very tall and

slender columnar cells are probably derived from endocervical glands and indicate a hyperactive and crowded epithelium with a possible formation of papillary projections or tufts.

No. 21 illustrates cells of a type repeatedly noted in cervical erosions. Such cells have a columnar form but are stouter and show cellular and nuclear hypertrophy and often vacuolation and multinucleation. The mucoid content of cells of this type may be demonstrated by special staining.

A group of hypertrophic endocervical cells from an endocervical smear of a pregnant woman is illustrated in No. 22. The cluster in No. 23, from a case of incomplete abortion, shows columnar endometrial cells exhibiting a lesser degree of hypertrophy. Pregnancy sometimes results in a marked enlargement of cells and their nuclei. This may be an underlying factor in the relatively frequent appearance of atypical cytologic and histologic pictures during pregnancy.

SERIES PLATE

A IV

Female Genital System

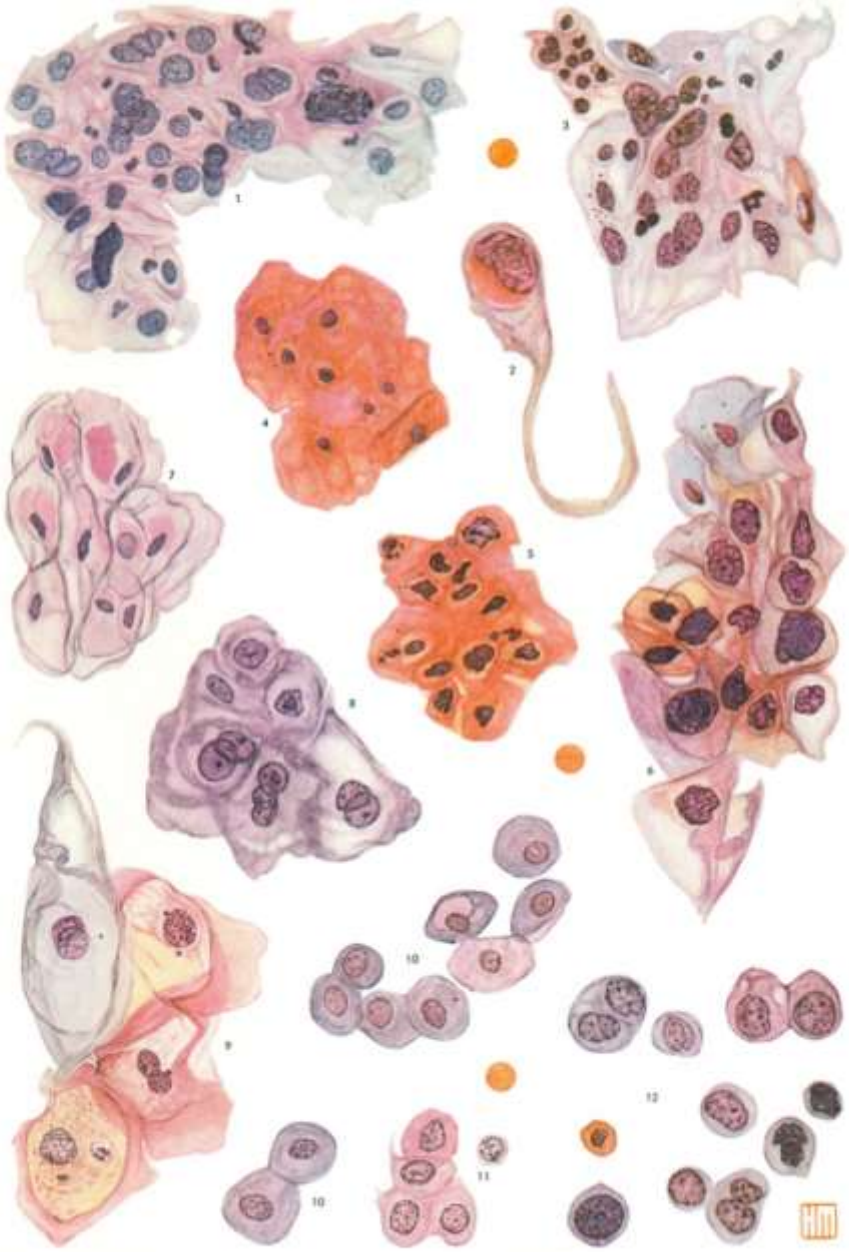
Normal epithelial cells and corresponding cells of the early malignant (dyskaryotic) type found in vaginal and cervical smears

AIV
DESCRIPTION

Normal epithelial cells and corresponding cells of the early malignant (dyskaryotic) type found in vaginal and cervical smears. Drawings x 525.

1. Superficial cell dyskaryosis. Vaginal smear. Eosin–Water blue series. Age 54. No biopsy was performed, and smears taken 10 years later showed no abnormal cells. This is considered a reversible case.
2. Tadpole cell with nuclear enlargement and binucleation. Cervical aspiration smear. Glycogen series. Age 48. Still menstruating. Pathologic diagnosis: intraepithelial carcinoma of the cervix. (Same case as A VII, 12.)
3. Cluster of superficial squamous cells showing dyskaryotic changes. (Same case as No. 2.)
4. Normal superficial squamous cells. Cervical aspiration smear. Glycogen series. Age 42. Sixteenth day of cycle.
5. Cells showing characteristics of superficial cell dyskaryosis (compare with No. 4). Cervical aspiration smear. OG–EA series. Age 47. Supracervical hysterectomy, 3 years prior to smear. Cervical stump removed following smear report. Pathologic diagnosis: epidermoid carcinoma in situ of cervix with downward growth along gland ducts. (Same case as A V, 6, 7 and 8.)
6. Superficial squamous cells exhibiting changes characteristic of dyskaryosis. Cervical smear. OG–EA series. Age 34. Pathologic diagnosis: preinvasive carcinoma of the cervix. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
7. Normal navicular cells, containing glycogen. Cervical aspiration smear. Glycogen series. Age 44. Fourteenth day of cycle.
8. Cells of the navicular type with characteristics of dyskaryosis (compare with No. 7). Vaginal smear. Glycogen series. Age 44. Biopsy negative.
9. Cluster of cells displaying dyskaryotic changes. Their form indicates a transition from the navicular to the superficial type. Note the extreme "cavitation." Cervical aspiration smear. OG–EA series. Age 35. Pathologic diagnosis: epidermoid carcinoma in situ. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
10. Normal cervical parabasal cells. Cervical aspiration smear. Glycogen series. Age 61. Surgical menopause.
11. Cells of the parabasal type showing nuclear activation and hypertrophy. Vaginal smear. OG–EA series. Age 30. Biopsy performed 5½ years after the smear was taken showed the presence of an intraepithelial carcinoma of the cervix.
12. Dyskaryotic cells of the parabasal type (compare with No. 10). Cervical aspiration smear. Glycogen series. Age 48. Pathologic diagnosis: intraepithelial carcinoma of the cervix.

AIV
FEMALE GENITAL SYSTEM



A IV
DISCUSSION

Normal epithelial cells and corresponding cells of the early malignant (dyskaryotic) type found in vaginal and cervical smears

Normal squamous cells of the superficial type are illustrated in No. 4, intermediate or navicular in No. 7 and parabasal in No. 10. Cells of the above three types exhibiting dyskaryotic changes are shown in Nos. 1, 2, 3, 5, 6, 8, 9 and 12.

These changes affect chiefly the nucleus and consist in enlargement, irregularity in form, hyperchromasia and bi- or multinucleation (page 30). In the superficial and navicular dyskaryotic cells a perinuclear "cavitation" is often present (Nos. 8 and 9). A very high nuclear-cytoplasmic ratio and sometimes an atypical vacuolation are characteristic of the dyskaryotic cells of the parabasal type (No. 12 and A V, 4, 6 and 8).

The cells shown in No. 11 were originally evaluated as suspicious but inconclusive. Since an intraepithelial carcinoma was found in a biopsy performed 5 1/2 years later, it is likely that these atypical parabasal cells represent early dyskaryotic changes.

It is not infrequent to find dyskaryotic cells of more than one type intermixed in smears from one case (page 30). No. 5 shows dyskaryotic cells of the superficial type. Dyskaryotic parabasal cells from the same case are illustrated in A V, 6, 7 and 8.

In some instances, particularly in cases of superficial cell dyskaryosis, a gradual disappearance of the abnormal cells, indicating a regressive course, has been noted in repeated smears. Two cases of this type are illustrated in Nos. 1 and 8. In the case of No. 1 no biopsy was performed. The abnormal cells disappeared and there was no recurrence over a period of 10 years. This case may be considered an example of spontaneous reversibility of a superficial cell dyskaryotic pattern.

In the case illustrated in No. 8, smears taken before the biopsy contained dyskaryotic cells of the superficial type, but in smears taken after a cervical biopsy no abnormal cells were present. The biopsy showed no evidence of carcinoma. However, in view of the fact that complete absence of abnormal cells has been repeatedly noted in smears taken after positive as well as negative biopsies, it is questionable whether such cases can be cited as conclusive proof of spontaneous regression. It is, of course, possible that in some of these cases the lesion was limited to a small intraepithelial area and was either removed by biopsy or subsequently desquamated in an accentuated exfoliative process caused by the biopsy procedure.

SERIES PLATE

A V

Female Genital System

Normal epithelial cells and cells of the early malignant (dyskaryotic) type found in cervical smears

AV
DESCRIPTION

Normal epithelial cells and cells of the early malignant (dyskaryotic) type found in cervical smears. Drawings x 525.

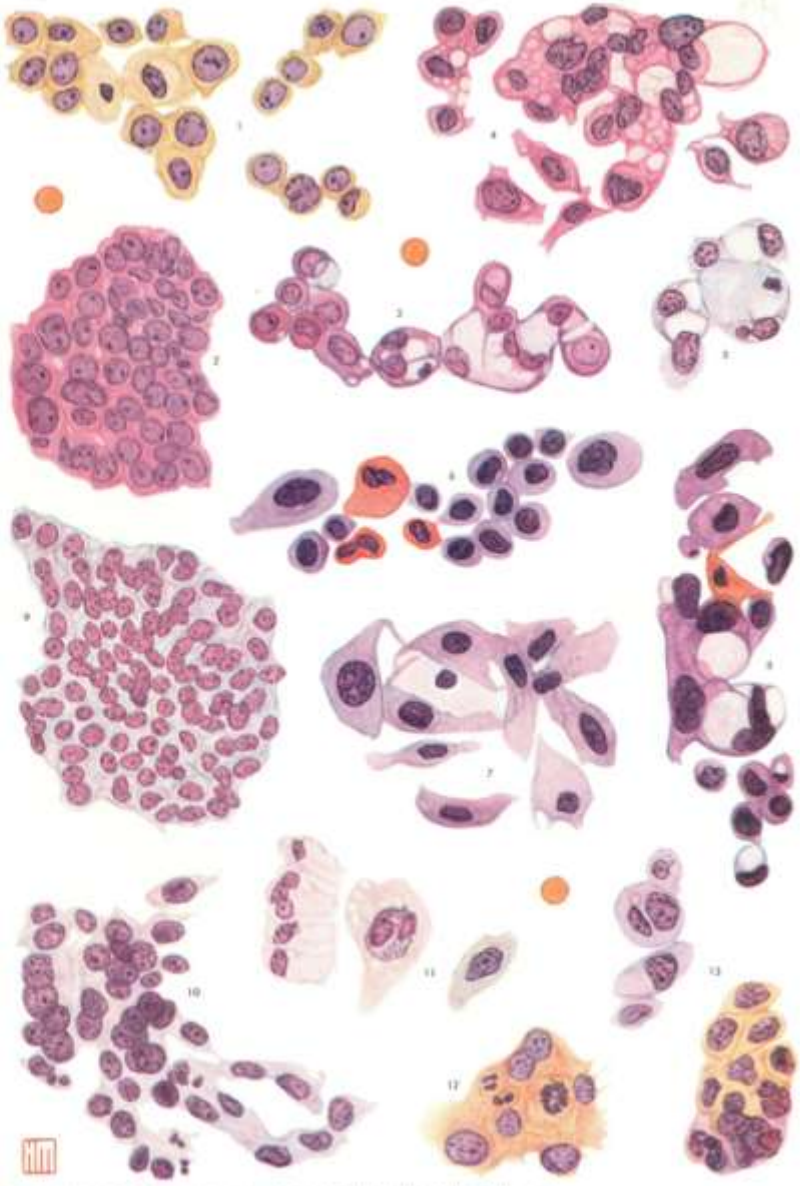
1. Parabasal cells showing nuclear enlargement and binucleation (compare with Nos. 6, 7 and 8). Cervical smear. OG–EA series. Age 45. Pathologic diagnosis: chronic cervicitis. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
2. Endocervical cells, showing some variation in the size of the nuclei. Cervical aspiration smear. Glycogen series. Age 48. Surgical menopause. Pathologic diagnosis: chronic cervicitis.
3. Cervical cells exhibiting pronounced vacuolation. Cervical aspiration smear. Glycogen series. Age 44. Pathologic diagnosis: chronic cystic cervicitis following cervical amputation.
4. Cervical cells showing nuclear changes characteristic of dyskaryosis and a highly vacuolated cytoplasm. Cervical aspiration smear. Glycogen series. Age 45. Pathologic diagnosis: intraepithelial epidermoid carcinoma of the cervix.
5. Dyskaryotic cells showing extreme vacuolation. Cervical aspiration smear. Glycogen series. Age 42. Biopsy—chronic cervicitis. Hysterectomy 14 months later. Pathologic diagnosis: intraepithelial squamous cell carcinoma of the cervix.

6–8. Dyskaryotic cells of the parabasal type. Some cells in No. 6 are acidophilic. Atypical vacuolation is shown in No. 8. Cervical aspiration smear. Glycogen series. Pathologic diagnosis: epidermoid carcinoma in situ of the cervix with downward growth along gland ducts. (Same case as A IV, 5.)

9. Cluster of normal endocervical cells. Cervical aspiration smear. Glycogen series. Age 48. Normal cervix. (Compare with Nos. 2 and 10.)
10. Endocervical cells showing dyskaryotic changes. Cervical smear. Glycogen series. Age 44. First biopsy negative; second, suspicious. A conization of the cervix 26 months after the first positive smear revealed a very early invasive epidermoid carcinoma of the cervix. (Same case as A XI, 6.)
11. One cluster of normal endocervical mucoid cells and two single atypical endocervical cells with nuclei showing early malignant changes. Cervical aspiration smear. Glycogen series. Age 57. Pathologic diagnosis: carcinoma in situ of the cervix.
12. Cluster of endocervical dyskaryotic cells. Note one mitotic figure. Cervical aspiration smear. OG–EA series. Age 67. Pathologic diagnosis: carcinoma in situ of the cervix. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
13. Cells showing changes characteristic of endocervical dyskaryosis. Cervical aspiration smear. OG–EA series. Age 35. Pathologic diagnosis: preinvasive squamous cell carcinoma of the cervix. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

AV

FEMALE GENITAL SYSTEM



A V
DISCUSSION

Normal epithelial cells and cells of the early malignant (dyskaryotic) type found in cervical smears

No. 1 shows nuclear enlargement and binucleation in non-malignant parabasal cells.

Marked vacuolation is illustrated in the cells of No. 3 (benign) and in Nos. 4, 5 and 8 (dyskaryotic). It is not the vacuolation alone, but its combination with nuclear atypia that reveals the malignant character of the cells.

A comparison of Nos. 2, 9 and 10 shows the difference in appearance between normal (No. 9), normal with hypertrophic nuclei (No. 2) and dyskaryotic (No. 10) endocervical cells.

The difference in the patterns of endocervical (No. 10) and parabasal (Nos. 6–8) cell dyskaryosis can be seen by comparison. The distinction between endocervical and parabasal dyskaryotic or even normal cells is at times difficult, as endocervical cells often exhibit metaplastic changes. In single cells the distinction is more difficult.

No. 11 illustrates, side by side, a cluster of normal endocervical columnar cells of the mucoid type and two slightly metaplastic endocervical dyskaryotic cells.

A mitosis within a cluster of endocervical dyskaryotic cells is shown in No. 12. It has no distinctly abnormal features and therefore should not be interpreted as giving evidence of malignancy by itself, but it indicates the presence of proliferative activity. Normal mitoses are seen in endocervical cells when there is regenerative activity as in healing erosions.

The cell clusters illustrated in Nos. 6–8 are from a case of mixed dyskaryosis (superficial and parabasal). Superficial dyskaryotic cells from the same case are shown in A IV, 5.

In two cases, Nos. 3 and 10, the biopsy performed when the first positive smears were taken was negative. The existence of an intraepithelial carcinoma was proved pathologically in one case 14 months later and in the other case about 2 years later.

SERIES PLATE

A VI

Female Genital System

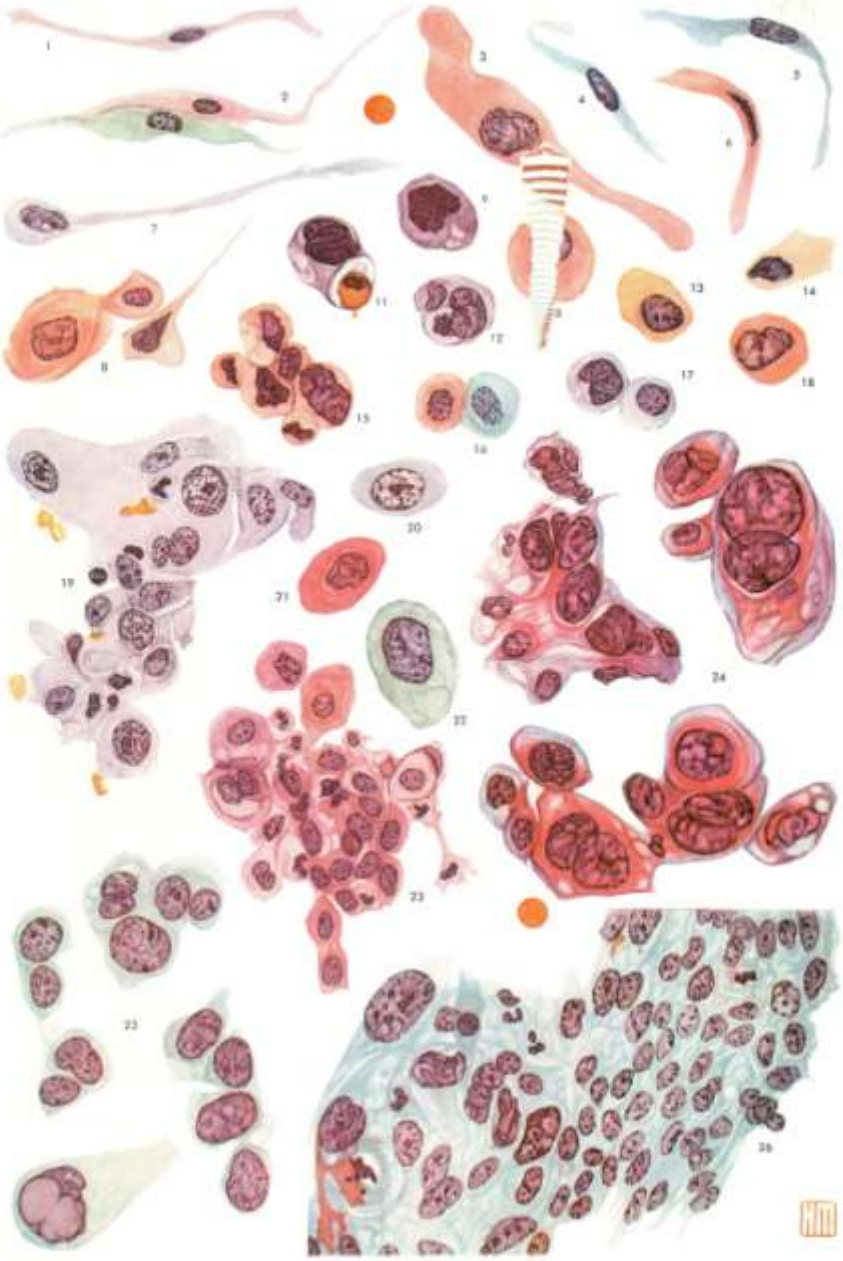
Cells found in vaginal and cervical smears from early and advanced cases of malignancy of the female genital organs

AVI
DESCRIPTION

Cells found in vaginal and cervical smears from early and advanced cases of malignancy of the female genital organs. Drawings x 525.

1. Malignant epidermoid cell (snake cell type). Vaginal smear. OG–EA series. Postmenopausal state. Pathologic diagnosis: carcinoma of the cervix, Grade II.
2. Malignant epidermoid cells (spindle cell type). Vaginal smear. OG–EA series. Age 38. Pathologic diagnosis: squamous cell carcinoma of the cervix.
3. Large malignant epidermoid cell. Vaginal smear. OG–EA series. (Same case as No. 2.)
4, 5 and 6. Malignant epidermoid cells (snake cell type). Vaginal smear. OG–EA series. (Same case as No. 2.)
7. Malignant epidermoid cell (tadpole type). Vaginal smear. OG–EA series. (Same case as No. 1.)
8. Malignant epidermoid cells. Vaginal smear. OG–EA series. (Same case as No. 1. Compare with C IV, 6.)
9–14. Malignant cells of the parabasal type. Vaginal smear. OG–EA series. Age 45. Pathologic diagnosis: carcinoma in situ of the cervix.
15. Malignant cells. Vaginal smear. OG–EA series. Age 28. Pathologic diagnosis: squamous cell carcinoma of the cervix, Grade II–III.
16. Malignant cells of the parabasal type. Vaginal smear. OG–EA series. (Same case as No. 2.)
17. Malignant cells of the parabasal type. Vaginal smear. OG–EA series. (Same case as No. 9.)
18. Malignant cell of the parabasal type. Vaginal smear. OG–EA series. (Same case as No. 1.)
19. A cluster of malignant cells of the endocervical type exhibiting the pattern of endocervical cell dyskaryosis. Cervical smear. OG–EA series. Age 50. Pathologic diagnosis: carcinoma in situ of the cervix. Primary diagnosis by smears. Patient alive and well 8 years after total hysterectomy.
20–22. Malignant cells from two cases of epidermoid carcinoma of the vulva, Grade II. Vaginal smears. OG–EA series.
23. Malignant cells. Vaginal smear. OG–EA series. Age 54. Pathologic diagnosis: squamous cell carcinoma of the cervix, Grade II.
24. Malignant cells. Vaginal smear. OG–EA series. Age 44. Pathologic diagnosis: adenoacanthoma of the endometrium with extension to the endocervix. Note extreme squamous metaplasia.
25. Malignant cells, one with a nucleus showing extreme vacuolation. Vaginal smear. OG–EA series. Age 50. Pathologic diagnosis: squamous cell carcinoma of the cervix.
26. Cells of the endocervical type showing dyskaryotic changes. Note pronounced anisokaryosis and two mitotic figures. Cervical aspiration smear. OG–EA series. Age 54. Pathologic diagnosis: carcinoma in situ of the cervix.

AVI
FEMALE GENITAL SYSTEM



A VI
DISCUSSION

Cells found in vaginal and cervical smears from early and advanced cases of malignancy of the female genital organs

Extremely elongated cells with abnormal hyperchromatic nuclei, characteristic of invasive epidermoid carcinoma of the cervix, are shown in Nos. 1–7. Nos. 1, 4, 5 and 6 represent the so-called snake type; No. 2, the spindle type. No. 7 has a tadpole form, while No. 3 pictures a large, bizarre cell. Many cells of the above types show acidophilia. Cytologic changes corresponding to those observed in carcinomas of the cervix may be seen also among cells exfoliated from epidermoid carcinomas of other organs such as the lung (C IV).

In Nos. 19 and 26 the endocervical type of dyskaryosis is clearly recognizable. In both there is evidence of proliferative activation of the nucleus and pronounced anisokaryosis. In No. 19 irradiation was administered before a biopsy was taken but a subsequent biopsy showed areas of abnormal epithelium, interpreted as malignant without evidence of invasion.

The relatively larger size of cells derived from epidermoid carcinomas of the vulva in comparison with corresponding cells from carcinomas of the cervix is shown in Nos. 20–22 (compare with No. 16).

In No. 23 the nuclear structure is not markedly abnormal, but there is an irregular pattern which is suggestive of malignancy.

The cells illustrated in No. 24 are from the vaginal smear of a case diagnosed as adenoacanthoma. They show extreme squamous metaplasia, marked nuclear abnormalities and degenerative changes. The tumor was endometrial in origin and extended into the endocervix, sections of which showed areas of squamous metaplasia adjacent to typical adenocarcinomatous areas.

The cells of No. 25, which are derived from a squamous cell carcinoma of the cervix, indicate an endocervical origin, although their type is not clearly defined. Note the extreme vacuolation of the nucleus of the lower cell.

SERIES PLATE

A VII

Female Genital System

Malignant cells found in vaginal and cervical smears from cases of carcinomas of the cervix and vagina

A VII
DESCRIPTION

Malignant cells found in vaginal and cervical smears from cases of carcinomas of the cervix and vagina. Drawings x 525 except Nos. 9, 10 and 11, which are x 1050.

1 and 2. Endocervical smear. Glycogen series. Age 50. Pathologic diagnosis: carcinoma of the cervix, Grade I. Primary diagnosis by smears.

3. Vaginal smear. OG–EA series. Age 70. Pathologic diagnosis: squamous cell carcinoma of the cervix. (Same case as A XI, 13.)

4. Vaginal smear. Glycogen series. Age 67. Pathologic diagnosis: squamous cell carcinoma of the cervix, Grade III. (Same case as Nos. 13–19.)

5. Vaginal smear. Glycogen series. Age 59. Pathologic diagnosis: squamous cell carcinoma of the cervix.

6. Malignant cell, snake cell form. Endocervical smear. OG–EA series. Age 42. Pathologic diagnosis: carcinoma of the endocervix.

7. Vaginal smear. OG–EA series. Age 51. Pathologic diagnosis: primary carcinoma of the vagina extending into the cervix, epidermoid type, Grade III. Primary diagnosis by smears. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

8. Vaginal smear. OG–EA series. Age 74. Pathologic diagnosis: squamous cell carcinoma of the cervix.

9. Same cluster of cells seen in No. 8, magnified (to 1050 diameters) to illustrate the pattern of chromatin distribution.

10 and 11. Vaginal smear. OG–EA series. Age 50. Pathologic diagnosis: squamous cell carcinoma of the cervix, Stage III. The cells are magnified (to 1050 diameters) to show the nuclear pattern. In No. 11 note the furrowing of the nucleus and the aggregation of chromatin in large clumps.

12. Epidermoid malignant cells showing stratification. Cervical aspiration smear. Glycogen series. Age 48. Pathologic diagnosis: intraepithelial squamous cell carcinoma of the cervix. (Same case as A IV, Nos. 2 and 3.)

13–19. Same case as No. 4. No. 13 shows typical stratification. No. 14 illustrates epidermoid malignant cells of the snake cell type. Two cells in No. 17 and one in No. 19 show karolysis. No. 19 illustrates stratification and many other cytoplasmic and nuclear characteristics of advanced malignancy.

A VII
DISCUSSION

Malignant cells found in vaginal and cervical smears from cases of carcinomas of the cervix and vagina

With the exception of Nos. 1, 2 and 12, the cells shown here are from advanced cases.

Nos. 1 and 2 illustrate a case of an early carcinoma of the cervix, Grade I, in which the diagnosis was made primarily by the examination of smears.

The cells illustrated in Nos. 6, 14, 15 and 18 show extreme elongation, which is frequently seen in epidermoid carcinomas. Typical stratification is illustrated in Nos. 12 and 13.

Malignant epidermoid cells should not be considered to be necessarily derived from the squamous epithelium of the ectocervix. Carcinomas originating in the endocervix may show marked squamous metaplasia resulting in the exfoliation of malignant cells of the epidermoid type.

Epidermoid cells often show eosinophilia (No. 6) or orangeophilia (No. 14), probably owing to cornification or keratinization.

No. 5 shows a group of dedifferentiated malignant cells. In spite of their deformation, their structural characteristics are suggestive of an endocervical origin.

No. 7 compares with A VI, 23. Despite the absence of striking nuclear abnormalities, both groups are interpreted as malignant on the basis of an abnormal pattern in combination with nuclear atypia. The case illustrated in No. 7 of this plate is of particular diagnostic interest. Repeated vaginal smears taken at the Kate Depew Strang Prevention Clinic of Memorial Center, New York, were persistently positive for a period of 11 months. Five biopsies performed during this period showed chronic cervicitis. Because of the positive cytologic findings, the patient was hospitalized and a thorough gynecologic examination revealed the presence of a concealed carcinoma of the vagina. Tissue sections examined after hysterectomy confirmed the malignant nature of the lesion and showed extension to the endocervix.

The structural characteristics of malignant nuclei are shown more clearly in the enlarged drawings (Nos. 9, 10 and 11). Such nuclear patterns are not infrequently seen in malignant cells and are fairly characteristic of malignancy.

A distinctly aberrant pattern with marked nuclear abnormalities is shown in No. 19.

SERIES PLATE

A VIII

Female Genital System

Non-malignant endometrial cells found in vaginal, cervical and endometrial smears

A VIII
DESCRIPTION

Non-malignant endometrial cells found in vaginal, cervical and endometrial smears. Drawings x 525.

1–10. Endometrial cells, menstrual phase. Vaginal smears. OG–EA series.
1–3 and 7. Early stage.
4. Early stage. Case of sterility.
5. Early stage. Sterility associated with periods of amenorrhea.
6 and 8–10. Late stage, 5th to 8th days.

11. Two clusters of endometrial cells, one showing leucocytic infiltration. Vaginal smear. Glycogen series. Age 44. Pathologic diagnosis: hyperplasia of the endometrium with metaplasia.

12 and 13. Endometrial columnar cells, ciliated (No. 12) and mucoid (No. 13). Endometrial smear. OG–EA series. Age 33. Pathologic diagnosis: hyperplasia of the endometrium.

14. Endometrial columnar cells. Endometrial smear. Glycogen series. Age 51. Menopause.

15. Endometrial columnar cells from a smear taken the 14th day of a normal period. Endometrial smear. Glycogen series. Age 30.

16. Endometrial cell cluster showing leucocytic infiltration. Vaginal smear. OG–EA series. Age 52. Pathologic diagnosis: chronic endometritis.

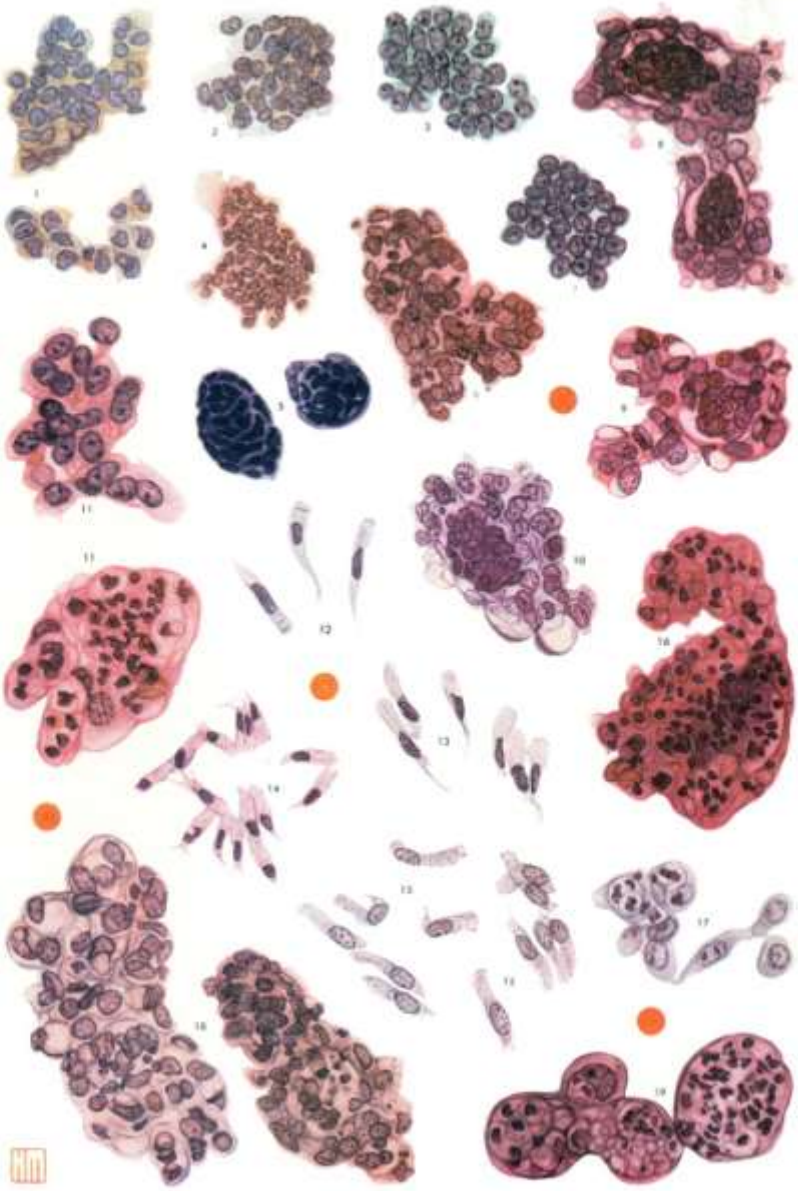
17. Atypical endometrial cells invaded by leucocytes. Cervical smear taken 2 days after curettage. Glycogen series. Age 50.

18. Clusters of endometrial cells. Tenth day of the period. Vaginal smear. OG–EA series. Age 44. Pathologic diagnosis: mild endometrial hyperplasia.

19. Endometrial cells with marked leucocytic infiltration. Vaginal smear taken on the 4th day after curettage. OG–EA series. Age 25. (Compare with A IX, 8.)

A VIII

FEMALE GENITAL SYSTEM



A VIII
DISCUSSION

Non-malignant endometrial cells found in vaginal, cervical and endometrial smears

Cells from the endometrial stroma and epithelium are found in vaginal and endocervical smears during the menstrual phase. The stroma cells are densely grouped and have a fairly uniform size (Nos. 1, 2, 3 and 7). Clusters of endometrial cells exfoliated during the later stages of the menstrual phase or postmenstrually exhibit characteristic patterns (Nos. 6, 8, 9 and 10). The cells tend to fall into denser secondary groups or to differentiate into two distinct zones within a cluster, a central zone consisting of densely grouped small cells and a peripheral one of larger, more loosely arranged cells. Some vacuolation, particularly of the peripheral cells, is noticeable.

It has recently been noted that clusters of endometrial cells observed in vaginal, cervical and endometrial smears in the late menstrual or postmenstrual stages may give rise to free phagocytic elements (histiocytes), the function of which is to cleanse the endometrial cavity, the cervical canal, and the lumen of the vagina from the degenerated and necrotic cellular elements accumulating during the menstrual phase (see page 26).

No. 4 shows a cluster of relatively small stroma cells, and No. 5 illustrates two compact groups of such cells. Both these clusters are from cases of sterility. However, such cells are an exception rather than a rule and should not be interpreted as characteristic of this condition.

The same remark applies to Nos. 11 and 18, which illustrate cells from two cases of endometrial hyperplasia. Such cell groups may be considered consistent with, but not diagnostic of, endometrial hyperplasia. The two clusters of No. 18 are from a smear taken on the 10th day of the cycle and show clearly the secondary grouping described above for the late menstrual or postmenstrual endometrial cell clusters.

The cells illustrated in Nos. 12–15 are of the columnar type. With the exception of the cells of No. 12, which are ciliated, they are of the mucoid, secretory type. Such columnar endometrial cells are only rarely seen in smears other than endometrial. Nos. 12 and 13 are from a case of endometrial hyperplasia; No. 14, from a smear after menopause; and No. 15, from a normal midmenstrual smear. These columnar endometrial cells are smaller and more slender than corresponding endocervical mucoid secretory cells (compare with A III, 3, 11, 18 and 19).

The cells illustrated in Nos. 13, 14 and 15, though all of the mucoid columnar type, show structural variations which probably reflect differences in the functional state of the endometrium. A more comprehensive comparative study of the various cell types found in normal endometrial smears will eventually make possible the use of such smears for an evaluation of the normal cyclic variations of the endometrium.

Nos. 16, 17 and 19 show marked infiltration by leucocytic elements. However, in No. 16, which is from a case of endometritis, the leucocytic infiltration is intercellular, whereas in Nos. 17 and 19, taken from smears obtained soon after a curettage, it is intracellular. Some diagnostic significance may be attributed to both types. Cell clusters of the type shown in Nos. 17 and 19 may often be misinterpreted because of their similarity to adenocarcinoma cells, which are frequently invaded by leucocytes (compare with A IX, 8 and 10). When such cells are found in smears one should always find out whether the patient has had a recent curettage.

SERIES PLATE

A IX

Female Genital System

Abnormal cells found in vaginal and endocervical smears in cases of malignant neoplasms of the endometrium

AIX
DESCRIPTION

Abnormal cells found in vaginal and endocervical smears in cases of malignant neoplasms of the endometrium. Drawings x 525.

1. Endometrial cells showing squamous metaplasia and nuclear atypia. Vaginal smear. OG–EA series. Age 53. Sections of the endometrium following hysterectomy were interpreted as adenoacanthoma by some pathologists and as extreme metaplasia by others. The patient had had prolonged estrogenic therapy.

2 and 3. Endometrial cells showing pronounced vacuolation of the peripheral cells and a somewhat atypical pattern. Vaginal smears. OG–EA series and glycogen series respectively. Age 35. Repeated curettages were diagnosed as hyperplasia and metaplasia of the endometrium. Hysterectomy performed at a later date. Pathologic diagnosis: early adenocarcinoma of the endometrium without evidence of metastasis.

4. Endometrial cells. Vaginal smear. OG–EA series. Age 55. Curettage, pathologic diagnosis: adenocarcinoma of the endometrium, Grade II. Insertion of radium 2 days after smear was taken. Panhysterectomy and bilateral salpingo-oophorectomy performed 2 months later. Patient alive and well 11 years after the smear was taken. (Same case as G I, 13.)

5. Adenocarcinoma cells intermixed with histiocytes. Vaginal smear. OG–EA series. Age 47. Pathologic diagnosis: adenocarcinoma of the endometrium, Grade I.

6 and 7. Endocervical aspiration smear. OG–EA series. Age 73. Pathologic diagnosis: adenocarcinoma of the endometrium. Note mitosis in No. 7.

8. Cluster of cells, typical of adenocarcinoma of the endometrium, showing marked vacuolation with leucocytic infiltration. Vaginal smear. OG–EA series. Age 62. Pathologic diagnosis: adenocarcinoma of the endometrium.

9. Vaginal smear. OG–EA series. Age 60. Pathologic diagnosis: carcinosarcoma of the uterus.

10. Vaginal smear. Glycogen series. Age 77. Pathologic diagnosis: adenocarcinoma of the endometrium.

11. Vaginal smear. Glycogen series. Age 55. Pathologic diagnosis: papillary adenocarcinoma of the endometrium.

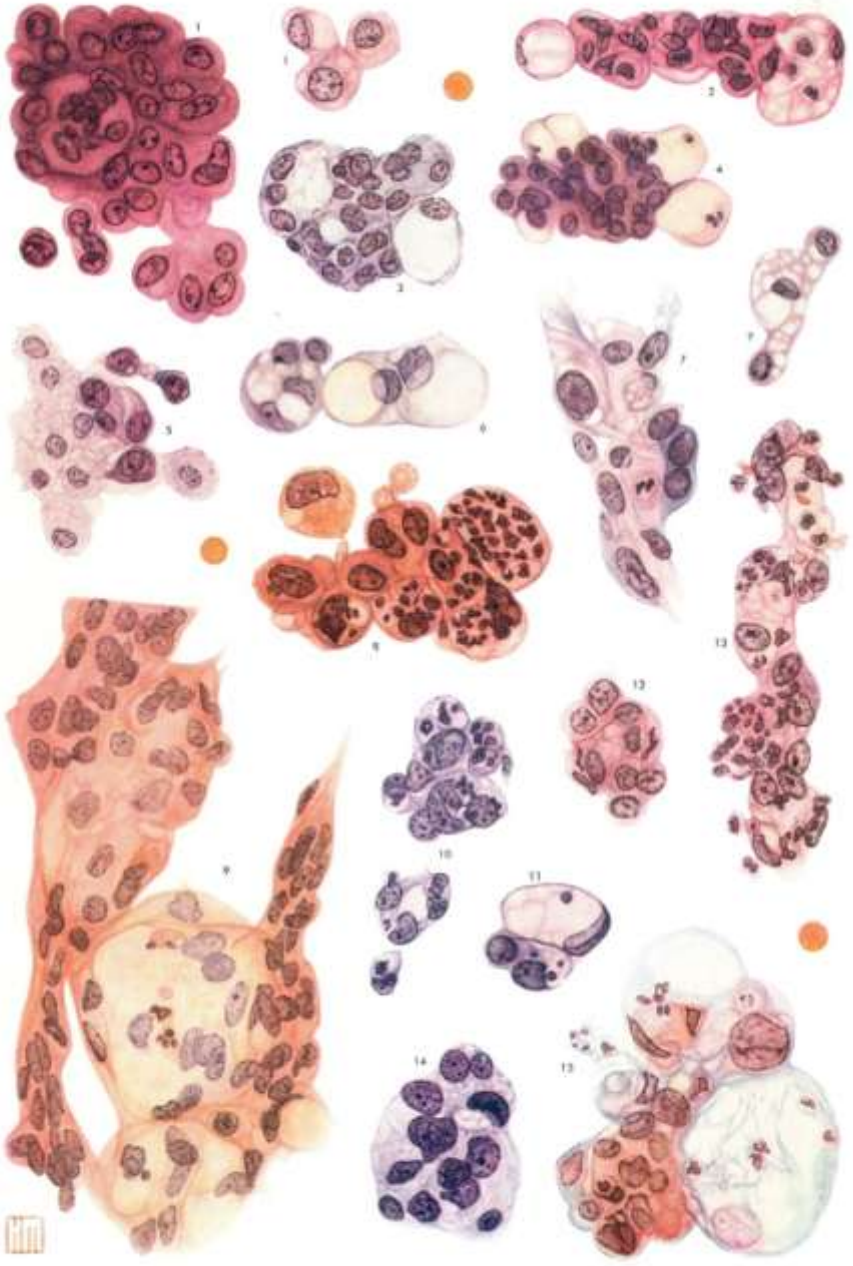
12 and 13. Vaginal smears. OG–EA series. Age 55. Pathologic diagnosis: adenocarcinoma of the endometrium. The cluster in No. 12 was found in a smear taken 14 months prior to the smear illustrated in No. 13.

14. Vaginal smear. Glycogen series. Age unrecorded. Pathologic diagnosis: mixed mesodermal tumor of the endometrium. Primary diagnosis by smears. (Courtesy of Dr. Thomas W. McElin, Evanston, Ill.)

15. Malignant cells showing extreme vacuolation. Vaginal smear. OG–EA series. Age 56. Pathologic diagnosis: adenocarcinoma of the endometrium.

AIX

FEMALE GENITAL SYSTEM



AIX
DISCUSSION

Abnormal cells found in vaginal and endocervical smears in cases of malignant neoplasms of the endometrium

The case illustrated in No. 1 is of particular cytologic and clinical interest. The patient had a history of prolonged estrogen therapy with relatively large dosages. Vaginal smears taken before hysterectomy contained clusters of endometrial cells showing pronounced squamous metaplasia, nuclear atypia and irregularity of pattern arousing a suspicion of a malignant neoplasm of the endometrium, possibly an adenoacanthoma. Sections of the endometrium following hysterectomy were interpreted by some pathologists as indicative of adenoacanthoma, while others considered it an extreme hyperplasia.

One year and a half prior to the time the suspicious vaginal smears were taken, the patient had had a radical mastectomy for a scirrhous carcinoma of the breast. The patient expired 3 years after hysterectomy with metastases, from the primary carcinoma of the breast, to skull, orbit, spine and pelvis. This case is of special interest because of its unique cytologic and histologic patterns. It is likely that the prolonged use of estrogens, though not necessarily a causative agent in the genesis of the tumor, was responsible for the particular cytologic pattern.

Nos. 2 and 3 show marked vacuolation of peripheral cells and a slightly irregular pattern but no nuclear changes characteristic of malignancy. The general cytologic picture in the smears was characteristic of a prolonged, relatively high estrogenic level. Because of atypical bleeding the patient had repeated curettages which showed hyperplasia and metaplasia of the endometrium. Hysterectomy performed at a later date showed an early adenocarcinoma of the endometrium without evidence of metastasis.

The great similarity in the cytologic picture of Nos. 2 and 3 with that of No. 4, which illustrates a cluster of adenocarcinoma cells, is apparent.

The relatively frequent presence of many histiocytes in smears of endometrial adenocarcinoma makes the recognition of the malignant cells sometimes difficult. When in close proximity to histiocytes as in No. 5, endometrial adenocarcinoma cells may be misinterpreted as young, incompletely differentiated histiocytes unless they possess distinctly abnormal features. The great similarity between endometrial cells and histiocytes can be better understood in the light of recent observations indicating the cytogenetic relationship of these two cell types (page 26).

The most characteristic criteria of advanced endometrial malignancy—i.e., marked vacuolation, leucocytic infiltration, nuclear atypia and irregularity of pattern—are shown in Nos. 6–8, 10–13 and 15. Leucocytic infiltration is apt to be more pronounced in cases with secondary infections.

No. 9 illustrates a cell cluster from a mixed tumor diagnosed as carcinosarcoma. No. 14 shows a cell group from a mixed mesodermal tumor of the endometrium. The cytology of these two cases (Nos. 9 and 14) presents some differences from that of the typical adenocarcinoma and some distinctive traits which, however, are not sufficient to permit an accurate evaluation of the histologic type of the tumors.

No. 12 illustrates the only atypical cell cluster found in an otherwise normal vaginal smear. It was overlooked during the first examination largely because of the relatively dark staining of the smear. Fourteen months later, smears taken from the same patient contained many typical clusters of adenocarcinoma cells (No. 13).[38] Because of these findings the original smear was reviewed after having been destained and restained, and it was then that the cluster illustrated in No. 12, which fits into the cytologic picture of early endometrial malignancy, was noted.

This case proved the unsuitability of the eosin–water blue staining procedure in use at that time and led to new experimentation with alcoholic solutions of various dyes, resulting in the development of the OG–EA staining method.

SERIES PLATE

A X

Female Genital System

Malignant endocervical, endometrial and ovarian cells found in vaginal, cervical and endometrial smears

AX
DESCRIPTION

Malignant endocervical, endometrial and ovarian cells found in vaginal, cervical and endometrial smears. Drawings x 525.

1 and 2. Vaginal smear. OG–EA series. Age 66. Pathologic diagnosis: adenocarcinoma of the fundus of the uterus, mucoid type.

3 and 4. Vaginal smear. Glycogen series. Age 50. Pathologic diagnosis: adenocarcinoma of the cervix.

5–8. Vaginal smear. OG–EA series. Age 58. Pathologic diagnosis: adenocarcinoma of the cervix. This patient had a history of prolonged estrogen therapy. Two cells in No. 7 show karyorrhexis.

9. Cervical aspiration smear. OG–EA series. Age 65. Pathologic diagnosis: adenoacanthoma of the fundus of the uterus.

10 and 11. Smears prepared from scrapings of the tumor surface. OG–EA series. Age 40. Pathologic diagnosis: adenocarcinoma of the endocervix.

12. Endometrial smear. Glycogen series. Age 63. Pathologic diagnosis: cystadenocarcinoma of the ovary. No evidence of metastasis to the uterus. (Same case as A XII, 11 and 12.)

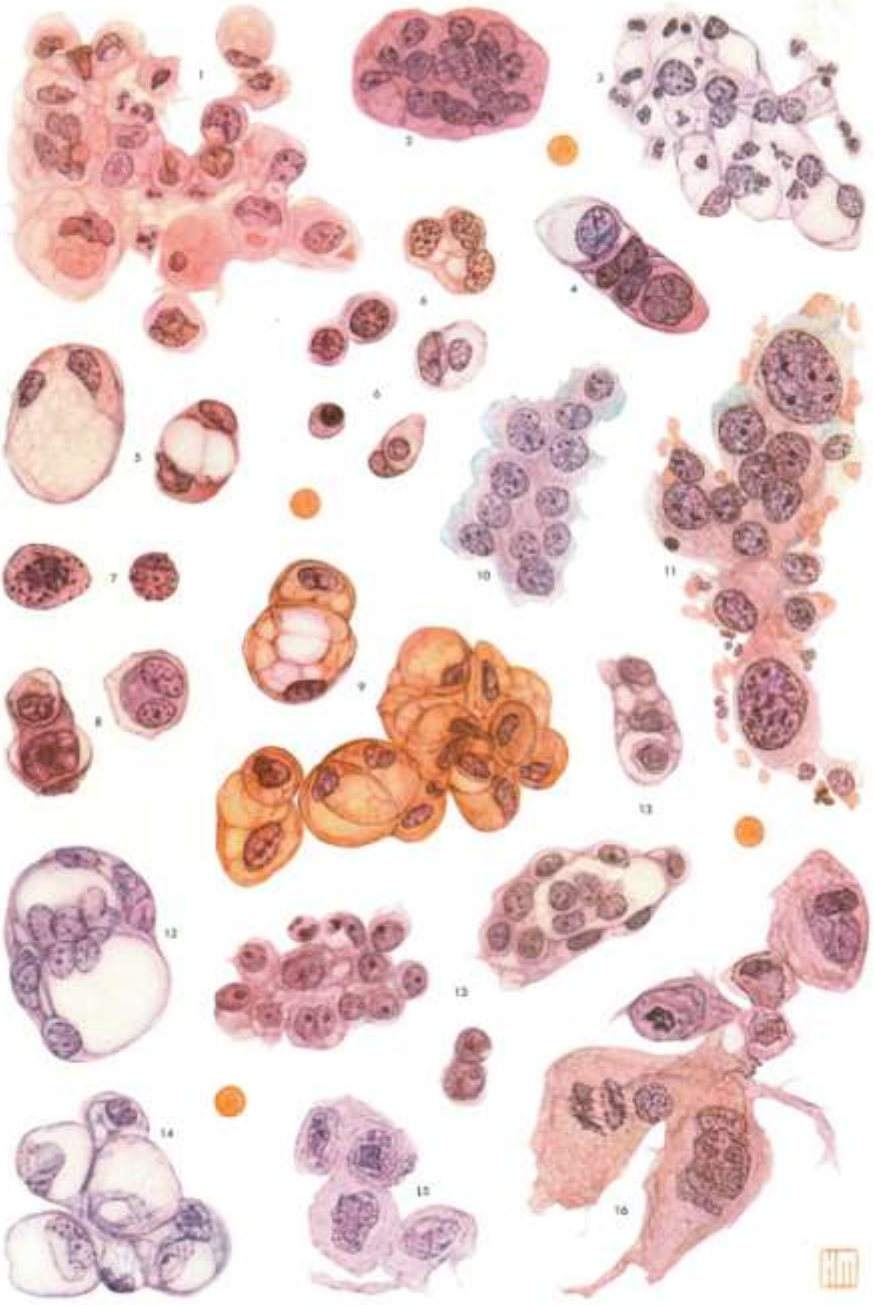
13. Vaginal smear. OG–EA series. Age 52. Pathologic diagnosis: cystadenocarcinoma of the ovary with metastasis to the uterus.

14. Endocervical aspiration smear. Glycogen series. Age 76. Pathologic diagnosis: adenocarcinoma of the cervix.

15 and 16. Smears prepared from scrapings of the surface of the tumor. OG–EA series. Age 27. Pathologic diagnosis: chorioepithelioma.

AX

FEMALE GENITAL SYSTEM



AX
DISCUSSION

Malignant endocervical, endometrial and ovarian cells found in vaginal, cervical and endometrial smears

Malignant endometrial cells are shown in Nos. 1 and 2, 9, and 15 and 16. The case illustrated in Nos. 1 and 2 was diagnosed as adenocarcinoma of the mucoid type, No. 9 as adenoacanthoma and Nos. 15 and 16 as chorioepithelioma. The differences in the cytologic patterns of these three cases are apparent. The cells in Nos. 1 and 2 show vacuolation and nuclear eccentricity. The cells in No. 9 show vacuolation but also squamous metaplasia, whereas those of Nos. 15 and 16 exhibit the extreme cellular and nuclear abnormalities and the degenerative changes which characterize the chorioepitheliomas.

Cells from adenocarcinomas of the endocervix are seen in Nos. 3 and 4, 5–8, 10 and 11, and 14. The clusters of Nos. 3, 5 and 14 consist of cells which may be linked with the endocervical cell type. In the other figures the endocervical origin of the cells is less apparent.

The cells illustrated in No. 7 show complete breakdown of the nucleus and an irregular distribution of the chromatin throughout the cytoplasm in the form of coarse granules of unequal size (see also A XII, 13). Such necrotic cells are often seen in the smears of adenocarcinoma cases and have a high diagnostic value.

Nos. 10 and 11 are adenocarcinoma cells from a smear prepared by scraping the tumor surface at operation. The cytologic type of the tumor is shown in No. 10, whereas No. 11 exhibits marked anisokaryosis and nuclear gigantism. One may expect a larger number and a greater variety of abnormal cells in smears prepared in this fashion.

Cells from ovarian carcinomas are illustrated in Nos. 12 and 13. The first case is one in which there was no evidence of metastasis to the uterus. The cluster shown in No. 12 was found in an endometrial smear. In such smears there is a much better chance of recovering malignant cells of ovarian origin than in endocervical or vaginal smears. The manner in which the malignant cells reach the cavity of the uterus in the absence of metastasis to the uterus or tubes has not been definitely established as yet. The cytology of clusters of ovarian cystadenocarcinoma differs in many respects from that of the primary adenocarcinomas of the endometrium. A differential diagnosis between these two types is thus possible in some cases. The ovarian cystadenocarcinoma cells tend to form well-outlined rosettes and show considerable crowding and overlapping. Vacuolation is very pronounced, but leucocytic infiltration within the vacuoles is not as common as in the adenocarcinomas of the endometrium. The cells illustrated in No. 13 are less characteristic for the type. They were found in a vaginal smear of a case showing metastasis to the uterine wall.

SERIES PLATE

A XI

Female Genital System

Non-malignant and malignant cells of various types found in vaginal and cervical smears

A XI
DESCRIPTION

Non-malignant and malignant cells of various types found in vaginal and cervical smears. Photomicrographs x 600.

1 and 2. Endocervical columnar cells filled with mucoid secretion. Endocervical smear. Glycogen series. Age 43. Cervical erosion.

3. Large endocervical mucoid cells of the goblet type. Cervical smear. Glycogen series. Age 49. Chronic cystic cervicitis.

4. Endocervical cells of the stout type showing hypertrophy and multinucleation. Endocervical smear. Glycogen series. Age 44. Cervical erosion.

5. Endocervical mucoid cells showing marked vacuolation as in cells of the goblet type. Cervical smear. OG–EA series. Age 51. Cervical polyp. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

6. Cells characteristic of endocervical cell dyskaryosis. Cervical smear. Glycogen series. Final pathologic diagnosis: very early invasive carcinoma of the cervix. (Same case as A V, 10.)

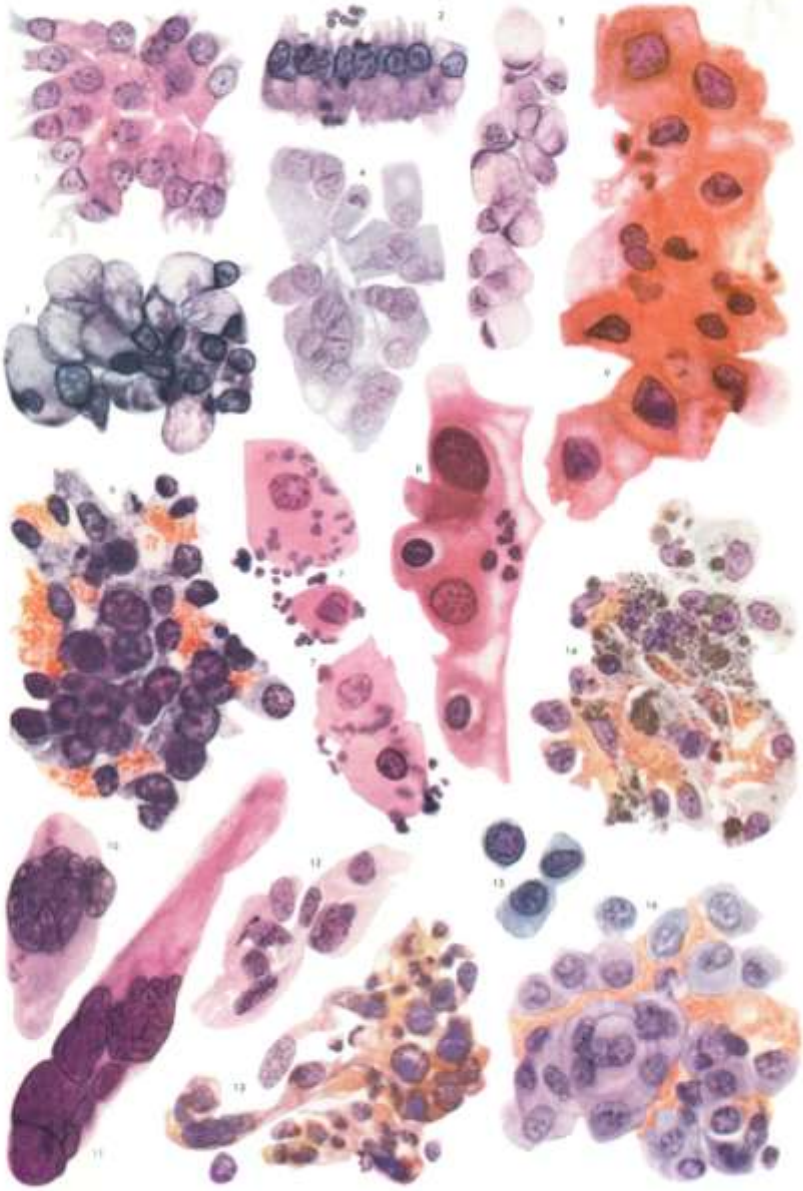
7. Superficial squamous cells showing nuclear enlargement ("karyomegaly"). Cells of this type resemble closely those of superficial cell dyskaryosis. Vaginal smear. OG–EA series. Age 32. Early pregnancy. Cervical biopsy: questionable carcinoma of the cervix. (Courtesy of Dr. Paul Kimmelstiel and Sara Hodges, Charlotte Memorial Hospital, Charlotte, N.C.)

8 and 9. Clusters of superficial squamous cells, showing karyomegaly and early dyskaryotic changes. Cervical swab smear. OG–EA series. Age 27. Pregnancy. Biopsy, pathologic diagnosis: carcinoma of the cervix in situ. Six weeks after delivery by Cesarian section, the abnormal cells had disappeared from the smears. (Courtesy of Dr. Benjamin Jacobson, New Brunswick, N.J.)

10–12. Malignant cells. Endocervical smear. Glycogen series. Age 49. Pathologic diagnosis: fibromyosarcoma of the endometrium recurrent in the endocervix. Subtotal hysterectomy had been performed 2½ years prior to the date the smears were taken.

13. Malignant cells. Vaginal smear. OG–EA series. Age 70. Pathologic diagnosis: squamous cell carcinoma of the cervix. (Same case as A VII, 3.)

14–16. Malignant cells. Vaginal smear. OG–EA series. Age 18. Pathologic diagnosis: melanosarcoma, metastatic. Primary site mole in midscapular area. Note numerous melanin granules in No. 14. (Courtesy of Dr. Paul Kimmelstiel, Charlotte Memorial Hospital, Charlotte, N.C.)



A XI
DISCUSSION

Non-malignant and malignant cells of various types found in vaginal and cervical smears

Cervical erosion is often characterized by distinctive cells of the endocervical type. Some of these are columnar cells stouter than the average endocervical cells and filled with mucoid secretion (Nos. 1 and 2). A palisade arrangement is often seen (No. 2). Multinucleation is another characteristic feature (No. 4). This latter feature may be partly the result of a fusion of adjacent cells or of karyokinesis without cytokinesis. The cells of No. 4 show great resemblance to those of A III, 21, which are also from a case of cervical erosion. Increased mitotic activity is often observed in actively regenerating erosions.

Cells like those illustrated in Nos. 3 and 5 are also of the columnar mucoid type, but their form is closer to that of the goblet cell. They are very probably of glandular origin. Cluster No. 3 was found in a case of chronic cystic cervicitis and may be considered consistent with, but not diagnostic of, this condition. This last remark applies also to No. 5, which was found in a case of cervical polyp.

No. 6 illustrates a group of cells characteristic of endocervical cell dyskaryosis (see A IV, Discussion). Cells from the same case are also shown in A V, 10. This case is of particular clinical interest in that five sets of smears taken over a period of 26 months showed progressive dyskaryotic changes. Of the two biopsies performed, the one taken a year after the first positive smear was negative and the other 13 months later was suspicious. A subsequent ring biopsy proved the presence of a carcinoma in situ with indication of early invasion.

The cells illustrated in Nos. 7–9 are from two cases of pregnancy and display abnormal features bordering on dyskaryosis of the superficial cell type. In No. 7 the cells show chiefly nuclear enlargement, "karyomegaly," whereas in Nos. 8 and 9 in addition to nuclear enlargement there is atypia suggestive of superficial cell dyskaryosis (compare with A IV, 6). A biopsy taken at the same time as the smears in No. 7 was interpreted as a questionable carcinoma of the cervix. No further follow-up is available beyond the information that gestation ended in a miscarriage. In the other case (Nos. 8 and 9) a biopsy was interpreted as a carcinoma in situ. Smears taken after the biopsy were also positive; however, the abnormal cells disappeared within six weeks after Cesarian section and subsequent smears have continued to be negative. A close comparison of these two cases with cases of typical superficial and parabasal cell dyskaryosis suggests the existence of some structural differences. An adequate comparative study and analysis of these abnormal patterns and an evaluation of their diagnostic and prognostic significance will require well-coordinated laboratory and clinical observations and a close follow-up over a long period.

The two giant cells in Nos. 10 and 11 are from a case of fibromyosarcoma of the endometrium recurring in the endocervix after subtotal hysterectomy and show abnormal forms, multinucleation and nuclear gigantism. Less aberrant cells from the same case are illustrated in No. 12.

A cluster of cells with features characteristic of an advanced epidermoid carcinoma of the cervix is shown in No. 13.

Nos. 14–16 illustrate cells from a metastatic melanosarcoma found in a vaginal smear. The cells of No. 15 are closer to the original cell type of the tumor. One of these cells has an unusually high nuclear cytoplasmic ratio and shows great resemblance to cells of a metastatic melanosarcoma of the lung found in a bronchial aspiration smear (C V, 18 and 19). Characteristic melanin granules are present in No. 14.

SERIES PLATE

A XII

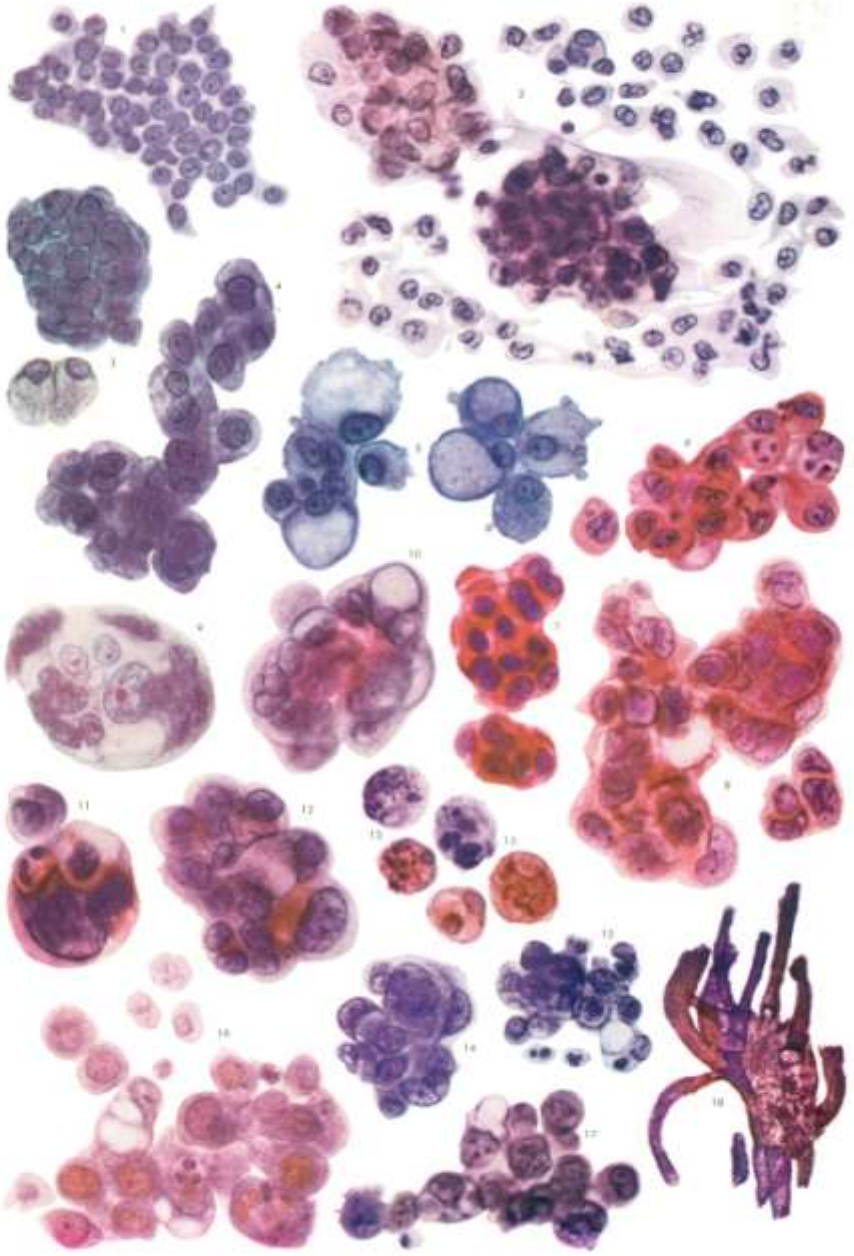
Female Genital System

Non-malignant and malignant cells found in vaginal, cervical and endometrial smears

A XII
DESCRIPTION

Non-malignant and malignant cells found in vaginal, cervical and endometrial smears. Photomicrographs x 600, except No. 18, which is x 50.

1. Normal endometrial cells. Endometrial smear. Glycogen series. Age 60. Menopause.
2. Clusters of endometrial cells in the process of histiocytogenesis (page 28). Vaginal smear. OG–EA series. Age 43. Tenth day of the sex cycle. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
3 and 4. Abnormal cells. Endometrial smears. Glycogen series. Age 45. Pathologic diagnosis: early adenocarcinoma of the endometrium. Primary diagnosis by smear. Irradiation therapy was initiated 11 days after the first smear (No. 3) was taken. The cells illustrated in No. 4 were found in a smear taken 35 days later.
5. Clusters of atypical cells, interpreted as histiocytic, showing pronounced vacuolation. Endometrial smear. Glycogen series. Age 55. Postmenopausal bleeding.
6. Cluster of endometrial cells, suggestive of adenocarcinoma. This was the only cluster of abnormal cells found in the smear. Curettage performed 1 year later did not provide sufficient tissue for diagnosis. Hysterectomy 11 months after curettage proved the presence of an adenocarcinoma of the endometrium. Cervical smear. Glycogen series. Age 44.
7 and 8. Endometrial cell clusters found in cervical smears taken from the same patient at an interval of 3 years and 2 months. Glycogen series. Age 61 at last smear. Final diagnosis at the time the second smear was taken: adenocarcinoma of the endometrium. The patient had Graves' disease and received prolonged treatment with 6-propyl thiouracil.
9. Cluster of malignant cells. Vaginal smear. OG–EA series. Age 40. Pathologic diagnosis: pseudomucinous cystadenocarcinoma of both ovaries with metastases to the serosal coat of the uterus and tubes. No evidence of metastasis to the endometrium or tubal mucosa. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
10. Cluster of malignant cells. Endometrial smear. Glycogen series. Age 56. Pathologic diagnosis: bilateral papillary cystadenocarcinoma of the ovary. No evidence of metastasis to the tubes or uterus.
11 and 12. Clusters of malignant cells. Endometrial smear. Glycogen series. Age 63. Pathologic diagnosis: adenocarcinoma of the ovary with metastasis to the liver. No evidence of metastasis to the tubes or uterus. (Same case as A X, 12.)
13. Malignant cells of endometrial origin showing karyorrhexis and other necrotic changes. Uterine smear. Glycogen series. Age 66. Pathologic diagnosis: early adenocarcinoma of the endometrium. Primary diagnosis by smears.
14. Malignant endometrial cells. Vaginal smear. Glycogen series. Age 59. Pathologic diagnosis: adenocarcinoma of the endometrium. Primary diagnosis by smears.
15. Malignant tubal cells. Vaginal smear. OG–EA series. Age 42. Pathologic diagnosis: adenocarcinoma of the tube. Primary diagnosis by smears.
16 and 17. Malignant cells. Vaginal smear. OG–EA series. Age 28. Pathologic diagnosis: malignant hydatidiform mole, Hertig's Class IV, with marked invasion (chorionadenoma destruens). (Courtesy of Dr. Norbert Reicher and Eleanor Bechtold, Department of Gynecology, State University of New York, Syracuse, N.Y.)
18. Fragment of endometrial tissue with segments of glands, 10th day of the cycle. Vaginal smear. Glycogen series. Age 52. No evidence of malignancy. (Magnification of 50 diameters.)



A XII
DISCUSSION

Non-malignant and malignant cells found in vaginal, cervical and endometrial smears

Normal endometrial cells from an endometrial smear of a postmenopausal woman are shown in No. 1 (compare with A VIII, 1–3). The two clusters of endometrial cells surrounded by histiocytes in No. 2 are from a vaginal smear taken on the 10th day of the menstrual cycle, which corresponds to the postmenstrual histiocytogenetic stage described on page 26 and in the discussion of A VIII, 8–10. This is a picture often seen in smears of the postmenstrual and early proliferative stages of the normal sex cycle during which cells located at the periphery of the endometrial cell clusters apparently become detached and differentiate into phagocytes.

Nos. 3 and 4 illustrate cells from a case of an early adenocarcinoma of the endometrium. They were found in the endometrial smears of a woman with intermenstrual spotting as the only clinical symptom. In No. 3 the two cells are distinctly abnormal; the cells of the large cluster merely show nuclear hypertrophy and crowding, but the structural pattern and well-organized configuration, as well as the nuclear hyperchromasia, give evidence of its malignant character. The cells illustrated in No. 4 were present in an endometrial smear taken 35 days after irradiation. Cellular and nuclear hypertrophy and some metaplasia are apparent. The primary diagnosis of the adenocarcinoma in this case was by the endometrial smear. The patient is alive and apparently well 3 years after operation.

The criteria of early malignancy in the endometrial cells are not so clean-cut as those of the early malignancy of the cervix, in which the exfoliated cells display marked and easily recognizable modifications of the nuclear structure. Our hope for identifying malignant endometrial cells at an early stage lies in the more general use of the endometrial smear. In such smears atypical or abnormal cells can be recognized more readily than in the more cellular and heterogeneous vaginal or endocervical smears. The chance of recovering early adenocarcinoma cells is also much greater in an endometrial than in a vaginal or cervical smear. Vaginal smears are often negative in early endometrial malignancy.

The cells illustrated in No. 5 were found in an endometrial smear of a woman with postmenopausal bleeding. Many histiocytes were present in this smear, some showing pronounced vacuolation and other atypical features. The two clusters shown here are interpreted as atypical histiocytes.

The group of adenocarcinoma cells in No. 6 exhibits distinct nuclear and cytoplasmic criteria of malignancy. It was the only cluster of abnormal cells found in an otherwise normal endocervical smear of an asymptomatic woman. The presence of adenocarcinoma was proved 23 months later.

The case illustrated by Nos. 7 and 8 is of equal interest. The group shown in No. 7 was found in an endocervical smear of a postmenopausal woman 3 years and 2 months prior to the diagnosis of an adenocarcinoma. Smears taken at the time of operation contained many typical clusters of adenocarcinoma cells, as shown in No. 8. A comparison of No. 7 with No. 3 shows a striking similarity between the two clusters. Their grouping and configuration are practically the same. However, in the case of No. 3 the cluster was readily identified in a pure endometrial smear and was interpreted correctly as an atypical cluster of endometrial cells; whereas the cluster illustrated in No. 7 passed unnoticed in an endocervical smear in which it was intermixed with endocervical cell groups.

The cluster of cystadenocarcinoma cells shown in No. 9 was noted in a vaginal smear. No endometrial smear was available in this case. An exploratory operation performed 8 months later proved the presence of a pseudomucinous adenocarcinoma of both ovaries.

No. 10 is a typical cystadenocarcinoma cluster. It was found in an endometrial smear. Sections of the tumor were diagnosed as a

papillary cystadenocarcinoma of the ovary without metastasis to the tubes or uterus.

Nos. 11 and 12 show two clusters of cells found in an endometrial smear from a case of cystadenocarcinoma of the ovary. Both groups, like the one illustrated in No. 10, exhibit the characteristic crowding and overlapping of ovarian cystadenocarcinoma cells. No uterine or tubal metastasis was found in this case. Another cell group from this case is shown in A X, 12.

The cells of No. 13 show necrotic changes characteristic of degenerating endometrial adenocarcinoma cells. (Compare with A X, 7.) They were found in the endometrial smear of a case of early unsuspected adenocarcinoma of the endometrium. No abnormal cells were noted in the vaginal smear. Necrotic cells of this type are very characteristic and have a high diagnostic value.

No. 14 is a typical cluster of adenocarcinoma cells found in a vaginal smear of a case of a relatively early adenocarcinoma of the endometrium. The primary diagnosis was by smears.

The group of adenocarcinoma cells illustrated in No. 15 was present in an endocervical smear of a woman with a primary adenocarcinoma of the tube. Curettage performed because of the cytologic suspicion of an adenocarcinoma of the endometrium or endocervix was negative. Repeat smears taken 23 days after curettage gave further cytologic evidence of malignancy. Total hysterectomy performed 2 days later because of the persistent positive smear findings revealed a primary adenocarcinoma of the tube. It is to be noted that in both sets of smears the malignant cells were found in the endocervical smears, whereas the vaginal were negative.

Cells from a malignant hydatidiform mole are depicted in Nos. 16 and 17. The cells have an atypical structure but do not display the variations in size and extreme degenerative changes seen in tumors developing from trophoblastic or decidual elements. (Compare with A X, 16.)

No. 18 illustrates a bizarre fragment of endometrial tissue found in an endocervical smear on the 10th day of the menstrual cycle. The patient had excessive and prolonged menstrual bleeding. This cluster is interpreted as an exfoliated fragment of the endometrial mucosa with portions of several glands attached. Another possible explanation is that it represents an atypical exfoliation resulting from a micropolypoid hyperplasia of the endometrium.

SERIES PLATE

B I

Urinary and Male Genital Systems

Non-malignant and malignant epithelial cells found in urine sediment and prostatic secretion smears

B I
DESCRIPTION

Non-malignant and malignant epithelial cells found in urine sediment and prostatic secretion smears. Drawings x 525.

1. Cells probably from the urethral epithelium. Vaginal smear. Eosin-Water blue series. Female. Age 52. Clinical diagnosis: urinary incontinence.

2–4. Cells from the urethral epithelium. Note the enlargement of the cells in Nos. 3 and 4 and the presence of glycogen in No. 4. Direct smear from the urethral meatus. Glycogen series. Female. Age 48. Clinical diagnosis: urethritis and vaginitis.

5. Transitional cells from the bladder. Resectoscope specimen. OG–EA series. Male. Age 57. No evidence of disease.

6. Transitional cells from the bladder showing some variability in nuclear size. Resectoscope specimen. OG–EA series. Male. Age 63. No evidence of disease.

7. Metaplastic prostatic cells filled with glycogen. Patient receiving estrogenic therapy. Prostatic massage smear. Glycogen series. Male. Age 76. Pathologic diagnosis: fibrosis of the prostate. (Compare with A I, 8.)

8. Superficial squamous cells of the cornified type found in the same prostatic smear as No. 7. Probable origin: squamous portion of the urethra.

9. Small epithelial cells. The group on the right consists of columnar cells. Catheterized urine. OG–EA series. Female. Age 39. Clinical diagnosis: cystitis and urethritis.*

10. Small cells possibly of prostatic origin. Urine after prostatic massage. OG–EA series. Male. Age 52. No evidence of disease.*

11. Cells interpreted as prostatic. Prostatic massage smear. OG–EA series. Male. Age 63. No evidence of disease.*

12. Cells with unusual forms, probably atypical transitional cells. Catheterized urine. OG–EA series. Female. Age 28. Clinical diagnosis: endocrinopathy; also, early spontaneous abortion suspected.

13. Navicular cells characteristic of gestation. Catheterized urine. OG–EA series. Female. Age 24. Normal pregnancy. (Compare with G II, 1–4.)

14. Trichomonad. Note small, elliptic nucleus. Voided urine. OG–EA series. Male. Age 65.

15 and 16. Cells found in voided urine. Trichomonas infestation. The two cells of No. 15 are interpreted as transitional, whereas those of No. 16 have the appearance of squamous urethral cells. (Same case as No. 14.)

17. Highly vacuolated non-malignant bladder cells. Catheterized urine. OG–EA series. Female. Age 45. Diagnosis: sarcoma of the uterus; diabetes mellitus.

18. Atypical cells, probably of prostatic origin. Voided urine. OG–EA series. Male. Age 55. Pathologic diagnosis: adenomatous hyperplasia of the prostate. (Same case as B II, 16.)

19. Atypical cells, probably of prostatic origin, with large and somewhat hyperchromatic nuclei. Voided urine after prostatic massage. OG–EA series. Male. Age 45. Pathologic diagnosis on a transurethral resection specimen: atypical prostatic cells suggestive of malignancy.

20. Corpus amylaceum. Voided urine after prostatic massage. OG–EA series. Male. Age 49.

21. Columnar cells found in a sediment smear prepared from fluid aspirated from a left renal cyst. OG–EA series. Male. Age 53. Pathologic diagnosis: large solitary cyst, benign, of the left kidney.

22. Atypical cells, interpreted as malignant prostatic cells. Voided urine after prostatic massage. OG–EA series. Male. Age 65. Pathologic diagnosis: carcinoma of the prostate.

23. Atypical cells with large nuclei and a relatively small amount of cytoplasm. Origin and nature not yet fully determined. Voided urine. Glycogen series. Male. Age 56. No evidence of malignancy.

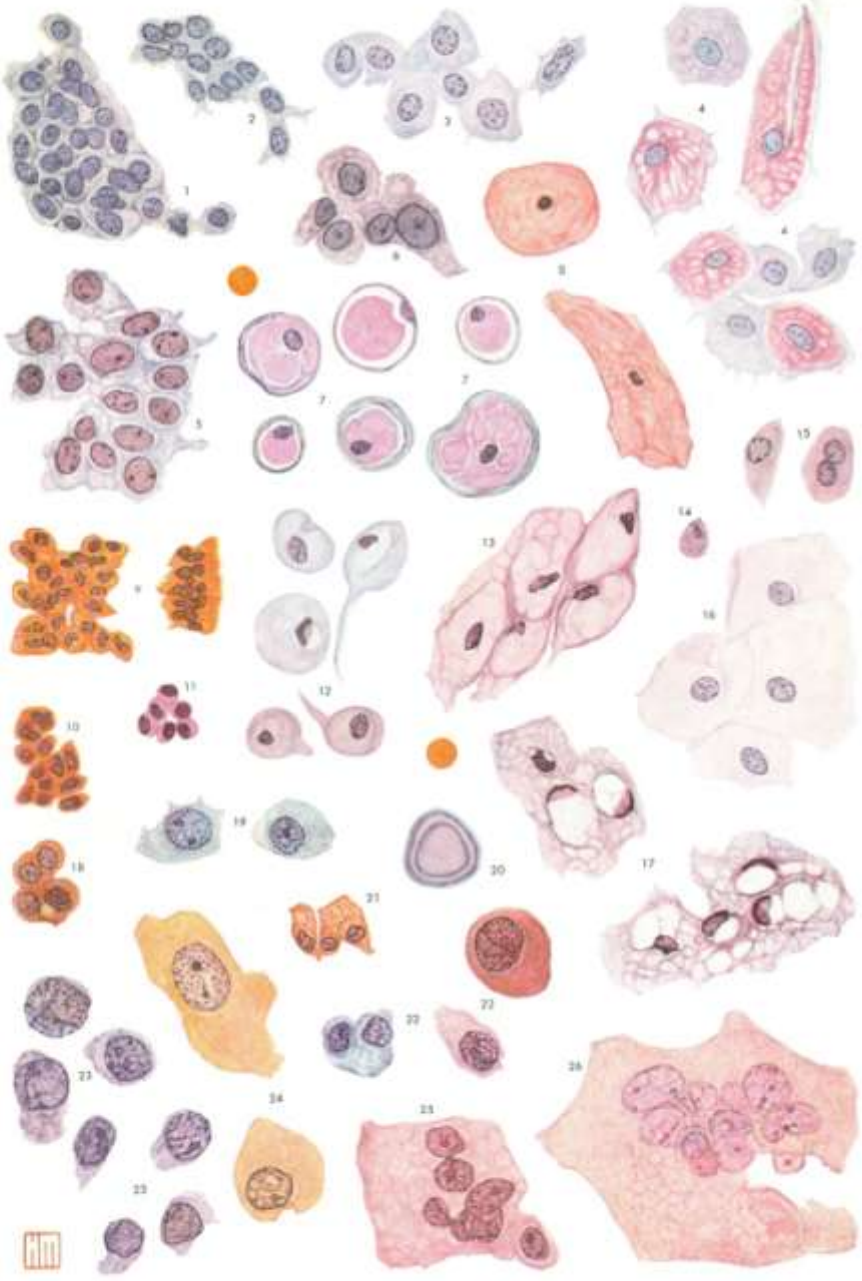
24. Cells with large nuclei showing no abnormal features. Prostatic massage smear. OG–EA series. Male. Age 57. Clinical diagnosis: prostatic hypertrophy.*

25. Multinucleated cell, interpreted as non-malignant. Catheterized urine from the pelvis of the right kidney. OG–EA series. Male. Age 51. Pathologic diagnosis: low-grade carcinoma of the bladder.

26. Multinucleated cell interpreted as a non-malignant cell of a type found in urine specimens of the renal pelvis. Voided urine after prostatic massage. (Same case as No. 22.)

*Courtesy of Strang Prevention Clinic, Memorial Center, New York.

URINARY AND MALE GENITAL SYSTEMS



Normal cells exfoliating from the epithelium of the female urinary and the male urogenital tracts show great variety in size and form depending on their site of origin and hormonal, inflammatory or other factors which may have a modifying effect upon them.

The cells illustrated in Nos. 1 and 2 are interpreted as cells derived from the stratified squamous epithelium of the female urethra. The cellular borders are distinctly seen in No. 1. The cells shown in Nos. 2, 3 and 4 were found in a direct smear from the urethral meatus and are considered to be squamous urethral cells. The larger cells are apparently derived from the more superficial layers and contain glycogen, as reflected in their staining reaction. Many intermediate forms between these cell types were observed in this smear. It should be noted that despite the marked difference in the size of the cells the nuclei show no significant variations in size and structure.

Nos. 5 and 6 represent normal transitional cells of the epithelium of the bladder from a urine specimen obtained by means of a resectoscope. They show marked vacuolation with perinuclear vacuoles. Certain variability in the size of the nuclei is evident, but their structure is characteristic of a normal interphase nucleus. These nuclei contain distinct though small nucleoli, or karyosomes, but the chromatin net is inconspicuous. A similar structure may be seen in the nuclei of the cells illustrated in Nos. 1–4.

No. 7 demonstrates cells loaded with glycogen found in a prostatic secretion smear (compare with corresponding cervical cells shown in A I, 8). Such glycogenic cells, though more frequently seen in prostatic secretions of patients receiving estrogen therapy, should not be considered the result of a specific action, since similar cells may occasionally be observed in cases with no history of estrogen therapy.

Cornified cells corresponding to the superficial squamous cells of the vaginal epithelium may also be found in the urine of male patients during the administration of an estrogen (No. 8). However, cells of this type are probably derived from the stratified squamous portion of the urethra rather than from the prostate or the bladder.

The cells illustrated in No. 9 are probably related to the existing chronic inflammatory condition of the bladder and urethra. However, their origin is not established.

Nos. 10 and 11 show small cells considered to be prostatic in origin. They were found in urine after prostatic massage and in prostatic secretion, respectively. The eccentric position of the nuclei as in No. 11 is not infrequent in prostatic glandular cells.

The cells of No. 10 show some similarity to those of No. 9, although the latter are somewhat larger and show distinct perinuclear vacuolation. Such a perinuclear vacuolation is also shown in the cells of B II, 4, which are from a case of renal calculus and are considered to be of ureteral origin. Structural differences permitting an accurate identification of the cell type and origin are much more difficult to recognize in cells of such small size.

The cells shown in No. 12 were found in the catheterized urine of a woman. They are apparently atypical cells from the superficial cell layers of the bladder. It is, however, difficult to ascertain whether this atypia is due to the hormonal effect of a possible early gestation or to an endocrine imbalance.

The cells of No. 13 are characteristic of pregnancy.[12] They are cells of the navicular type, which are usually filled with glycogen. Similar cells are also seen in vaginal smears during gestation.

A trichomonad found in the voided urine of a male patient is shown in No. 14. It can be identified by its typical form and its small elliptic nucleus. Exfoliated cells from the same case are shown in Nos. 15 and 16. The cells

of No. 15 are probably of the transitional type, whereas those of No. 16 are very likely of urethral origin.

The cells shown in No. 17 are considered to be superficial cells from the bladder showing extreme vacuolation, the cause and significance of which are not clear.

The cells of No. 18 are atypical cells found in a case of adenomatous hyperplasia of the prostate and are thought to be of prostatic origin (see B II, 16, Discussion).

The cells shown in Nos. 19 and 22 were found in voided urine collected after prostatic massage. The cells of No. 22 are distinctly abnormal and consistent with the diagnosis of a carcinoma of the prostate but those of No. 19 are equivocal and resemble cells which are often seen in cases of benign prostatic hypertrophy.

A corpus amylaceum is illustrated in No. 20.

The columnar cells shown in No. 21 were found in fluid aspirated from a cyst of the kidney and are probably lining cells or cells from a papillary projection within the cyst.

Cells of the type shown in No. 23 present certain characteristics suggestive of malignancy, such as an unusually high nuclear-cytoplasmic ratio. They have been found in cases of malignancy as well as in cases in which no evidence of malignancy could be obtained. Their nature, origin and significance are therefore still obscure. One of their distinctive traits is the scantiness of the cytoplasm and its frequent concentration toward one pole of the cell. Some have a resemblance to spermatogonia or spermatocytes, but the fact that similar cells have also been seen in the urine of women does not support the hypothesis of a testicular origin.

The cells illustrated in No. 24 exhibit cellular and nuclear hypertrophy; yet the nuclear structure is apparently normal. They were found in a prostatic secretion smear from a case of prostatic hypertrophy. Cells of this type should not be considered malignant unless they have distinctly abnormal nuclei.

No. 25 shows a multinucleated cell found in a urine specimen aspirated from the pelvis of the kidney. Though the patient had a low-grade carcinoma of the bladder, this cell appears to correspond to the aberrant though non-malignant multinucleated cells which are frequently encountered in ureteral specimens.

The multinucleated cell illustrated in No. 26 was found in a voided urine specimen collected after prostatic massage. Such cells resembling those found in ureteral specimens are sometimes seen in voided urine after massage. However, the fact that they are, as a rule, absent from corresponding prostatic secretion specimens indicates that they are of a ureteral or pelvic rather than a prostatic origin. The fading of the nuclei in this case is probably due to degeneration.

SERIES PLATE

B II

Urinary and Male Genital Systems

Non-malignant and malignant cells found in specimens from the female urinary and male urogenital tracts

B II
DESCRIPTION

Non-malignant and malignant cells found in specimens from the female urinary and male urogenital tracts. Drawings x 525.

1. Normal transitional cells. Catheterized left ureteral urine. OG–EA series. Female. Age 35. Clinical diagnosis: pyelonephritis.

2 and 3. Non-malignant cells. Ureteral urine. OG–EA series. Female. Age 38. Clinical diagnosis: hematuria, no evidence of malignancy.

4. Atypical, non-malignant cells with hyperchromatic nuclei. Voided urine. Glycogen series. Male. Age 48. Operative diagnosis: renal calculus.

5. Cluster of cells showing variations in size and structural detail of the nuclei bordering on malignancy. Right ureteral specimen. OG–EA series. Male. Age 59. Pathologic diagnosis: papillary adenoma of the right kidney.

6–8. Non-malignant multinucleated cells. Right ureteral specimen. Glycogen series. Female. Age 50. Pathologic diagnosis: nephrosis and hemorrhage in the tubules of the kidney. (Compare with G IV, 4.)

9–13. Non-malignant cells showing nuclear enlargement and multinucleation but normal nuclear structure. Right ureteral specimen. OG–EA series. Male. Age 53. Pathologic diagnosis: large, benign cyst of the kidney.

14 and 15. Cells found in ejaculated semen. Condom specimen. OG–EA series. Male. Age 40. No. 15 shows marked vacuolation of the cells. Pathologic diagnosis: carcinoma of the testis.

16. Atypical cells. Voided urine. OG–EA series. Male. Age 55. Pathologic diagnosis: adenomatous hyperplasia of the prostate. (Same case as B I, 18.)

17. Non-malignant multinucleated cell found in the epithelial lining of a collective tubule in a section of a normal kidney. (Compare with Nos. 6, 7 and 8.) Hematoxylin and eosin.

18. Malignant cells. Voided urine. OG–EA series. Male. Age 78. Pathologic diagnosis: carcinoma of pseudoepidermoid type originating in the penile urethra.

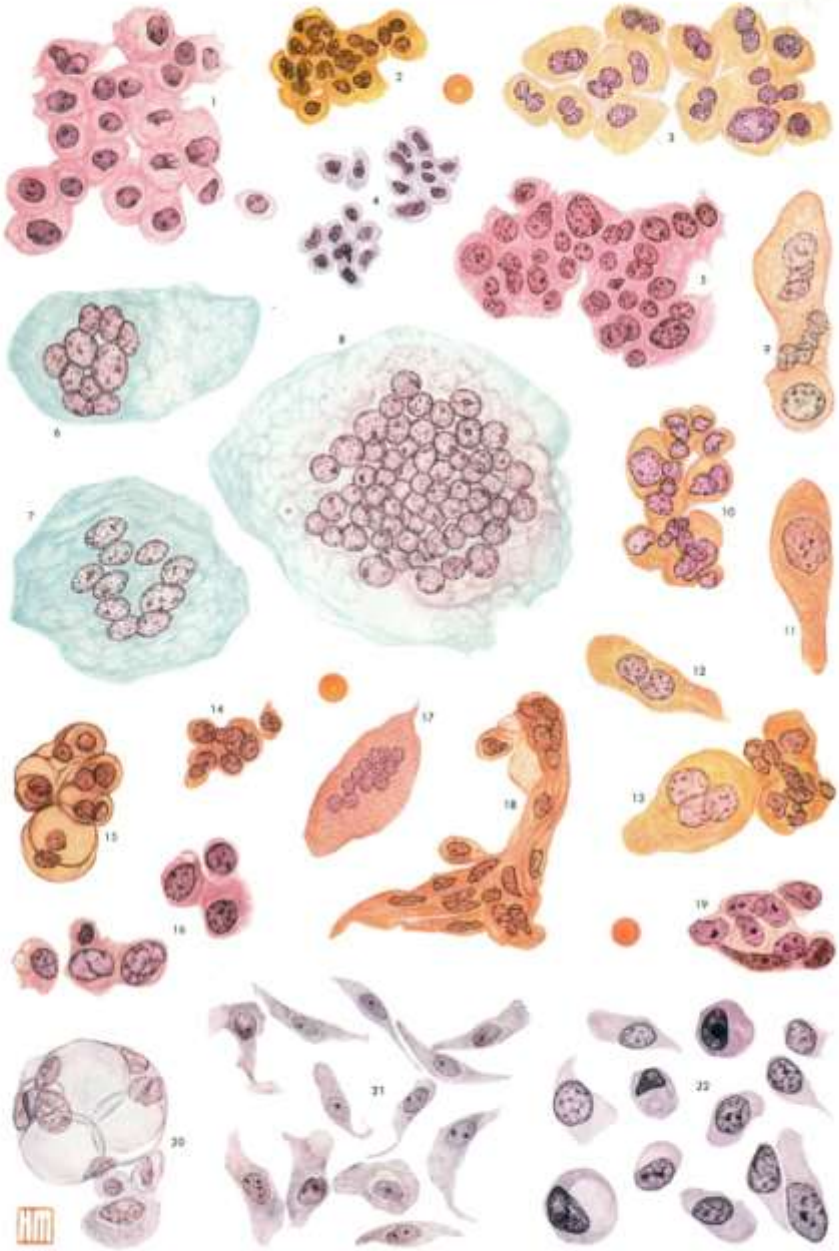
19. Malignant cells found in a smear from an aspirate of the corpus cavernosum of the penis. OG–EA series. Male. Age 39. Pathologic diagnosis: metastatic embryonal cell carcinoma originating in the right testicle.

20. Cells, interpreted as non-malignant, showing extreme vacuolation. Right ureteral specimen. Glycogen series. Male. Age 52. Clinical diagnosis: hematuria, no evidence of malignancy.

21. Cells, characteristic of a papillary growth, from a resectoscope urine specimen in which non-malignant cells of this type were intermixed with cells showing nuclear changes suggestive of malignancy. OG–EA series. Male. Age 74. Pathologic diagnosis: papillary carcinoma of the bladder.

22. Cells characteristic of a papillary growth with malignant transformation. Resectoscope specimen. OG–EA series. Male. Age 55. In this case, as in the previous, both benign and malignant cells were intermixed in the smear. The tumor was diagnosed originally as a papilloma. However, a recurrent tumor, 13 months later, was diagnosed as a papillary carcinoma of the bladder.

URINARY AND MALE GENITAL SYSTEMS



B II
DISCUSSION

Non-malignant and malignant cells found in specimens from the female urinary and male urogenital tracts

No. 1 shows normal transitional cells from the renal pelvis. Note the perinuclear vacuolation and heavy ectoplasmic zone of the cells.

Nos. 3 and 6–13 illustrate the marked variations in size and in the size and number of the nuclei of cells seen in ureteral specimens. The pronounced nuclear enlargement and the multinucleation are particularly characteristic of cells found in such specimens and may easily lead to a false positive evaluation. In each of the above cases except No. 5 the final diagnosis was definitely negative for malignancy and the nuclei, though varying greatly in size and form, show a distinctly normal structure.

In No. 5 the nuclei show more pronounced atypia and some hyperchromasia, suggesting the presence of a malignant neoplasm. A nephrectomy disclosed the presence of a papillary adenoma of the right kidney. However, in a study of serial sections, a malignant neoplastic cord growing into a lymph vessel was noted by Dr. Irena Koprowska. On the basis of this finding one cannot rule out the possibility of a malignant transformation of the adenoma or a coexisting neoplasm of metastatic origin.

No. 4 illustrates atypical cells present in the voided urine of a patient with a renal calculus. The nuclei are dense and stain deeply but show no other changes that would suggest malignancy.

Nos. 14 and 15 are cells found in ejaculated semen from a case of carcinoma of the testis. The detection of such cells in a specimen of semen in which clusters of epithelial cells are normally absent should arouse a suspicion of a neoplasm, although the cells appear to be well differentiated and without distinctly malignant features.

The cells of No. 16 have atypical and rather suspicious nuclei; yet the diagnosis of a transurethral resection specimen was adenomatous hyperplasia of the prostate. A year later an adenoma of the thyroid was excised; within the next two years two cervical node biopsies were diagnosed as metastatic carcinoma. However, the site of the primary tumor is still unknown.

No. 17 shows a multinucleated cell found in the epithelium of a collective tubule in a section of a normal kidney. The cell and its nuclei are considerably smaller than corresponding cells observed in ureteral urine.

The cluster illustrated in No. 18 consists of atypical epidermoid cells. It was found in the voided urine of a case of carcinoma of pseudoepidermoid type originating in the penile urethra.

No. 19 depicts a group of malignant cells found in an aspirate of the corpus cavernosum of the penis in a case of embryonal cell carcinoma metastatic from the right testicle.

No. 20 shows a cluster of highly vacuolated cells found in a ureteral specimen from a negative case. The normal structure of the nuclei indicates the benign nature of the cells despite their conspicuous vacuolation (compare with A X, 12).

Nos. 21 and 22 illustrate columnar cells found in resectoscope urine specimens from two cases of papillary growth of the bladder. The cells of No. 21 have normal nuclei and are interpreted as non-malignant though found intermixed with malignant cells in the smear of a case diagnosed pathologically as papillary carcinoma.

The cells of No. 22, which show definitely malignant characteristics, were found in the smear of a case diagnosed clinically and pathologically as a benign papilloma of the bladder. However, a recurrent tumor removed 13 months later was diagnosed pathologically as a papillary carcinoma. This case suggests that the malignant nature of a papilloma may sometimes be detected by cytologic smears which contain cells exfoliated from the entire surface of the tumor when a biopsy or section of the tumor may occasionally fail to include the malignant area.

SERIES PLATE

B III

Urinary and Male Genital Systems

Cells found in urine sediment smears, from cases of malignancy with the exception of No. 18

B III
DESCRIPTION

Cells found in urine sediment smears, from cases of malignancy with the exception of No. 18. Drawings × 525.

1–5. Voided urine. OG–EA series. Male. Age 58. Pathologic diagnosis: carcinoma of the bladder. Note crowding and engulfment of cells.

6. Voided urine. OG–EA series. Male. Age 73. Pathologic diagnosis: carcinoma of the bladder. Primary diagnosis by smears.

7. Ureteral urine. Glycogen series. Female. Age 53. Pathologic diagnosis: tubular carcinoma of the kidney, clear cell type.

8–10. Catheterized urine. OG–EA series. Male. Age 75. Pathologic diagnosis: metaplastic transitional cell carcinoma of the bladder.

11 and 12. Voided urine. Glycogen series. Male. Age 76. Pathologic diagnosis: carcinoma of the bladder. Two cells show cytoplasmic inclusions.

13. Catheterized urine. OG–EA series. Male. Age 66. Pathologic diagnosis: carcinoma of the bladder.

14. Voided urine. OG–EA series. Male. Age 71. Pathologic diagnosis: papillary carcinoma of the bladder.

15. Right ureteral urine. Glycogen series. Female. Age 70. Pathologic diagnosis: carcinoma in situ of the renal pelvis. Primary diagnosis by smears.

16. Ureteral urine. Glycogen series. Male. Age 62. Pathologic diagnosis: carcinoma of the left ureter, transitional cell type.

17. Voided urine. OG–EA series. Male. Age 57. Pathologic diagnosis: recurrent clear cell carcinoma of the kidney.

18. Normal mitotic figure. Right ureteral urine. Glycogen series. Female. Age 37. Clinical diagnosis: right ureteral calculus.

19. Voided urine. OG–EA series. Male. Age 75. Clinical diagnosis: carcinoma of the prostate.

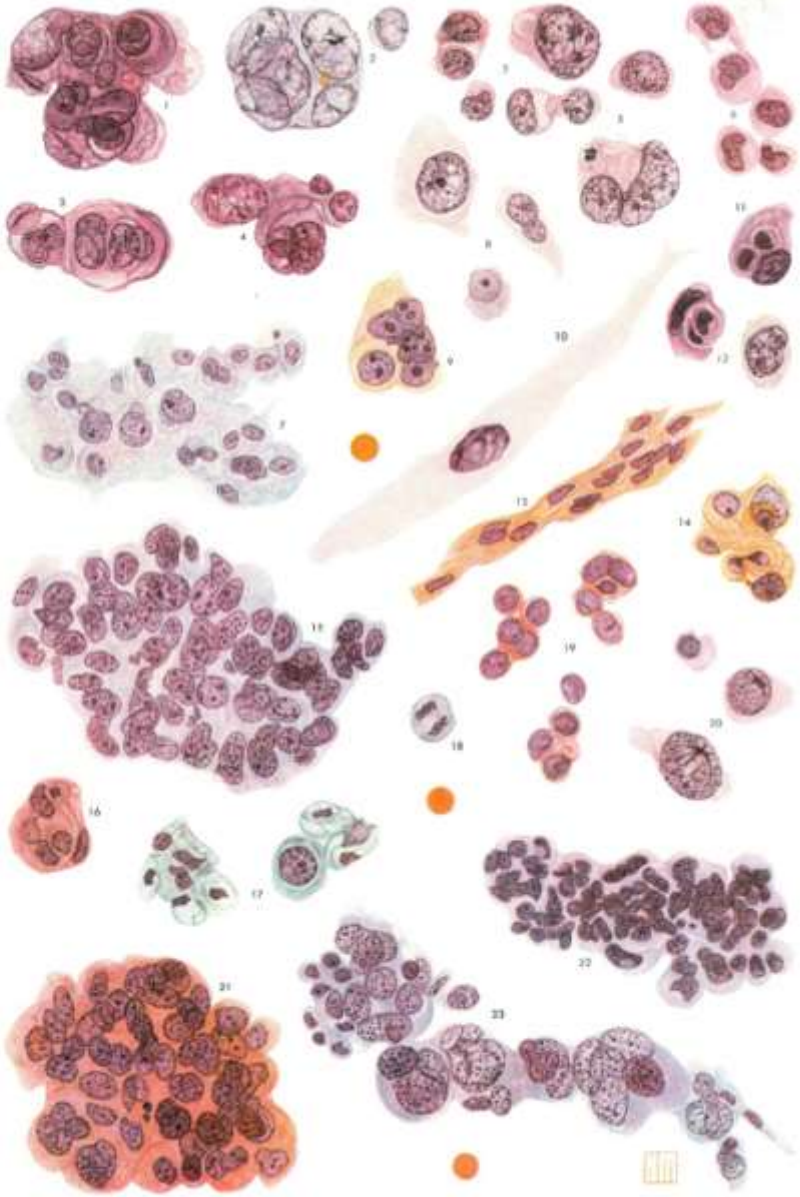
20. Voided urine after prostatic massage. Glycogen series. Pathologic diagnosis: multiacinar carcinoma of the prostate. Note the indication of chromatin polarization in the two large nuclei.

21. Voided urine. OG–EA series. Female. Age 56. Pathologic diagnosis: transitional cell carcinoma of the renal pelvis. Primary diagnosis by smears. Note similarity of cell cluster in No. 15.

22. Voided urine. Glycogen series. Male. Age 63. Pathologic diagnosis: adenocarcinoma of the prostate.

23. Same case as No. 22. Voided urine specimen taken 7 months later, during which period the patient was receiving estrogen therapy.

URINARY AND MALE GENITAL SYSTEMS



B III
DISCUSSION

Cells found in urine sediment smears, from cases of malignancy with the exception of No. 18

Nos. 1–6 and 8–14 show cells from carcinomas of the bladder. The cells exhibit great variability in size and form depending upon the type of the tumor and its developmental stage. Nos. 1–4 show marked nuclear enlargement, hyperchromasia, multinucleation, crowding and engulfment. The nuclei in No. 5 are well preserved and illustrate a characteristic nuclear pattern with prominent, irregular chromocenters confluent with a heavy, granular chromatin network. A similar nuclear pattern is shown in two cells of No. 20 with a chromatin arrangement suggestive of polarization and furrowing. The nuclei of No. 9 have a finely granular appearance with distinct true nucleoli. The highly hyperchromatic appearance of many of the malignant nuclei may be due to a degenerative change.

A metaplastic giant cell is shown in No. 10. The group illustrated in No. 13 exhibits stratification which is suggestive of an epidermoid change.

No. 6 is of particular clinical interest in that the tumor, which was small and concealed in a diverticulum of the bladder, was not noted at cystoscopy. The primary diagnosis of carcinoma was made on the basis of exfoliated cells found in a voided urine specimen.

No. 7 illustrates a cluster of malignant cells found in a sediment smear of ureteral urine. Subsequent nephrectomy proved the presence of a tubular carcinoma of the kidney of the clear cell type. At the last examination the patient was alive and well, approximately 5 years after operation.

Cells of a clear cell carcinoma of the kidney found in a voided urine 10 months after a right nephrectomy are shown in No. 17. Clinically there was a recurrence in the region of the right kidney. The cells of both Nos. 7 and 17 show pronounced vacuolation.

The clusters illustrated in Nos. 15 and 21 have a remarkable similarity in structure. Both are from cases of transitional cell carcinoma of the renal pelvis detected primarily by cytologic examination.

No. 15 was found in a ureteral specimen from a case of carcinoma in situ which was described by Foot and Papanicolaou.[17]

No. 21 was found in voided urine in what proved to be a more advanced case. Further cytologic exploration disclosed the presence of malignant cells in catheterized urine specimens. A provisional diagnosis of non-functioning right kidney and pyonephrosis was made on the basis of intravenous and retrograde pyelograms.

A nephrectomy performed largely on the repeated positive cytologic findings showed the presence of a transitional cell carcinoma of the renal pelvis. These cases as well as others of the same type indicate that exfoliation is fairly prolific in transitional cell carcinomas of the renal pelvis and that it begins early.

The cluster in No. 16 is from a ureteral specimen of a case of an early carcinoma of the left ureter, transitional cell type, with no evidence of invasion of the muscular coats. The primary diagnosis in this case was also on the basis of cytologic evidence.

No. 18 illustrates the anaphase stage of a normal mitotic division of a cell found in a ureteral specimen from a case of a right ureteral calculus.

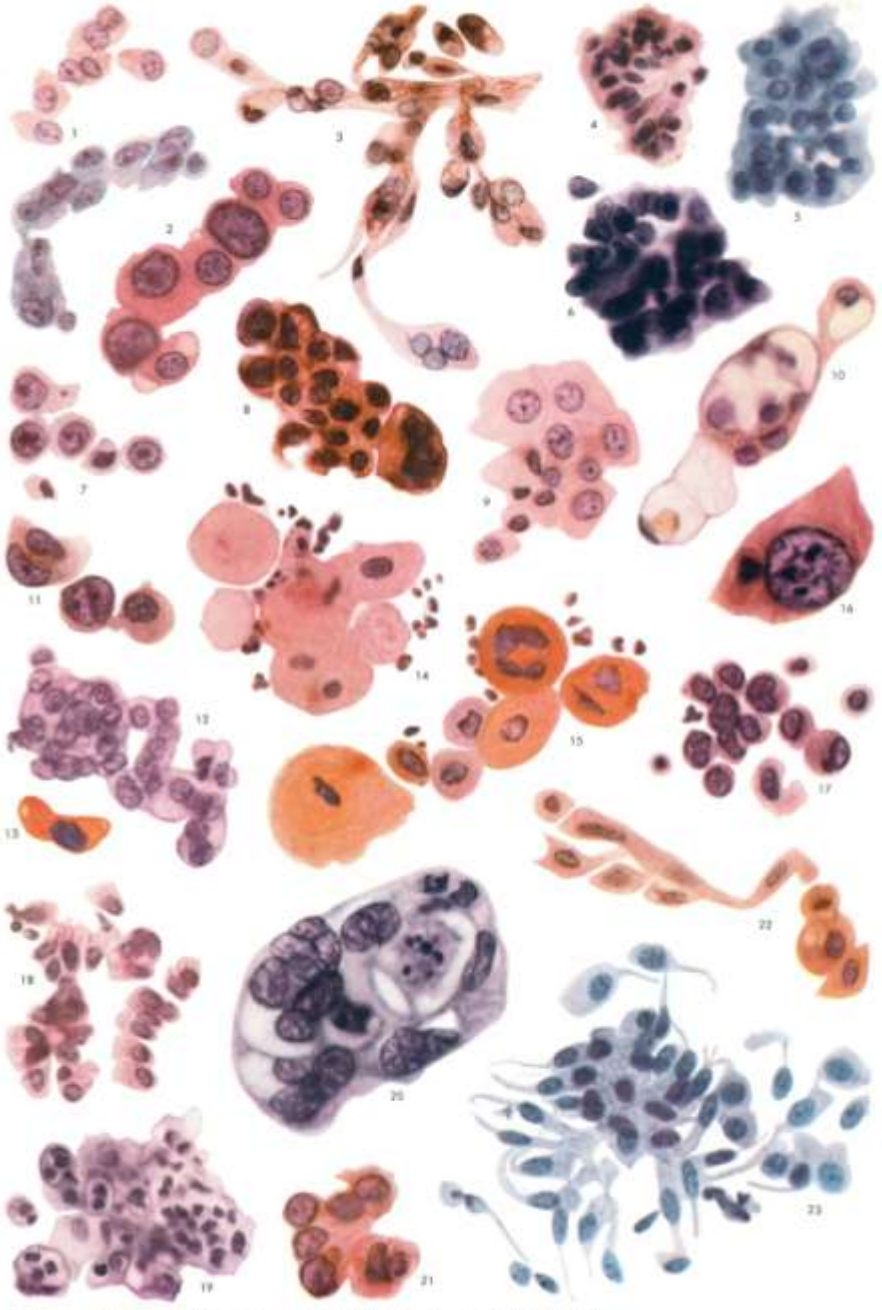
The wide variation in the cytology of prostatic carcinoma can be seen by comparison of Nos. 19, 20, 22 and 23. The case illustrated in Nos. 22 and 23 is of special interest in that smears were examined before (No. 22) and 7 months after (No. 23) the administration of estrogens. Extreme pleomorphism is shown in No. 23, with some cells within the cluster comparable in size with those of No. 22, while others exhibit marked hypertrophy. Although there is no direct evidence, one might be tempted to attribute the cytologic pattern of the second specimen to the stimulus of estrogen therapy.

SERIES PLATE

B IV

Urinary and Male Genital Systems

Malignant and non-malignant cells found in urine sediment smears



BIV
DISCUSSION

Malignant and non-malignant cells found in urine sediment smears

Cells originating in carcinomas of the kidney are shown in Nos. 1 and 3. In both cases a final diagnosis of primary carcinoma of the kidney was made. Although the configuration of the clusters differs in the two cases, the similarity in the type of the cells is evident. The cells are relatively small with some vacuolation and a tendency toward nuclear eccentricity. Those of No. 3 show irregularity in form and multinucleation.

The diagnosis of malignancy is very difficult when anisokaryosis and hyperchromasia or other general criteria of malignancy are absent as in the upper group of No. 1. The accurate evaluation of such a group of cells would be practically impossible for any one who had not acquired a familiarity with cells of this particular type.

No. 2 illustrates a cluster of malignant cells from a papillary epidermoid carcinoma of the ureter secondary to a papillary transitional cell carcinoma of the kidney, Grade II. The malignancy in this case is evidenced by general criteria. The difference in the cytology between the cells of this cluster and those of Nos. 1 and 3 is apparent.

The three groups of cells shown in Nos. 4, 5 and 6 were found in a smear from a voided urine specimen taken after prostatic massage. The pathologic diagnosis on a transurethral resection specimen was nodular hyperplasia of the prostate. The cytology of cluster No. 4 is consistent with this diagnosis; however, clusters Nos. 5 and 6 exhibit characteristics suggestive of a malignant transformation, such as nuclear enlargement and hyperchromasia, irregularity of pattern and crowding. A biopsy performed 6 years later because of persistent symptoms was diagnosed as carcinoma of the prostate.

Cells from various types of carcinoma of the prostate are shown in Nos. 7, 8 and 11–15.

In Nos. 7, 8 and 11 the cytologic picture is basically the same and is consistent with a glandular cell type. No. 12 consists of more differentiated cells and is another example of a neoplastic cell type which can be diagnosed by specific rather than by general criteria. The single cell from the same case shown in No. 13 exhibits more distinct general criteria of malignancy such as an atypical form and large, hyperchromatic nucleus.

The cells illustrated in Nos. 14 and 15 show extreme squamous metaplasia and closely resemble cells, including the characteristic ghost cells, found in sputum and bronchial specimens from cases of bronchogenic epidermoid carcinomas. In this particular case the diagnosis was primary squamous cell carcinoma of the prostate with extension to the bladder.

The cells of Nos. 16 and 17, which were found in voided urine, suggest a primary carcinoma of the prostate; the pathologic diagnosis was carcinoma of the bladder, secondary to the prostate. The close similarity of the cells of No. 17 to those of 7 and 11 is apparent.

The cells illustrated in No. 18 show no malignant characteristics and were interpreted as suggestive of a benign papillary growth. However, no thorough exploration of the urinary bladder was made, and the patient died 10 months later with a final diagnosis of hypertension and multiple myeloma. No autopsy was performed.

A group of cells heavily infiltrated by leucocytes is shown in No. 19 and is consistent with a diagnosis of chronic cystitis. In this case, however, a bladder biopsy was performed 9 days before the urine specimen was taken and might have caused the heavy leucocytic infiltration. Such an infiltration has been noted in smears taken from other organs shortly after an operative procedure, as in smears from the female genital tract following curettage (A VIII, 17 and 19).

The cluster in No. 20 is from a papillary carcinoma of the bladder and exhibits many of the general characteristics of malignancy.

The group of malignant cells shown in No. 21 is from a case of carcinoma of the rectum extending to the bladder, with a fistula opening from the rectum into the bladder. These cells were observed in voided urine, and it would be difficult to determine whether they were derived from the bladder lesion or came, instead, indirectly from the rectum through the fistula.

The cells of No. 22, found in a catheterized urine specimen, are distinctly of the epidermoid type. The patient had a proved epidermoid carcinoma of the cervix with no evidence of extension to the bladder. However, the possible origin of these cells from the cervical lesion cannot be ruled out, as they could have been introduced into the bladder at catheterization.

The cells illustrated in No. 23 are of the columnar type and were found in a direct urethral smear. They are consistent with the clinical diagnosis of a benign urethral polyp.

SERIES PLATE

C 1

Respiratory System

Non-malignant cells found in sputum and bronchial washings

C1
DESCRIPTION

Non-malignant cells found in sputum and bronchial washings. Drawings x 525.

1, 4, 7, 10 and 15. Ciliated columnar cells. Bronchial washings. OG–EA series except No. 4, which is glycogen series.

2 and 11. Binucleated ciliated cells. Bronchial washings. OG–EA series.

3. Ciliated columnar cells found in a direct smear from the vocal cords. OG–EA series.

5, 8 and 9. Ciliated columnar cells showing polarization and protrusion of chromatin. Bronchial washings. OG–EA series except No. 5, glycogen series.

6. One ciliated and one goblet cell. Bronchial washing. Glycogen series.

12. Ciliated columnar cell with relatively large and atypical nucleus. Bronchial washing. OG–EA series.

13, 14 and 18. Multinucleated ciliated cells. In No. 18 there is indication of a fusion of the nuclei. Bronchial washings. Glycogen series except No. 18, which is OG–EA series. Clinical diagnosis: No. 13, bronchiectasis and pneumonitis; No. 14, multiple lung abscesses; No. 18, bleeding of undetermined origin.

16. Atypical cell interpreted as a degenerated ciliated cell showing more advanced fusion of multiple nuclei. Sputum. OG–EA series. Clinical diagnosis: cyanosis and dyspnea, undetermined etiology.

17. Two ciliated cells, one showing three atypical nuclei and a prominent nucleolus. Bronchial washing. Glycogen series. Pathologic diagnosis: bronchiectasis and chronic interstitial pneumonia.

19. Columnar cells of the goblet type. Bronchial washing. OG–EA series.

20–22. One ciliated and two mucoid cells from a nasopharyngeal washing. The nucleus of the ciliated cell (No. 20) shows a chromatin protrusion ("nipple"). OG–EA series.

23. Ambiguous cell which might be interpreted either as a smooth muscle cell or an extremely elongated histiocyte. Bronchial washing. Glycogen series. Pathologic diagnosis: chronic bronchitis.

24. Dense group of cells of the ciliated type. Sputum. OG–EA series.

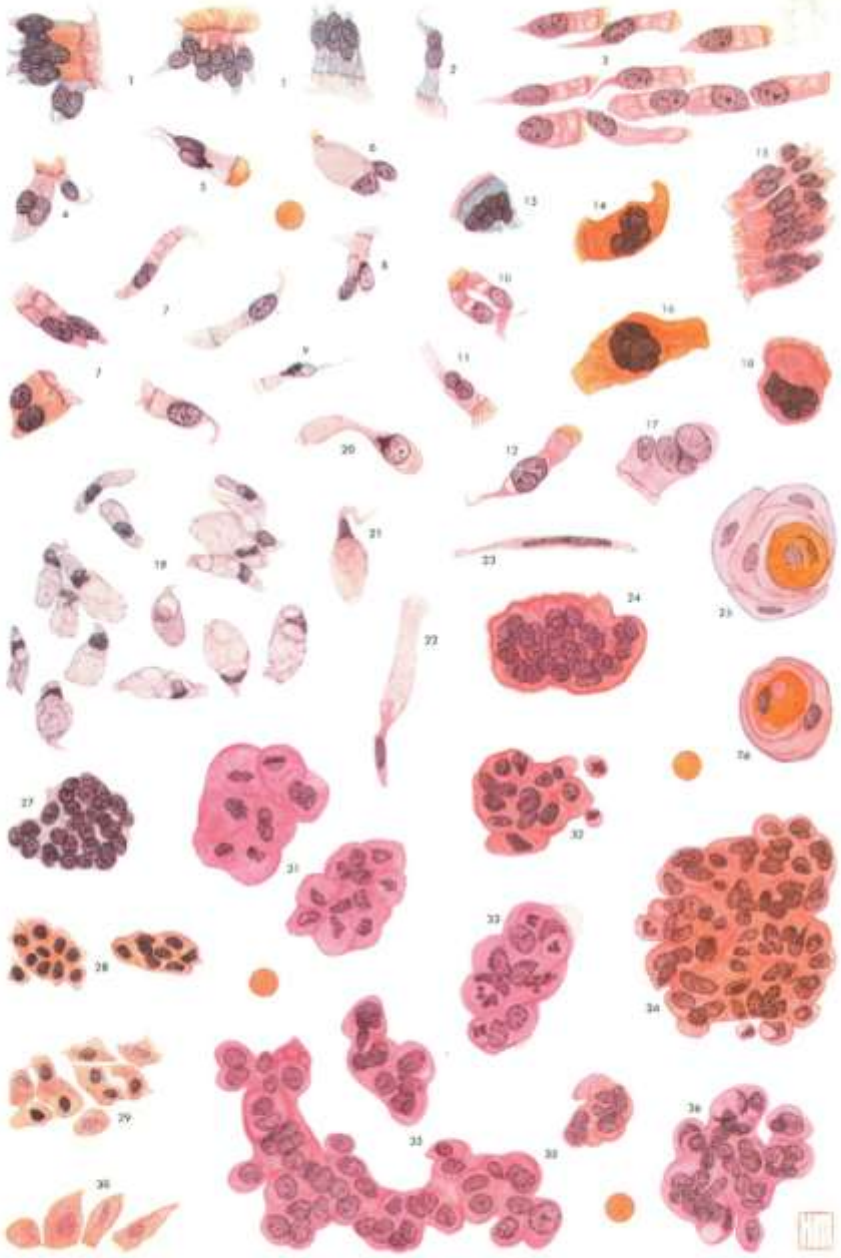
25 and 26. Epithelial pearls. Sputum. Glycogen and OG–EA series respectively. No. 25, no definite diagnosis; No. 26, no evidence of malignancy.

27. A group of cells interpreted as basal, undifferentiated cells of the bronchial mucosa. Bronchial washing. Glycogen series. Final diagnosis: bronchiectasis and pneumonitis.

28. Small elliptic cells with pyknotic nuclei designated in our laboratory as "Pap" cells. Sputum. OG–EA series. Age 65. Pathologic diagnosis: upper respiratory tract infection.

29 and 30. Cells of the same type as those of No. 28. The fading of the nuclei is interpreted as an early necrotic change. Sputum. OG–EA series. Age 59. Pathologic diagnosis: bronchopneumonia of unknown origin.

31–36. Clusters of cells from cases of bronchiectasis.
31. Sputum. Age 47.
32. Bronchial washing. Age 50.
33 and 34. Sputum. Age 69. Also a history of recurrent pneumonia.
35 and 36. Bronchial secretion. Age 7.



C I
DISCUSSION

Non-malignant cells found in sputum and bronchial washings

Normal ciliated cells from the bronchial mucosa are seen in Nos. 1, 4, 7, 10 and 15. The most characteristic features of these cells are the cilia, the heavy cuticular border, the round to oval granular nucleus, and the perinuclear and distal cytoplasmic vacuolation. Binucleation (Nos. 2 and 11) and multinucleation (Nos. 13, 14 and 18) are not infrequent, the latter possibly resulting from the fusion of cells and, sometimes, of their nuclei. Cells of this type which have lost their ciliated border (No. 16) may be easily misinterpreted as malignant because of the large size and deep staining of the nucleus. However, the multiple nature of the nucleus is indicated by its distinct lobulation. Multinucleation may be observed in malignant as well as non-malignant cells. Its specific diagnostic significance has not as yet been determined. Multinucleated cells sometimes possess anisometric nuclei (No. 17). However, such anisokaryosis in a well-differentiated ciliated cell is not necessarily indicative of malignancy.

Ciliated cells from other regions of the respiratory tract such as the laryngeal (No. 3) and the nasopharyngeal (No. 20) exhibit forms which differ from that of the ciliated cells of the bronchial mucosa. Those of the laryngeal area appear to be stouter, whereas those from the nasopharynx have a tendency to be taller.

The distal nipple-like protrusion of the chromatin in the ciliated cells of the respiratory tract (Nos. 5, 8, 9 and 20) is similar to that seen in ciliated cells of the female genital tract (see A III, 1, 6 and 9).

Columnar cells of the mucoid type are shown in Nos. 19, 21 and 22. Those illustrated in 19 and 21 are characteristic of the goblet cell type.

The cell illustrated in No. 23 shows a certain similarity to a smooth muscle fibre, although the possibility that it may be an elongated histiocyte cannot be ruled out. Histiocytes exhibiting extreme elongation often resemble fibrous connective tissue cells or smooth muscle fibers.

Dense clusters of ciliated cells (No. 24) can easily be misinterpreted as clusters of the type found in bronchiectasis (Nos. 32, 34, 36 and C V, 1) or even as neoplastic cells (C V, 3). Their peripheral ciliation and the normal and uniform structure and size of the nuclei are criteria by which the type and benign character of the cells can be recognized.

Epithelial pearls are a relatively rare occurrence in sputum and bronchial washing specimens but may be observed in non-malignant as well as malignant cases. Although some morphologic differences appear to exist between the malignant and non-malignant types (compare Nos. 25 and 26 with C II, 15 and 16), a distinction between the two is not always possible.

Clusters of reserve cells from the deeper layers of the bronchial mucosa (No. 27) are also apt to be misinterpreted as malignant cells of the anaplastic or oat cell type (see C V, 9–12). The normal nuclear structure and size are the best criteria for their identification.

A very characteristic cell type often encountered in sputum and bronchial washing smears is that designated in our laboratory as the "Pap" cell. The name owes its origin to the fact that cells of this type were first observed in the author's sputum taken during a period of a prolonged chronic inflammation of the upper respiratory tract. The most typical of these cells are relatively small and have an elliptic form and an oval pyknotic nucleus. They may appear singly or in compact clusters (No. 28). Fading of the nucleus is not an infrequent occurrence and is a rather characteristic feature contributing to the identification of cells of this type (Nos. 29 and 30).

The origin and diagnostic significance of "Pap" cells is still obscure. They may be squamous parakeratotic cells or may represent a metaplasia occurring in localized regions of

the bronchial mucosa as a result of irritation caused by chronic inflammation or virus and mycotic infections. Cells of this type, as a rule, show little variation in size, although close relationship with somewhat larger squamous cells has been noted in smears from some cases, giving the impression of a metaplastic process. In still other cases, "Pap" cells have been found in the same smear with malignant cells in proved cases of malignancy, but no definite indication of transition from the "Pap" cell to the malignant cell type has been noted.

The groups of cells illustrated in Nos. 31–36 were found in cases of bronchiectasis. The grouping pattern of the clusters resembles that of neoplastic cells. The normal structure and relatively uniform size of the nuclei are features revealing the benign nature of the cells. Infiltration by leucocytes may be observed in some cases in which there is a superimposed infection. When metaplasia is present (C I, 35) the evaluation is more difficult (compare with C II, 3 and C III, 6). It should be noted that characteristic cell groups such as the ones which are illustrated here are found in only a relatively small number of cases of bronchiectasis.

SERIES PLATE

C II

Respiratory System

Malignant cells and foreign cells found in sputum and bronchial washings or in tissue sections

C II
DESCRIPTION

Malignant cells and foreign cells found in sputum and bronchial washings or in tissue sections. Drawings x 525.

1 and 2. Clusters of cells found in sputum (No. 1). Clusters of cells from a histologic section of the tumor (No. 2). Pathologic diagnosis: alveolar cell carcinoma. (Courtesy of Dr. Nathan Freedman and Dr. Morris Simon, Jewish General Hospital, Montreal, Canada.)

3. Sputum. OG–EA series. Male. Age 68. Pathologic diagnosis: anaplastic epidermoid carcinoma.

4 and 5. Sputum. OG–EA and glycogen series respectively. Male. Age 53. Pathologic diagnosis: oat cell carcinoma. (Same case as C V, 11 and 12.)

6. Sputum. OG–EA series. Male. Age 52. Pathologic diagnosis based on a biopsy of a supraclavicular node: Malignant thymoma.

7. Sputum. OG–EA series. Male. Age 52. Pathologic diagnosis: adenocarcinoma of the lung. (Same case as C V, 33 and 34.)

8. Sputum. OG–EA series. Female. Age 28. Pathologic diagnosis: chorioepithelioma metastatic to the lung.

9. Sputum. Glycogen series. Female. Age 52. Pathologic diagnosis based on a biopsy of a supraclavicular node: Hodgkin's disease.

10. Bronchial washing. OG–EA series. Female. Age 75. Pathologic diagnosis: adenocarcinoma of the kidney metastatic to the lung.

11. Sputum. OG–EA series. Male. Age 27. Pathologic diagnosis: adenocarcinoma of the rectum metastatic to the lung.

12. Sputum. Cell-like structures, probably vegetable cells. OG–EA series.

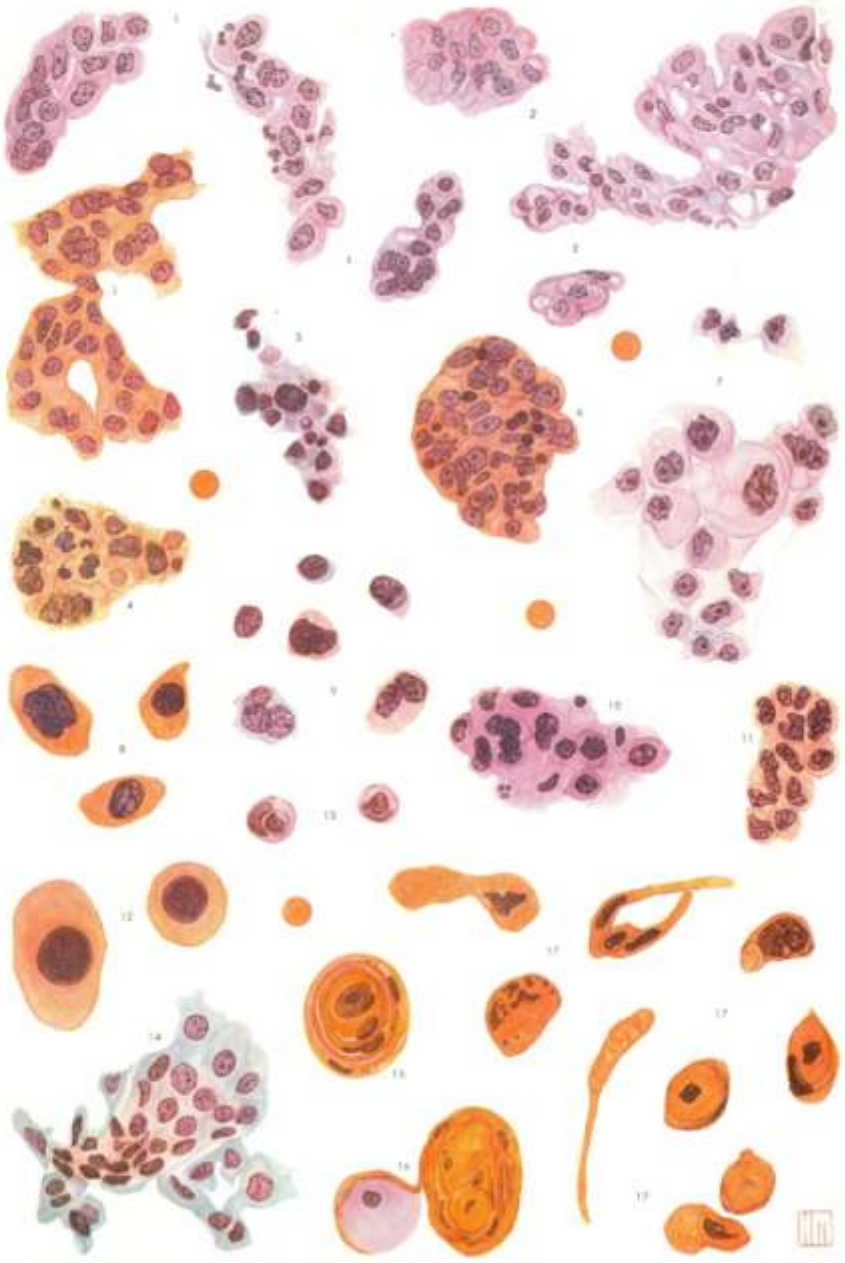
13. Sputum. OG–EA series. Male. Age 50. Pathologic diagnosis: alveolar cell carcinoma, primary diagnosis by smears. (Same case as C III, 4, and G IV, 16.)

14. Sputum. Glycogen series. Male. Age 63. Pathologic diagnosis: epidermoid carcinoma of the epiglottis.

15. Epithelial pearl. Sputum. OG–EA series. Male. Age 65. Pathologic diagnosis: bronchogenic epidermoid carcinoma.

16. Epithelial pearl. Sputum. OG–EA series. Male. Age 73. Clinical diagnosis at death: carcinoma of the lung.

17. Antral washing. OG–EA series. Female. Age 55. Pathologic diagnosis: carcinoma of the left maxillary sinus.



C II
DISCUSSION

Malignant cells and foreign cells found in sputum and bronchial washings or in tissue sections

The three clusters of No. 1 were found in a sputum specimen from a case of alveolar cell carcinoma.[42] No. 2 shows corresponding cells in a tissue section from the same case. The similarity in the cytologic pattern between smears and sections is apparent.

A group of cells from an anaplastic epidermoid carcinoma is shown in No. 3. The malignant character of the cells is not so obvious in this type of tumor as in the oat cell carcinoma, cells of which are illustrated in Nos. 4 and 5. In the latter type one usually finds marked hyperchromasia, irregularities in the size and form of the nuclei, and plasmolysis.

The cluster illustrated in No. 6 was found in the sputum of a case diagnosed as a malignant thymoma. The abnormal pattern and the lymphocytic infiltration may be considered suggestive, but not diagnostic, of the type of the tumor.

A group of cells typical of adenocarcinoma is shown in No. 7. The vacuolation of the cytoplasm and the eccentricity of the nuclei are criteria by which adenocarcinoma cells can be identified.

The cells of No. 8 were found in the sputum of a woman with a proved chorioepithelioma metastatic to the lung, liver and brain. The type of these cells concurs with the pathologic diagnosis.

In neoplasms of the lymphoid type such as Hodgkin's disease, malignant cells often appear singly, scattered throughout the smear. A variety of such cells are illustrated in No. 9. The three upper cells are representative of the lymphocytic type, while the other three are closer to the reticular type (Reed-Sternberg cells). Their malignant character is apparent.

No. 10 represents cells from an adenocarcinoma of the kidney metastatic to the lung. The malignant character of the cells is obvious, but their type is not clearly indicated.

The same remark applies to No. 11, which illustrates a case of adenocarcinoma of the rectum metastatic to the lung.

The two cell-like structures shown in No. 12 were seen in a sputum smear and are probably foreign cells from some vegetable foodstuff. Superficially they show great resemblance to malignant squamous cells, but by closer observation they can be distinguished from human cells by the homogeneity of their "nucleus" and of the "cytoplasm" which surrounds it.

The cells of No. 13 show engulfment of one cell by another, giving the impression of an early pearl formation. Such cells may occasionally be seen in cases of carcinoma of the lung.

No. 14 illustrates a cluster of epidermoid cells from a carcinoma of the epiglottis.

Two typical pearls from cases of carcinomas of the lung are shown in Nos. 15 and 16. Their advanced keratinization as well as their atypical pattern contrast with the two pearls illustrated in C I, 25 and 26, which are interpreted as benign.

Very characteristic malignant epidermoid cells found in antral washings from a case of carcinoma of the left maxillary sinus are illustrated in No. 17. They exhibit a striking similarity to malignant epidermoid cells found in carcinomas of the lung (C IV). The bizarre form and orangeophilia of the cells and the distinctive degenerative pattern of the nuclei give convincing evidence of their malignant nature. Two of the cells show engulfment, like those of No. 13. Two others reveal complete resorption of the nucleus like that of the so-called "ghost" cells found in carcinomas of the lung.

SERIES PLATE

C III

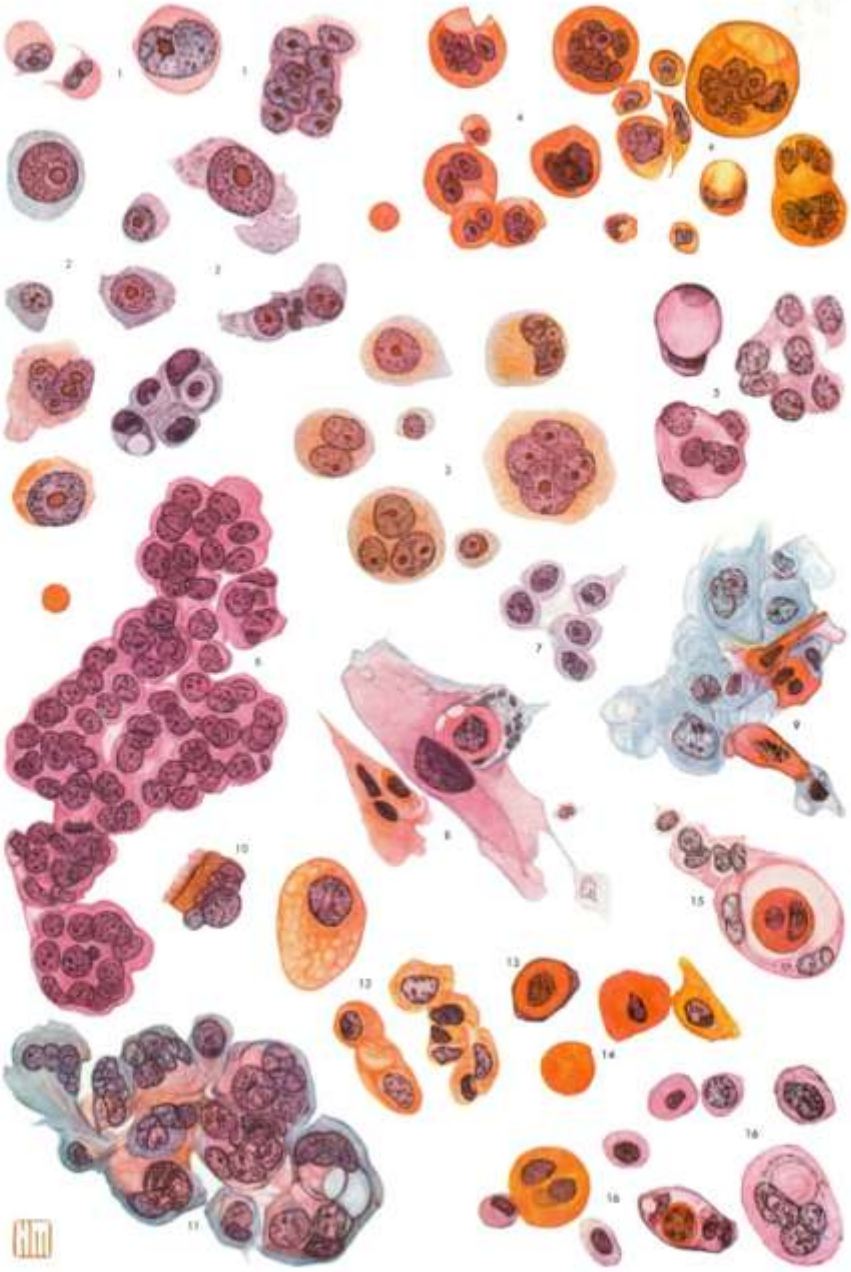
Respiratory System

Cells found in sputum and bronchial washings from cases of malignancy

C III
DESCRIPTION

Cells found in sputum and bronchial washings from cases of malignancy. Drawings x 525.

1. Sputum. Glycogen series. Male. Age 62. Clinical diagnosis based on x-ray and sputum smears: bronchogenic carcinoma (type undetermined).
2. Bronchial washing. Glycogen series. Female. Age 71. Pathologic diagnosis: bronchogenic carcinoma (type undetermined). Primary diagnosis by smears.
3. Sputum. OG–EA series. Male. Age 65. (Case at the Massachusetts General Hospital. Courtesy of Ruth M. Graham and Dr. Robert H. Fennell, Jr.) Sections of the resected right lower lobe were discussed at a conference of the New England Pathological Society. Opinions were divided as to whether the lesion was neoplastic or inflammatory.
4. Sputum. OG–EA series. Male. Age 50. Pathologic diagnosis: alveolar cell carcinoma. Primary diagnosis by smears. (Same case as C II, 13.)
5. Bronchial washing. OG–EA series. Female. Age 31. Pathologic diagnosis: alveolar cell carcinoma. Primary diagnosis by smears.
6. Sputum. Glycogen series. Female. Age 51. Clinical and x-ray diagnosis: bronchogenic carcinoma with metastases. Note one mitotic figure.
7. Sputum. Glycogenic series. Female. Age 73. Clinical and x-ray diagnosis: carcinoma of the lung. (Same case as E I, 30 and 31.)
8 and 9. Sputum. OG–EA series. Male. Age 69. Pathologic diagnosis: bronchogenic epidermoid carcinoma.
10. Cluster of ciliated cells (presumably nonmalignant) showing marked anisokaryosis. Bronchial washing. OG–EA series. Male. Age 56. Pathologic diagnosis: epidermoid carcinoma of the lung. Grade III. (See Discussion of this plate.)
11. Sputum. OG–EA series. Male. Age 43. Pathologic diagnosis: bronchogenic carcinoma, undifferentiated. Primary diagnosis by smears.
12. Sputum. OG–EA series. Male. Age 57. Pathologic diagnosis: carcinoma in situ of bronchus. Primary diagnosis by smears.
13–16. Sputum. OG–EA series. Male. Age 66. Pathologic diagnosis: bronchogenic epidermoid carcinoma.



C III
DISCUSSION

Cells found in sputum and bronchial washings from cases of malignancy

The cells illustrated in Nos. 1, 2 and 3 show certain structural similarities, such as the presence of large true nucleoli and multinucleation. The relationship between the size and number of nucleoli in each nucleus and the relationship between the size of the nucleoli and that of the nuclei are shown in the various cells of No. 2. A similar cytologic picture may be seen in E I, 30 and 31, illustrating cells observed in a specimen of pleural fluid from a patient with a metastatic carcinoma of the lung. Malignant cells from the latter case found in a sputum specimen are shown in No. 7, but the cells are smaller and the nucleoli less prominent.

No definite pathologic diagnosis of the type of the neoplasm was made in any of the above cases. It is therefore impossible to ascertain whether their cytology is representative of the same type of bronchogenic carcinoma. In No. 1 the final diagnosis of carcinoma was based on clinical and x-ray evidence, in addition to that furnished by the cytologic examination of the sputum. In No. 2 two different pathologic interpretations were given to a bronchoscopic biopsy specimen. One was anaplastic squamous carcinoma and the other, adenocarcinoma.

The case illustrated in No. 3 remains practically undiagnosed, as the pathologists were divided in their opinion as to whether it was malignant or not. From the cytologic standpoint there is good evidence in favor of the malignant character of the cells. If one compares them with typical histiocytes (see E I, 2) a histiocytic origin appears to be unlikely. Furthermore, the large number of abnormal mitotic figures noted in cells of this case (see H II, 9, 10 and 11) and a comparison of some of the multinucleated cells from the same case (G IV, 17) with multinucleated histiocytic cells (G IV, 2) give further evidence of the malignant character of this case.

Multinucleation and a relative prominence of nucleoli are also shown in cells of an alveolar cell carcinoma in No. 4. Vacuolation is apparent in some of the cells as well as in some cells of No. 5, which are from another case of alveolar cell carcinoma. The latter case is of particular diagnostic interest in that the lesion, which was diffused throughout both lungs, was originally diagnosed as miliary tuberculosis. The cytologic examination of a bronchial aspiration specimen gave the first indication of the malignant nature of the disease.

The specific type of the carcinoma from which a cell cluster is illustrated in No. 6 has not been determined. On the basis of an aspiration biopsy of a metastatic area in the ilium and the clinical evidence of lung involvement, the pathologic diagnosis was primary bronchogenic carcinoma metastatic to the ilium. The skull and other bones also showed metastatic areas. The cytologic picture may be interpreted as suggestive of a bronchogenic carcinoma of an undifferentiated type.

No. 11 is another example of cells from an undifferentiated (pleomorphic) carcinoma. This is a case of historic interest in that a sputum specimen from this case was the first positive one examined in our laboratory. It was collected by Dr. Henry Cromwell from an undiagnosed case. The evidence of malignancy furnished by the smear was later confirmed by an exploratory thoracotomy which revealed an inoperable carcinoma.

The cytology of the undifferentiated type of bronchogenic carcinoma is in striking contrast to that of the bronchogenic epidermoid type shown in Nos. 8 and 9 and 13–16, as well as in C IV. The variation in size and form of malignant cells of the epidermoid type is much more pronounced, as shown by the presence of extremely aberrant and bizarre forms (C IV). The acidophilia or orangeophilia of the cytoplasm in some cells and the characteristic degeneration, pyknosis and final resorption of the nuclei are other distinguishing criteria of the malignant epi-

dermoid type (Nos. 14–16). Engulfment of one cell by others is shown in Nos. 15 and 16. In No. 15 the engulfing cells appear to be histiocytes phagocytosing malignant cells.

The cluster of ciliated cells in No. 10 was found in a bronchial washing from a case of proved epidermoid carcinoma. It shows one markedly enlarged nucleus; yet the normal structure of the nuclei and the well-differentiated type of the cells give no support to the interpretation of these cells as malignant. Ciliated cells with disproportionate nuclear enlargement are often seen in bronchial washings from non-malignant cases (C I, 17).

The cells illustrated in No. 12 are from a case of carcinoma in situ of the lung.[38] It was through the evidence furnished by the examination of smears that this very early lesion was detected. The clinical picture was that of chronic bronchitis which finally caused the death of the patient. No visible lesion was found at autopsy. The presence of a tumor was established through the examination of serial sections of random samples of the bronchial mucosa. The cytologic picture is somewhat comparable to that observed in smears from cases of early epidermoid carcinoma of the cervix.

SERIES PLATE

C IV

Respiratory System

Malignant cells exfoliated from epidermoid carcinomas of the lung and larynx

CIV
DESCRIPTION

Malignant cells exfoliated from epidermoid carcinomas of the lung and larynx. Drawings x 525.

1. Large epidermoid tadpole cell with cytoplasmic processes showing striation. Sputum. OG–EA series. Male. Age 70. Pathologic diagnosis: bronchogenic epidermoid carcinoma.

2–4. Various types of epidermoid cells, tadpole, "snake" and others showing nuclear degeneration and resorption. Sputum. OG–EA series. Male. Age 73. Pathologic diagnosis: bronchogenic epidermoid carcinoma. (Compare with A VII, 6 and 14.)

5–8. Various types of epidermoid cells. Laryngeal swab smear. OG–EA series. Male. Age 62. Pathologic diagnosis: epidermoid carcinoma of the larynx, Grade III. Primary diagnosis by smears.

9. Epidermoid cell showing a thin cytoplasmic process. Sputum. Glycogen series. Male. Age 59. Pathologic diagnosis: bronchogenic epidermoid carcinoma.

10–14. Epidermoid cells showing elongation, cytoplasmic processes and degeneration and resorption of nuclei. Sputum. Glycogen series. Female. Age 52. Pathologic diagnosis: bronchogenic epidermoid carcinoma.

15. Epidermoid cells found in a smear of a skull tumor aspirate. OG–EA series. Male. Age 60. Pathologic diagnosis: metastatic carcinoma from a primary bronchogenic epidermoid carcinoma. (Compare with A VII, 15.)

16. Large, elongated "snake" cells. OG–EA series. Pathologic diagnosis: bronchogenic epidermoid carcinoma. Sputum smear from same case as No. 15.

CIV
DISCUSSION

Malignant cells exfoliated from epidermoid carcinomas of the lung and larynx

The morphologic characteristics of malignant epidermoid cells may be summarized as follows:

1. Enlargement and hyperchromasia of the nucleus (Nos. 5, 6, 8 and C V, 13).
2. Enlargement of the cells beyond normal limits (Nos. 1, 6, 15, 16 and C V, 13).
3. Extreme elongation of cells resulting in bizarre forms such as the tadpole cells (Nos. 1–4, 15), "snake" cells (Nos. 3, 10, 16) or other weird shapes.
4. Tendency to formation of cytoplasmic processes (Nos. 1, 9–15).
5. Compactness of the cytoplasm and change from a basophilic to an acidophilic or distinctly orangeophilic staining reaction due possibly to progressive keratinization.
6. Degeneration, pyknosis and gradual disappearance of the nuclei manifested in a sequence of characteristic patterns.
7. Engulfment of one cell by another (No. 5). Such an engulfment may represent the beginning of pearl formation (C II, 17).
8. Occasional multinucleation (No. 5 and C III, 16).

Corresponding changes can be seen in cells of epidermoid carcinomas of the cervix (A VI and A VII).

SERIES PLATE

C V

Respiratory System

Malignant and non-malignant cells found in sputum, bronchial aspirates and antral washings

CV
DESCRIPTION

Malignant and non-malignant cells found in sputum, bronchial aspirates and antral washings. Photomicrographs x 600.

1 and 2. Sputum. OG–EA series. Female. Age 52. Final diagnosis: bilateral bronchiectasis.

3. Sputum. OG–EA series. Male. Age 62. Pathologic diagnosis: metastatic adenocarcinoma of both lungs presumably of rectosigmoid origin.

4–7. Sputum and bronchial aspirate. Male. Age 60. Pathologic diagnosis: pulmonary adenomatosis.

8. Sputum. OG–EA series. Male. Age 64. Final diagnosis: chronic bilateral pulmonary tuberculosis with cavitation. Pulmonary silicosis.

9 and 10. Cells interpreted as normal reserve cells of the bronchial mucosa. Bronchial aspirate. OG–EA series. Male. Age 64. Pathologic diagnosis: bronchial cyst, benign.

11 and 12. Bronchial aspiration. OG–EA series. Male. Age 53. Pathologic diagnosis: oat cell carcinoma of the lung. (Same case as C II, 4, 5.)

13. Sputum. OG–EA series. Male. Age 54. Final diagnosis: bronchogenic epidermoid carcinoma.

14 and 15. Bronchial aspirate. OG–EA series. Female. Age 50. Final diagnosis: carcinoma of the breast, metastatic to lung, pleura and mediastinum. The cells in No. 15 are normal ciliated cells included for comparison with the malignant cells.

16–19. Bronchial aspirate. OG–EA series. Female. Age 42. Pathologic diagnosis: pigmented malignant melanoma of the right lung metastatic from the hard palate.

20 and 21. Sputum. Glycogenic series. Male. Age 35. Pathologic diagnosis: bronchial adenoma, right lung.

22. Sputum. OG–EA series. Male. Age 56. Final diagnosis: rhabdomyosarcoma of the thigh with multiple pulmonary metastases.

23. Sputum. OG–EA series. Female. Age 53. Pathologic diagnosis: papillary adenocarcinoma of the lung, possibly metastatic.

24. Bronchial aspirate. OG–EA series. Male. Age 66. Pathologic diagnosis: alveolar cell carcinoma of the lung.

25. Sputum. OG–EA series. Female. Age 48. Pathologic diagnosis: adenocarcinoma of the lung.

26. Bronchial aspirate. OG–EA series. Male. Age 46. Clinical diagnosis: mild bronchiectasis.

27. Sputum. Glycogenic series. Male. Age 30. Pathologic diagnosis: malignant lymphoma (reticulum cell sarcoma).

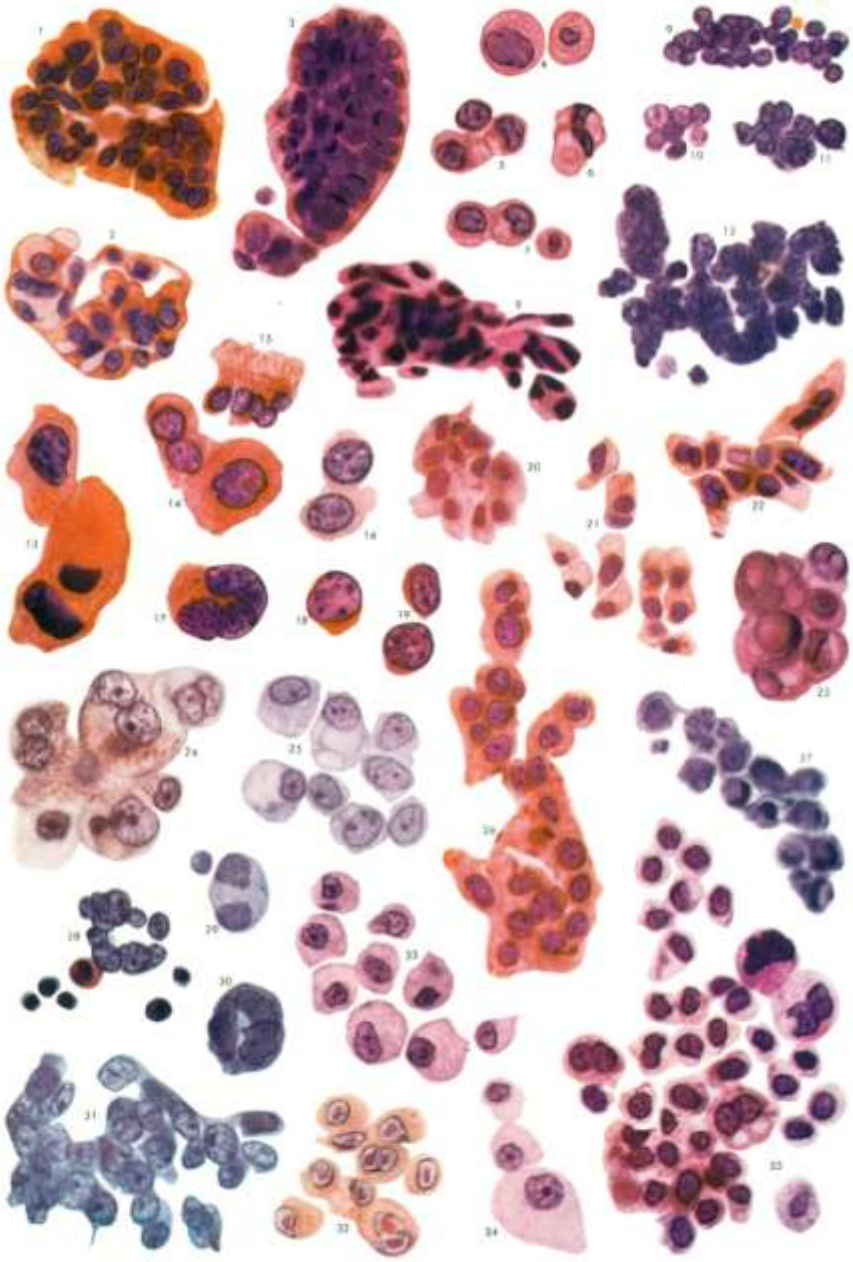
28–30. Antral washings. OG–EA series. Male. Age 49. Pathologic diagnosis: reticulum cell sarcoma with some characteristics of transitional cell carcinoma. (Courtesy of Dr. Paul Kimmelstiel, Charlotte Memorial Hospital, Charlotte, N.C.)

31. Antral washings. OG–EA series. Male. Age 45. Pathologic diagnosis: unclassified carcinoma with features of glandular and stratified squamous epithelium. (Courtesy of Dr. Paul Kimmelstiel, Charlotte Memorial Hospital, Charlotte, N.C.)

32. Sputum. Glycogen series. Male. Age 66. Pathologic diagnosis: carcinoma of the lung metastatic from the pancreas.

33 and 34. Sputum. OG–EA series. Male. Age 52. Pathologic diagnosis: adenocarcinoma of the lung. (Same case as C II, 7.)

35. Sputum. OG–EA series. Male. Age 53. Pathologic diagnosis: adenocarcinoma of the lung (bilateral miliary).



CV
DISCUSSION

Malignant and non-malignant cells found in sputum, bronchial aspirates and antral washings

Groups of cells characteristic of bronchiectasis are shown in Nos. 1, 2 and 26. The cells of Nos. 1 and 2 are of the columnar type, whereas those of No. 26 show some degree of squamous metaplasia. Other illustrations of bronchiectasis are given in C I and C II. A more detailed discussion of the exfoliative cytology of bronchiectasis is given in the general remarks on C I.

Cells of metastatic neoplasms are seen in Nos. 3 (rectosigmoid), 14 (breast), 16–19 (melanoma of hard palate), 22 (rhabdomyosarcoma), and 32 (pancreas). Differences in the cytologic patterns of these cell groups are obvious and have a diagnostic value for the tumor type, but this does not imply that each one of these represents a specific and unique pattern to be found in all cases of the same primary origin. It should be realized that, at the present state of our knowledge, our ability to differentiate various types of neoplasms by cytologic criteria is still limited and based on observations of a relatively small number of cases.

The cells shown in Nos. 16–19 are from a case of metastatic melanoma. Melanin granules, as seen in No. 17, are usually present in larger cells and constitute a conclusive criterion of the tumor type. Cells of the type illustrated in Nos. 18 and 19 as well as larger cells richer in cytoplasm and containing melanin from a case of metastatic melanosarcoma can be seen in A XI, 14–15.

Cells from a case diagnosed pathologically as pulmonary adenomatosis are shown in Nos. 4–7. The patient died 1 1/2 years later and no autopsy was performed to determine the final state of the disease. The similarity of the cells to those of adenocarcinomas, as illustrated in Nos. 23, 25, 33, 34 and 35, is apparent. The eccentricity of the nucleus, vacuolation of the cytoplasm and the range of variability in cell size are characteristics common to all these cases and suggest an adenomatous neoplastic pattern.

In the case of No. 23 the tumor was suspected to be of metastatic origin, possibly from one of the organs of the digestive system.

The cytologic features of No. 24, which is from a case diagnosed pathologically as an alveolar cell carcinoma, compare with those of the adenocarcinomas. Cytologically a differential diagnosis between an alveolar cell carcinoma and an adenocarcinoma of the lung is difficult.

The cells from Nos. 20 and 21 are from a case of adenoma. The nuclear eccentricity and the distinctly columnar form of the cells are indicative of an adenomatous type; yet the normal appearance and relative uniformity of the nuclei do not suggest malignancy.

Nos. 9–12 afford a comparison between normal undifferentiated reserve cells of the bronchial mucosa (9 and 10; see also C I, 27) and cells from an oat cell carcinoma (11 and 12). In smears overstained with hematoxylin, normal reserve cells may be misinterpreted as malignant, particularly if there is some anisokaryosis as in No. 10. The nuclei of the cells of oat cell carcinoma show, as a rule, some variability in size, irregularity in form and marked hyperchromasia. The cytoplasm may be dispersed (see C II, 5) or totally absent.

The cells of No. 8 were found in the sputum of a case of tuberculosis and silicosis and show a resemblance to those described previously as "Pap" cells (see C I, 28), which are considered indicative of a chronic inflammatory condition.

Typical cells from an epidermoid carcinoma of the lung are illustrated in No. 13.

No. 14 shows malignant cells from a metastatic carcinoma of the breast. A group of normal ciliated cells (No. 15) from the same case is included for comparison.

The cells in No. 27 were seen in the sputum of a case diagnosed as malignant lymphoma (reticulum cell sarcoma) involving the lungs as well as many other organs of the body.

C V DISCUSSION

The cytologic features of the cluster are consistent with a diagnosis of a neoplasm of lymphoid origin; however, a diagnosis of its specific type could not be established on the basis of this cluster.

The cells illustrated in Nos. 28–30 were found in antral washings from a case diagnosed as reticulum cell sarcoma with some characteristics of transitional cell carcinoma. They show great resemblance to those illustrated in C II, 9, which are from a case diagnosed as Hodgkin's disease. Those of No. 28 are closer to the lymphocytic type, while those of 29 and 30 closely resemble the Reed-Sternberg cells.

The cells in No. 31 are also from an antral washing and show distinct malignant criteria, but their type is not clearly indicated. Smears prepared from antral washings are of greater value than nasopharyngeal washings in the diagnosis of malignant neoplasms of the sinuses.

SERIES PLATE

D I

Digestive System

Non-malignant and malignant cells found in smears from esophageal, gastric, duodenal, rectal and colonic aspiration and washing specimens

D I
DESCRIPTION

Non-malignant and malignant cells found in smears from esophageal, gastric, duodenal, rectal and colonic aspiration and washing specimens. Drawings x 525.

1 and 2. Gastric aspirate. OG–EA series. Male. Age 61. Pathologic diagnosis: chronic ulcer of the stomach.

3. Gastric aspirate. OG–EA series. Male. Age 48. Clinical diagnosis: chronic ulcer of the stomach.

4. Gastric aspirate. Glycogen series. Female. Age 51. Clinical and x-ray diagnosis: recurrent gastric ulcer on the lesser curvature of the stomach.

5. Gastric aspirate. Glycogen series. Female. Age 55. Pathologic diagnosis: benign adenomatous gastric polyp. Same case as D IV, 5.

6 and 7. Gastric aspirate. OG–EA series. Male. Age 56. Negative gastric findings. In No. 7 some cells at one edge of the cluster are tilted, and reveal their columnar form.

8. Gastric balloon specimen. Glycogen series. Male. Age 59. Pathologic diagnosis: hypertrophic gastritis.

9. Gastric aspirate. Glycogen series. Male. Age 47. Clinical and x-ray diagnosis: chronic duodenal ulcer.

10. Esophageal washing. Glycogen series. Male. Age 65. Pathologic diagnosis: ulcer of the esophagus.

11. Duodenal drainage from a site near the ampulla. OG–EA series. Male. Age 69. Clinical diagnosis: homologous serum hepatitis.

12. Gastric aspirate. OG–EA series. Female. Age 62. Clinical and x-ray diagnosis: gastric ulcer.

13. Gastric aspirate. OG–EA series. Female. Age 65. Operative diagnosis: low-grade obstruction of the duodenum and pylorus.

14 and 15. Gastric aspirate. OG–EA series. Male. Age 72. Negative gastric findings.

16. Gastric aspirate. OG–EA series. Male. Age 62. Clinical diagnosis: Negative gastric findings.

17. Rectal washing. OG–EA series. Female. Age 57. Clinical diagnosis: benign polyp. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

18. Duodenal aspirate. Glycogen series. Male. Age 62. Negative findings.

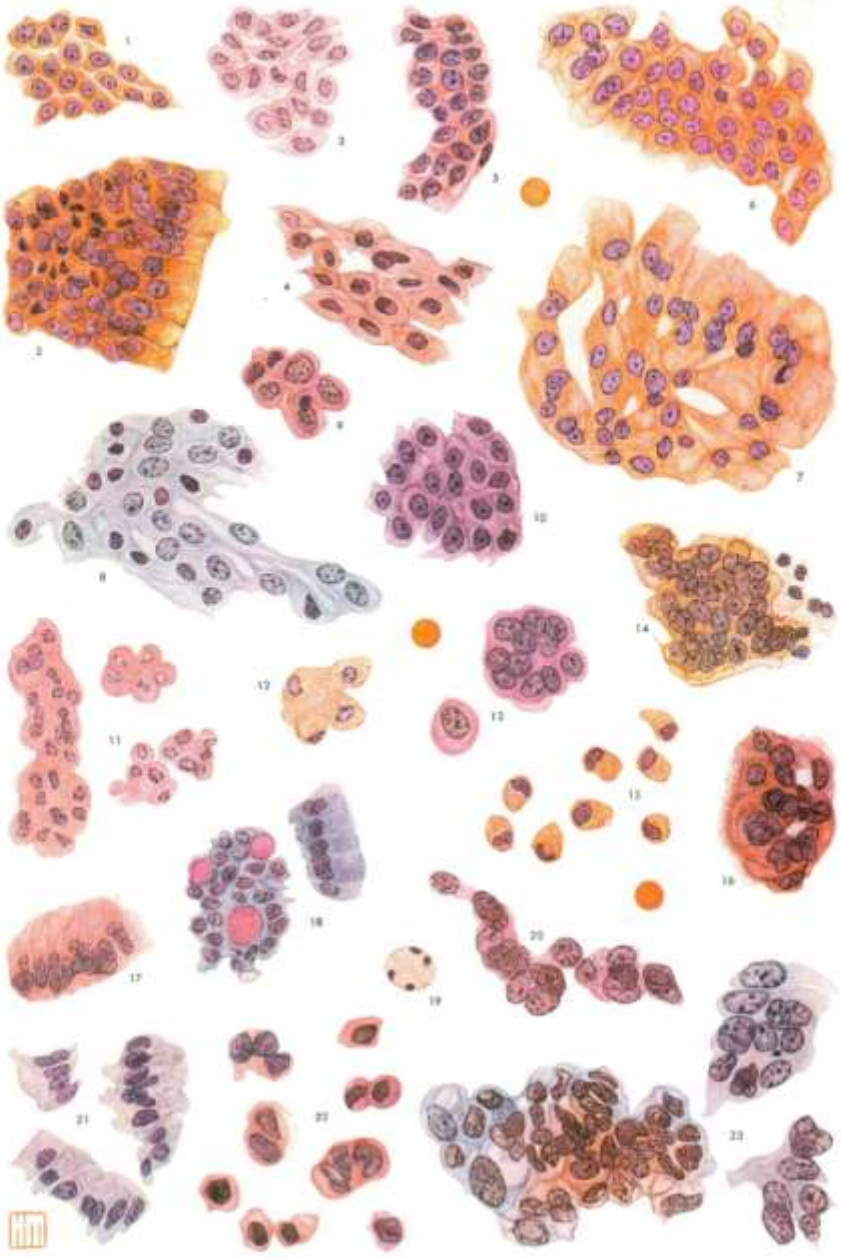
19. Amoeba histolytica found in a rectal washing smear. OG–EA series. Female. Age 49. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

20. Rectal washing. OG–EA series. Female. Age 66. Pathologic diagnosis: adenocarcinoma of the rectum, Grade III. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

21. Direct smear of the sigmoid mucosa. Glycogen series. Female. Pathologic diagnosis: carcinoma of the sigmoid colon.

22. Rectal lavage. OG–EA series. Male. Age 67. Pathologic diagnosis: carcinoma of the sigmoid colon.

23. Rectal washing. OG–EA series. Female. Age 57. Pathologic diagnosis: adenocarcinoma of the rectum, Grade II. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)



DI
DISCUSSION

Non-malignant and malignant cells found in smears from esophageal, gastric, duodenal, rectal and colonic aspiration and washing specimens

Clusters of benign epithelial cells exfoliated from the gastric mucosa are illustrated in Nos. 1–3, 6 and 7. In surface view some show a pavement cell arrangement with individual cells separated by distinct borders (Nos. 1, 3 and 6) while others have a more irregular arrangement of the cells and their nuclei (Nos. 2 and 7). Cells presenting a side view, such as those found singly or at the edge of a cluster (Nos. 2 and 7) reveal their columnar form and mucoid type.

The differences in the structural pattern of detached fragments of gastric epithelium are not always easy to reconcile with the histologic pattern of the normal gastric mucosa. Larger well-preserved clusters, as found in some gastric specimens (D III, 1 and 2), are more uniform and more representative of the normal exfoliative pattern of the epithelium of the stomach.

In Nos. 1, 2 and 3 a diagnosis of gastric ulcer was made. It is possible that the slight atypia shown in No. 2, such as the irregular distribution and crowding of the nuclei and the presence of some nuclei undergoing degeneration and pyknosis, is due to the presence of an ulcer. In the case shown in Nos. 6 and 7 the clinical findings were essentially negative.

Nuclear degeneration and pyknosis are also shown in cluster No. 4, which is from another case of gastric ulcer.

More pronounced atypia with some anisokaryosis is shown in No. 5, from a case of gastric polyp. However, this structural atypia should not be interpreted as specifically diagnostic of a polypoid growth.

The clusters shown in Nos. 8 and 9, from cases of gastritis and duodenal ulcer respectively, exhibit some nuclear degeneration and pyknosis.

The cells illustrated in No. 10 are interpreted as being of esophageal origin. They were found in an esophageal washing from a case of ulcer of the esophagus. Their structure is essentially normal.

The three groups of cells of No. 11 represent a cytologic type often found in duodenal specimens. In this particular case, the fluid was aspirated from a site near the ampulla and the cells are considered to be normal cells exfoliated from the epithelium of the common duct. Their pattern of nuclear degeneration is rather characteristic. The chromatin is granular and accumulates at the periphery of the nucleus, and in optical section forms a ring around a relatively clear center.

The cells of No. 12, found in a gastric aspirate, show a similar pattern of nuclear degeneration. Although these cells are somewhat larger than those of No. 11, they probably are derived from the common duct and entered the stomach by reflux action. That cells may reach the lumen of the stomach in this manner has been established by evidence obtained in other cases. In one instance the presence of a carcinoma of the pancreas was revealed by cells found in a gastric aspirate. No metastasis to the stomach was found at autopsy.

It should be borne in mind that the stomach is a receptacle for extraneous cells of diverse origin such as cells from the oral, nasopharyngeal and bronchial mucosae, the esophageal epithelium and the upper portion of the intestinal tract. The cells illustrated in Nos. 14, 15 and 16 are probably examples of such extraneous cells. The distribution of the nuclei and the presence of ciliation in clusters 14 and 16 suggest a nasopharyngeal origin. Nasopharyngeal cells are not infrequently noted in gastric aspirates, particularly when the Levine tube has been introduced into the stomach through the nose. The cells of No. 15 do not seem to correspond to exfoliated cells of the gastric mucosa, but their origin is not clear.

D I DISCUSSION

The cells in No. 13 were found in a gastric aspirate from a case of pyloric obstruction and offer another example of the variety of atypical cells of undetermined origin found in gastric fluid.

The two clusters in No. 18, which were found in a duodenal aspirate, represent mucoid cells the origin of which is not clear. Their mucoid type is proved by the columnar form of the cells of the upper right group and by the presence of a mucinous secretion in the lower left cluster, as indicated by its staining reaction. Cells found in duodenal aspirates are particularly difficult to interpret because the normal exfoliative cytology of the duodenum and of the pancreatic and hepatic ductal systems has not, as yet, been adequately explored.

The group of columnar cells shown in Nos. 17 and 21 are both suggestive of a polypoid growth. The cluster in No. 17 has normal nuclei and was found in the rectal washing of a case of a benign rectal polyp. The cell clusters in No. 21 exhibit distinct nuclear atypia and were found in a swab smear from the sigmoid mucosa in a case of carcinoma of the sigmoid colon. On close comparison of the illustrations of both cell groups (Nos. 17 and 21), the malignant character of the cells of No. 21 is more apparent than if these cells were seen by themselves in a smear.

The cells shown in No. 22 are also from a carcinoma of the sigmoid colon. The nuclear eccentricity and the form of the cells suggest a cuboidal or columnar cell origin.

The cell illustrated in No. 19 was found in a rectal washing smear. A competent parasitologist identified it as an Amoeba histolytica.

Malignant cells found in rectal washing smears from two cases of adenocarcinoma of the rectum are shown in Nos. 20 and 23. The malignant nature of the cells is revealed by the fulfillment of many general criteria of malignancy.

SERIES PLATE

D II

Digestive System

Cells found in esophageal, gastric, duodenal and gall bladder aspirates, from malignant cases with the exception of No. 16

D II
DESCRIPTION

Cells found in esophageal, gastric, duodenal and gall bladder aspirates, from malignant cases with the exception of No. 16. Drawings x 525.

1 and 2. Gastric aspirate. OG–EA series. Male. Age 60. Pathologic diagnosis: mucous carcinoma of the stomach with metastasis to regional lymph nodes.

3–5. Gastric aspirate. OG–EA series. Female. Age 50. Pathologic diagnosis: adenocarcinoma of the stomach with multiple metastases.

6. Gastric aspirate. OG–EA series. Male. Age 55. Pathologic diagnosis: adenocarcinoma of the cardia of the stomach invading esophagus and liver.

7. Gastric aspirate. OG–EA series. Male. Age 60. Pathologic diagnosis: anaplastic carcinoma of the cardiac end of the stomach invading the esophagus.

8. Duodenal drainage. OG–EA series. Male. Age 52. Pathologic diagnosis: carcinoma of the pancreas with metastasis to adjacent nodes.

9. Gastric aspirate. OG–EA series. Male. Age 70. Exploratory operation showed no gastric tumor. Patient expired. Autopsy diagnosis: carcinoma of the pancreas extending into the medial wall of the descending portion of the duodenum and involving the mucosa. (Courtesy of Dr. George Romberg, White Plains Hospital, White Plains, N.Y.)

10. Esophageal aspirate. OG–EA series. Female. Age 53. Pathologic diagnosis: adenocarcinoma of the stomach, scirrhous type, recurrent in the esophagus 5 months after total gastrectomy.

11. Gastric aspirate. Glycogen series. Female. Age 72. Pathologic diagnosis: epidermoid carcinoma of the esophagus.

12 and 13. Gastric balloon specimen. Glycogen series. Male. Age 63. Clinical diagnosis: carcinoma of the stomach (operation refused).

14 and 15. Duodenal drainage. OG–EA series. Female. Age 63. Pathologic diagnosis: primary carcinoma of the pancreas with metastasis to the omentum and lymph nodes and greater curvature of the stomach. (Same case as D V, 9.)

16. Esophageal aspirate. OG–EA series. Female. Age 40. Diagnosis: cardiospasm, no evidence of malignancy.

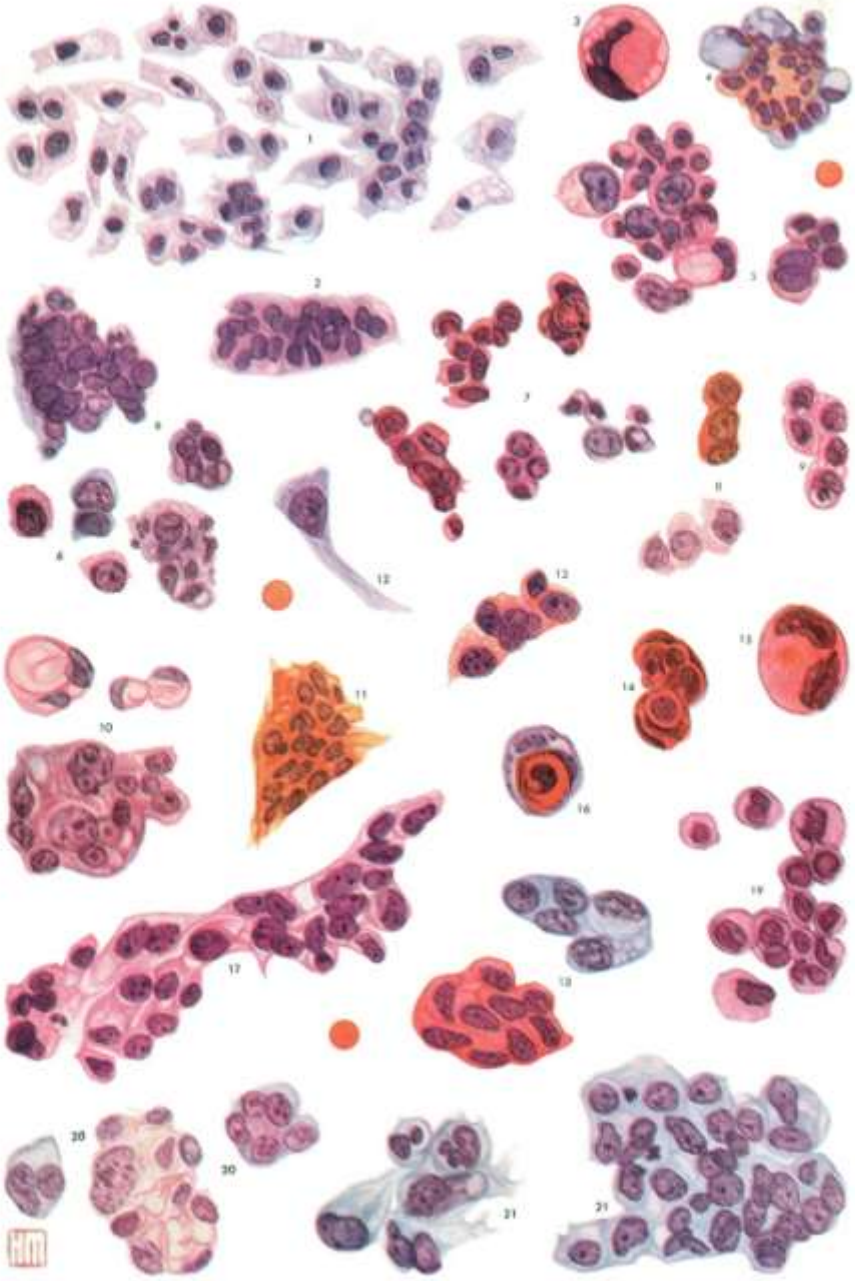
17. Gastric balloon specimen. OG–EA series. Male. Age 31. Pathologic diagnosis: adenocarcinoma of the stomach with metastasis to liver and omentum.

18. Gastric balloon specimen. OG–EA series. Male. Age 67. Pathologic diagnosis: adenocarcinoma of the stomach, Grade II.

19. Gastric aspirate. Glycogen series. Male. Age 63. Pathologic diagnosis: mucous carcinoma of the stomach with widespread metastasis.

20. Gall bladder aspirate. Glycogen series. Female. Age 59. Pathologic diagnosis: adenocarcinoma presumably arising in bile ducts.

21. Gastric aspirate. Glycogen series. Male. Age 70. Pathologic diagnosis: pleomorphic adenocarcinoma of the cardia of the stomach with invasion of the esophagus.



D II
DISCUSSION

Cells found in esophageal, gastric, duodenal and gall bladder aspirates, from malignant cases with the exception of No. 16.

Clusters of cells found in gastric specimens in cases of carcinoma of the stomach are shown in Nos. 1–2, 3–5, 6, 7, 12–13, 17, 18, 19 and 21. Of these, Nos. 1–2 and 19 were diagnosed as mucous carcinomas; 3–5, 6, 17, 18 and 21 as adenocarcinomas; 7 as an anaplastic carcinoma; and 12–13 as carcinoma without specification of the type. The malignant character of the cells is apparent in all these clusters but it is in only a few that the type of the tumor can be recognized.

The two cases diagnosed pathologically as mucous carcinomas (Nos. 1–2 and 19) present different cytologic pictures. In No. 1 the cells are of the columnar, mucoid cell type; whereas in No. 19 the cells are rounded and their glandular mucoid character is less easily recognizable.

Differences in the cytologic pattern are also noticeable in the group of adenocarcinoma cases. In Nos. 3–5 the vacuolation of the cells and the eccentricity of the nuclei are clearly shown. There are also binucleation and marked nuclear enlargement, as well as nuclear degeneration. The adenomatous type of the cells is apparent. In No. 6, which illustrates cells from an adenocarcinoma of the cardiac end of the stomach, the cytologic picture is somewhat different. These cells show great similarity to those of an adenocarcinoma of the endometrium shown in A IX, 5.

In the clusters of Nos. 17, 18 and 21, the cells and nuclei are larger and the glandular type of the cells is less distinct. The cell groups of No. 21 are from a case diagnosed as a pleomorphic adenocarcinoma of the cardia of the stomach. Nos. 17 and 18 were found in gastric balloon specimens, and 21 in an aspirate.

Cells from a case diagnosed as an anaplastic carcinoma of the cardiac end of the stomach are seen in No. 7. These cells, though smaller, show a certain resemblance to those of No. 6.

Another case of carcinoma of the stomach is illustrated in Nos. 12 and 13. An unusual tadpole cell is shown in No. 12. No pathologic diagnosis of the type of the tumor is available, since the patient refused operation.

Cells from two cases of carcinoma of the esophagus are shown in Nos. 10 and 11. No. 10 is from an adenocarcinoma of the stomach recurrent in the esophagus. Its cytology resembles closely that of the adenocarcinoma of the stomach illustrated in Nos. 3–5. No. 11 is from a primary epidermoid carcinoma of the esophagus and has an altogether different pattern.

The two groups of cells in No. 8 were found in a duodenal drainage from a patient with carcinoma of the pancreas. This is of particular interest since it was the first case in which a carcinoma of the pancreas was diagnosed by the cytologic examination of duodenal drainage smears.[24]

Cells from a duodenal drainage specimen of another case of carcinoma of the pancreas are illustrated in Nos. 14–15.

The cluster of No. 9 was found in a gastric aspiration specimen. The cells were interpreted as malignant. An exploratory operation revealed no gastric tumor. The patient expired 3 weeks later and an autopsy revealed the presence of a carcinoma of the pancreas extending into the medial wall of the descending portion of the duodenum and involving the mucosa. It is likely that the abnormal cells reached the stomach by reflux from the duodenum.

A pearl-like structure found in an esophageal aspirate is shown in No. 16. On the basis of the abnormal appearance of the central cell one might be led to suspect malignancy, but this case proved to be negative. A certain similarity in structure exists between this and the pearl in C I, 26, found in the sputum of a case with no evidence of malignancy.

No. 20 illustrates malignant cells found in a gall bladder aspirate from a patient with a pathologic diagnosis of adenocarcinoma presumably arising in the biliary duct system. The malignant character as well as the adenomatous type of the cells are evident.

SERIES PLATE

D III

Digestive System

Non-malignant and malignant cells found in gastric and esophageal specimens

D III
DESCRIPTION

Non-malignant and malignant cells found in gastric and esophageal specimens. Photomicrographs x 600.

1. Normal gastric epithelium. Gastric balloon specimen. OG–EA series. Male. Age 46. Pathologic diagnosis: carcinoma of the pancreas with metastasis to the liver. Normal stomach. (Same case as D IV, 9.)
2. Large group of mucoid columnar cells viewed from the basal surface and showing a honeycomb appearance. A smaller cluster seen from the side shows the columnar form of the cells. Gastric aspirate. OG–EA series. Male. Age 61. Pathologic diagnosis: chronic inflammatory process of the lung. No gastric disease.
3. Cluster of cells of the mucoid type exhibiting a papillary pattern. Gastric balloon specimen. OG–EA series. Female. Age 70. Pathologic diagnosis: adenocarcinoma of the lesser curvature of the stomach. (Same case as D IV, 7.)
4. Cluster of malignant cells retaining a papillary pattern. Gastric balloon specimen. OG–EA series. Male. Age 65. Pathologic diagnosis: annular carcinoma of the stomach.
5. Multinucleated malignant cell. Esophageal washing. OG–EA series. Male. Age 58. Pathologic diagnosis: epidermoid carcinoma of the esophagus, Grade III.
6. Malignant cells showing crowding and overlapping of nuclei. Esophageal washing. OG–EA series. Male. Age 63. Pathologic diagnosis: epidermoid carcinoma of esophagus, Grade III.
7–9. Clusters of malignant cells. Gastric balloon specimen. OG–EA series. Male. Age 65. Pathologic diagnosis: adenocarcinoma of the stomach.

D III
DESCRIPTION

Non-malignant and malignant cells found in gastric and esophageal specimens. Photomicrographs x 600.

1. Normal gastric epithelium. Gastric balloon specimen. OG–EA series. Male. Age 46. Pathologic diagnosis: carcinoma of the pancreas with metastasis to the liver. Normal stomach. (Same case as D IV, 9.)
2. Large group of mucoid columnar cells viewed from the basal surface and showing a honeycomb appearance. A smaller cluster seen from the side shows the columnar form of the cells. Gastric aspirate. OG–EA series. Male. Age 61. Pathologic diagnosis: chronic inflammatory process of the lung. No gastric disease.
3. Cluster of cells of the mucoid type exhibiting a papillary pattern. Gastric balloon specimen. OG–EA series. Female. Age 70. Pathologic diagnosis: adenocarcinoma of the lesser curvature of the stomach. (Same case as D IV, 7.)
4. Cluster of malignant cells retaining a papillary pattern. Gastric balloon specimen. OG–EA series. Male. Age 65. Pathologic diagnosis: annular carcinoma of the stomach.
5. Multinucleated malignant cell. Esophageal washing. OG–EA series. Male. Age 58. Pathologic diagnosis: epidermoid carcinoma of the esophagus, Grade III.
6. Malignant cells showing crowding and overlapping of nuclei. Esophageal washing. OG–EA series. Male. Age 63. Pathologic diagnosis: epidermoid carcinoma of esophagus, Grade III.
7–9. Clusters of malignant cells. Gastric balloon specimen. OG–EA series. Male. Age 65. Pathologic diagnosis: adenocarcinoma of the stomach.

D III
DISCUSSION

Non-malignant and malignant cells found in gastric and esophageal specimens

Two clusters of normal gastric cells are shown in Nos. 1 and 2. The normal character of the cells and their nuclei is apparent. The two clusters differ somewhat in their pattern. The larger cluster (No. 2) has the honeycomb appearance which is often distinct in cell clusters of the mucoid type when they are viewed from their proximal (basal) surface (see Discussion, A III). The columnar form of these cells is evident in the smaller group of No. 2, which is seen from the side. In No. 1 the distinctive form and type of the individual cells cannot be determined, as is often the case in flat sheets of exfoliated epithelium viewed from the distal surface. In examining epithelial fragments one sometimes finds, at their periphery, cells in a side view revealing their type, as in the upper edge of No. 2.

Nos. 3 and 4 are both from cases diagnosed as carcinoma of the stomach. The malignant character of the cells can be easily recognized in No. 4 by the marked nuclear and cytoplasmic atypia. The cluster illustrated in No. 3 is more difficult to evaluate. It is strongly suggestive of a papillary growth, but the absence of marked nuclear and other abnormalities gives no support to its interpretation as malignant. In this case the possibility of malignancy was indicated by the presence of other clusters exhibiting nuclear atypia and an irregular pattern.

A multinucleated malignant cell from a case of epidermoid carcinoma of the esophagus is shown in No. 5. Such monstrous cells are sometimes seen in carcinomas of the epidermoid type. A smaller squamous cell appears to be engulfed within the cytoplasm of the giant cell.

The malignant nature of the cluster illustrated in No. 6, which is from another case of epidermoid carcinoma of the esophagus (Grade III), is clearly indicated by the crowding and overlapping of the nuclei and the irregular pattern.

Distinct criteria of malignancy are also exhibited in Nos. 7, 8 and 9, which are from a case of adenocarcinoma of the stomach. Nuclear abnormalities are present in all three clusters and are particularly striking in No. 8. No. 9 illustrates a fragment of the tumor in which some of the cells and their nuclei show marked enlargement and elongation, giving an impression of transition from a benign to a malignant area. This cluster serves to illustrate the adequacy of the cytologic material obtained by the gastric balloon method, which in some instances may be comparable to a histologic specimen.

SERIES PLATE

D IV

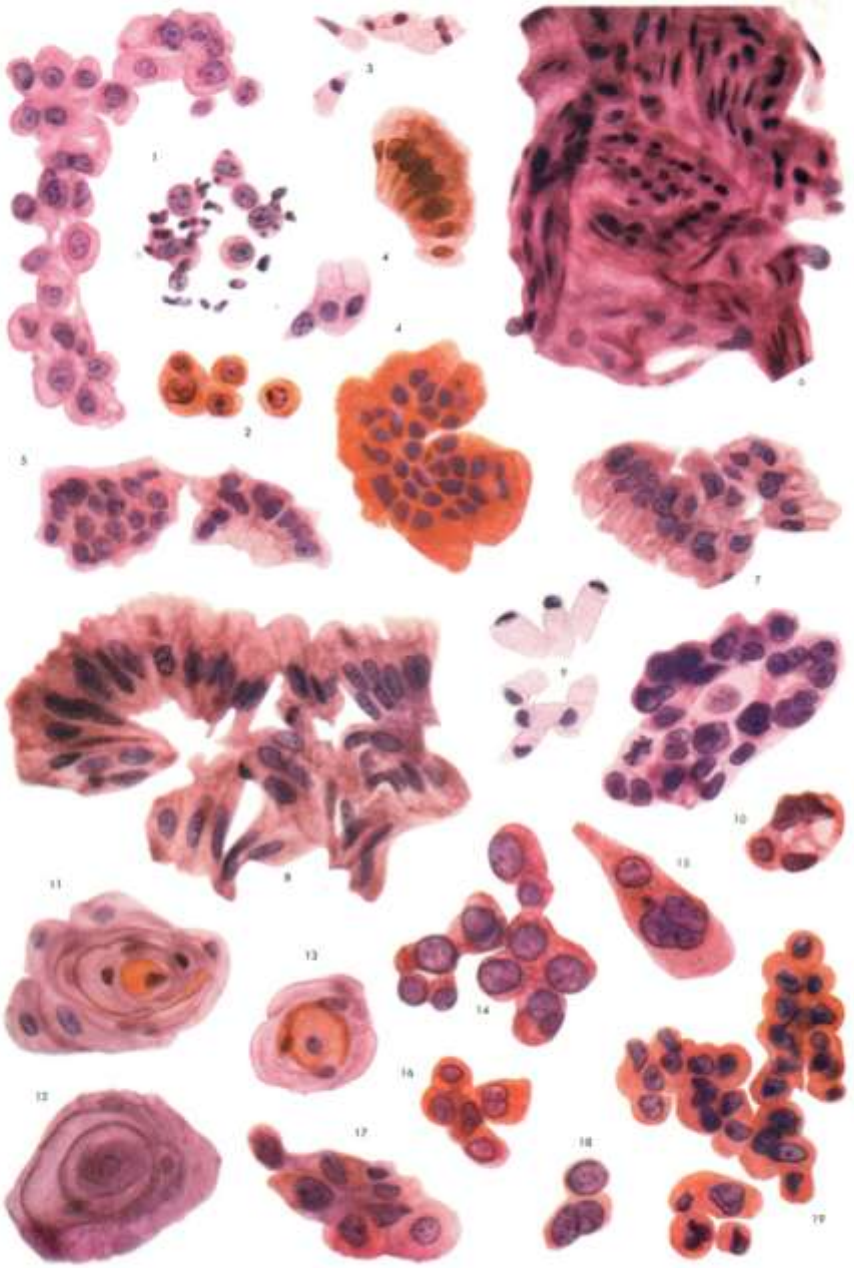
Digestive System

Non-malignant and malignant cells found in gastric and esophageal specimens and in a bile duct aspirate

DIV
DESCRIPTION

Non-malignant and malignant cells found in gastric and esophageal specimens and in a bile duct aspirate. Photomicrographs x 600.

1. Gastric balloon specimen. OG–EA series. Male. Age 56. Pathologic diagnosis: chronic gastritis.
2. Gastric balloon specimen. OG–EA series. Male. Age 49. Clinical and gastroscopic diagnosis: hypertrophic gastritis.
3. Non-malignant columnar cells. Gastric balloon specimen. OG–EA series. Male. Age 52. Pathologic diagnosis: papilloma of the stomach.
4. Clusters of non-malignant columnar cells. Aspirate from esophagus and stomach. OG–EA series. Male. Age 73. Pathologic diagnosis: papilloma of the stomach, esophagitis and gastritis.
5. Gastric aspirate. OG–EA series. Female. Age 55. Pathologic diagnosis: benign adenomatous gastric polyp. (Same case as D I, 5.)
6. Gastric balloon specimen. OG–EA series. Male. Age 49. Pathologic diagnosis: ulcerating carcinoma of the stomach, Grade IV.
7. Gastric balloon specimen. OG–EA series. Female. Age 70. Pathologic diagnosis: adenocarcinoma of the lesser curvature of the stomach. (Same case as D III, 3.)
8. Gastric balloon specimen. OG–EA series. Male. Age 52. Pathologic diagnosis: adenocarcinoma of the stomach.
9. Cells of the goblet type. Gastric balloon specimen. OG–EA series. Male. Age 48. Pathologic diagnosis: carcinoma of the pancreas with metastasis to the liver, and partial obstruction of the third portion of the duodenum. (Same case as D III, 1.)
10. Gastric balloon specimen. OG–EA series. Male. Age 60. Pathologic diagnosis: adenocarcinoma of the stomach, Grade II. Note one mitosis.
11. Epithelial pearl. Esophageal aspirate taken approximately 1 year after x-ray therapy. OG–EA series. Male. Age 69. Clinical diagnosis: carcinoma of the esophagus.
12. Epithelial pearl. Esophageal aspirate. OG–EA series. Male. Age 77. Pathologic diagnosis: epidermoid carcinoma of the esophagus, Grade III. (Same case as No. 14.)
13. Epithelial pearl. Gastric balloon specimen. OG–EA series. Male. Age 52. Pathologic diagnosis: papilloma of stomach.
14. Group of malignant cells from same case as No. 12.
15. Esophageal aspirate. OG–EA series. Male. Age 73. Operative diagnosis: inoperable carcinoma of the esophagus with metastases.
16. Esophageal aspirate. OG–EA series. Male. Age 64. Pathologic diagnosis: squamous carcinoma of the esophagus, Grade II.
17. Esophageal aspirate. OG–EA series. Male. Age 73. Pathologic diagnosis: epidermoid carcinoma of the esophagus.
18. Esophageal aspirate. OG–EA series. Male. Age 56. Pathologic diagnosis: squamous cell carcinoma of the esophagus, Grade III.
19. Aspirate from the biliary duct at time of operation. OG–EA series. Male. Age 63. Pathologic diagnosis: cholangiocarcinoma of the bile ducts with obstructive jaundice. Patient had adenocarcinoma of the rectum 6 1/2 years previously.



DIV
DISCUSSION

Non-malignant and malignant cells found in gastric and esophageal specimens and in a bile duct aspirate

Cells from gastric balloon specimens in two cases of gastritis are shown in Nos. 1 and 2. The similarity in the type of the cells from the two cases is apparent. The individuality of the cells and the structural uniformity of the cells and their nuclei reveal their non-malignant character.

The cells illustrated in Nos. 3 and 4 are from two cases of papilloma of the stomach. No. 3 was from a gastric balloon specimen and No. 4 from an aspirate taken at an esophagoscopy in which gastric mucosa was seen and biopsied. The benign appearance and columnar form of the cells are consistent with the diagnosis of papilloma. Cells of this type should, however, be considered suggestive, rather than diagnostic, of this condition.

The cells in No. 5, obtained by gastric aspiration in a case of benign adenomatous gastric polyp, are apparently columnar and may be considered compatible with a diagnosis of a polypoid structure. Another cluster of cells from the same case is shown in D I, 5.

No. 6 represents approximately one-fourth of a large tissue fragment found in a gastric balloon specimen from a case of an ulcerating carcinoma of the stomach, Grade IV. At first glance one might be led to interpret it as smooth musculature with an irregular fiber pattern. However, closer examination and a comparison with other tissue fragments and clusters of cells present in the same smear as well as with a section of the tumor prove that this is a fragment of neoplastic tissue with a structure corresponding to that of the tumor itself. Such large pieces of tissue cannot be expected to be seen except in cases of tumors with a large ulcerated surface area.

Clusters of columnar cells obtained by the gastric balloon technique from two cases of adenocarcinoma of the stomach are depicted in Nos. 7 and 8. The malignant character of the cells is demonstrated more clearly in cluster No. 8 by the irregularity of its pattern and the distinct enlargement and elongation of the cells and their nuclei. In the cluster of No. 7, which is from the same case as D III, 3, the atypia of the cells is much less pronounced. A cluster of this type may arouse a suspicion of malignancy but is not sufficient to warrant a definite diagnosis. A comparison of cells in Nos. 3–5 and 7 and 8 of this plate and Nos. 3 and 4 of D III illustrate the transition from the normal to the malignant columnar cell type with intermediate forms, the benign or malignant nature of which is difficult to recognize.

Examples of a mucoid, goblet cell type are given in No. 9. These cells were found in a gastric balloon specimen from a case of carcinoma of the pancreas with metastasis to the liver and partial obstruction of the third portion of the duodenum. They are interpreted as non-malignant, but their exact nature and site of origin are not clear, since cells of this type are rarely seen in gastric specimens.

The two clusters of cells in No. 10 recovered in a gastric balloon specimen from an adenocarcinoma of the stomach, Grade II, exhibit distinct criteria of malignancy. Proliferative activity is revealed by the presence of a mitotic figure in the larger cluster.

Epithelial pearls from three different cases are shown in Nos. 11–13. The ones illustrated in 11 and 12 were found in esophageal aspirates; that in 13 was seen in a gastric balloon specimen. In the case of No. 11 a clinical diagnosis of carcinoma of the esophagus was made; however, since the patient had previously received x-ray therapy, the interpretation of this pearl-like structure is very difficult. In the case of No. 12 there was a diagnosis of epidermoid carcinoma of the esophagus, Grade III. The pronounced atypia of this pearl offers strong evidence of its origin from the malignant lesion. In the case of No. 13 the diagnosis was papilloma of the stomach; however, it is likely that this is a benign pearl originating in the esophageal epithelium. Benign and malignant pearls

found in sputum specimens are shown in C I, 25 and 26, and C II, 15 and 16.

Malignant cells found in esophageal aspirates from cases of carcinomas of the esophagus are shown in Nos. 14–18. All these clusters exhibit distinctly abnormal characteristics which permit a ready evaluation of their malignant nature.

The cells illustrated in No. 19 were found in an aspirate from the biliary duct of a man with cholangiocarcinoma of the biliary ducts. The patient also had a recurrent adenocarcinoma of the rectum. Since this specimen was obtained by direct aspiration from a bile duct during operation, the site of origin and nature of the cells are well established.

SERIES PLATE

D V

Digestive System

Malignant and non-malignant cells found in bile aspirate, duodenal drainage and rectal and colonic washing specimens

DV
DESCRIPTION

Malignant and non-malignant cells found in bile aspirate, duodenal drainage and rectal and colonic washing specimens. Photomicrographs x 600.

1. Fluid obtained from the common bile duct at exploratory celiotomy. Glycogen series. Female. Age 62. Pathologic diagnosis: chronic cholecystitis.

2 and 3. Bile obtained from the common duct at operation. OG–EA series. Male. Age 50. Pathologic diagnosis: adenocarcinoma of the common duct.

4–6. Bile obtained from the common duct at operation. OG–EA series. Male. Age 62. Pathologic diagnosis on a biopsy of a lymph node from the gall bladder: chronic lymphadenitis.

7 and 8. Bile aspirated from the gall bladder at operation. OG–EA series. Female. Age 83. Pathologic diagnosis: carcinoma of the gall bladder with metastasis to liver and gastrohepatic ligament.

9. Duodenal drainage. Glycogen series. Female. Age 63. Pathologic diagnosis: carcinoma of pancreas, metastatic to omentum, lymph nodes and greater curvature of stomach. (Same case as D II, 14 and 15.)

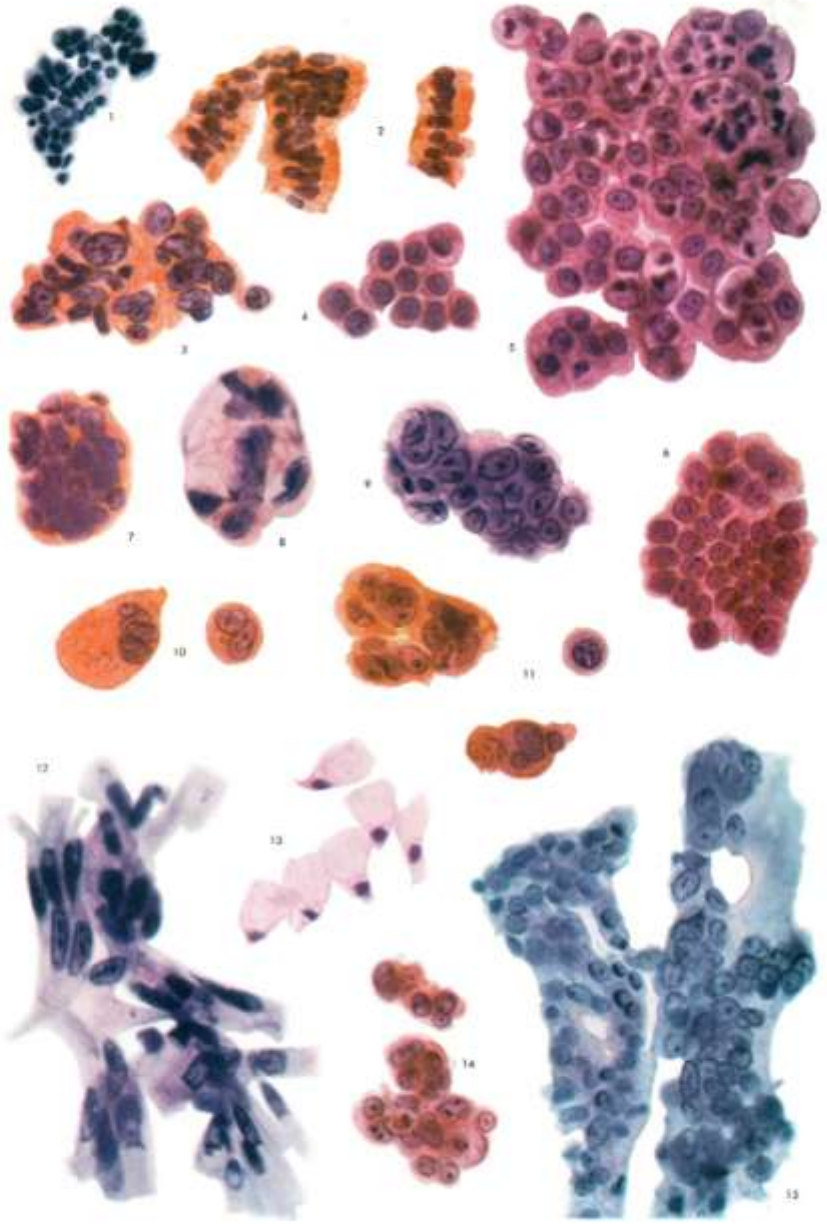
10 and 11. Duodenal aspirate. OG–EA series. Female. Age 67. Pathologic diagnosis: adenocarcinoma of the head of the pancreas, metastatic to the liver and lungs and with infiltration of the duodenum and obstruction of common and cystic ducts.

12. Rectal washing. OG–EA series. Male. Age 42. Pathologic diagnosis: adenocarcinoma of the rectum, Grade II. Also adenomatous polyp showing atypical epithelium. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

13. Colonic washing. OG–EA series. Female. Age 52. Pathologic diagnosis: pedunculated adenoma of the sigmoid. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

14. Rectal washing. OG–EA series. Female. Age 70. Pathologic diagnosis: adenocarcinoma of the sigmoid, Grade II. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)

15. Colonic washing. OG–EA series. Male. Age 62. Pathologic diagnosis: adenocarcinoma of the descending colon, Grade I. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)



DV
DISCUSSION

Malignant and non-malignant cells found in bile aspirate, duodenal drainage and rectal and colonic washing specimens

The group of cells shown in No. 1 was found in fluid aspirated from the common bile duct during an exploratory operation. The cells are interpreted as normal lining cells of the duct. A pathologic diagnosis of chronic cholecystitis was made in this case.

The cell clusters illustrated in Nos. 2 and 3 are from an adenocarcinoma of the common duct. They were found in smears prepared from fluid aspirated during an exploratory operation. The cells of No. 3 are evidently exfoliated tumor cells, and their malignant character is apparent. Those of No. 2 show no distinct abnormal features and give the impression of normal ductal lining cells, although the possibility that they are well-differentiated cells from another part of the tumor cannot be ruled out.

The cell clusters of Nos. 4, 5 and 6 were also found in a specimen aspirated from the common duct at the time of operation. The cells are large and do not give the impression of being normal lining cells, although the uniformity in size and structure of the nuclei and the regularity of the pattern do not suggest malignancy. The invasion of many cells by leucocytes suggests a chronic inflammatory process. A diagnosis of chronic lymphadenitis was made on the basis of a biopsy of a lymph node from the gall bladder.

Cells from a carcinoma of the gall bladder are shown in Nos. 7 and 8. The specimen was obtained by aspiration from the gall bladder at operation. There is a marked difference in the appearance of the two clusters, although both exhibit distinct criteria of malignancy.

A group of malignant cells found in a duodenal drainage specimen from a case of carcinoma of the pancreas is illustrated in No. 9. Since there was evidence of metastasis to the stomach it is difficult to determine the site of origin of these cells. Among other criteria of malignancy one may note the prominence of the nucleoli.

Cells found in a duodenal aspirate from another case of carcinoma of the pancreas are shown in Nos. 10 and 11. In this case, too, the site of origin of the cells cannot be determined because of the involvement of the duodenum.

No. 12 shows cells found in a rectal washing from a case of an adenocarcinoma of the rectum, Grade II. The enlargement, hyperchromasia and elongation of the nuclei and the irregular pattern compare with those of D IV, 8 which illustrates a cluster of cells from an adenocarcinoma of the stomach. Elongation of the nuclei and irregularity of pattern may also be seen in D IV, 6, which represents cells from an ulcerated carcinoma of the stomach. Such an elongation of cells and their nuclei in association with nuclear enlargement and hyperchromasia is a good criterion of malignancy in gastrointestinal neoplasms.

A group of columnar cells of the goblet type found in a colonic washing from a case of pedunculated adenoma of the sigmoid are shown in No. 13. The type of the cells is consistent with the pathologic diagnosis.

No. 14 illustrates cells found in a rectal washing from a case of adenocarcinoma of the sigmoid, Grade II. Prominent nucleoli, anisokaryosis and crowding of the cells are some of the malignant features to be seen, particularly in the larger cluster.

Benign and malignant cells found side by side in a colonic washing smear from a case of an adenocarcinoma of the descending colon, Grade I, are illustrated in No. 15. The group on the left consists of cells which have a normal appearance, whereas the one on the right is made up of cells showing enlargement, hyperchromasia and elongation of the nuclei, crowding of the cells and a somewhat irregular pattern. Here again, in a case which proved to be a relatively early case of malignancy, the enlargement, hyperchromasia and elongation of the nuclei are in evidence, though not so pronounced and extreme as in other more advanced cases (see discussion of No. 12).

SERIES PLATE

E I

Exudates

Non-malignant and malignant cells found in sediment smears of pleural and peritoneal exudates

E I
DESCRIPTION

Non-malignant and malignant cells found in sediment smears of pleural and peritoneal exudates. Drawings x 525.

1–3. Histiocytes from pleural exudates. OG–EA series. Clinical diagnoses:
1. Resolving bronchopneumonia with pleural effusion. Female. Age 38.
2. Acute pericarditis with effusion. Female. Age 62. (Same case as E II, 2, and G IV, 2.)
3. Right pleural effusion, undetermined etiology. Note one mitosis. Male. Age 66.

4. Mesothelial cells. Pleural fluid. OG–EA series. Male. Age 44. Clinical diagnosis: pulmonary congestion with pleural effusion.

5. Signet ring cell. (Same case as No. 4.)

6. Mesothelial cells. Pleural fluid. OG–EA series. Male. Age 55. Autopsy diagnosis: cirrhosis of the liver, Laennec's type.

7. Cluster of cells interpreted as mesothelial. Note one mitosis. Pleural fluid. OG–EA series. Female. Age 17. Clinical diagnosis: pulmonary tuberculosis with pleural effusion.

8 and 9. Mesothelial cells. Note crowding, binucleation and anisocytosis. Pleural fluid. OG–EA series. Female. Age 20. Diagnosis: hemopneumothorax of unknown origin.

10–12. Mesothelial cells. Note binucleation, engulfment and one mitotic figure. Peritoneal fluid. OG–EA series. Female. Age 52. Autopsy diagnosis: cirrhosis of the liver, Laennec's type.

13–15. Cells showing extreme vacuolation with signet ring formation. Peritoneal fluid. OG–EA series. Female. Age 43. Autopsy diagnosis: portal cirrhosis of the liver with ascites.

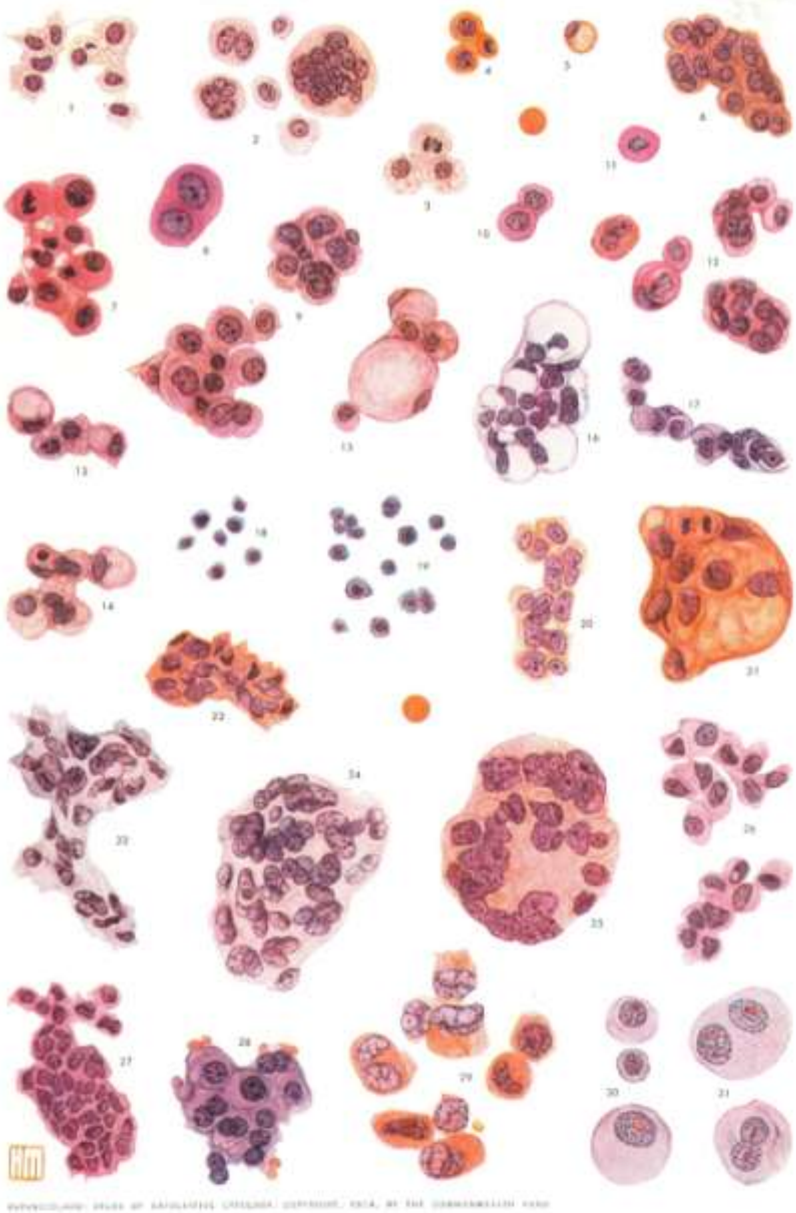
16 and 17. Cells showing pronounced vacuolation, formation of signet ring types, binucleation and engulfment. Pleural fluid. OG–EA series. Male. Age 60. Clinical diagnosis: cirrhosis of the liver with ascites.

18. Lymphocytes. Pleural fluid. OG–EA series. Male. Age 58. Pathologic diagnosis: bronchogenic epidermoid carcinoma.

19. Anisometric lymphocytes showing atypical nuclear structure. Pleural fluid. Glycogen series. Female. Age 24. Autopsy diagnosis: acute myelogenous leukemia.

20. Pleural fluid. OG–EA series. Male. Age 42. Pathologic diagnosis: solitary pleural mesothelioma.

21–31. Cells from metastatic carcinomas.
21. Generalized carcinomatosis with history of carcinoma of the breast (operated 1928), adenocarcinoma of the colon (operated 1935 and recurrent 1950), and carcinoma of the uterus (autopsy June 30, 1950). Peritoneal sediment smear of January 6, 1950. OG–EA series. Age 72.
22. Pleural fluid. Glycogen series. Female. Age 75. Pathologic diagnosis: epidermoid carcinoma of the cervix.
23. Peritoneal fluid. OG–EA series. Female. Age 37. Pathologic diagnosis: adenocarcinoma of the breast with metastasis to the liver.
24. Peritoneal fluid. OG–EA series. Female. Age 64. Pathologic diagnosis: papillary serous cystadenocarcinoma of the ovary with metastases.
25. Peritoneal fluid. OG–EA series. Female. Age 50. Pathologic diagnosis: adenocarcinoma of the ovary with extensive metastases.
26. Peritoneal fluid. OG–EA series. Female. Age 63. Pathologic diagnosis: mucous carcinoma of the stomach with abdominal carcinomatosis.
27. Lower abdominal fluid. OG–EA series. Male. Age 47. Pathologic diagnosis: carcinoma of the prostate. The patient had had adenocarcinoma of the stomach 12 years earlier.
28. Pleural fluid. Glycogen series. Female. Age 75. Autopsy diagnosis: bronchogenic adenocarcinoma of a well-differentiated, extremely desmoplastic type with metastasis to the vertebrae.
29. Pleural fluid. OG–EA series. Male. Age 54. Pathologic diagnosis: epidermoid carcinoma of the lung.
30 and 31. Pleural fluid. Glycogen series. Female. Age 73. Pathologic diagnosis: carcinoma of the lung. (Same case as C III, 7.)



EI
DISCUSSION

Non-malignant and malignant cells found in sediment smears of pleural and peritoneal exudates

Histiocytes are commonly present in exudates, sometimes in large numbers, and exhibit great variability in size and form. The foamy appearance of the cytoplasm, the eccentric position and the shape of the nucleus, and the loose arrangement of the cells when in clusters are features by which the histiocytes usually can be differentiated from mesothelial cells. Such a differentiation is often difficult because of the presence of intermediate forms.

A group of typical histiocytes from a case of bronchopneumonia with pleural effusion is shown in No. 1. The histiocytes of No. 2 from a case of acute pericarditis range from the small typical form to the large multinucleated type. In the group illustrated in No. 3, from a pleural effusion of unknown etiology, one cell is in mitosis. Mitoses are not infrequently seen in exudates in normal histiocytic and mesothelial cells as well as in malignant cells. Other mitotic figures are illustrated in this plate in Nos. 7, 11 and 21.

Mesothelial cells vary greatly in size and form. The typical ones (No. 10), have a round or slightly oval outline and a relatively large, centrally located, vesicular nucleus with a distinct chromatin net and one to several small karyosomes. Distinctly outlined nucleoli may also be seen in some nuclei (No. 8). Multinucleation is rather rare in mesothelial cells but binucleation is not infrequent (Nos. 9 and 12). Engulfment of one cell by another also occurs (Nos. 12 and 17). The cellular outlines are usually distinct in clusters except in those with crowded, poorly differentiated cells, as in No. 12. The nuclear-cytoplasmic ratio is fairly constant, and an increase in the size of the nucleus is usually associated with a corresponding increase in the size of the cell, as in Nos. 7 and 8. In No. 7, which is from a case of tuberculous pleurisy, there is some atypia, such as enlargement of some of the cells and their nuclei, hyperchromasia and the presence of a mitotic figure. However, the fact that the cells are well differentiated and retain their individuality is evidence of their non-malignant nature.

Vacuolation is often pronounced in cells found in exudates, and there are many instances in which one is unable to decide whether some of the highly vacuolated and enlarged cells are of mesothelial or histiocytic origin. The cells in No. 16 are probably of the mesothelial type, as they form an integrated cluster with marked vacuolation of the peripheral cells. The interpretation of the cells in Nos. 13, 14 and 15 is equivocal, since some of the cells show more resemblance to mesothelial cells while others seem to be closer to the histiocytic type. An extreme vacuolation and displacement of the nucleus to the periphery of the cell result in the formation of the so-called "signet ring" cell. Pronounced vacuolation is frequently seen in cases of cirrhosis of the liver (Nos. 13–16).

A group of normal lymphocytes found in the pleural fluid of a case of bronchogenic epidermoid carcinoma is shown in No. 18. Lymphocytes when present in large numbers in an exudate may justifiably arouse a suspicion of malignancy.

The lymphocytic cells illustrated in No. 19 show distinctly abnormal features such as the central clumping of the chromatin which has been repeatedly noted in malignant cells of the lymphoid type. Since this case was diagnosed as acute myelogenous leukemia complicated by miliary tuberculosis, the diagnostic significance of these cells is obscure in this case.

An illustration of a cell cluster from the pleural fluid of a case of mesothelioma may be seen in No. 20. This cluster shows irregularity of pattern and a relative prominence of the nucleoli and may be considered compatible with the diagnosis of a mesothelioma but not pathognomonic of this type of tumor.

Groups of cells from various metastatic tumors are shown in Nos. 21–31. The malignant nature of the cells is shown in all groups,

although the cytologic changes are more obvious in some than in others. The recognition of the type and site of the primary tumors is in most instances very difficult. In the clusters illustrated in this plate, one may suspect an adenocarcinoma of unknown origin in Nos. 21 and 26, an ovarian carcinoma in 24 and 25, and a probable carcinoma of the lung in 29–31. Our present knowledge of the exfoliative cytology of metastatic tumors is still too fragmentary to permit an accurate diagnosis of their type and origin.

The cells illustrated in Nos. 30 and 31 resemble those found in sputum and bronchial washings from cases of pulmonary carcinomas (C III, 1, 2 and 3). (Cells from the same case as Nos. 30 and 31, found in sputum, are shown in C III, 7.)

SERIES PLATE

E II

Exudates

Malignant and non-malignant cells found in sediment smears of pleural, peritoneal and hydrocele fluids

E II
DESCRIPTION

Malignant and non-malignant cells found in sediment smears of pleural, peritoneal and hydrocele fluids. Photomicrographs x 600.

1. Mesothelial cells. Peritoneal fluid. OG–EA series. Male. Age 73. Final clinical diagnosis: cirrhosis of the liver.

2. Mesothelial cell. Pleural fluid. OG–EA series. Female. Age 62. Clinical diagnosis: acute pericarditis with effusion. (Same case as E I, 2, and G IV, 2.)

3. Peritoneal fluid. OG–EA series. Female. Age 69. Pathologic diagnosis: primary adenocarcinoma of the endometrium, Grade II. Biopsy of peritoneum: metastatic adenocarcinoma.

4. Peritoneal fluid. OG–EA series. Female. Age 73. Pathologic diagnosis: primary adenocarcinoma of the endometrium.

5. Pleural fluid. OG–EA series. Female. Age 50. Pathologic diagnosis: metastatic carcinoma, probably from an epidermoid carcinoma of the cervix, Grade II, removed 6 years earlier.

6. Pleural fluid. OG–EA series. Female. Age 42. Pathologic diagnosis: primary carcinoma of the breast.

7–9. Peritoneal fluid. OG–EA series. Female. Age 44. Pathologic diagnosis: carcinoma of the ovary.

10. Pleural fluid. OG–EA series. Female. Age 45. Pathologic diagnosis: adenocarcinoma of the lung with pleural metastases.

11. Atypical cell structure found in hydrocele fluid of a case diagnosed as seminoma. Male. Age 46.

12 and 13. Pleural fluid. OG–EA series. Female. Age 72. Pathologic diagnosis: primary carcinoma of the breast.

14. Pleural fluid. OG–EA series. Female. Age 38. Pathologic diagnosis: infiltrating duct carcinoma of the breast, Grade III.

15. Pleural fluid. OG–EA series. Male. Age 19. Pathologic diagnosis: malignant lymphoma, Hodgkin's type.

16. Pleural fluid. Glycogen series. Male. Age 62. Pathologic diagnosis: follicular lymphosarcoma.

17. Pleural fluid. OG–EA series. Female. Age 72. Pathologic diagnosis: lymphosarcoma, reticulum cell type.

18. Pleural fluid. OG–EA series. Female. Age 56. Clinical diagnosis: lymphoma of mediastinal lymph nodes, probably Hodgkin's disease.

19. Pleural fluid. OG–EA series. Male. Age 79. Pathologic diagnosis: carcinoma of the kidney, metastatic to lung and pleura.

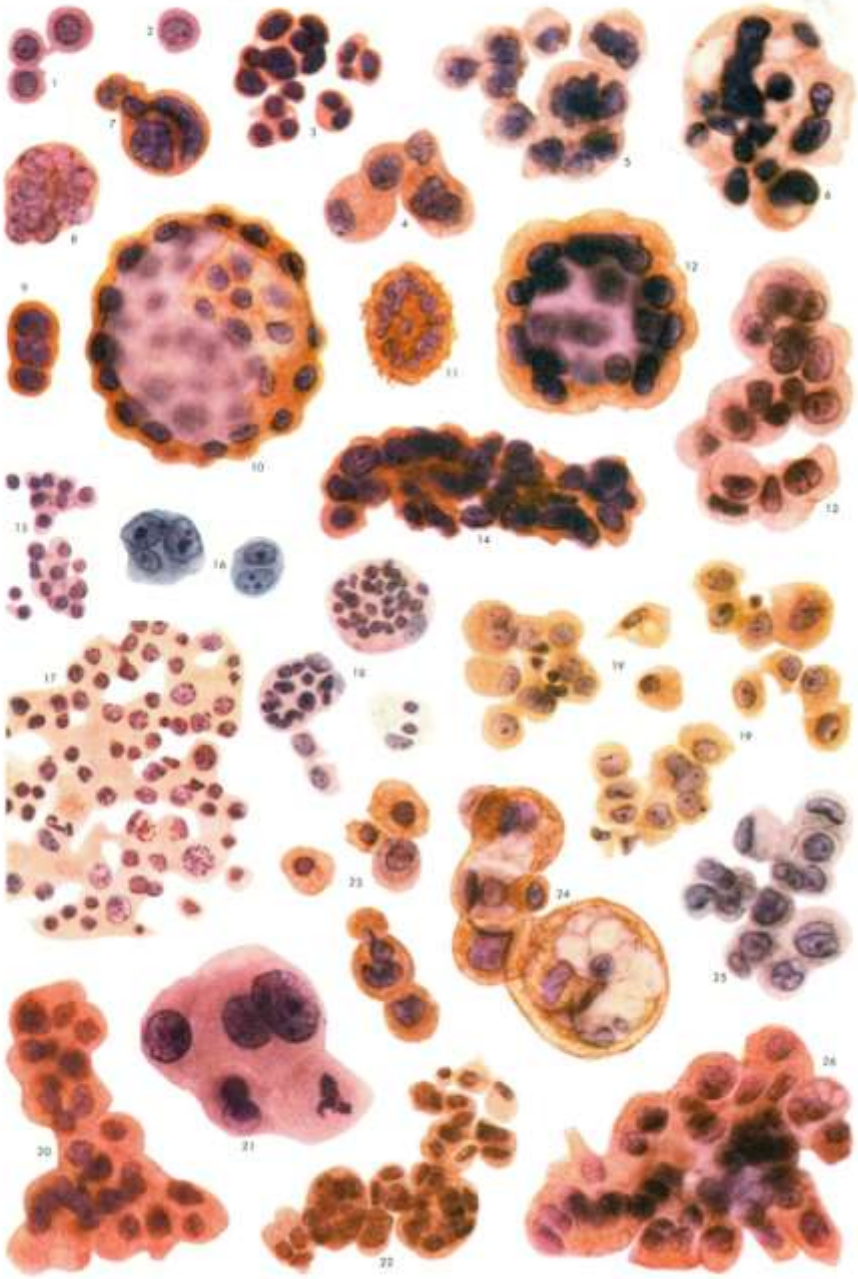
20 and 21. Peritoneal and pleural fluids. OG–EA series. Female. Age 58. Final diagnosis: carcinoma of the pancreas with evidence of widespread carcinomatosis.

22. Cluster of cells from a case diagnosed as leiomyosarcoma of the stomach. Pleural fluid. OG–EA series. Female. Age 50. (Same case as G IV, 10.)

23. Pleural fluid. OG–EA series. Female. Age 49. Pathologic diagnosis: carcinoma of the lung with metastases to pleura.

24 and 25. Pleural fluid. OG–EA series. Male. Age 53. Pathologic diagnosis: epidermoid carcinoma of the lung metastatic to the pleura.

26. Pericardial fluid. OG–EA series. Male. Age 24. Pathologic diagnosis: alveolar cell carcinoma of the lung. (Courtesy of William Umicker, Comdr., M.C., U.S.N., St. Albans Naval Hospital, Long Island, N.Y.)



E II
DISCUSSION

Malignant and non-malignant cells found in sediment smears of pleural, peritoneal and hydrocele fluids

Normal mesothelial cells are shown in Nos. 1 and 2 for comparison with the malignant cell types. For a discussion of the structure of the normal mesothelial cells see the Discussion of E I.

Cell types found in pleural and peritoneal fluids in cases of metastatic neoplasms from various organs are shown in Nos. 3-26, with the exception of Nos. 11 and 22. The type and the probable site of the primary tumors in these cases are as follows: adenocarcinoma of the endometrium, Nos. 3 and 4; carcinoma of the cervix, No. 5; carcinoma of the ovary, Nos. 7–9; carcinoma of the breast, Nos. 6, 12–13, 14; carcinoma of the lung, No. 10 (adenocarcinoma), No. 23 (unknown type), Nos. 24–25 (epidermoid), No. 26 (alveolar cell); carcinoma of the kidney, No. 19; carcinoma of the pancreas (unknown type), Nos. 20–21; tumors of lymphoid origin, Nos. 15, 18 (malignant lymphoma, Hodgkin's type), No. 16 (follicular lymphosarcoma), and No. 17 (reticulum cell sarcoma).

A survey of all these cell varieties shows distinct structural differences which permit, in some cases, the recognition of the tumor type, if not its primary site. Numbers 6, 10, 12–13, 14, 20 and 26, though representing cells from tumors originating in various organs, exhibit patterns suggestive of an adenocarcinoma, but the primary sites cannot be distinguished with a reasonable expectation of accuracy. The arrangement of cells in elongated clusters as in No. 14 is often seen in exudates in cases of metastatic adenocarcinoma of the breast. The arrangement of cells in balls as in Nos. 10 and 12 is quite characteristic of adenocarcinomas.

The cells illustrated in Nos. 3, 4 and 5 do not disclose the type and origin of the primary tumor. However, those of Nos. 7 and 8 resemble round clusters and rosettes found in endometrial smears from cases of cystadenocarcinomas of the ovary. (See A XII, 9–12 and E I, 24 and 25.) A striking similarity exists between the cluster of No. 7 and that of A XII, 11.

Cells from lymphoid tumors (Nos. 15–18) can generally be recognized as such, but the particular type of neoplasm cannot always be ascertained. No. 17 gives a good illustration of the exfoliative cytology of lymphosarcoma, with aggregations of lymphocytic cells showing distinct abnormal features and some mitotic activity. Cells undergoing degeneration exhibit a characteristic pattern with the nucleus breaking into chromatin clumps of unequal size, as may be seen in one cell of No. 17. This degenerative pattern may also be seen in other tumor types. The engulfed cells of No. 18 show a similarity to the cells of No. 15 and are interpreted as lymphocytes ingested by histiocytes. The cells of No. 16 are larger and show a resemblance to cells interpreted as Reed-Sternberg cells (C II, 9 and C V, 29 and 30).

The cells illustrated in Nos. 23, 24 and 25 are consistent with a diagnosis of primary carcinoma of the lung but are not sufficient to make possible a definition of the type. However, the cluster in No. 24 has a pattern compatible with an epidermoid carcinoma of the lung.

The cells of No. 19 show some similarity to cells found in urine sediment smears in cases of carcinoma of the kidney (see B IV, 1). Despite this similarity, however, the accurate identification of the type and origin of such cells found in an exudate smear would be very difficult.

No. 22 illustrates a cluster with rather unusual cytology and a pattern which cannot be definitely linked with the diagnosis of leiomyosarcoma of the stomach. The malignant character of the cluster is debatable because of the absence of distinct general criteria of malignancy.

No. 11 illustrates a cell structure found in fluid aspirated from a hydrocele in a case of seminoma. Should one rule out the possibility

that this may be a multinucleated histiocyte, the other interpretation is that it is a cluster of epithelial cells which, however, show no distinct malignant characteristics and therefore cannot be definitely linked with the testicular tumor.

SERIES PLATE

F 1

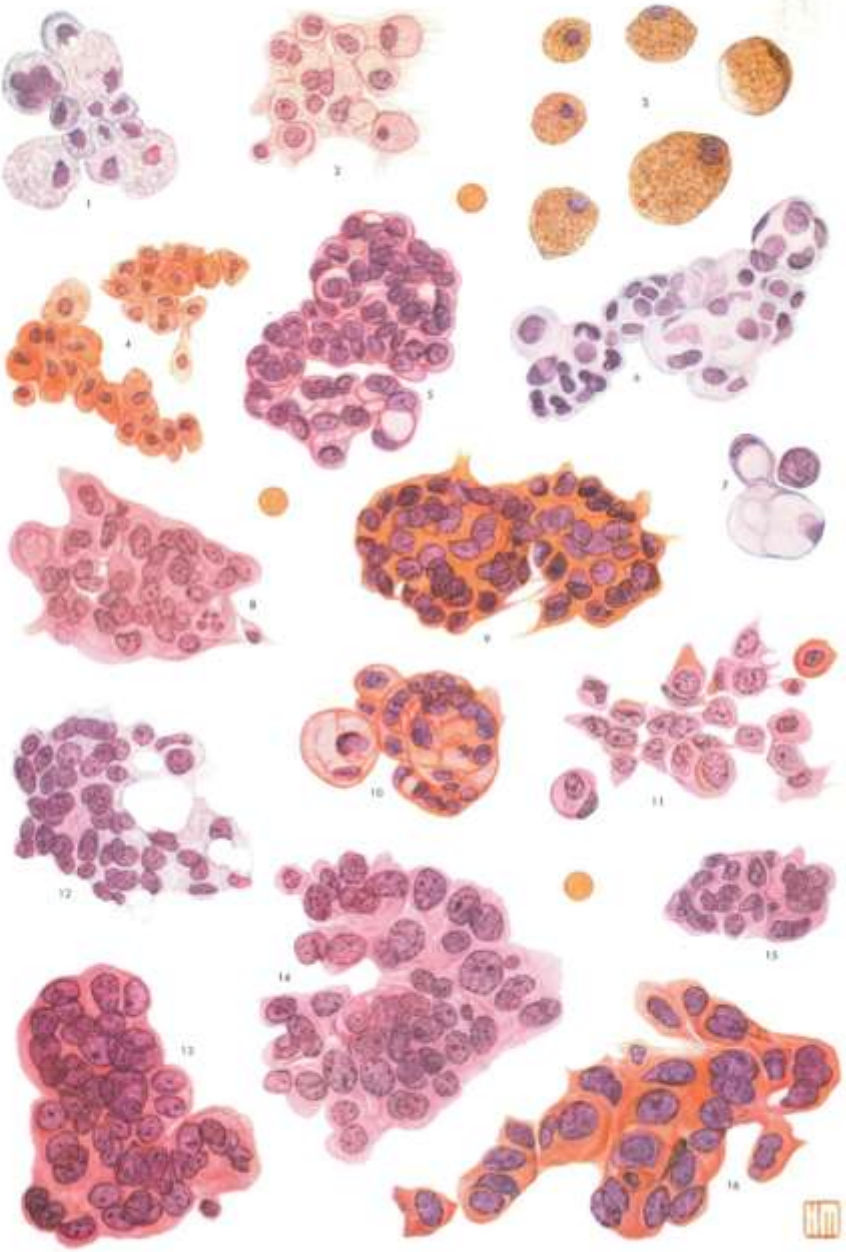
Breast

Non-malignant and malignant cells found in breast secretion or aspiration smears

FI
DESCRIPTION

Non-malignant and malignant cells found in breast secretion or aspiration smears. Drawings x 525.

1. Histiocytes. Breast smear. OG–EA series. Age 43. No evidence of breast disease. Secretion attributed to overdosage of estrogens.
2. Histiocytes. Breast smear. OG–EA series. Age 47. Pathologic diagnosis: non-infiltrating duct carcinoma. A group of carcinoma cells from the same case is shown in No. 8. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
3. Histiocytes filled with blood pigment. Breast smear. Glycogen series. Age 72. (Same case as No. 5.)
4. Epithelial cells found in fluid aspirated from a cyst. OG–EA series. Age 45. Clinical diagnosis: cyst of left breast.
5. Breast smear. Glycogen series. Biopsy and two excisions of the tumor performed within a period of 26 months were diagnosed pathologically as intraductal papilloma. Simple mastectomy 2 1/2 years after smear examination revealed the presence of carcinoma. (Same case as No. 3.)
6. Breast smear. Glycogen series. Age 36. Pathologic diagnosis: intraductal papilloma.
7. Breast smear. Glycogen series. Age 54. Pathologic diagnosis: intraductal papilloma. Note pronounced vacuolation.
8. Breast smear. Pathologic diagnosis: non-infiltrating duct carcinoma. (Same case as No. 2. Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
9. Breast smear. Age 55. Pathologic diagnosis: infiltrating comedo carcinoma, Grade II. Nodes at all levels negative and no palpable mass. Primary diagnosis by smears. (Same case as F II, 28. Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
10 and 11. Breast smear. OG–EA series. Age 49. Pathologic diagnosis: carcinoma in situ of the breast, comedo type. Primary diagnosis by smears, no palpable mass. (Courtesy of Dr. George Romberg, White Plains Hospital, White Plains, N.Y.)
12. Breast smear. OG–EA series. Age 51. Pathologic diagnosis: adenocarcinoma of the breast, Grade II. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
13 and 14. Breast smear. OG–EA series. Age 50. Pathologic diagnosis: adenocarcinoma of the breast with axillary metastasis. Primary diagnosis by smears. (Same case as F II, 17–19.)
15. Breast smear. Glycogen series. Age 64. Pathologic diagnosis: carcinoma of the breast.
16. Breast smear. OG–EA series. Age 48. Pathologic diagnosis: intraductal carcinoma of the mammary gland. (Courtesy of Dr. Leslie R. Grams and Alexander W. Rakosy, Englewood Hospital, Chicago, Ill.)



DISCUSSION

Non-malignant and malignant cells found in breast secretion or aspiration smears

Histiocytes are depicted in Nos. 1 and 2. In breast secretion smears they appear singly or in groups consisting of cells of varying size. The cytoplasm has a fine vacuolation which gives it a foamy appearance, and the nuclei have an irregular, wrinkled form. Some cells show nuclear eccentricity and multinucleation. The histiocytes of No. 1 have round or oval nuclei and finely vacuolated cytoplasm. Histiocytes of this type are commonly seen in breast secretion smears and are particularly numerous in cases of chronic cystic mastitis. The histiocytes in No. 2 have better preserved nuclei and the vacuolation of the cytoplasm is less pronounced.

The two groups in No. 4 consist of well-preserved epithelial cells which are interpreted as normal lining cells or small papillary projections of the lining of a cyst. They were found in fluid aspirated from a ductal cyst.

Cells from two cases of intraductal papilloma of the breast are shown in Nos. 6 and 7. The pronounced vacuolation, particularly of the peripherally located cells, is a frequent cytologic feature in exfoliated cell clusters in this condition. The boundaries of the individual cells are usually well preserved, and the cells are grouped in characteristic, compact and sharply outlined clusters. The nuclei are, as a rule, of uniform size and normal structure, but a certain irregularity in their distribution within the cluster may be noted.

The cluster illustrated in No. 5 has a grouping pattern suggestive of papilloma. However, because of the crowding and irregular distribution of the cells and the hyperchromasia of the nuclei it was interpreted as strongly suggestive of malignancy. A biopsy performed 5 days later on the strength of the cytologic report was diagnosed as chronic fibrocystic disease of the breast and intraductal papilloma. Three months later a tumor of the right breast was excised and was also diagnosed as an intraductal papilloma.

Thirteen months after the first biopsy a tumor was excised from the left breast and was diagnosed as intraductal papilloma. A recurrent tumor of the left breast was removed thirteen months later and was diagnosed pathologically as intraductal papilloma. After 4 months the patient was rehospitalized because of bleeding from the left nipple. On a specimen of blood and extruded tissue from the nipple a pathologic diagnosis of carcinoma of the breast was made which was confirmed at simple mastectomy. The final diagnosis of carcinoma of the left breast was thus established in this case approximately 2 1/2 years after the cytologic smear in which the cluster illustrated in No. 5 was found.

Histiocytes from the same case, filled with blood pigment and with fragmented erythrocytes indicating active phagocytosis of old blood, are illustrated in No. 3. They were found in the breast secretion in a case of intraductal papilloma.

The clusters illustrated in Nos. 8 to 11 are from cases of early carcinoma of the breast. Nuclear atypia and irregularity of pattern are apparent in all clusters. The cells are rather densely grouped and their boundaries are indistinct. In some cases there is a resemblance in the arrangement of the cells to that seen in papillomas, as in Nos. 9–10. These clusters are from early carcinomas of the comedo type diagnosed first on the basis of smears. In neither case was there a palpable mass or any evidence of glandular involvement. It is significant that active exfoliation may occur at such a relatively early stage.

Nos. 12–16 represent more advanced cases of carcinoma of the breast. All groups exhibit distinct criteria of malignancy; these are, however, more pronounced in the clusters of Nos. 13, 14 and 16. Since most primary carcinomas of the breast are of glandular origin, there is not as great variation in the structure and form of exfoliated malignant cells in breast smears as is observed in smears of some other organs.

SERIES PLATE

F II

Breast

Malignant and non-malignant cells found in breast secretion and aspiration smears

F II
DESCRIPTION

Malignant and non-malignant cells found in breast secretion and aspiration smears. Photomicrographs x 600.

1. Histiocytes. Breast smear. OG–EA series. Age 58. Provisional diagnosis: ductal papillary growth.

2. Breast smear. OG–EA series. Age 42. Pathologic diagnosis: duct papillomatosis and duct ectasia.

3–6. Breast smear. OG–EA series. Age 52. Pathologic diagnosis: atypical papillomatosis, borderline malignancy.

7. Aspirate from breast cyst. Glycogen series. Age 37. Clinical diagnosis: benign cyst.

8 and 9. Aspirate from breast cyst. OG–EA series. Age 41. Clinical diagnosis: ductal cyst.

10. Aspirate from breast cyst. Glycogen series. (Same case as No. 7.)

11–16. Breast smear. Glycogen series. Age 49. Pathologic diagnosis: duct carcinoma in the subareolar area of the breast. Lymph nodes revealed no evidence of metastatic involvement.

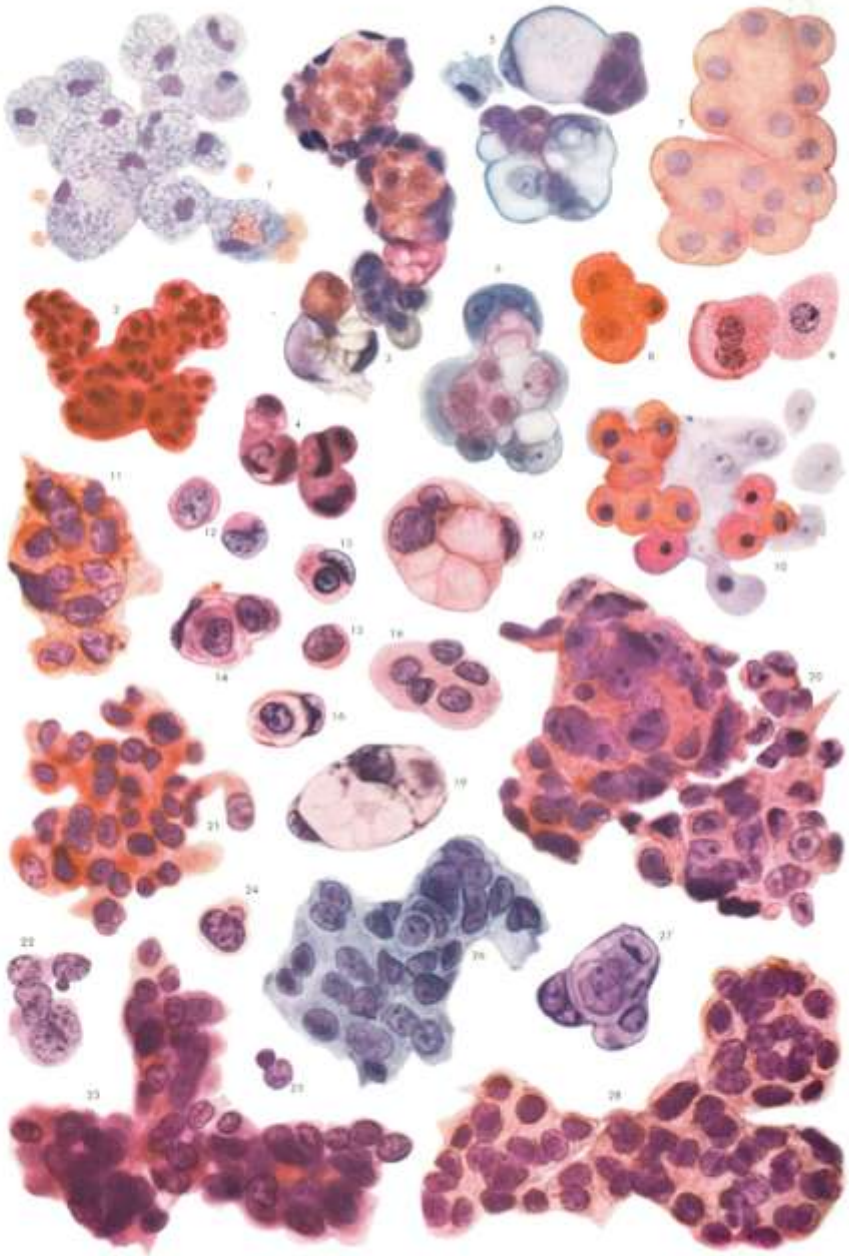
17–19. Breast smear. OG–EA series. Age 50. Pathologic diagnosis: adenocarcinoma of the breast with axillary metastasis. (Same case as F I, 13 and 14.)

20. Aspirated fluid from a recurrent tumor mass on the anterior chest wall. The patient had had a radical mastectomy 1 year earlier. OG–EA series. Age 36. Pathologic diagnosis: carcinoma of the breast, recurrent.

21–25. Breast smear. OG–EA series. Age 50. Clinical diagnosis: inoperable carcinoma of the breast with pulmonary metastasis.

26 and 27. Direct smear from an ulcerated lesion of the left breast. OG–EA series. Age 50. Pathologic diagnosis: carcinoma of the breast, Paget's disease of the nipple.

28. Breast smear. OG–EA series. Female. Age 55. Pathologic diagnosis: infiltrating comedo carcinoma, Grade II. Nodes at all levels negative and no palpable mass. Primary diagnosis by smears. (Same case as F I, 9. Courtesy of Strang Prevention Clinic, Memorial Center, New York.)



F II
DISCUSSION

Malignant and non-malignant cells found in breast secretion and aspiration smears

The histiocytes in No. 1 are of the same type as those in F I, 1. Ingestion of erythrocytes is shown in a few cells.

Cells from two cases of intraductal papillary growths are illustrated in Nos. 2 and 3–6. In both cases the exfoliated cell clusters exhibit the pattern characteristic of such growths (see F I, 6 and 7). However, the cytology differs in the two cases. In No. 2, which was diagnosed as duct papillomatosis with duct ectasia, the cells are small and more densely grouped, and have a uniform appearance. Those of the second case (Nos. 3–6) are larger and display atypical morphologic features with pronounced vacuolation and an irregular pattern. Corresponding atypia was found in the operative specimen, which was diagnosed as atypical papillomatosis, borderline malignancy.

Nos. 7–10 illustrate cells found in fluid aspirated from ductal cysts of the breast. They have the appearance of normal epithelial cells and are interpreted as cells exfoliated from the lining epithelium or from small papillary projections of the epithelium of the cysts. Some of the cells show degenerative changes such as fading or pyknosis of the nuclei. Others are better preserved (Nos. 8 and 9) and have large vesicular nuclei. Their cytoplasm is finely vacuolated and contains an acidophilic secretion. The characteristic lobulation of the clusters, the round shape of the cells and the densification of their free borders are distinctive features of cells of this type.

All remaining numbers (11–28) are from carcinomas of the breast. Nos. 11–16 and 28 are from two relatively early cases. The cells from the first case (11–16) were found in the breast secretion of a 49-year-old woman who consulted her physician because of an excoriation about the left nipple. A biopsy performed 3 months before the smears were taken showed only chronic inflammatory disease. Smears prepared from a small amount of fluid obtained from the nipple contained abnormal cells and cell groups conclusive for malignancy. On the basis of the smear findings a local excision of the nipple and areola was performed and the specimen was reported pathologically as duct carcinoma. A subsequent radical mastectomy revealed no invasion or extension to the lymph nodes.

Single cells with disproportionately large and hyperchromatic nuclei (Nos. 12, 15, and 24) or even stripped abnormal nuclei (No. 25) are not infrequently seen in smears from cases of carcinoma of the breast. They constitute a valuable criterion of malignancy as they can be spotted more readily in breast smears than in smears of other types because of the relative uniformity of the normal breast cytology. The enclosure of one cell into another often occurs in carcinomas of the breast as well as in carcinomas of other organs and gives the impression of a phagocytic process, although in larger clusters in which the cells are crowded (No. 26) it is probably the result of the pressure exerted around actively growing cells.

The pattern of the early duct carcinoma of the comedo type illustrated in No. 28 resembles greatly that of an intraductal papilloma. The enlargement and hyperchromasia of the nuclei, however, reveal the malignant nature of the cells.

Nos. 17–19 are from an advanced carcinoma of the breast with metastasis to lymph nodes. Some highly vacuolated cells are shown in Nos. 17 and 19. Their differentiation from non-malignant vacuolated cells found in papillomas (F I, 6 and 7) is based chiefly on nuclear criteria.

All remaining clusters (Nos. 20–27) were found in advanced cases and exhibit distinctive cytologic criteria of malignancy. One of the nuclei of No. 22 and those of Nos. 24 and 25 show a structural atypia which simulates a budding process.

SERIES PLATE

G I

Miscellaneous

Histiocytes found in smears of various body fluids, with the exception of No. 1, which illustrates plasma cells

GI
DESCRIPTION

Histiocytes found in smears of various body fluids, with the exception of No. 1, which illustrates plasma cells. Drawings x 525.

1. Plasma cells. Section of cervix. Special stain. Age 34. Pathologic diagnosis: fibroma of the cervix with infiltration of inflammatory cells.

2–15. Histiocytes.

2 and 3. Vaginal smear. OG–EA series. Age 29. Normal cycle, 7th day.

4. Sputum. OG–EA series. Male. Age 75. Negative case.

5. Normal histiocytes of the dust cell type. Sputum. OG–EA series. Male. Age 69. Case of bronchogenic epidermoid carcinoma. (Same case as C III, 8 and 9.)

6. Normal histiocytes of the dust cell type. Sputum. Experimental stain. Female. Age 52. Case of adenocarcinoma of the lung.

7. Endocervical smear. OG–EA series. Age 62. Menopause, 10 years. Negative case.

8. Histiocyte with pseudopodia. Endocervical smear. OG–EA series. Age 41. Normal cycle, 25th day. Negative case.

9. Histiocytes showing phagocytosis of polymorphonuclear leucocytes and erythrocytes. Vaginal smear. OG–EA series. Age: postmenopausal. Case of squamous cell carcinoma of the cervix.

10. Histiocytes containing engulfed erythrocytes and round bodies which give the impression of nuclei exhibiting karyorrhexis. Catheterized urine. OG–EA series. Female. Age 28. Case of chorioepithelioma with metastasis to the lung.

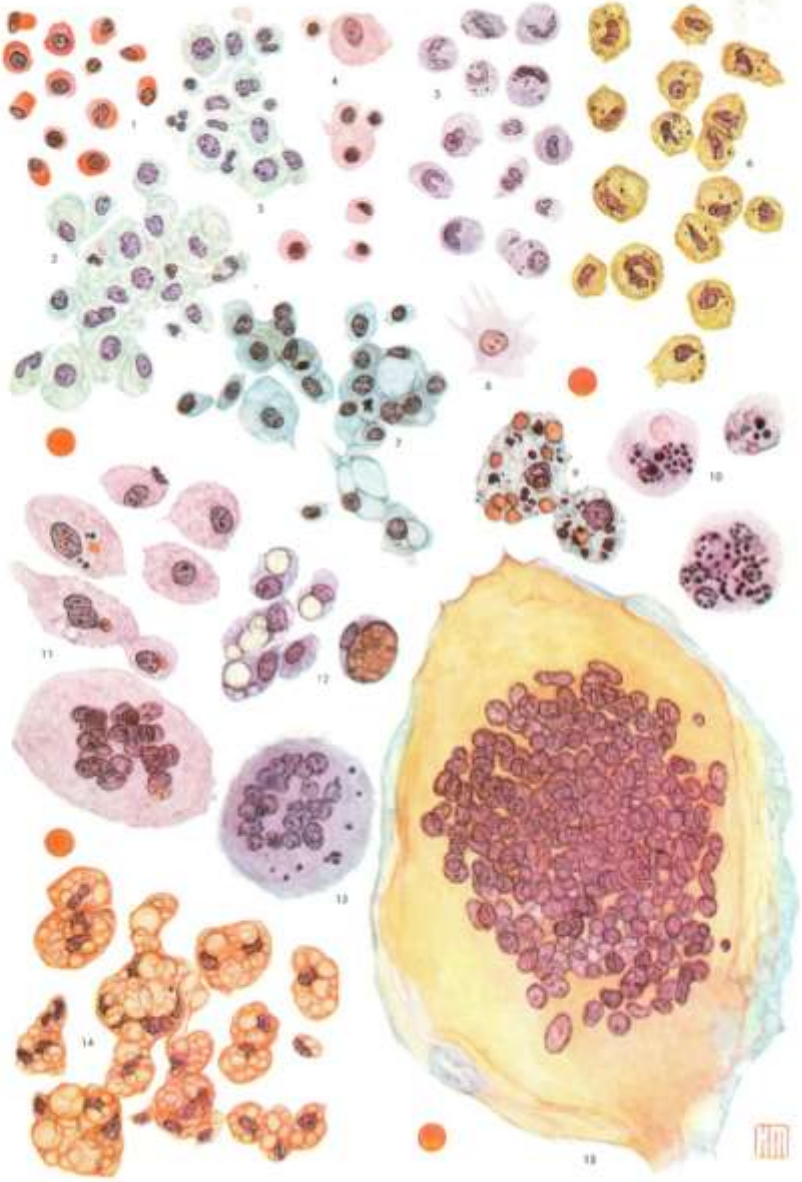
11. Histiocytes phagocytosing erythrocytes and leucocytes. Endocervical smear. OG–EA series. Age 59. Postmenopausal bleeding.

12. Histiocytes phagocytosing blood. Endocervical smear. OG–EA series. Age 38. Normal cycle, 25th day.

13. Large histiocyte with inclusions of nuclear fragments of inflammatory cells. Vaginal smear. OG–EA series. Age 55. Case of adenocarcinoma, Grade II. Menopause, 5 years. (Same case as A IX, 4.)

14. Histiocytes phagocytosing lipoid droplets. Sputum. OG–EA series. Male. Age 50. Diagnosis: chronic lipoid pneumonia.

15. Giant histiocyte. Endocervical smear. OG–EA series. Age 58. Menopause, 5 years. Negative case.



G1
DISCUSSION

Histiocytes found in smears of various body fluids, with the exception of No. 1, which illustrates plasma cells

Plasma cells, found in a section of a chronically inflamed cervix, are shown in No. 1. Such cells are seen in smears more rarely than other inflammatory cells. They are included here for the sake of comparison with the histiocytes. Their characteristic, frequently eccentric nucleus and the homogeneity and compactness of the cytoplasm are distinguishing features. The intensely acidophilic appearance is the result of special staining.

Some of the criteria by which histiocytes can be identified are nuclear eccentricity, a kidney- or horseshoe-shaped nucleus, fine vacuolation of the cytoplasm causing a foamy appearance, frequent presence of inclusions, and variation in size and loose arrangement of the cells in aggregates or clusters. Normal histiocytes from the female genital tract are illustrated in Nos. 2 and 3. In vaginal smears histiocytes are especially numerous during the postmenstrual stage (Discussion, A VIII). The cells illustrated here were found on the 7th day of the cycle.

The absence of the typical cytoplasmic vacuolation in histiocytes, as in those of No. 4, makes their identification difficult. Their recognition is possible, however, by some of the other criteria enumerated above.

Typical histiocytes of the lung, known as "dust" cells, are shown in Nos. 5 and 6. Many of these contain dust or carbon particles. Kidney- and horseshoe-shaped nuclei are more frequently seen in the dust cells of the lung than in the histiocytes of the genital tract. The differences in color in the various types of histiocytes illustrated in this plate are the result of staining with various procedures and modifications of the OG–EA series and are of no particular significance. Normally the histiocytes are basophilic and their cytoplasm stains faintly.

No. 7 illustrates two clusters of histiocytes with large vacuoles. Their grouping resembles somewhat that of endometrial cells.

A normal histiocyte with pseudopodia is shown in No. 8. Such forms are rarely seen, since histiocytes, like all exfoliated epithelial cells, tend to become rounded in fixed smears.

Two histiocytes loaded with erythrocytes and nuclear fragments of inflammatory cells are illustrated in No. 9. Such cells may be seen in malignant as well as non-malignant conditions, and indicate the presence of a chronic inflammatory process with atypical bleeding. Fresh erythrocytes, such as are found during menstruation, are not as a rule attacked by histiocytes. The phagocytosis of erythrocytes and leucocytes implies that they are, to a certain degree, degenerated and necrotic.

Phagocytosis of erythrocytes by histiocytes is shown also in No. 10. These histiocytes, however, contain some round and oval bodies with coarse chromatin granules which are probably nuclei exhibiting an unusual pattern of degeneration. They were found in the urine of a woman with chorioepithelioma with no evidence of metastasis to the urinary organs.

Histiocytes phagocytosing blood are also seen in Nos. 11 and 12. In both instances they were found in endocervical smears from women with atypical bleeding but no evidence of malignancy.

Two multinucleated histiocytes, one from a non-malignant case and one from a case of adenocarcinoma of the endometrium, are shown in Nos. 11 and 13 respectively. Multinucleation of histiocytes is not a criterion of malignancy; it may be observed in malignant and non-malignant conditions when there is a marked histiocytic reaction.

The phagocytosis of lipoid substances causes a characteristic, bubble-like vacuolation of the histiocytes which can be readily recognized. Histiocytes of this type are known as "lipophages." When found in sputum, as those of No. 14, they are diagnostic of lipoid pneumonia.

A giant histiocyte with an unusually large

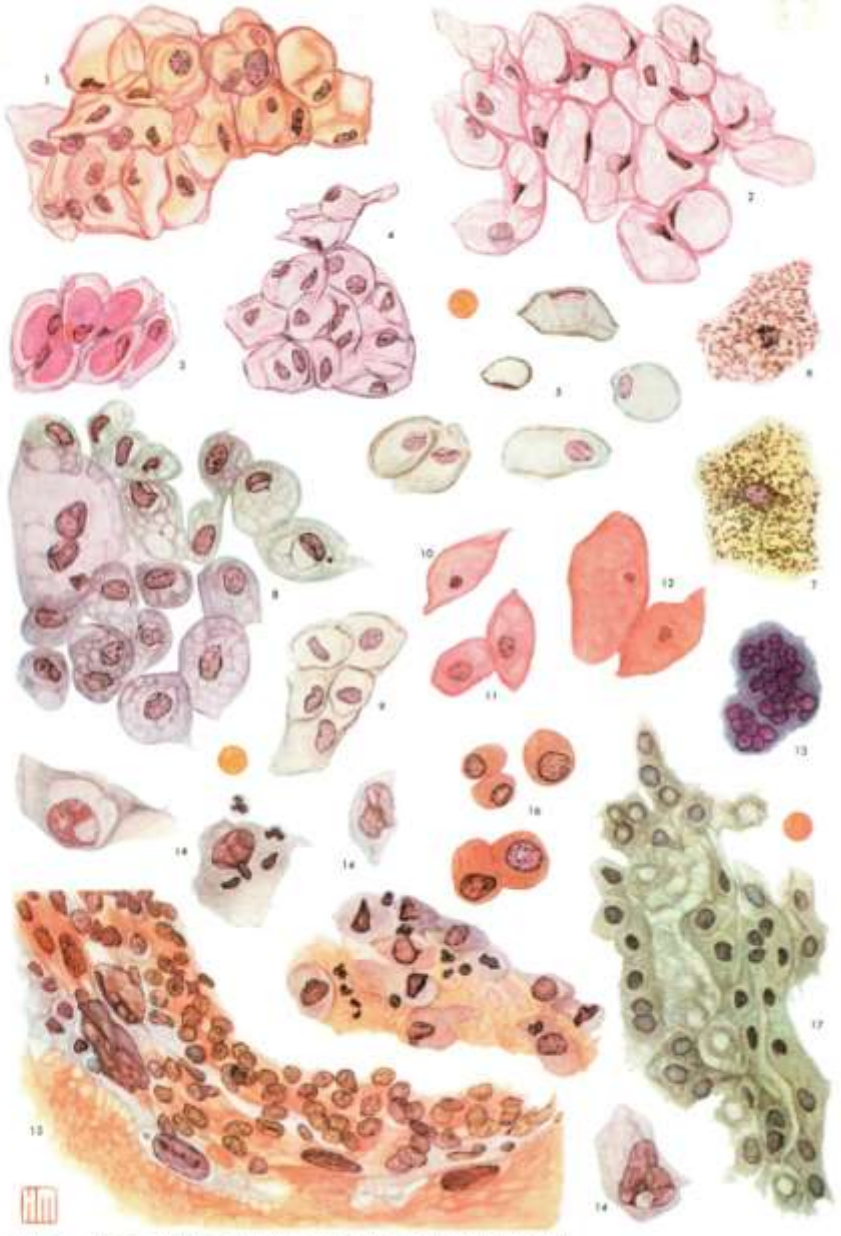
number of nuclei, found in an endocervical smear of a postmenopausal woman, is shown in No. 15. A cell of this type has a certain resemblance to the multinucleated cells found in ureteral urine specimens as illustrated in B II, 8. However, a closer inspection reveals great differences in the structural patterns of these two cell types.

SERIES PLATE

G II

Miscellaneous

Cells from vaginal, endocervical and urine sediment smears in cases of pregnancy and from an amnion scraping smear



G II
DISCUSSION

Cells from vaginal, endocervical and urine sediment smears in cases of pregnancy and from an amnion scraping smear

Nos. 1, 3 and 5 illustrate cells of the navicular type found in vaginal smears in cases of pregnancy. Cells of this type are usually filled with glycogen which may be clearly demonstrated in well-preserved cells stained specifically for glycogen (No. 3). Navicular cells may be seen frequently, in smears not only from pregnant but also from non-pregnant women, and cannot therefore be considered specifically diagnostic of pregnancy. However, navicular cells of pregnancy may show certain distinguishing characteristics, such as larger nuclei, heavier cell outlines and a more compact grouping.

Similar cells are also found in the urine of pregnant women (Nos. 2 and 4).[22] Their form resembles closely that of the navicular cells of the vagina, although they are cells differentiated from the transitional epithelium of the bladder. The cluster in No. 3 was found in a vaginal smear and that of No. 4 in a urine sediment smear from the same woman on the 229th day of pregnancy. The eccentricity of the nuclei is due to the pressure of accumulated glycogen and is a characteristic feature of navicular cells in general, including those of pregnancy, which as a rule are rich in glycogen. Navicular cells are usually basophilic. The differences in the staining reaction in Nos. 1, 2 and 5 are due to the use of different formulae within the OG–EA series and therefore are of no particular significance.

Superficial squamous cells filled with blood pigment are illustrated in Nos. 6 and 7. They were found in a case of missed abortion. Similar cells have been noted also in smears from normal women, chiefly during the late menstrual stage (A I, 17 and 18), or in cases of atypical bleeding. They cannot therefore be considered specifically diagnostic of missed abortion. The consideration of the age and menstrual history of the patient is necessary for the proper interpretation of such cases.

Some characteristic cell types may also be found in postpartum smears (Nos. 9, 10 and 11). The navicular form of the cells is still discernible, although the cells have a tendency to become rounded. A distinct acidophilia is shown by some cells (Nos. 10 and 11).

The group illustrated in No. 8 is from a case of abortion occurring on the 117th day of pregnancy. The type of the cells corresponds to that of the outer layers of the parabasal zone, which become highly hypertrophied during pregnancy and undergo extensive exfoliation at the end of gestation. The vacuolation pattern suggests a rich glycogen content. One of the cells is abnormally enlarged and is binucleated.

The two cells of No. 12 are from a case of tubal abortion. In such cases as well as in incomplete abortions and ectopic pregnancies there is a definite increase in the number of acidophilic cells reflecting an increased estrogenic stimulation. The two cells illustrated here are of a type that one might be tempted to call an "abortion" cell type, as it is encountered chiefly in cases of abortion. The cells are distinctly acidophilic but retain their navicular form and have a small pyknotic nucleus which shows signs of fading.

A syncytial knot, found in a case of incomplete abortion, is shown in No. 13. Such syncytial knots are only rarely seen in vaginal smears but have a specific diagnostic value. Their relatively small nuclei of normal appearance and uniform size and their dense grouping are characteristics by which they can be differentiated from multinucleated histiocytic and epithelial cells (G I, 11 and 13; and G IV, 2, 9, 10 and 12).

The cells in No. 14 are interpreted as decidual cells from the maternal placenta. They were found in a vaginal smear from a case of incomplete abortion. Cells of this type can be identified by the enlargement, irregular form, lobulation and vacuolation of the nuclei,

and the degenerate appearance of the cells in general. Because of these features they show a great resemblance to malignant cells and may be easily misinterpreted as such. The history of the patient is of great help in the proper evaluation of cells of this type.

Nuclei corresponding to those of the cells in No. 14 are shown also in No. 15, which illustrates a tissue fragment found in an endocervical smear from a case of retained decidua. The characteristic lobulation and vacuolation are shown distinctly in some of the nuclei. A comparison of No. 15 with A VI, 26 shows the structural differences between the nuclei of abnormal decidual cells and those of typical malignant cells.

The cells of No. 16 were found in a vaginal smear from a case of abortion and are also interpreted as decidual cells. The identification of their type and origin is, however, more difficult. In this case, in which there was a strong clinical suspicion of malignancy, they were misinterpreted as adenocarcinoma cells.

The tissue fragment shown in No. 17 was obtained by the scraping of a normal amnion. Cells from the amnion cannot be expected to be present in the vaginal smear except at the time of the rupture of the amniotic sac. Such cells found in vaginal smears appear in clusters and have a characteristic polygonal form, making possible their identification.[7]

SERIES PLATE

G III

Miscellaneous

Exfoliated non-malignant and malignant cells showing effects of irradiation

G III
DESCRIPTION

Exfoliated non-malignant and malignant cells showing effects of irradiation. Drawings x 525.

1–8. Cells from a vaginal smear taken 11 days after the beginning of x-ray treatment (400 R daily). OG–EA series. Age 41. Pathologic diagnosis: squamous cell carcinoma of the cervix, Grade II.

1. Cells of the parabasal type interpreted as non-malignant.
2. Cluster consisting of degenerated cells (non-malignant?) and polymorphonuclear leucocytes.

3–8. Aberrant superficial and parabasal squamous cells interpreted as malignant.

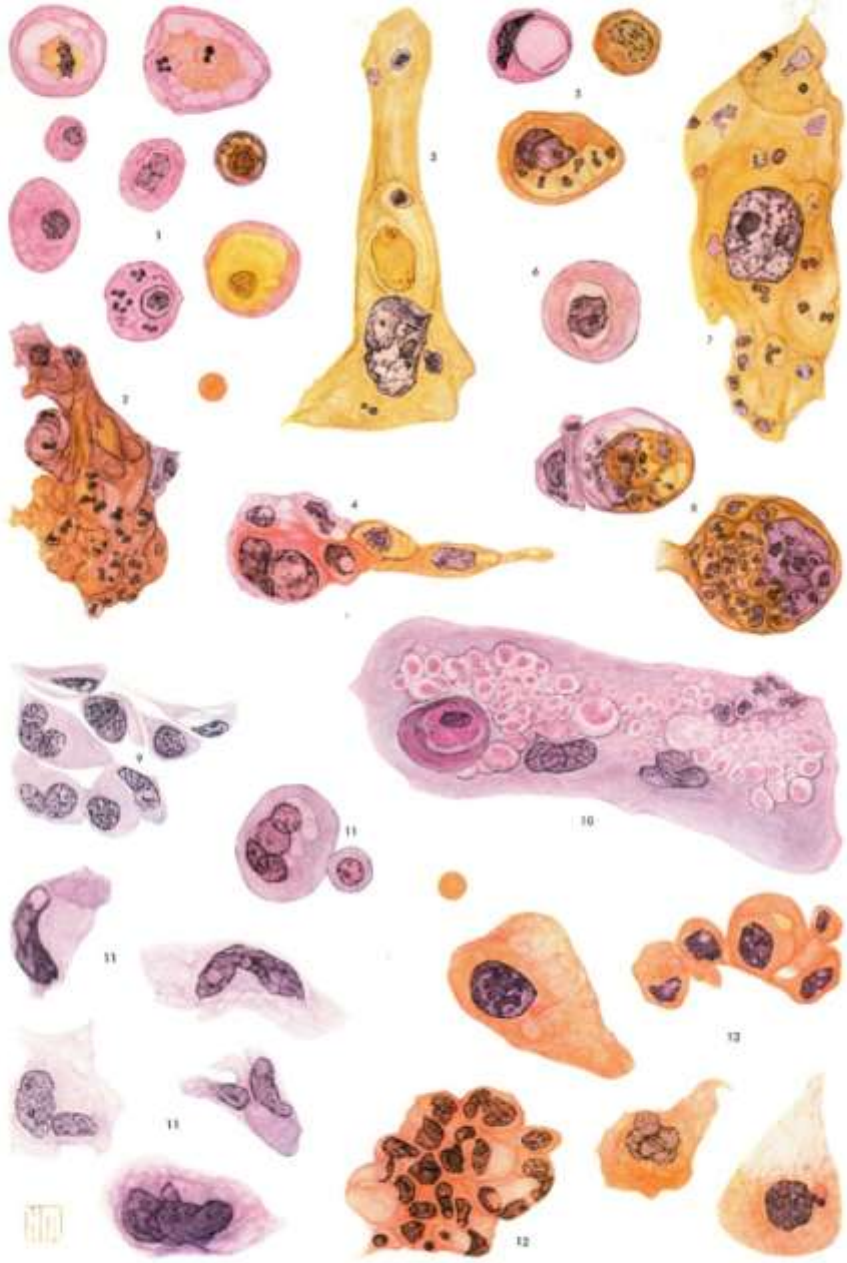
9. Cells from a patient who had had x-ray and radium therapy 6 years earlier for carcinoma of the cervix (proved by biopsy). They are interpreted as atypical but not suggestive of a recurrence. Cervical smear. Glycogen series. Age 41. Negative clinical findings. (Same case as G IV, 9.)

10. Cervical epithelial fragment consisting of degenerated cells found 41 days after the completion of x-ray therapy for squamous cell carcinoma of the cervix, Stage III. The substance seen in the vacuoles appears to be glycogen which remained after the degeneration of glycogen-filled cells. Vaginal smear. Glycogen series. Age 63.

11. Abnormal cells found in an endometrial smear 6 months after radium therapy for menorrhagia. At the time of irradiation, curettage did not disclose malignancy. Glycogen series. Age 53.

12. Malignant cells. Sputum. OG–EA series. Female. Age 65. Clinical diagnosis: carcinoma of the lung. Autopsy diagnosis 1 year later: alveolar cell carcinoma. The patient had leukemia also.

13. Malignant cells from the same case as No. 12, showing marked postradiation effects. Sputum specimen taken 11 days after the completion of a 12-day course of x-ray therapy (1500 R). OG–EA series.



G III
DISCUSSION

Exfoliated non-malignant and malignant cells showing effects of irradiation

The differentiation between malignant and non-malignant exfoliated cells becomes difficult after irradiation because of the extreme morphologic changes which occur not only in the malignant cells but also in normal irradiated cells. In cells of the squamous type which have high growth potentialities these changes are particularly pronounced and consist of an excessive growth of the cells and their nuclei and of marked structural modifications followed by degenerative and necrotic changes.

The cells illustrated in Nos. 1–8 were found in a vaginal smear from a case of carcinoma of the cervix, Grade II. The smear was taken 11 days after the beginning of x-ray treatment (400 R daily). The cells shown here were selected to represent normal and malignant cells after irradiation. However, this selection was, in a way, arbitrary, since the accurate determination of the benign or malignant character of a cell after it has been irradiated is very difficult.

The cells of No. 1 are considered to be normal cervical cells of the parabasal type showing degenerative radiation effects such as fading and nuclear resorption, and atypical vacuolation as well as invasion of the cells by inflammatory elements. Leucocytic infiltration is commonly seen in exfoliated irradiated cells, both normal and malignant. It may be indicative of a phagocytic process, probably an endophagocytosis, as it is unlikely that the leucocytic elements would be actively ingested by these necrotic cells. Such an infiltration is shown in many of the illustrated cells, as in No. 2, which is considered to be a cluster of necrotic benign cells, and in Nos. 7 and 8, which have the appearance of degenerating malignant cells.

The extreme enlargement of the cells and the structural abnormality of the nuclei are distinctly seen in Nos. 3 and 7. Vacuolation of the nucleus, which is considered a degenerative change, is shown in No. 4 and may be observed also in other cells undergoing necrosis, malignant (A VI, 24; A X, 14 and 15) as well as benign (C II, 14). Nuclear vacuolation is not a specific effect of irradiation and may be caused by other factors such as the drying of cells prior to fixation.

After irradiation for carcinoma of the cervix, aberrant cell forms may persist in the smear for a relatively long period of time. It is often difficult to decide whether the presence of such aberrant cells represents extreme postradiation atypia, a recurrence, or the existence of a residual lesion. No sharp lines of distinction exist between some of the extremely atypical forms and the truly malignant cells.

A group of abnormal endocervical cells from a smear taken 6 years after irradiation are illustrated in No. 9. Repeat smears from the same case taken 2 years later showed persistent atypia but no evidence of a recurrence. An atypical multinucleated cell from the same case is illustrated in G IV, 9.

The abnormal mass shown in No. 10 probably represents a fusion of degenerated cells of the parabasal glycogenic type. The presence of a well-preserved glycogenic cell and the high content of glycogen within the mass are in favor of such an interpretation.

The cells in No. 11 were found in an endometrial smear of a patient who had received radium therapy for menorrhagia approximately 6 months before the smear was taken. No evidence of malignancy was found in the curettings obtained at the time of the insertion of radium. It is therefore likely that the cells illustrated here represent extreme structural aberrations of irradiated normal endometrial cells or their descendants. Vacuolation of nuclei is well demonstrated in some of the cells of this group.

The evaluation of sputum or bronchial aspiration smears from patients subjected to irradiation for carcinoma of the lung is somewhat less difficult than that of smears from

irradiated epidermoid carcinomas of the cervix. Normal cells of the bronchial mucosa, either ciliated or goblet, which have been exposed to irradiation do not exhibit the extreme aberrations in size and form found in the irradiated normal squamous cells of the cervix and therefore can be differentiated with greater accuracy from the irradiated malignant cells. No. 13 illustrates irradiated malignant cells found in a sputum specimen taken 11 days after the completion of a 12-day course of x-ray therapy. Malignant cells found in the smear of the same patient 2 months prior to irradiation are shown for comparison (No. 12).

Malignant cells usually disappear within 2 to 4 weeks after effective irradiation. Their persistence indicates that the effect of treatment was unsatisfactory and implies a rather unfavorable prognosis. In the case illustrated in Nos. 12 and 13, the patient died 8 months after the initiation of radiation therapy and autopsy revealed an alveolar cell carcinoma. In addition to carcinoma cells, the preradiation sputum smears contained numerous lymphocytic cells which were attributed to the coexisting chronic lymphatic leukemia. Whereas the carcinoma cells persisted in smears taken at intervals until death, the lymphocytes disappeared soon after irradiation and never reappeared in any significant numbers.

SERIES PLATE

G IV

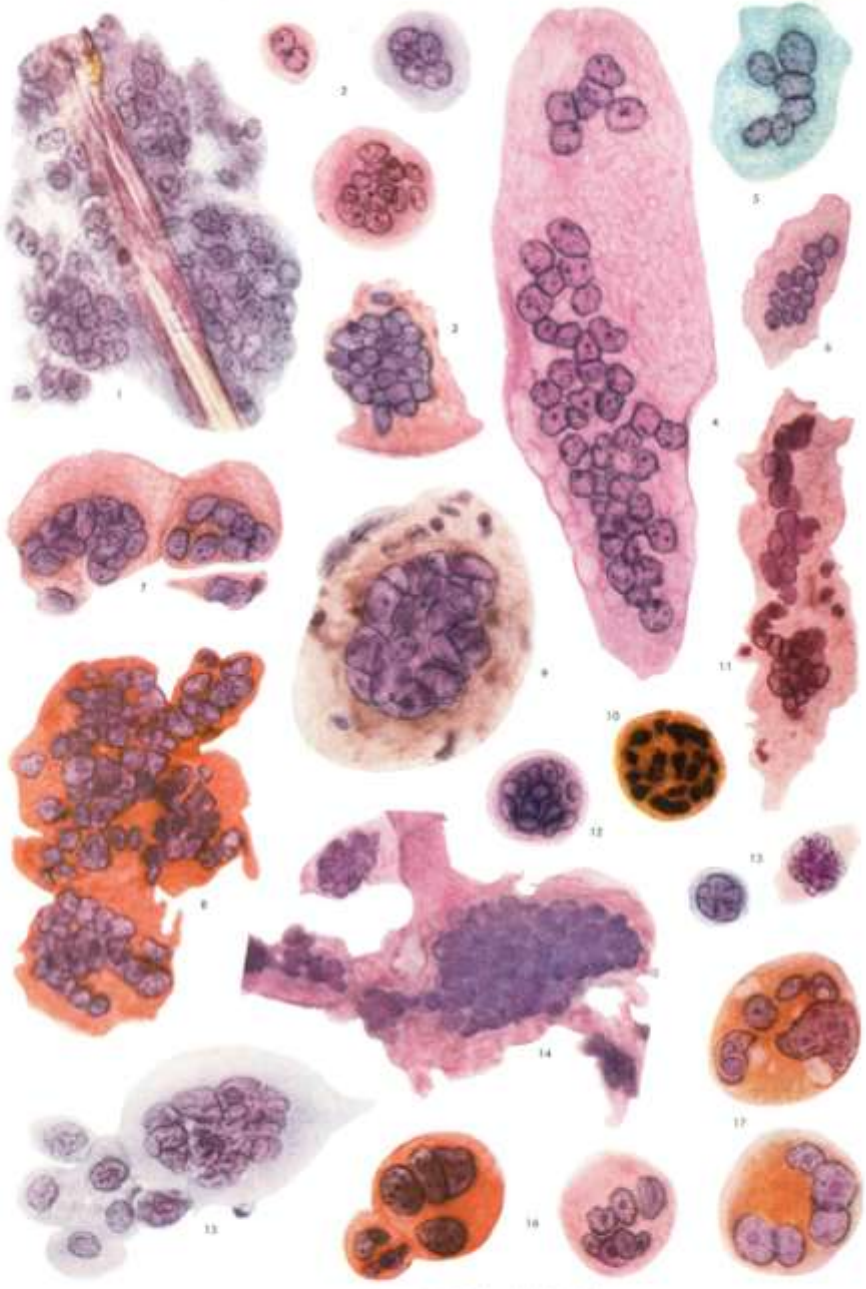
Miscellaneous

Non-malignant and malignant multinucleated cells from various organs

GIV
DESCRIPTION

Non-malignant and malignant multinucleated cells from various organs. Photomicrographs x 600.

1. Cells interpreted as histiocytes. Vaginal smear. OG–EA series. Female. Age 43. Pathologic diagnosis: intraepithelial squamous cell carcinoma of the cervix.
2. Cells interpreted as multinucleated histiocytes. Pleural fluid. OG–EA series. Female. Age 62. Final diagnosis: acute pericarditis with effusion. (Same case as E I, 2 and E II, 2.)
3. Multinucleated cell of the ciliated type. Bronchial washing. OG–EA series. Male. Age 44. Pathologic diagnosis: carcinoma of the lung.
4. Multinucleated cell of the transitional type. Ureteral specimen. OG–EA series. Male. Age 63. Clinical diagnosis: suspicion of a renal cyst. No evidence of malignancy.
5. Multinucleated cell of the transitional type. Ureteral specimen. Glycogen series. Female. Age 50. Pathologic diagnosis: nephrosis and hemorrhage in the renal tubules, and chronic pyelitis of the follicular type. (Same case as B II, 6–8.)
6. Non-malignant multinucleated cell found in the epithelial lining of a collective tubule in a section of a normal kidney. (See B II, 17.)
7. Multinucleated cells of cervical origin. Cervical smear. Glycogen series. Female. Age 40. Clinical diagnosis: chronic cervicitis, and a questionable cervical erosion.
8. Histiocytic syncytium. Sputum. OG–EA series. Male. Age 32. Clinical diagnosis: bronchitis, and a questionable bronchiectasis.
9. Abnormal multinucleated epithelial cell of the squamous type. Cervical smear taken 6 years after x-ray and radium therapy for carcinoma of the cervix. No evidence of recurrence. Glycogen series. Age 41. (Same case as G III, 9.)
10. Interpreted as a multinucleated histiocyte. Pleural fluid. OG–EA series. Female. Age 50. Pathologic diagnosis: leiomyosarcoma of the stomach. (Same case as E II, 22.)
11. Histiocytic syncytium. Sputum. OG–EA series. Male. Age 49. Pathologic diagnosis: bronchogenic epidermoid carcinoma, Grade III.
12. Multinucleated histiocyte. Pleural fluid. OG–EA series. Female. Age 56. Clinical diagnosis: cirrhosis of the liver.
13. Multinucleated malignant cells. Pleural fluid. OG–EA series. Female. Age 63. Pathologic diagnosis: reticulum cell sarcoma.
14. A syncytial mass, probably of histiocytic origin. Esophageal washing. OG–EA series. Male. Age 61. Clinical diagnosis: peptic ulcer of the esophagus.
15. Group of dyskaryotic cells including a large multinucleated cell. Cervical smear. OG–EA series. Age 39. Pathologic diagnosis: epidermoid carcinoma in situ of the cervix. (Courtesy of Strang Prevention Clinic, Memorial Center, New York.)
16. Malignant cells. Sputum. OG–EA series. Male. Age 50. Pathologic diagnosis: alveolar cell carcinoma of the lung. Primary diagnosis by smears. (Same case as C II, 13, and C III, 4.)
17. Cells interpreted as malignant. Sputum. OG–EA series. Male. Age 65. For a more detailed description and discussion of this case see C III, 3. (Courtesy of Ruth Graham and Dr. Robert H. Fennell, Jr., Massachusetts General Hospital, Boston, Mass.)



G IV
DISCUSSION

Non-malignant and malignant multinucleated cells from various organs

Multinucleation is commonly seen in histiocytes found in various fluids, particularly in pleural and peritoneal exudates and in secretions of the breast, female genital tract and prostate. Multinucleated histiocytes are also known as foreign body giant cells. They are usually found in chronic inflammatory conditions and in cases of malignancy in which secondary infections are present. The histiocytes illustrated in this plate are from the pleural fluids of cases of acute pericarditis (No. 2), leiomyosarcoma of the stomach (No. 10), and cirrhosis of the liver (No. 12); the sputum of cases of bronchitis (No. 8) and bronchogenic epidermoid carcinoma (No. 11); and an esophageal washing from a case of peptic ulcer of the esophagus (No. 14).

In some instances the formation of histiocytic syncytia may be the result of a group phagocytosis occurring when cells or foreign bodies too large for engulfment by a single histiocyte have to be disposed of. No. 1 gives a picture of group phagocytosis of a hair within the vagina of a woman with an intraepithelial carcinoma of the cervix. The histiocytes were numerous in this case and there was evidence of an increased phagocytic activity. In both benign and malignant cases the appearance and structure of the nuclei within the histiocytic syncytia is relatively uniform, although their grouping pattern may differ in the individual cases.

In normal epithelial cells multinucleation is observed more frequently in certain cell types, such as the ciliated cells (No. 3) and the giant transitional cells found in specimens of urine aspirated from the renal pelvis (Nos. 4 and 5). Multinucleated cells may also be encountered in endocervical smears in certain pathologic conditions, such as chronic cervicitis, cervical erosion, etc. (No. 7). The non-malignant nature of cells of this type is indicated by the uniformity and normal structure of the nuclei and the well-differentiated state of the cells.

In exfoliated malignant cells the presence of multinucleation is not an infrequent occurrence. In this plate such cells are illustrated in Nos. 13, 15 and 16. The malignant nature of the cells can be easily recognized by the distinct nuclear atypia in Nos. 13 (pleural fluid, reticulum cell sarcoma), 15 (carcinoma in situ of the cervix) and 16 (sputum, alveolar cell carcinoma of the lung).

The cell shown in No. 9 was found in a cervical smear of a woman who had received x-ray therapy for carcinoma of the cervix 6 years earlier. This cell has distinctly abnormal features; yet, as stated in the discussion of the same case in G III, 9, it is difficult to say whether it is a malignant cell or an aberrant postradiation form. In this case there was no evidence of recurrence or of a persistent residual lesion; therefore this cell is interpreted as an aberrant postradiation form.

The cells of No. 17 are from a case in which an agreement as to the pathologic diagnosis was never reached. However, from the cytologic standpoint a diagnosis of malignancy seems to be justifiable. The appearance of the cells and the structure of the nuclei are strongly suggestive of malignancy. Their interpretation as cells of histiocytic origin can be ruled out by comparison with typical multinucleated histiocytes such as those shown in No. 2. A more detailed comment on this same case is given in the Discussion of C III, 3.

SERIES PLATE

H I

Mitosis

Normal and abnormal mitotic figures found in smears of various body fluids

H I
DESCRIPTION

Normal and abnormal mitotic figures found in smears of various body fluids. Photomicrographs x 1600. The material included in this plate was described and discussed by Dr. Doris G. Holmquist.

1–4. Cells interpreted as histiocytes in various stages of normal mitotic division.
   1. Metaphase. Pleural fluid. OG–EA series. Female. Age 36. Pathologic diagnosis: Hodgkin's disease.
   2. Anaphase. Peritoneal fluid. OG–EA series. Female. Age 44. Clinical diagnosis: cirrhosis of the liver, Laennec's type.
   3. Telophase. Peritoneal fluid. OG–EA series. Female. Age 52. Pathologic diagnosis: cirrhosis of the liver, Laennec's type. (Same case as E I, 10–12.)
   4. Metaphase. Cervical smear. Glycogen series. Age 53. No evidence of malignancy.

5. Abnormal metaphase. Pleural fluid. OG–EA series. Female. Age 52. Clinical diagnosis: carcinoma of undetermined primary site. No follow-up possible.*

6. Abnormal metaphase. (Same case as No. 5.)*

7. Cell filled with micronuclei. (Same case as No. 5.)*

8. Ring of small nuclei, some with nucleoli. (Same case as No. 5.)*

9. Late anaphase stage with a chromatin bridge remaining between the separating daughter chromosomes. Cervical smear. OG–EA series. Age 76. Pathologic diagnosis: sarcoma of the uterus.

10. Telophase with chromosome bridge. Cytokinesis is almost complete. Peritoneal fluid. OG–EA series. Male. Age 46. Pathologic diagnosis: reticulum cell sarcoma with involvement of the serosa and wall of transverse colon.

11. Distorted cell which may be interpreted as the result of an incomplete division of chromosomes resulting in a dumbbell-shaped nucleus and blocked cytokinesis. Endocervical aspiration smear. Glycogen series. Age 76. Pathologic diagnosis: adenocarcinoma of the cervix. (Same case as A X, 14, and H II, 13 and 14.)

12. Chromatin bridge between nuclei within a multinucleated cell. (Same case as No. 5.)*

13. Stripped nuclei connected by a chromatin bridge. (Same case as No. 9.)

14. Metaphase stage of a tripolar mitotic figure. Peritoneal fluid. OG–EA series. Female. Age 70. Clinical diagnosis at death: carcinoma of the gastrointestinal tract, site undetermined, with metastasis to the liver and peritoneum. (Same case as H II, 3, 7 and 15.)

15. Metaphase stage of a tripolar mitosis with irregular distribution of chromosomes in relation to the poles. (Same case as No. 9.)

16. Late anaphase of a tripolar division with lagging chromosomes. Pleural fluid. OG–EA series. Female. Age 63. Clinical diagnosis: cardiac failure with pulmonary congestion and effusion.

17. Two cells, one of which is in tripolar mitotic division. Ascitic fluid. OG–EA series. Female. Age 61. Clinical diagnosis: carcinoma of the ovary with metastases.

18. Irregular multipolar division in late anaphase showing chromosome bridges. (Same case as No. 9.)

19. Anaphase of a pentapolar mitotic division. Pleural fluid. OG–EA series. Male. Age 62. Pathologic diagnosis: follicular lymphosarcoma. (Same case as E II, 16.)

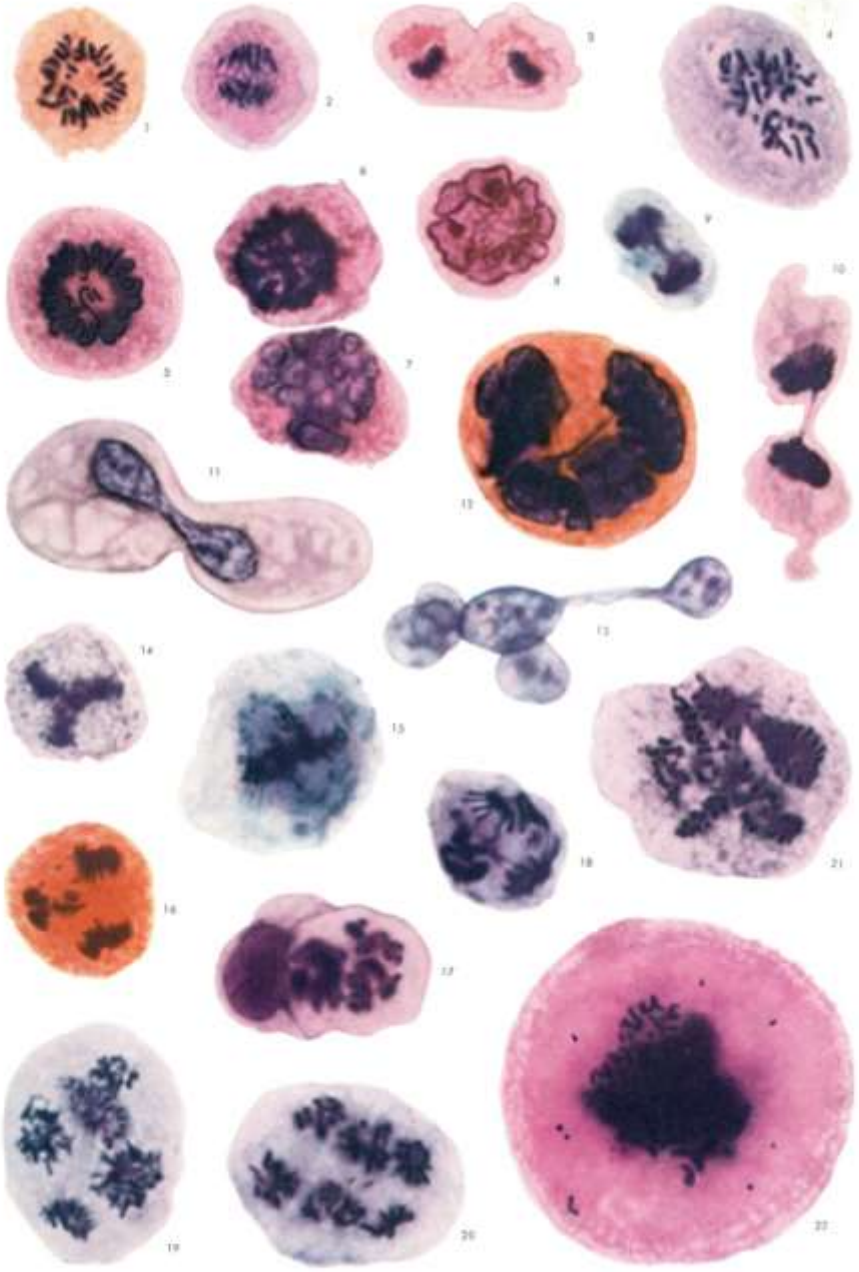
20. Late anaphase of a hexapolar mitotic division showing chromosomes lagging between the main chromosomal groups. (Same case as No. 19.)

21. Cell in division showing a very irregular arrangement of the chromosomes, the exact number of which cannot be ascertained but is greater than the normal diploid complement. (Same case as No. 14.)

22. Large polyploid cell in mitosis. Fifty-four chromosomes could be counted in the loosely arranged area at the periphery of the dense chromosomal mass. Pleural fluid. Female. Age 50. Pathologic diagnosis: carcinoma of the left breast with lymph node involvement.

* Courtesy of Dr. Malcolm Mackenzie and Elizabeth Gray, Herbert Reddy Hospital, Montreal, Canada.

MITOSIS



HI
DISCUSSION

Normal and abnormal mitotic figures found in smears of various body fluids

Mitotic figures have been noted among exfoliated cells in smears from every application of the cytologic method.[21a] Except for occasional cases, however, they are rather rare in smears from the respiratory, urinary and genital tracts, and are found with highest frequency in pleural and peritoneal fluids.

Differences have been observed in both the frequency and character of the mitoses found in smears from non-malignant as compared with malignant conditions. In smears from non-malignant cases, mitotic figures are rather rare, but when present they are usually normal in appearance. In smears from various cases of malignancy, on the other hand, mitoses may be more frequent, in some instances representing as much as 5 per cent of the total cell number. Even more striking is the great variety of mitotic abnormalities which are encountered. The changes in some instances appear to be chromosomal in nature and in others to take origin in disturbances in the spindle mechanism.

It is difficult to assess the possible diagnostic significance to be attached to the finding of mitoses in smears. In those cases in which many strikingly abnormal mitotic figures have been seen, the cytology as a whole has been that of a frankly malignant case. In such instances, the presence of abnormal mitotic figures is only a contributing factor in the final evaluation of the smear. However, cytologic smears from a few cases of proved malignancy have been observed in which the occurrence of a few abnormal division figures has been the only criterion by which the presence of malignancy might be suspected.

Because of the multiplicity of agents effective in inducing mitotic abnormalities experimentally, and because of the occurrence of abnormal mitoses in at least two cases in this series in which no malignancy could be found, it would be unwise to consider the presence of abnormal mitotic figures as an absolute criterion of malignancy. It is valid only when corroborated by other cytologic findings in the smear, as is any single criterion.

The cells illustrated in Nos. 1–4 are in various stages of normal mitotic division. In 1 and 4, which show polar views of the metaphase stage, the chromosomes are well defined and easily countable.

An abnormality generally referred to as "stickiness," in which the chromosomes lose their individuality and adhere to one another, may be noted in the metaphases shown in Nos. 5 and 6. It seems probable that such mitoses may frequently fail to proceed into anaphase, but may instead go directly into the interphase stage. The cell in No. 7 is filled with small nuclear blebs which are connected with one another by chromatin strands. In No. 8 small nuclei, retaining the metaphase ring arrangement with chromatin strands traversing the center, may conceivably have been derived by the arrest of mitosis in a metaphase such as that illustrated in No. 6. It may be noted that two of the small nuclei contain nucleoli. The cells in Nos. 5–8 are from the same case.

Another result of the "stickiness" of chromosomes may be the formation of chromatin bridges in anaphase (No. 9) which may persist through telophase (No. 10) and in some cases result in interphase nuclear bridges (Nos. 11 and 13). In No. 12, a chromatin bridge remaining between two nuclei of a multinucleated cell indicates their origin from a single chromosomal division not accompanied by cytoplasmic division.

Various degrees of multipolarity of the spindle are indicated by the chromosome arrangements in Nos. 14–21. The multipolar spindles were found in cases of malignancy, with the exception of No. 16. This was encountered in the pleural fluid of a patient having a clinical diagnosis of cardiac failure with pulmonary congestion and effusion.

The cell in No. 22 is greatly hypertrophied and probably highly polyploid. In addition to the main mass of chromosomes concentrated in the center of the cell, many chromosomes or chromosome fragments are scattered throughout the cytoplasm.

SERIES PLATE

H II

Mitosis

Abnormal mitotic figures and cells showing abnormalities interpreted as the result of disturbances in the mitotic mechanism

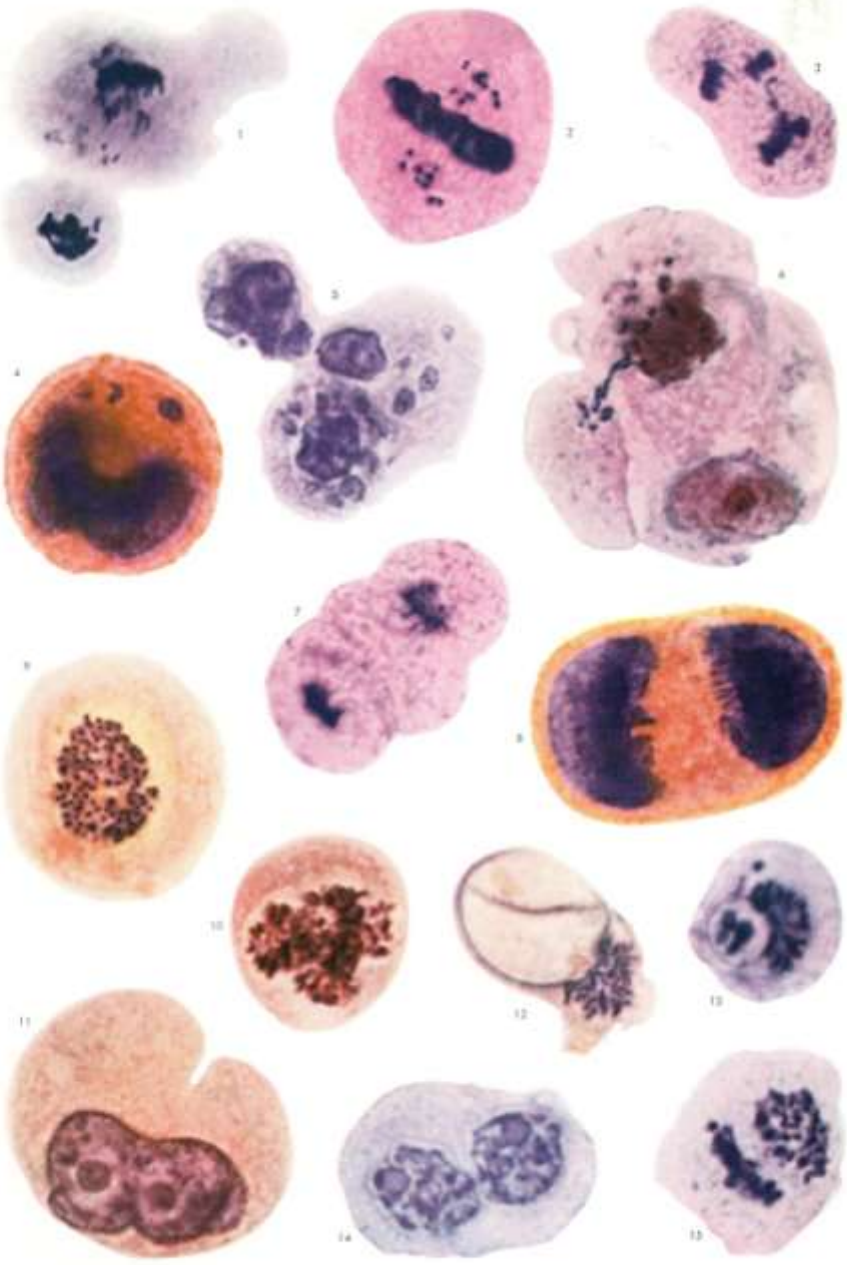
H II
DESCRIPTION

Abnormal mitotic figures and cells showing abnormalities interpreted as the result of disturbances in the mitotic mechanism. Photomicrographs x 1600. The material included in this plate was described and discussed by Dr. Doris G. Holmquist.

1. Abnormal division with lagging and unequal distribution of the chromosomes and unequal cytoplasmic division. Pleural fluid. Glycogen series. Male. Age 56. Clinical diagnosis: carcinoma of the lung with pleural effusion.
2. Abnormal metaphase stage seen in side view. Gastric balloon specimen. Glycogen series. Male. Age 65. Pathologic diagnosis: adenocarcinoma of the stomach.
3. Tripolar division with unequal distribution of chromosomes. Peritoneal fluid. OG–EA series. Female. Age 70. Clinical diagnosis: carcinoma of the gastrointestinal tract metastatic to the liver and peritoneum. (Same case as H I, 14 and 21.)
4. Large cell showing three small chromosome vesicles or micronuclei. Pleural fluid. OG–EA series. Female. Age 52. (Same case as H I, 5–8 and 12.)*
5. Large multinucleated cells with many micronuclei. Pleural fluid. OG–EA series. Female. Age 47. Pathologic diagnosis: carcinoma of the ovary and stomach with extensive metastasis to pleural and peritoneal surfaces.
6. Cluster of cells showing an abortive mitosis. (Same case as No. 5.)
7. Bipolar mitotic division accompanied by the simultaneous formation of two cytoplasmic furrows. (Same case as No. 3.)
8. Large interphase nuclei probably derived from a bipolar chromosomal division without cytoplasmic division. (Same case as No. 4.)*
9. Abnormal metaphase. Sputum. Male. Age 65. Pathologic diagnosis: sections of the resected right lower lobe were interpreted by some as malignant and by others as benign. (Same case as C III, 3 and G IV, 17.)†
10. Irregular arrangement of chromosomes judged to be in metaphase. (Same case as No. 9.)†
11. Large cell interpreted as the result of an arrested mitotic division. (Same case as No. 9.)†
12. Mitotic division of a signet ring cell. Pleural fluid. OG–EA series. Female. Age 36. Pathologic diagnosis: Hodgkin's disease. (Same case as H I, 1.)
13. Mitotic division in a malignant cell with leucocytic inclusions. Endocervical smear. Glycogen series. Age 76. Pathologic diagnosis: adenocarcinoma of the cervix. (Same case as A X, 14, and H I, 11.)
14. Synchronous early prophase condensation of the chromosomes in a binucleate cell. (Same case as No. 13.)
15. Simultaneous formation of two metaphase configurations within a single cell. One chromosomal group is seen in side view, the other in polar view. (Same case as No. 3.)

* Courtesy of Dr. Malcolm Mackenzie and Elizabeth Gray, Herbert Boddy Hospital, Montreal, Canada.
† Courtesy of Ruth Graham and Dr. Robert H. Fennell, Jr., Massachusetts General Hospital, Boston, Mass.

HII
MITOSIS



H II
DISCUSSION

Abnormal mitotic figures and cells showing abnormalities interpreted as the result of disturbances in the mitotic mechanism

The cells illustrated in this plate are interpreted as malignant with the exception of the signet ring cell in No. 12.

Unequal chromosomal and cytoplasmic division as well as lagging chromosomes are illustrated in No. 1. The cell in No. 2 is in the metaphase stage and the spindle fibers are faintly discernible. At each pole of the spindle, there are chromosomes which have failed to be incorporated in the main mass at the equatorial plate.

The chromosome arrangement seen in No. 3 is suggestive of a tripolar division, although the spindle is not distinguishable. There is one well-defined chromosome interposed between two of the chromatin masses, and beginning cytoplasmic division into two cells is suggested.

The cell in No. 4 exhibits one large hyperchromatic nucleus and three small nuclear blebs, two of which are clearly seen in the photograph. In No. 5, many such nuclear blebs or small nuclei are present. In general, they have the same internal structure as the larger nuclei, and some have been observed to contain nucleoli. Such miniature nuclei probably arise from chromosomes which lag on the spindle during division, as in No. 1, or in some manner lose their spindle fiber attachment and fail to be incorporated in the daughter nuclei at division.

In the very bizarre cluster illustrated in No. 6, three cells varying greatly in size are present. The largest cell contains an interphase nucleus and above that a large mass of poorly defined chromosomes. The cell to the left and the one above the chromosomal mass are devoid of nuclei, and contain only a few chromosomes still connected with the main mass by a chromatin bridge, as may be seen clearly in the cell to the left. This cluster is interpreted as probably resulting from a tripolar mitotic division with extreme inequality in the distribution of the chromosomes, abnormal chromatin clumping and bridge formation.

The chromosomal division is in the telophase stage in the cell illustrated in No. 7. Apparently cytokinesis has begun at two levels and, if continued, would result in the formation of three cells, only two of which would contain nuclei.

The large orangeophilic cell shown in No. 8 contains two interphase nuclei presumably formed by karyokinesis without cytokinesis. The medial borders of the nuclei retain the rodlike pattern of chromosomes entering telophase, although interphase transformation has occurred.

No. 9 shows what appears to be the metaphase stage of a cell containing more than the normal diploid number of chromosomes, not all of which could be included in one plane of focus. The chromosomes are greatly shortened as compared with the usual length of human chromosomes at this stage.

In No. 10, another abnormal metaphase arrangement is depicted. The vertical group of chromosomes, as seen in the photograph, actually forms a ring through the center of which the horizontal group of chromosomes passes. This aberrant configuration indicates a disturbance in the spindle mechanism.

It is difficult to interpret such a figure as that seen in No. 11. The cytoplasmic indentation which coincides with the plane of constriction of the nucleus may possibly be an indication of an incomplete mitotic division. This, of course, may be considered by some as suggestive of an amitotic division. Nos. 9, 10 and 11 were from the same case, which is discussed more fully in C III, 3.

No. 12 illustrates an eccentric metaphase configuration in a signet ring cell. The cell is not considered malignant, although it was found in the pleural fluid of a case of Hodgkin's disease. No. 13 is interpreted as a malignant cell in mitosis with an inclusion of

two polymorphonuclear leucocytes. It is from a case of adenocarcinoma of the cervix.

Nos. 14 and 15 show the onset of mitosis simultaneously in both nuclei of binucleate cells. In No. 14 the nuclei are in early prophase; whereas in the cell in No. 15 the metaphase stage has been attained. In the latter cell, the equatorial plates of the two spindles are apparently in planes perpendicular to one another.

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INDEX

Numerals preceded by a letter (a 1, a 2, etc.) refer to pages in the Plates section.
Numerals standing alone (1, 2, etc.) refer to pages in the general text.

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SUPPLEMENT 1

Atlas of Exfoliative Cytology

GEORGE N. PAPANICOLAOU, M.D., Ph.D.

Published for The Commonwealth Fund by
Harvard University Press, Cambridge, Mass., 1956

Publication of the *Atlas* in loose-leaf form was motivated by the realization that a periodic addition of new plates would be needed to keep abreast of new advances in this rapidly growing field. One need, felt more acutely since the general adoption of the cytologic method of cancer diagnosis by pathologists, is the correlation of cytologic with histopathologic findings in cases of benign or malignant tumors from various organs. Fourteen of the 16 plates in Supplement 1, therefore, present such comparative illustration of cells as seen in a tissue section and in a smear, thus bringing out clearly not only similarities but dissimilarities, which are often marked. A discussion of this topic and of other features of the plates appears on page 58. All of the Supplement plates are to be filed in the various present series, as indicated below and on the reverse of this sheet.

Female Genital System

A XIII	Cytology
AP I	Pathology-Cytology
AP II	
AP III	
AP IV	

Urinary and Male Genital Systems

BP I	Pathology-Cytology
BP II	

Respiratory System

C VI	Cytology
CP I	Pathology-Cytology
CP II	
CP III	

Digestive System

DP I	Pathology-Cytology
DP II	

Exudates

EP I	Pathology-Cytology
EP II	

Breast

FP I	Pathology-Cytology

TURN THE PAGE for detailed contents and instructions for insertion in the Atlas.

SUPPLEMENT 1

Contents and Instructions for Insertion in

ATLAS OF EXFOLIATIVE CYTOLOGY

SUPPLEMENT 1 MATERIAL	WHERE TO INSERT
Acknowledgments, pp. ix and x	**Substitute for Acknowledgments, pp. ix and x**
List of PLATES, pp. xv–xvii	**Substitute for list of PLATES, pp. xv and xvi**
[See note below about inserting new pp. xix–xx]	
Comment on PLATES, pp. 57 and 58	**Substitute for comment on PLATES, following p. 56**
Plate units: A XIII and AP I–AP IV	**Insert after plate unit A XII**
BP I and BP II	**Insert after plate unit B IV**
C VI and CP I–CP III	**Insert after plate unit C V**
DP I and DP II	**Insert after plate unit D V**
EP I and EP II	**Insert after plate unit E II**
FP I	**Insert after plate unit F II**
Index, pp. *xvii–xx*	**Insert after Index, p. *xvi***

NOTE. Insert this sheet (pp. xix and xx) after the new Plates list (pp. xv-xvii), to serve as a permanent record of the contents of Supplement 1.

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I am particularly grateful to Dr. Doris G. Holmquist for her invaluable cooperation in the writing of the text and the preparation of the plates and for selecting and describing the material in the plates of Series H. I am also indebted to Dr. Xenophon Callas for his helpful cooperation during the early formulation of the text material; to Dr. Nelson D. Holmquist and Constantine Bailey for their assistance in the final reading and correction of the manuscript; and to my wife, Mary G. Papanicolaou, a faithful and untiring companion and volunteer assistant throughout the years of my scientific research.

December, 1953 G. N. P.

SUPPLEMENT 1

I wish to express my gratitude to the American Cancer Society for the generous support given to our laboratory which enabled us to proceed with the preparation of Supplement 1.

For the tissue sections placed at my disposal for use in the pathology-cytology correlation plates my deep appreciation goes to the pathology laboratories of Cornell University Medical College, The New York Hospital, and Lying-In Hospital and to the pathology laboratories of Memorial Center for Cancer and Allied Diseases, Doctor's, French and Flower–Fifth Avenue Hospitals of Manhattan, Beth-El Hospital of Brooklyn, and F. C. Smith Clinic of Columbus, Ohio.

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April, 1956 G. N. P.

The following figures also appeared in *Diagnosis of Uterine Cancer by the Vaginal Smear* by George N. Papanicolaou and Herbert F. Traut:

Series	Plate	No.
A	I	3 and 16
	II	1, 2, 5, 6, 10, 11, 12, 15, 17 and 19
	IV	1
	VI	1–15 and 20–23
	VIII	1–8, 16 and 18
	IX	4 and 9
	X	1, 2, 5, 6, 7, 11 and 13
C	I	2, 3, 9 and 13
	II	5–8, 13 and 14

Figures which appeared in *The Epithelia of Woman's Reproductive Organs* by George N. Papanicolaou, Herbert F. Traut and Andrew A. Marchetti are:

Series	Plate	No.
A	III	1, 2, 4, 8, 12 and 16

PLATES

PLATES

Plates

CYTOLOGY PLATES

To assist the reader in making a more profitable use of this atlas, some comment on the selection of the illustrations and their organization into plates is in order. The plan of arrangement as originally conceived provided for groups or series of plates based on various smear types, with separate plates for non-malignant and malignant cell forms within each series.

Such an arrangement was deemed desirable and would have been possible if all the material from which representative cells and cell clusters were to be chosen for illustration had been at hand at the initial stage of the work. This, however, was not the case. The selection and illustration of representative cytologic types were conducted gradually over a period of many years, during which time exfoliative cytology continued to advance steadily and to expand into new applications, made possible through the introduction of improved technical procedures.

In order to cover the new material which was being accumulated, the original plan for twenty-four plates of drawings was expanded to include twelve plates of photomicrographs. This called for an addition of new plates in each of the old series. In the digestive system (Series D), for example, the number of plates was increased from two, as originally planned, to five. This was considered necessary because of new and important advances, chiefly the gastric balloon technique and newer procedures for obtaining rectal and colonic washings, which made possible the procurement of more satisfactory and better-preserved material. As the new photomicrographic plates were added after many of the original drawing plates had been engraved, some repetition was unavoidable if the better material available was to be illustrated.

On the other hand, the segregation of the non-malignant from the malignant cells in the new plates was found to be impracticable because of space limitations. Exceptions to the segregation plan were also made for the sake of a close comparison of normal and malignant cell types, as in the plates concerning dyskaryosis (A IV and A V), in which normal and corresponding malignant cells were illustrated side by side.

Series A through F deal with the exfoliative cytology of various body systems. Series G (Miscellaneous) includes four plates, each of which is composed either of groups of cells found in various smears under many different forms, such as histiocytes (G I) and multinucleated cells (G IV), or of specific cell types found in particular conditions such as pregnancy (G II) or after irradiation (G III). Series H is devoted to the illustration of mitosis as seen in smears prepared from various body fluids. These special subjects were treated on separate plates in order to provide a more comparative picture of their cytology.

PATHOLOGY-CYTOLOGY PLATES

A need which has been felt more acutely since the general adoption of the cytologic method of cancer diagnosis by pathologists is the correlation of cytologic and histopathologic findings in cases representative of

various types of benign and malignant tumors from different organs. It was therefore decided to devote 14 of the 16 plates comprising Supplement 1 of the *Atlas* to the combined illustration of the cytology and corresponding histopathology in 30 cases of neoplasms involving various organs. Such a comparative illustration may serve a double purpose by pointing not only to the similarities but also to the dissimilarities between cells of the same type as seen in a tissue section and in a smear. Dissimilarities are often very marked and may be explained by the fact that cells are seen *in toto* in a smear and only in part in a tissue section. As a result of this, cells and their nuclei look, as a rule, larger in smears than in corresponding sections and are more deeply stained, particularly when in dense clusters. A greater variety of individual cell types and cell cluster patterns is also evident in the smears. It is thus apparent that the evaluation of tissue sections and cytologic smears should be based not only on general criteria common to both but also on criteria more specific for each diagnostic approach. To interpret cytologic smears efficiently a pathologist would therefore need special training in exfoliative cytology, just as a cytologist should be adequately trained in pathology for interpreting tissue sections.

Differences in the coloration of the cells in tissue sections and in corresponding smears on the pathology-cytology plates are due to the fact that the sections, with the exception of AP II, 1–3,* were stained in various pathologic laboratories with hematoxylin-eosin and other methods differing from those used in the staining of the smears. For the latter our routine OG 6-EA staining method (procedures 268 and 267 described on pages 6 and 12) was used, with the exception of some smears which were stained with experimental glycogen stains† differing only slightly from our standard stain. The following exceptions in the staining of the smears may be mentioned: The cells in BP II, 4, 5, 7–10 and 13, and CP II, 5–8 were stained with a modified procedure emphasizing basophilia. The cells illustrated in CP I, 5–11 are from a smear stained with Shorr's method.

One of the difficulties in the staining of smears is the maintenance of uniformity. Thick or poorly preserved smears or smears containing much mucus or blood do not stain as evenly as those which are thinly spread and well preserved. The standardization of the stains is also difficult. Repeated use of a staining solution tends to weaken it and may result in the understaining of the smears. On the other hand, when the staining solution, particularly hematoxylin, is fresh, the cells and the nuclei may be so overstained as to give a false impression of hyperchromasia. Counterstaining with cytoplasmic stains that emphasize basophilia usually tends to darken the hematoxylin-stained nuclei. Staining procedures overemphasizing acidophilia may also be misleading. The evaluation of such discrepancies is especially difficult in reading smears stained in other laboratories.

A false impression of hyperchromasia (pseudohyperchromasia) is sometimes conveyed by pyknosis, particularly in relatively large nuclei. A uniformly stained dark nucleus usually indicates degeneration rather than true hyperchromasia. The latter is more typically expressed in well-preserved, active nuclei with an increased chromatin content but no loss of fine structural details (see page 14).

The description and discussion of the sixteen new plates follows essentially the same plan used in the original plates of the *Atlas*. The diagnosis stated for the tissue section of each case is that of the pathologist of the laboratory where the section was read. The discussion of each tissue section is our own, although always formulated with due consideration of the original pathologic report. However, the cytologic descriptions and discussions reflect the author's views.

* Stained with Procedure No. 268 (page 6).

† Investigative work is still continued in this series, the results of which will be published at a later date. However, the general cytologic picture is similar to that obtained with the OG–EA procedures.

SERIES PLATE

A XIII

Female Genital System

Non-malignant and malignant cells found in vaginal and cervical smears

A XIII
DESCRIPTION

Non-malignant and malignant cells found in vaginal and cervical smears. Photomicrographs x 900 except Nos. 2 and 3, which are x 180.

1. Normal endocervical cells. Cervical smear. Age 43. Normal cervix.
2. Cluster of endocervical cells. Cervical smear. Age 34. Clinical diagnosis: cervical erosion.
3. Endocervical cells. Cervical smear. Age 53. Clinical diagnosis: cervical erosion.
4. Endocervical columnar cells. Cervical smear. Age 28. Trichomonas infestation. Clinical diagnosis: cervical erosion. (Same case as Nos. 14 and 15.)
5. Endocervical cells. Cervical smear. Age 53. Clinical diagnosis: cervical erosion.
6. Endocervical cells. Cervical smear. Age 46. Clinical diagnosis: cervical erosion. (Same case as No. 13.)
7. Endocervical cell. Cervical smear. Age 45. Clinical diagnosis: cervical erosion.

8–10. Endocervical columnar cells. Cervical smear. Age 28. Clinical diagnosis: endocervical erosion.

11–12. Endocervical columnar cells. Cervical smear. Age 53. Clinical diagnosis: endocervical polyp.

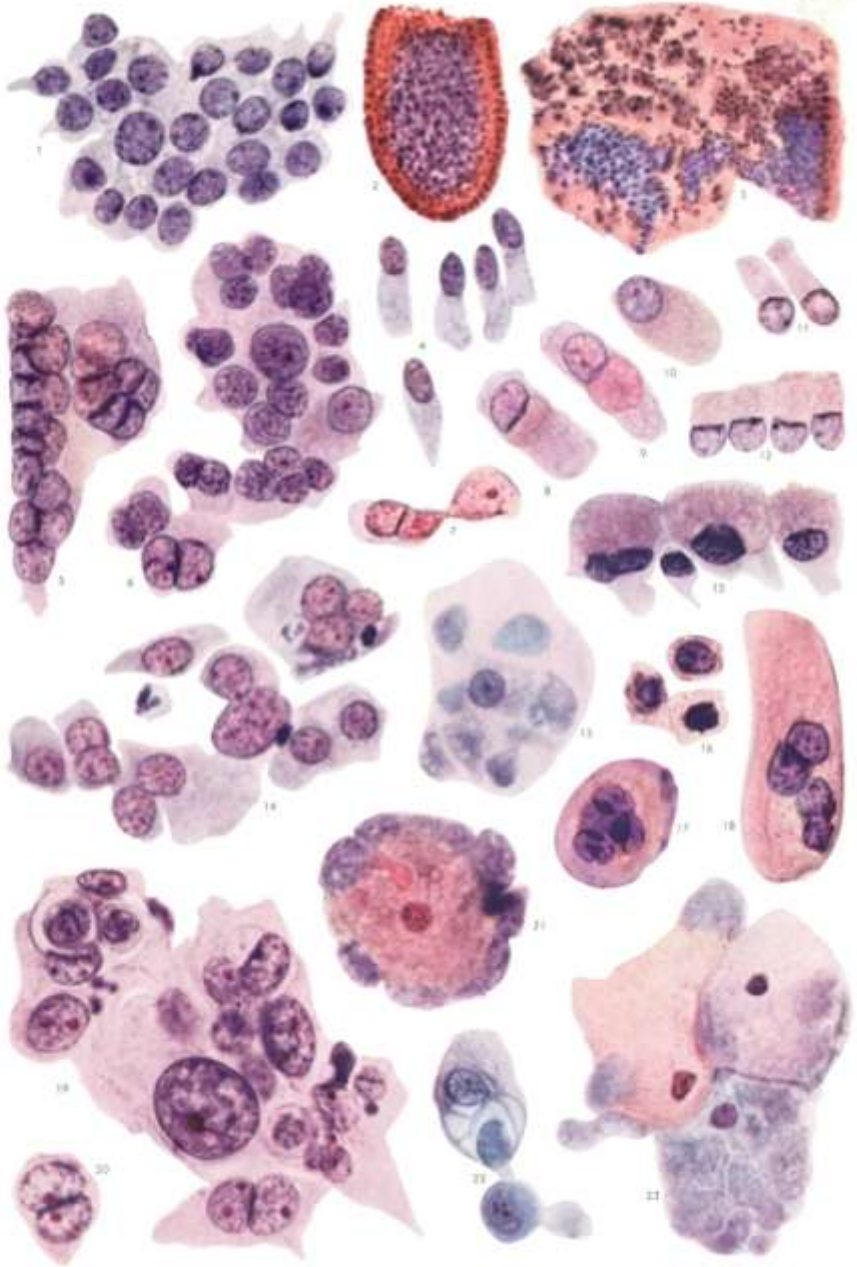
13. Endocervical columnar cells. Cervical smear. Age 46. Clinical diagnosis: cervical erosion. (Same case as No. 6.)
14. Endocervical cells. Cervical smear. Age 28. Trichomonas infestation. Clinical diagnosis: cervical erosion. (Same case as Nos. 4 and 15.)
15. Superficial squamous cell infiltrated by trichomonads. Cervical smear. Age 28. Trichomonas infestation. Clinical diagnosis: cervical erosion. (Same case as Nos. 4 and 14.)

16–18. Cervical cells, malignant. Cervical smear. Age 39. Clinical diagnosis: cervical erosion. Pathologic diagnosis: carcinoma in situ. Primary diagnosis by smears.

19–20. Endocervical cells, malignant. Draghi tampon smear. Age 55. Pathologic diagnosis: epidermoid carcinoma of the cervix, Grade II.

21. Superficial squamous cell being invaded by trichomonads. Vaginal smear. Age 48. Trichomonas infestation.
22. Cervical parabasal cells, one being invaded by a trichomonad. Cervical smear. Age 50. Clinical diagnosis: Trichomonas cervicitis.
23. Superficial squamous cells invaded by trichomonads. Cervical smear. Age 44. Trichomonas infestation.

NOTE: Nos. 2–5, 8–12 and 14–19: OC–EA series. Nos. 1, 6–7, 13 and 20–23: Glycogen series.



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A XIII
DISCUSSION

Non-malignant and malignant cells found in vaginal and cervical smears

Erosion of the uterine cervix is one of the lesions which may sometimes be diagnosed cytologically by exfoliated cells exhibiting characteristic features. The cytologic criteria of erosion are given on page 29 and also are discussed on pages a 12 and a 43. No. 3 illustrates at a low magnification the relatively rich exfoliation of endocervical cells which is sometimes observed in cervical and endocervical smears from cases of cervical erosion. Most of the cells are of the columnar type and often have a palisade arrangement (Nos. 3 and 13; see also A XI, 1 and 2). A papilloid structure like the one illustrated at a low magnification in No. 2 is not an infrequent finding in smears from cases of erosion. It apparently corresponds to one of the papillary projections of the endocervical epithelium which are often seen in tissue sections of eroded areas of the uterine cervix. The columnar cells of No. 4 have been selected to show the normal endocervical type. The cellular and nuclear hypertrophy of the columnar cells, characteristic of erosion, and the rich content of mucus within the cytoplasm are evident in Nos. 8–10 and 13. (See also A III, 21.) In Nos. 8 and 9 the secretion appears in the form of a ball adjacent to the distal pole of the nucleus. Columnar endocervical cells with relatively rich mucoid content are also occasionally found in smears from cases with endocervical polyps. Nos. 11 and 12 show such cells, which, like some of the erosion cells, sometimes exhibit a palisade arrangement (No. 12). The flattening or caving in of the distal end of the nucleus (Nos. 9, 11 and 12) is apparently the result of pressure from an increased amount of mucoid secretion within the cytoplasm. No. 7 represents an atypical cell with three contiguous nuclei giving the impression of an incomplete mitotic or amitotic division.

Clusters of exfoliated normal epithelial cells of the endocervix viewed from their distal surface are shown in No. 1. The secretory activity of the cells as indicated by the cytoplasmic vacuolation, the tendency to nuclear enlargement, and the active appearance of the nuclei as revealed by the chromatin pattern and the presence of knob-like chromatin protrusions may be attributed to the relatively high estrogenic level prevailing on the 17th day of the period when the smear was taken. The clusters of endocervical epithelial cells illustrated in Nos. 5 and 6 are from two cases with erosion of the cervix and demonstrate the increased range of variability in the size of the nuclei and the presence of many multinucleated cells. Multinucleation is undoubtedly one of the most characteristic smear changes observed in cervical erosion (see also A XI, 3).

Nuclear enlargement and multinucleation are also shown in the cells of No. 14, which are from a case of erosion with a concomitant Trichomonas infestation. The cells of Nos. 16–18 are from a case of carcinoma in situ with cervical erosion. The structural characteristics of the cells and of their nuclei suggest a malignant lesion; however, the type of the multinucleation resembles that seen in benign erosions. Frankly malignant cells of the endocervical type are shown in Nos. 19 and 20 for comparison. The cellular and nuclear abnormalities are quite obvious in this case.

One of the characteristics of Trichomonas vaginalis has been its tenacity and its frequent recurrence, even in cases which have been subjected to a thorough and apparently effective treatment. As far as I have been able to ascertain, no evidence of encysted sexual forms which would account for an endogenous reinfection has been brought forth thus far. On the basis of a study of smears from numerous cases of Trichomonas vaginalis infestation, evidence has been accumulated that there is an invasion of squamous epithelial cells by trichomonads which may be of significance in that it affords a means by which such invaded vaginal and ectocervical squamous cells of the host may become centers of continual reinfection. An explanation is thus offered, not

only for the perpetuation of the infection, but also for the apparent periodicity in the numerical representation of the trichomonads within the vagina which seems to be related to the various stages of the menstrual cycle.

This phenomenon of invasion is particularly noticeable in cases with a history of prolonged infestation. The process of penetration of the parasites into the squamous cells is illustrated in Nos. 21 and 23. The trichomonads may enter into the cytoplasm of the cell or may remain attached to the cell surface and draw their nourishment through foot-like projections directed toward the center of the cell, as indicated faintly in the cell of No. 21 at about 10 and 11 o'clock. The nucleus, which is usually pyknotic, is, as a rule, unaffected even in cells which have been totally invaded, as has the lower cell of No. 23. Whether the parasites enter the cells before and/or after exfoliation has not as yet been established. The cornified squamous cells are the most common hosts of the trichomonads. Their penetration into the deeper parabasal cells as shown in No. 22 is rather rare.

This description, which is based on the observation of this phenomenon in approximately 50 cases, is given here for the first time. A more detailed report will be presented in a special article which is in preparation.

SERIES AP Pathology-Cytology

PLATE 1

Female Genital System

Adenocarcinoma of the endometrium and cystadenocarcinoma of the ovary

AP I
DESCRIPTION AND DISCUSSION

Adenocarcinoma of the endometrium and cystadenocarcinoma of the ovary. Photomicrographs.

1–9. Papillary adenocarcinoma of corpus uteri. Age 61. Primary diagnosis by smears.

1–2. Section of the endometrial tumor. x 150 and x 600. Some papillary projections are clearly outlined. The process of exfoliation of cells and of cell clusters from the tips of the papillae is also indicated. A papilla consisting of columnar neoplastic cells with prominent nucleoli is shown at a higher magnification in No. 2.

3–9. Exfoliated clusters of normal and malignant cells. Endometrial aspiration smear. x 150 (No. 3); x 600 (Nos. 4–9): All cell clusters are from the same endometrial smear. Nos. 5, 7, 8 and 9 are of malignant cell clusters with a configuration characteristic for this type of tumor. Their form closely resembles that of the papillary projections within the tumor. The nuclei compare in size and form with those shown in No. 2, but are more deeply stained, largely because of their compact grouping within the clusters. A few nuclei in Nos. 7 and 9 show large central nucleoli. Engulfment with formation of cup-shaped nuclei in the outer cells can be seen in Nos. 4 and 7. For the sake of comparison a cluster of non-malignant endometrial cells is shown in No. 6. A large fragment exfoliated from the tumor is illustrated at a low magnification in No. 3 to demonstrate the growth pattern of the tumor.

(See also A XII, 8 and 14.)

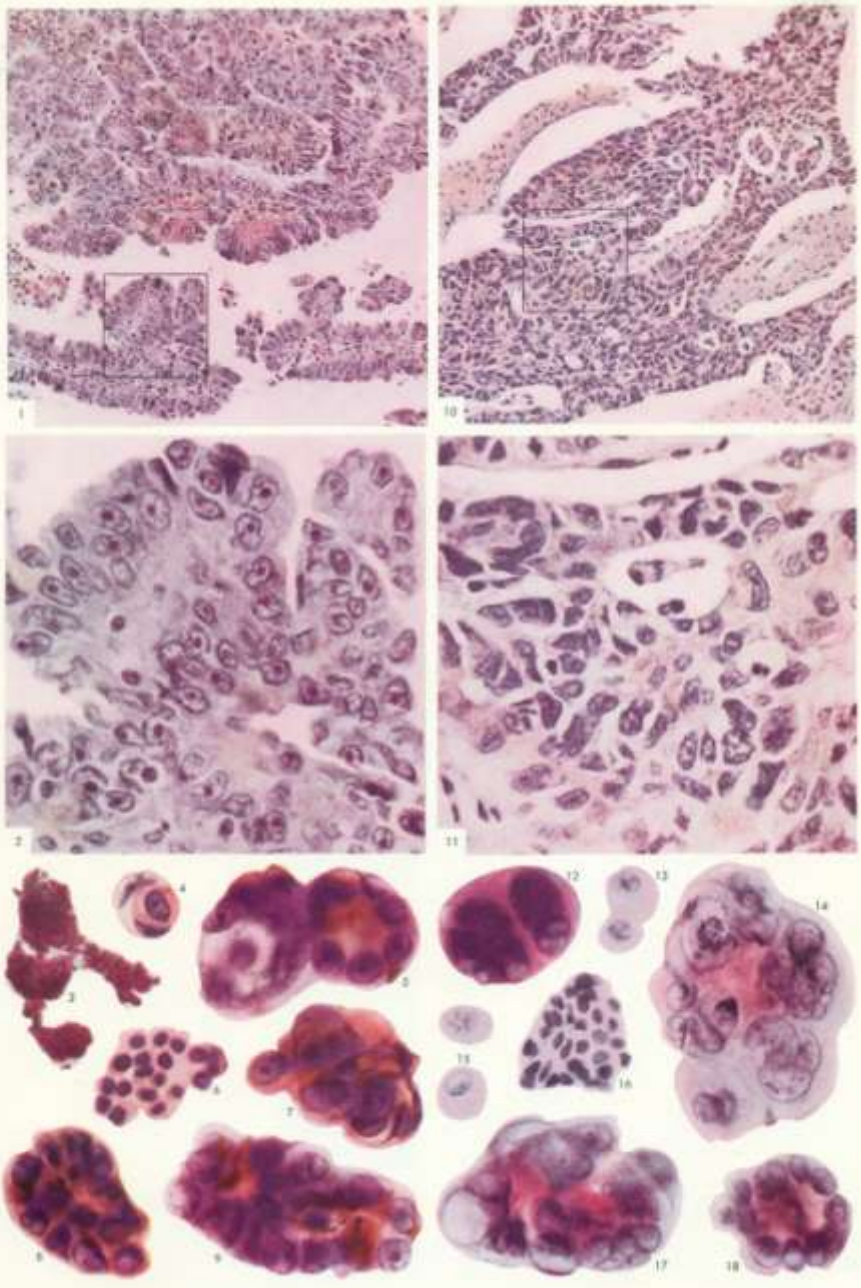
10–18. Bilateral papillary cystadenocarcinoma of the ovary. No evidence of metastasis to the tubes or uterus. Age 56. (Same case as A XII, 10.)

10–11. Section of the ovarian tumor. x 150 and x 600. The type of the tumor is indicated in No. 10. Its cellular components are shown at a higher magnification in No. 11.

12–18. Exfoliated normal and malignant cells. Endometrial aspiration smear. x 600. All cells and cell clusters are from the same endometrial smear. No. 16 illustrates a cluster of non-malignant endometrial cells for the sake of comparison. Nos. 12, 14, 17 and 18 show a pattern consistent with a papillary adenocarcinoma. Their configuration resembles that of Nos. 5, 7, 8 and 9. However, the cells show more pronounced vacuolation and their nuclei are larger. Some of the cells are multinucleated. One abnormal mitosis is shown in No. 14. Four relatively smaller malignant cells are illustrated in Nos. 13 and 15. Since in this case no evidence of metastasis to the tubes or uterus was found at autopsy, it is assumed that all the malignant cells found in the endometrial smear are of ovarian origin.

(See also A XII, 9–12.)

NOTE: Nos. 3–9: OG–EA series. Nos. 12–18: Glycogen series.



SERIES AP Pathology-Cytology PLATE II

Female Genital System

Carcinomas of the cervix

AP II
DESCRIPTION AND DISCUSSION

Carcinomas of the cervix. Photomicrographs.

1–13. Epidermoid carcinoma in situ of the uterine cervix with downward growth along gland ducts. Age 47. Supracervical hysterectomy 3 years prior to smear examination. Cervical stump removed on the strength of the smear report. (Same case as A IV, 5 and A V, 6–8.)

1–3. Section of the cervical stump through two areas of the malignant lesion within the epithelium of the ectocervix. x 75 (Nos. 1 and 2) and x 600 (No. 3). The transition from the non-malignant to the malignant epithelium is shown in No. 1. The exfoliation of cells from the superficial squamous zone that is still intact at this early developmental stage of the lesion is clearly indicated in Nos. 1 and 2. The abnormal features of the cells throughout the superficial and parabasal cell zones and the irregular pattern of the epithelium are apparent in No. 3. A few mitoses are shown within the parabasal zone in No. 3.

4–13. Exfoliated malignant cells and cell clusters. Cervical smear. x 600. The recognition of the origin and type of the malignant cells is still possible at the intraepithelial stage of malignancy, as in this case. Nos. 4 and 5 illustrate cells of the superficial squamous zone characteristic of "superficial cell dyskaryosis."* The nuclear abnormalities and the irregularity in the grouping of the cells within cluster No. 4 are evident. These cells, which obviously are of the same type as those in the superficial zone of the lesion shown in No. 3, afford a good example of the difference in the appearance of cells of the same origin and type in smears and in tissue sections. The cellular and particularly the nuclear abnormalities are much more clearly indicated in the cells preserved *in toto* in the smear than in those of the cross-section of the cervical lesion. The same remark applies to the cells of the parabasal zone. Malignant cells from this zone are illustrated in Nos. 7–13. The disproportionate enlargement and the marked hyperchromasia of the nuclei, which typify the pattern of "parabasal cell dyskaryosis," are obvious. Some cells show vacuolation, as in Nos. 8 and 13. Those of No. 13 are highly vacuolated, giving a false impression of adenocarcinoma. It is possible that these are cells of endocervical origin, which usually exhibit higher vacuolation than parabasal cells (see A V, 3). Acidophilic parabasal cells resembling the one illustrated in No. 11 are often seen in cases of cervical malignancy, early as well as advanced (see A V, 6 and 8 and A VI, 10, 13, 16 and 18); although they may be seen also in other instances, as in atrophic postmenopausal smears or in chronic inflammatory conditions. The cell of No. 6 is of an intermediate type. It has the approximate size of a superficial but the form of a parabasal cell.

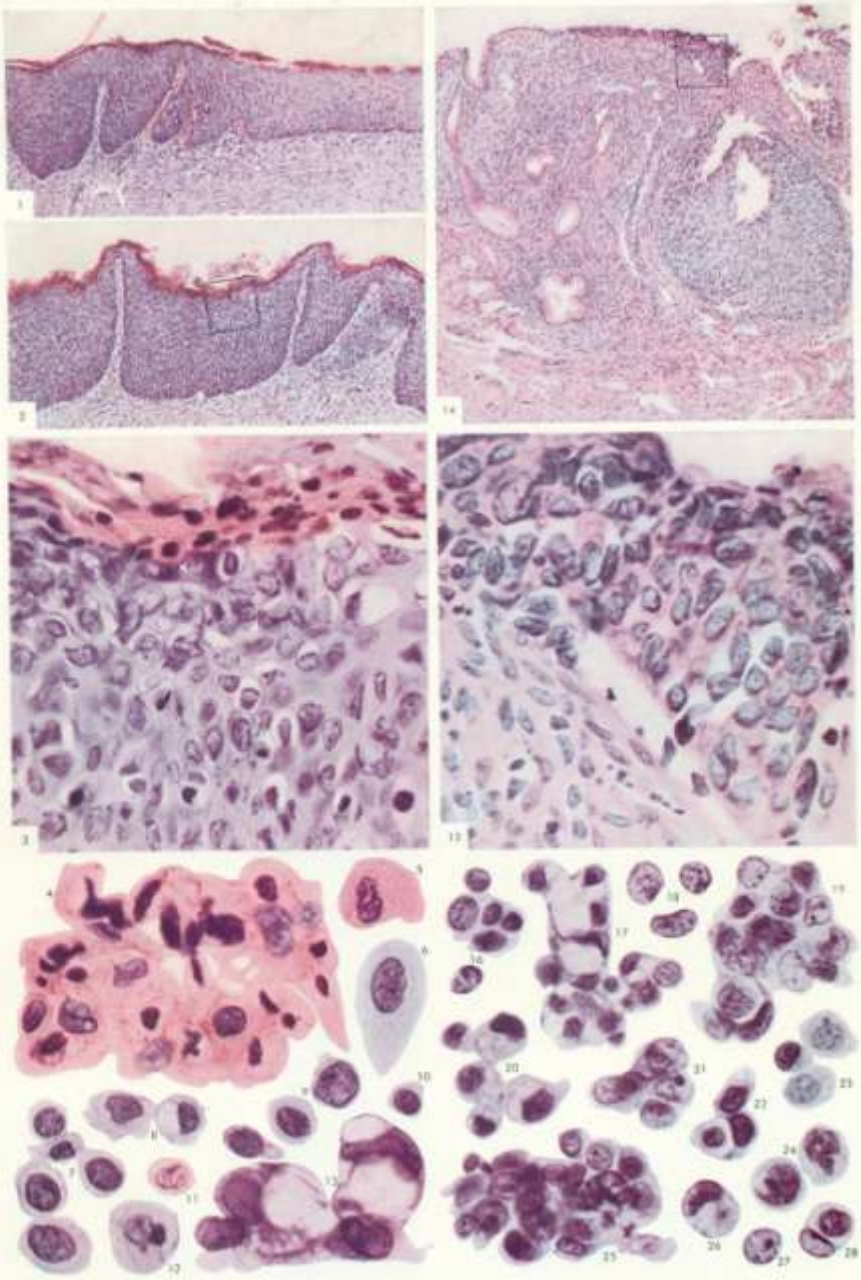
(See also Plates A IV and A V.)

14–28. Carcinoma in situ of the cervix. Age 57. Primary diagnosis by smears.

14–15. Section of the endocervix through an area of the malignant lesion. x 75 and x 600. The transition from the normal endocervical columnar cells to the malignant and the extension of the latter into an endocervical gland are shown in No. 14. There are also adjacent uninvolved glands and evidence of inflammatory cell reaction. The malignant features of the cells and their disorientation are seen in No. 15. The structural differences between the epithelial cells of the ectocervix and those of the endocervix, which are so evident in the normal state, become less and less distinct with the onset of a cancerous change. Thus, the malignant cells illustrated in the section in No. 15 of this case, though apparently of endocervical origin, closely resemble those of the ectocervical parabasal layers of the malignant lesion shown in No. 3. In the more advanced stages of carcinomas, the differences between cells of ectocervical and endocervical origin are even less apparent, making a distinction between the two cell types very difficult if at all possible.

16–28. Exfoliated malignant cells and cell clusters. Cervical smear. x 600. The malignant cells are closer to the endocervical than the ectocervical type. The eccentricity of the nucleus and the rather widespread occurrence of cytoplasmic vacuolation (Nos. 17 and 20)

* There are at least four distinct types of dyskaryosis corresponding to the four types of cells found in the epithelial lining of the uterine cervix: the superficial, intermediate and parabasal squamous and the endocervical. Each of these four dyskaryotic patterns appears to have a different diagnostic and prognostic significance. Lesions with an endocervical or parabasal squamous cell pattern are much more likely to follow a progressive course than those of the superficial or intermediate squamous cell types. The latter two types have a more favorable prognosis, as they have been shown in some instances to regress or even to disappear (see pages 30–31).



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are two fairly reliable differential criteria. The mucoid type of some cells (No. 20) is indicated by the presence of multiple cytoplasmic vacuoles. Engulfment (Nos. 22 and 28) and leucocytic infiltration of the cells (No. 24) are not uncommon. Variation in the size and form of the nuclei is fairly marked. (See also A VI, 28.) Another distinctive feature of this type of tumor is the occasional presence of large and hyperchromatic stripped nuclei (No. 18). Size, however, is not as reliable a criterion of malignancy in such nuclei as is structure. Marked variation in size may be seen also in endocervical stripped nuclei found in smears from non-malignant cases (see A III, 6), more specifically at the peak of the follicular activity or during estrogenic therapy.

NOTE: Nos. 1–4, 13 and 16–28: OG–EA series. Nos. 5–12: Glycogen series.

SERIES AP Pathology-Cytology

PLATE III

Female Genital System

Carcinomas of the cervix

AP III
DESCRIPTION AND DISCUSSION

Carcinomas of the cervix. Photomicrographs.

1–11. Squamous cell carcinoma of the cervix uteri. Age 70.

1–2. Section through the malignant tumor. x 150 and x 600. The structural pattern of the tumor is that of a well-differentiated epidermoid carcinoma with areas of keratinization and pearl formation. Cells of this type exfoliated from such areas of keratinization occurring at the surface of the tumor may be recovered in smears.

3–11. Exfoliated malignant cells and cell clusters. Vaginal tampon smear. x 600. Many of the cells from this type of tumor are very large and show bizarre forms and marked eosinophilia or orangeophilia, which greatly facilitate their identification (Nos. 3, 7, 9, 10). Some assume the shape of a tadpole (Nos. 3 and 9) or other characteristic forms (Nos. 7 and 10). Others are round or oval (engulfed cell in No. 5). The nuclei show enlargement and hyperchromasia. Pyknosis, which is a sign of degeneration, is seen mostly in the acidophilic cells. There is a striking similarity between some of the exfoliated acidophilic epidermoid cells and those shown within the exfoliative centers of the sections (Nos. 1 and 2). Cells of this type are also illustrated in previous plates (A VI and A VII). In addition to these highly differentiated cells, less differentiated cells appear in the smears either singly or in clusters. No. 11 shows cells closely resembling those seen in the section of the tumor (No. 2). Three round basophilic cells with large and hyperchromatic nuclei are illustrated in Nos. 6, 7 and 8. Vacuolated cells are shown in Nos. 4 and 7. One of these (No. 4) has a large, distinctly malignant nucleus. Engulfment is shown in No. 5. The well-differentiated cells of the epidermoid carcinoma of the cervix tend to appear in the smears singly or in relatively small groups. This remark applies also to cells exfoliated from tumors of the same type in other organs such as the epidermoid carcinomas of the lung (see C III and C IV).

(See also plates A VI and A VII.)

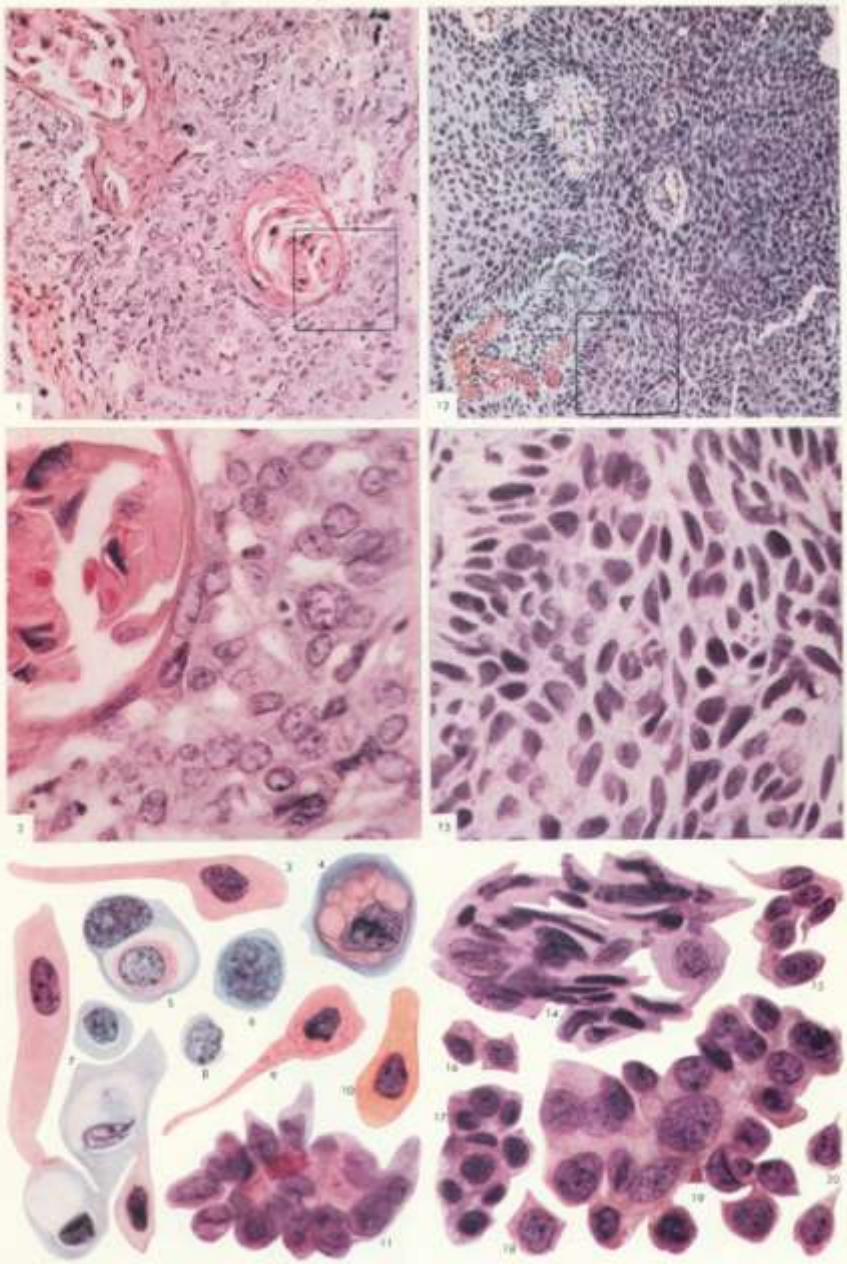
12–20. Carcinoma of the cervix, Grade III. Age 40.

12–13. Section of the biopsy of the tumor. x 150 and x 600. The structural pattern of the tumor contrasts with that of Nos. 1 and 2. The cells are more densely grouped and show a tendency to elongation but not to an extreme epidermoid differentiation and keratinization. The disorientation of the cells is very marked.

14–20. Exfoliated malignant cells and cell clusters. Vaginal smear. x 600. There is a very close similarity in the appearance of the exfoliated cells and those seen in the section of the tumor. The elongation of the cells into spindle-like forms is clearly indicated in the section (No. 13) and in the exfoliated state in No. 14. Though the exfoliated cells and their nuclei appear to be larger than those of the section (No. 13), as is the general rule (see page 58), the uniformity in their type in both section and smears is apparent. The difference between the histopathology and the exfoliative cytology of this tumor and the one illustrated in Nos. 1–11 of the same plate is as marked as that between the two intraepithelial carcinomas of the cervix representing the endocervical and ectocervical type shown in AP II. These structural differences may be interpreted as indicating a different origin for each of the two types, one probably originating in the basal cells of the stratified squamous epithelium of the ectocervix, and the other in the reserve cells of the columnar epithelium of the endocervix. It is, however, likely that such cytologically pure tumor types as those illustrated in this plate are in the minority and that the great majority of cervical carcinomas, which develop at the squamo-columnar junction, are of mixed cytologic texture.

The marked structural differences between cells exfoliating from preinvasive or very early invasive carcinomas of the uterine cervix and cells found in smears from advanced cases of invasive lesions* may be visualized by a comparison of Nos. 4–13 and 16–28 of plate AP II with Nos. 3–11 and 14–20 of AP III. In the early, preinvasive stages of a cervical carcinoma the structural changes observed in the exfoliated malignant cells are confined for the most part to the nucleus, whereas in the advanced invasive

* Similar noteworthy differences are evident in a comparison of cells derived from early and advanced cases of carcinoma of the cervix as illustrated in plates A IV–A VII.



stages many malignant cells exhibit very aberrant and bizarre forms. A careful observer may thus be able to determine with a fair degree of accuracy the developmental stage of a cervical carcinoma by the cytology of the vaginal and cervical smears (see pages 30 and 31).

NOTE: Nos. 3–11 and 14–20: OG–EA series.

SERIES AP Pathology-Cytology

PLATE IV

Female Genital System

Papilloma of the cervix and spindle cell sarcoma of the uterus

APIV
DESCRIPTION AND DISCUSSION

Papilloma of the cervix and spindle cell sarcoma of the uterus. Photomicrographs.

1–16. Papilloma of the cervix with squamous metaplasia and anaplasia. Age 44.

1–2. Section through the region of the papilloma. x 150 and x 600. No. 2 illustrates an area with typical squamous metaplasia. The metaplastic cells as well as cells of the basal layers show no malignant features. However, a number of atypical cells "suggestive of anaplasia" and of cells "with variation in size and shape of the nuclei" were mentioned in the pathology report on the histologic sections of the cervix.

3–16. Exfoliated cells exhibiting atypical features. Vaginal and cervical smears. x 600. The cluster in No. 14 consists of cells which are close to the basal type. Some nuclear hyperactivity is indicated by the chromatin pattern, the binucleation, and the protrusion of the chromatin in some of the nuclei. Metaplastic cells are shown in No. 5. The cells in Nos. 3, 6, 9 and 10 may also be considered metaplastic. The last one (No. 10) is binucleated. Other cells, such as Nos. 4, 7, 8, 9, 11, 12, 15 and 16, show marked acidophilia probably due to cornification or keratinization in certain areas of the tumor not shown in the sections in Nos. 1 and 2. Some of these acidophilic cells show no distinctly abnormal features (No. 15) while others exhibit structural atypia, degeneration, and nuclear pyknosis (Nos. 4, 7, 8 and 11) or karyorrhexis (No. 12). Several of the small degenerated acidophilic cells are engulfed by other larger cells (Nos. 7 and 8). Their degenerative pattern presents some distinctive traits. No. 8 gives the impression of the splitting of a cell, possibly as a result of a mitotic or amitotic division, with a chromatin bridge apparently stretched between the nuclei of the two engulfed cells. Vacuolation is distinctly shown in one of the two cells of No. 13. In comparing this cell with Nos. 7 and 8 one gets the impression of a relationship between this type of vacuolation and the process of engulfment. No. 16 illustrates a rather ambiguous cell. Its large and hyperchromatic nucleus may arouse a suspicion of malignancy. Whether this highly atypical cell should be considered as benign or malignant is an open question. Because of the presence of this and a few other ambiguous cells in the smears a Class III report was given in this case. The mention of a number of atypical cells "suggestive of anaplasia" and of cells "with variation in size and shape of the nuclei" in the pathologic report (see discussion of No. 2) suggests the possibility of an early malignant change in this case.

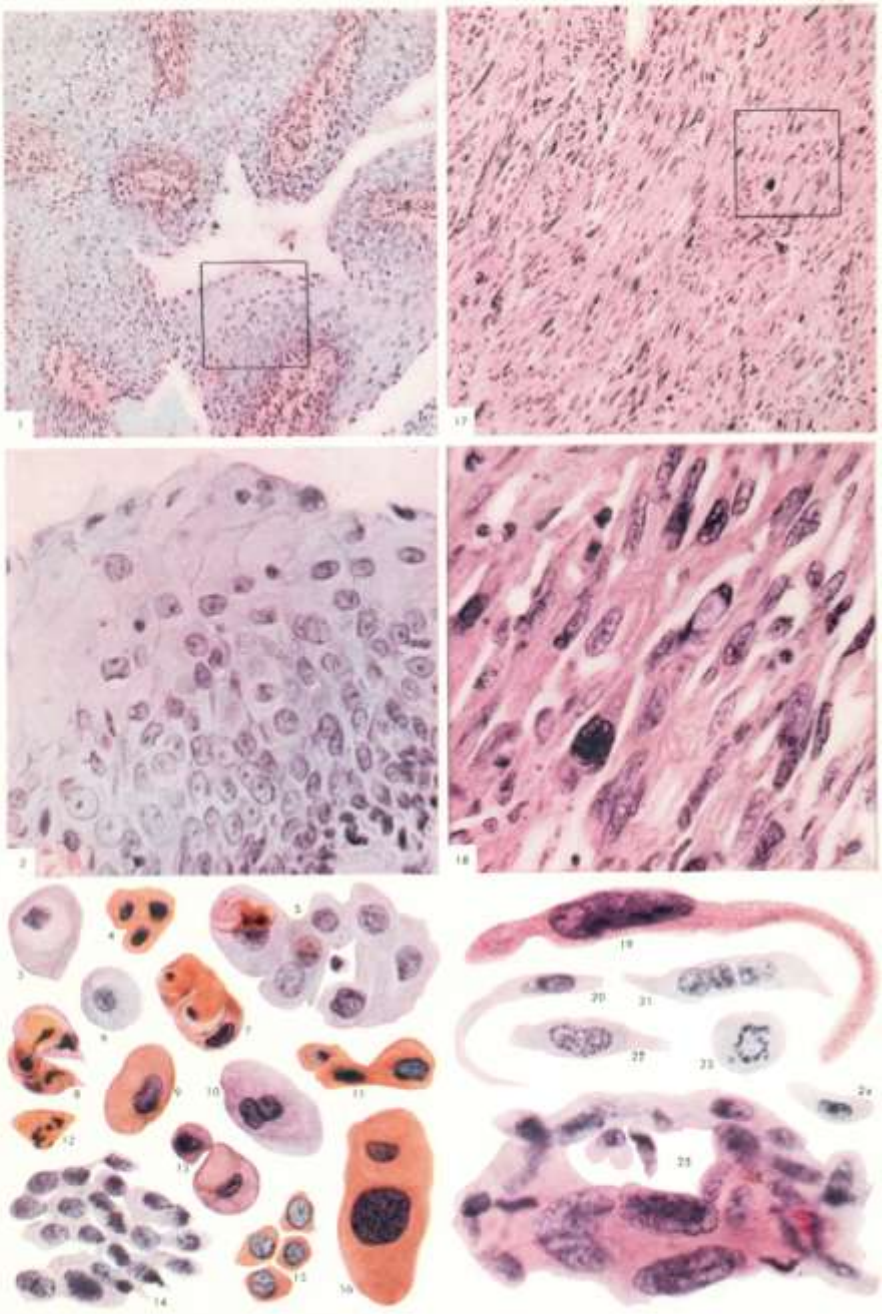
17–25. Spindle cell sarcoma of the uterus. Age 34. (Courtesy of Dr. Louis Wolfe, Cytology Laboratory, Harlem Hospital, New York.)

17–18. Section of the tumor biopsy. x 150 and x 600. The spindle-like form and gigantism of the cells and the marked enlargement and hyperchromasia of some of the nuclei are shown in both low and high magnification.

19–25. Malignant cells. Scraping smear from an extension of the tumor into the vagina. x 600. The cells illustrated in No. 25 give a clear picture of the pattern and type of the tumor. No. 19 shows one of the many gigantic cells with bizarre and hyperchromatic nuclei found in the smear, which, owing to their preservation *in toto*, appear to be much larger than those shown at the same magnification in No. 18 (see above). Other abnormal cells are illustrated in Nos. 20–24. No. 23 shows a malignant cell in mitosis.

(See also A XI, 10–12.)

NOTE: Nos. 3–16 and 19–25: OG–EA series.



SERIES BP Pathology-Cytology PLATE I

Urinary and Male Genital Systems

Carcinoma of the bladder and carcinoma of the kidney pelvis

BP I
DESCRIPTION AND DISCUSSION

Carcinoma of the bladder and carcinoma of the kidney pelvis. Photomicrographs.

1–15. Carcinoma of the urinary bladder. Male. Age 63.

1–2. Section through an area of the tumor. x 150 and x 600. Several nests of malignant cells penetrating deeply into the wall of the bladder can be seen.

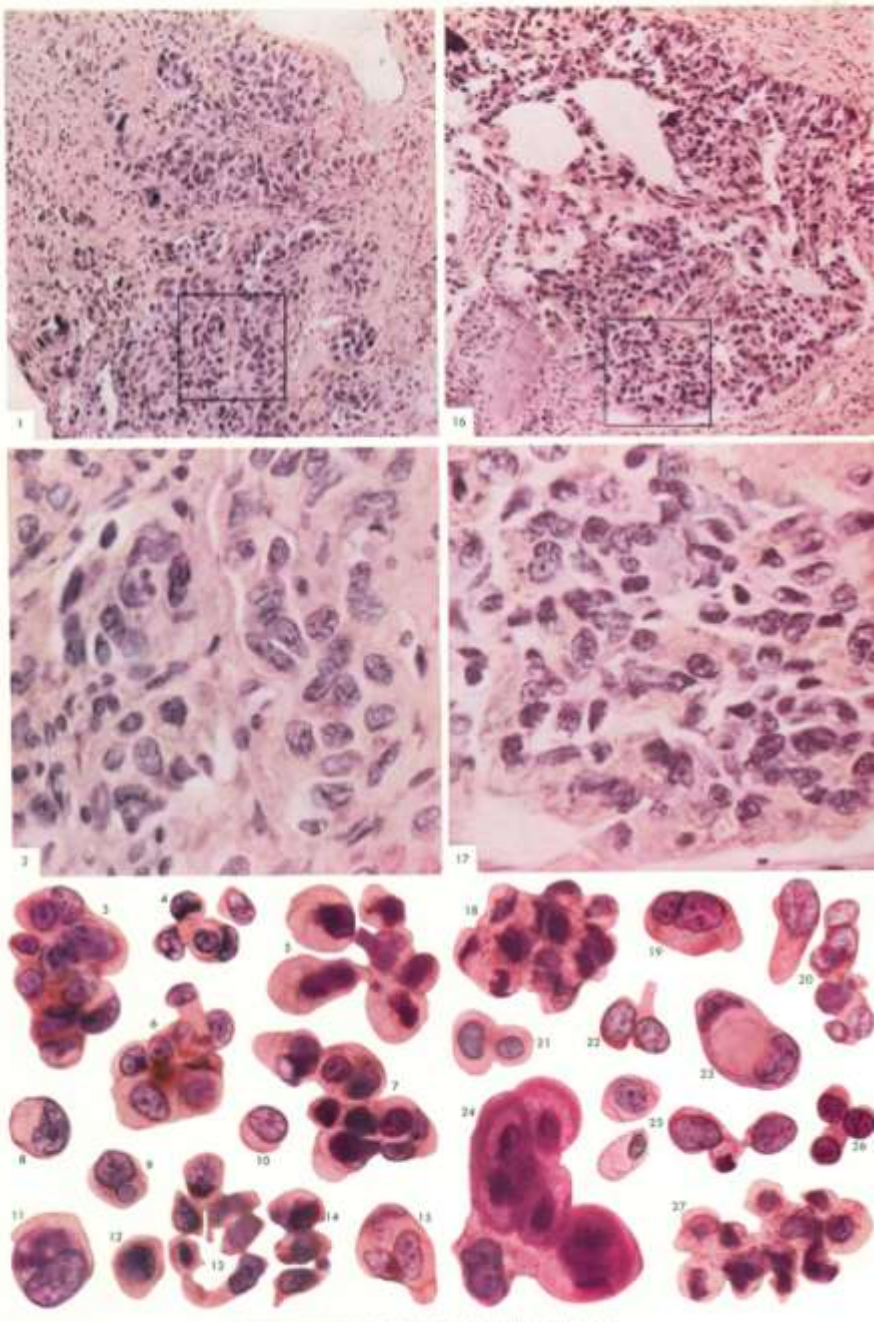
3–15. Exfoliated malignant cells. Voided urine. x 600. The cells and their nuclei show greater variation in size and form than those illustrated in section No. 2. The dark staining of some nuclei and the lack of structural detail are apparently due to degeneration and pyknosis (Nos. 5, 7, 12 and 13). A few cells show vacuolation (No. 8) and engulfment (No. 4). An unequal division of the nucleus, simulating a budding process, is illustrated in No. 9. Marked nuclear enlargement is evidenced in No. 11 and multinucleation in No. 15 and in one cell of No. 13. This last cell has a distinctly columnar form. Such columnar cells are seen more frequently in urine specimens in cases of papillomas, either benign or malignant. The grouping pattern of the clusters and the type of the individual cells may be considered consistent with the diagnosis of carcinoma of the bladder, but not indicative of the type of the tumor. Engulfment, disproportionate nuclear enlargement, multinucleation and nuclear eccentricity with or without cytoplasmic vacuolation may often be seen in carcinoma of the bladder (see B III, 1–5 and B IV, 20).

16–27. Carcinoma of the pelvis of the kidney. Male. Age 77. Primary diagnosis by smears.

16–17. Section of the kidney in the area of the tumor. x 150 and x 600. The pattern of the tumor appears to be characteristic of a primary carcinoma originating in the transitional epithelium. A comparison of the two tumors illustrated in Nos. 16–17 and 1–2 shows great similarity in their structural pattern.

18–27. Exfoliated malignant cells. Ureteral urine. x 600. The exfoliated cells exhibit certain similarities to those illustrated in Nos. 3–15. However, some differences are apparent, such as a greater variation in the size and form of the cells, ranging from undifferentiated forms (Nos. 20, 22, 25 and 26) to highly differentiated types, as in the cell cluster illustrated in No. 24. The structure of the cells of this cluster and more specifically the heavy cytoplasmic rim of some of the cells are very characteristic of well-differentiated cells of the transitional type as seen in smears from urine aspirated from the pelvis of the kidney (see B II, 1). Dark staining of the nuclei (pseudohyperchromasia), apparently due to degeneration and pyknosis, is evident in Nos. 18 and 27; and cytoplasmic vacuolation with ensuing peripheral displacement of the nuclei and change to a cup-shaped form may be seen in No. 23. Nuclear enlargement beyond normal limits is shown in the columnar cell illustrated in No. 20 and in one cell of cluster No. 24, which also shows true hyperchromasia. Bi- and multinucleation are evident in Nos. 19 and 24. The cells of No. 21 and the lower left cell of No. 25 are atypical but show no distinctly malignant features.

NOTE: Nos. 3–15 and 18–27: OG–EA series.



SERIES BP Pathology-Cytology PLATE II

Urinary and Male Genital Systems

Carcinoma of the kidney and carcinoma of the prostate

BP II
DESCRIPTION AND DISCUSSION

Carcinoma of the kidney and carcinoma of the prostate. Photomicrographs.

1–13. Carcinoma of the left kidney. Male. Age 52. (Same case as B IV, 1.)

1–2. Section through the renal tumor. x 150 and x 600. Nests of malignant cells can be seen within the tumor area. A glomerulus is shown in the upper right corner of No. 1. The tumor originated in the kidney parenchyma and was described as consisting of highly anaplastic cells with abundant pale cytoplasm and markedly pleomorphic nuclei.

3–13. Exfoliated malignant cells. Voided urine. x 600. These cells were found in two smears stained with different staining procedures, a fact which accounts for the difference in their coloration (see page 58). Cell clusters in Nos. 6, 11 and 12 show more clearly the structural characteristics of the cells and their nuclei. (No. 11 is also illustrated in B IV, 1.) The pattern of these clusters and of those of Nos. 4, 5, 8, 9 and 13 suggests their neoplastic origin, although the cells themselves, owing to their anaplastic type, do not show any of the more conclusive general characteristics of malignancy. Some suspicion could be based on the relative prominence of nucleoli in a number of cells (Nos. 4, 6, 9, 11, 12 and 13). The deeper staining of the nuclei in Nos. 5, 8, 9 and 13 in comparison with Nos. 6, 11 and 12 should be attributed to the difference in the staining procedure employed. In anaplastic or other carcinomas in which the exfoliated cells do not display conspicuously abnormal characteristics, a suspicion of malignancy may still be aroused by the fact that their distinctly atypical features do not fit into the normal cytologic pattern of the involved organ. A positive cytologic diagnosis may often be arrived at on the basis of specific criteria evolved through experience with the particular tumor in question. No. 3 represents an aggregate rather than a cluster of cells. The larger cells correspond to those seen in the various clusters. Some of the smaller ones give the impression of degenerating cells, whereas others have the size and form of lymphocytes. Inflammatory cells were rather numerous in the smears of this case, indicating an inflammatory reaction with infiltration of some cell clusters by polymorphonuclears, as indicated in No. 9. A degenerating cell is shown in No. 10. Degenerating cells are also present in Nos. 4 and 6.

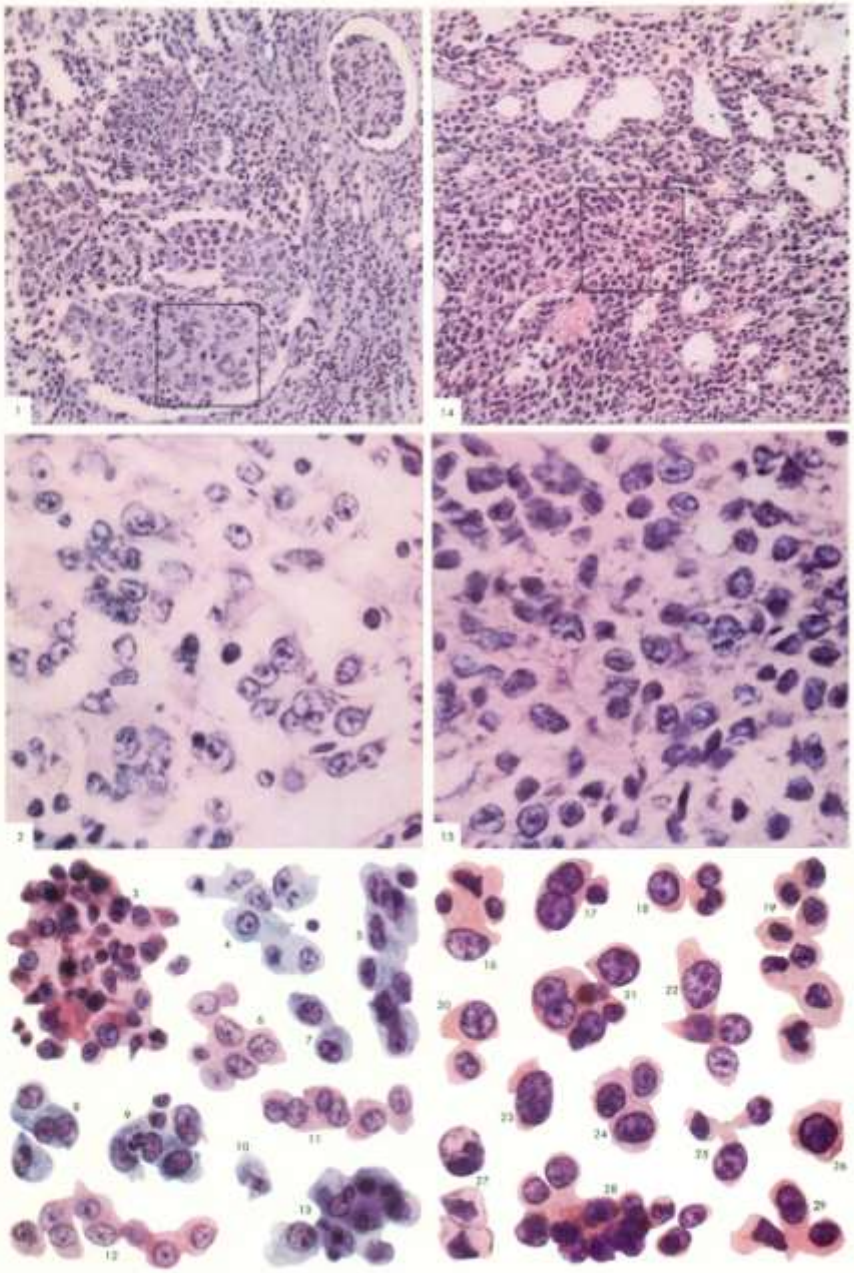
14–29. Carcinoma of the prostate. Male. Age 63.

14–15. Section through the malignant tumor of the prostate. x 150 and x 600. The tumor is composed of small round cells with nuclei exhibiting moderate variation in size, hyperchromasia, and relatively prominent nucleoli.

16–29. Exfoliated malignant cells. Voided urine. x 600. The exfoliated cells show close resemblance to those of the section (No. 15), although displaying greater variation in size and form. The nuclei are distinctly hyperchromatic, and some show fairly large nucleoli (Nos. 16, 18, 20, 21 and 22). The frankly malignant character of the cells is evident. Nuclear degeneration and pyknosis are indicated in several clusters (Nos. 16, 17, 18, 21, 25, 27 and 29). Nuclear enlargement is shown in Nos. 16–18 and 20–26 and binucleation in Nos. 17 and 21. The pattern of clusters Nos. 19 and 27 is strongly suggestive of an adenomatous type of tumor.

(Compare with B III, 19 and B IV, 7 and 11.)

NOTE: Nos. 3, 6, 11–12 and 16–29: OG–EA series. Nos. 4, 5, 7–10 and 13: Glycogen series (procedure emphasizing basophilia).



SERIES PLATE

C VI

Respiratory System

Non-malignant and malignant cells found in sputum and bronchial aspirates

CVI
DESCRIPTION

Non-malignant and malignant cells found in sputum and bronchial aspirates. Photomicrographs x 900.

1–4. Sputum. Male. Age 56. Final diagnosis: virus pneumonia.

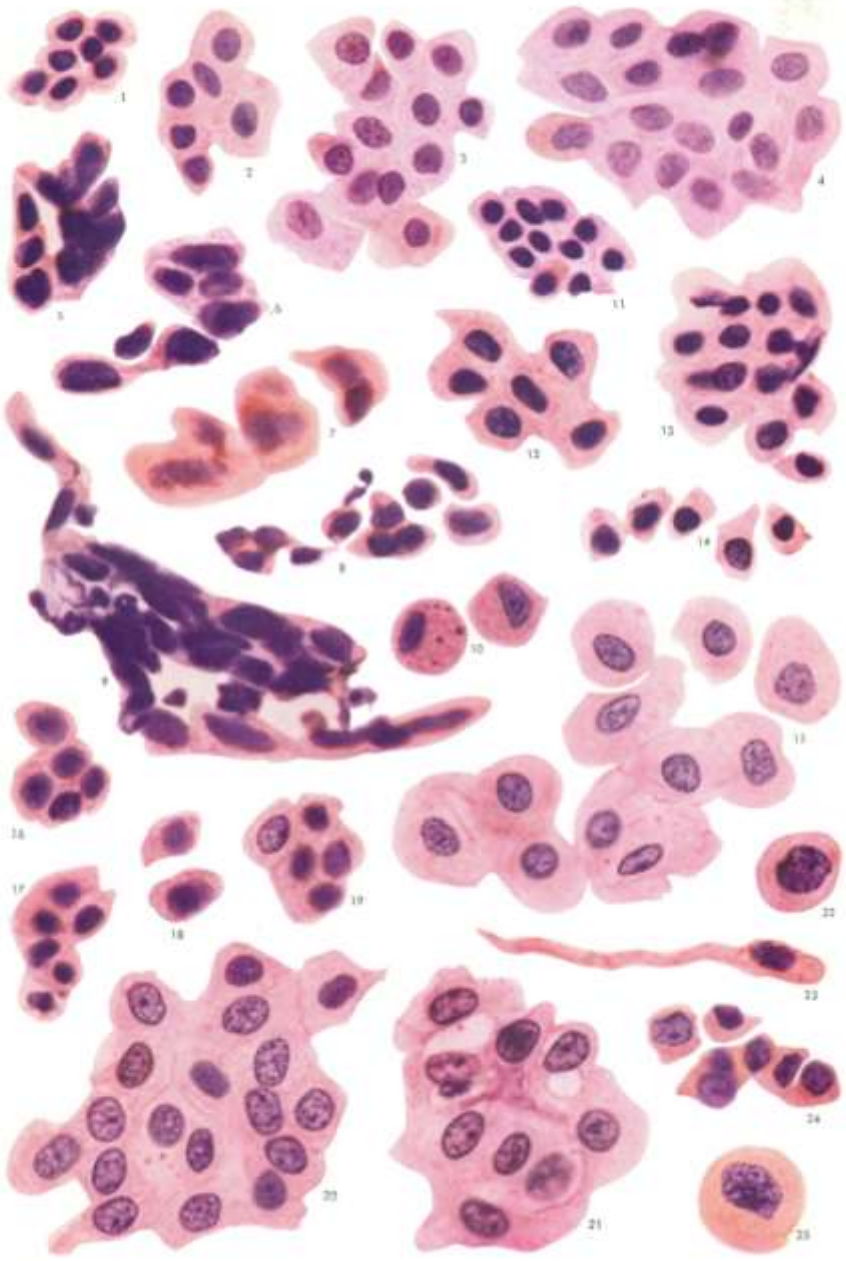
5–9. Sputum (Nos. 5, 6, 8 and 9) and bronchial aspirate (No. 7). Female. Age 81. Final diagnosis: pneumonia.

10. Sputum. Male. Age 57. Pathologic diagnosis: carcinoma of bronchus. (Same case as Nos. 16–25.)

11–15. Sputum. Male. Age 60. Pathologic diagnosis: carcinoma of the lung.

16–25. Sputum. Male. Age 57. Pathologic diagnosis: carcinoma of the bronchus. (Same case as No. 10.)

NOTE: Nos. 1–25; OG-EA series.



CVI
DISCUSSION

Non-malignant and malignant cells found in sputum and bronchial aspirates.

The cell clusters illustrated in Nos. 1–4 are from sputum smears of a case diagnosed as virus pneumonia. The cells of No. 1 are small metaplastic cells known as "Pap" cells, which are frequently found in both acute and chronic inflammatory conditions (see pages 43 and c 3; also C I, 28–30 and C V, 8). The cells of clusters Nos. 3 and 4 are larger cells showing typical squamous metaplasia. The presence of intermediate forms in the smears of this case, as indicated in No. 2, is suggestive of a transition from the small metaplastic ("Pap" cell) to the large metaplastic type. These metaplastic cells, though atypical, do not exhibit morphologic characteristics consistent with a definitely malignant change. The deep staining of the "Pap" cells should be attributed to pyknosis rather than to true hyperchromasia.

Small, intermediate, and large metaplastic cells are also seen in the cell clusters of Nos. 11–15, which are from a case diagnosed pathologically as carcinoma of the lung. The process of metaplasia as indicated in the exfoliated cells seems to follow the same pattern as in the previous case, but the medium- and large-sized metaplastic cells show fairly active and distinctly hyperchromatic nuclei, warranting a suspicion of malignancy.

The same remarks apply to the cell clusters illustrated in Nos. 16–25, which are from a case diagnosed pathologically as carcinoma of the bronchus. The malignant characteristics of the cells and of their nuclei are even more pronounced in this case, particularly in the cells shown in Nos. 22–25, which are further differentiated toward the malignant epidermoid type. Two histiocytes (dust cells) from a smear of this case are illustrated in No. 10. Of these the one to the left can be easily identified by the ingested dust particles; the other shows a certain similarity to some of the metaplastic cells, but not to the fully differentiated malignant epidermoid cells of Nos. 22 or 25. Atypical histiocytes probably constitute the most frequent source of error in the cytologic interpretation of sputum specimens.

The cell clusters illustrated in Nos. 5–9 exhibit a pattern resembling that of the "Pap" cells, but the individual cells show distinctly atypical features which might well be interpreted as suspicious, if not positive, for malignancy. In fact, the cytologic findings in this case were reported as suspicious for malignancy. However, the clinical work-up failed to substantiate this suspicion and the patient was discharged with a final diagnosis of pneumonia. A follow-up about three years later was essentially negative for the respiratory tract. This case is very instructive in that it points to the need of making a distinction between cells with enlarged and hyperchromatic nuclei of a pyknotic type indicating degeneration, as in this case, and cells with active nuclei showing true hyperchromasia, as in the two malignant cases illustrated in this plate. Nuclear pyknosis in itself should not be considered as definite evidence against malignancy, as it may be observed in both benign and malignant cells. The same remark applies to the fading of the nuclei (shown in No. 7), which also represents a degenerative change.

The cases illustrated in this plate were selected for the purpose of demonstrating some of the similarities as well as the dissimilarities of cells of the bronchial epithelium undergoing squamous metaplasia as revealed by the study of exfoliated cells found in smears. In the light of the cytologic changes observed in the cases illustrated in this plate, metaplasia does not appear to be necessarily a sign of malignancy or even of a definitely malignant potentiality. In individual cases its course may vary from one leading to a benign cytologic type, as in the first case of this plate (Nos. 1–4), to one culminating in a distinctly malignant type, as in the cases of Nos. 11–15 and 16–25. It may also evolve toward a strikingly

abnormal and suspicious yet not malignant pattern, as in the case illustrated in Nos. 5–9. It is hoped that the study of exfoliated cells and their correlation with the clinical and pathologic findings will contribute to a better understanding and analysis of some of the obscure borderline changes observed in cells of this type.

SERIES CP Pathology-Cytology

PLATE I

Respiratory System

Bronchial adenoma and carcinoma of the bronchus

CP I
DESCRIPTION AND DISCUSSION

Bronchial adenoma and carcinoma of the bronchus. Photomicrographs.

1–11. Bronchial adenoma. Male. Age 63. (Courtesy of Dr. Bernard Pierson, Department of Pathological Anatomy, University of Nancy, Nancy, France.)

1–2. Section of aspirated bronchial tissue. x 150 and x 600. The tumor consists of cords of well-differentiated cells with benign appearance forming acini lined by cuboidal or low columnar cells. Debris of exfoliated cells can be seen within the lumina.

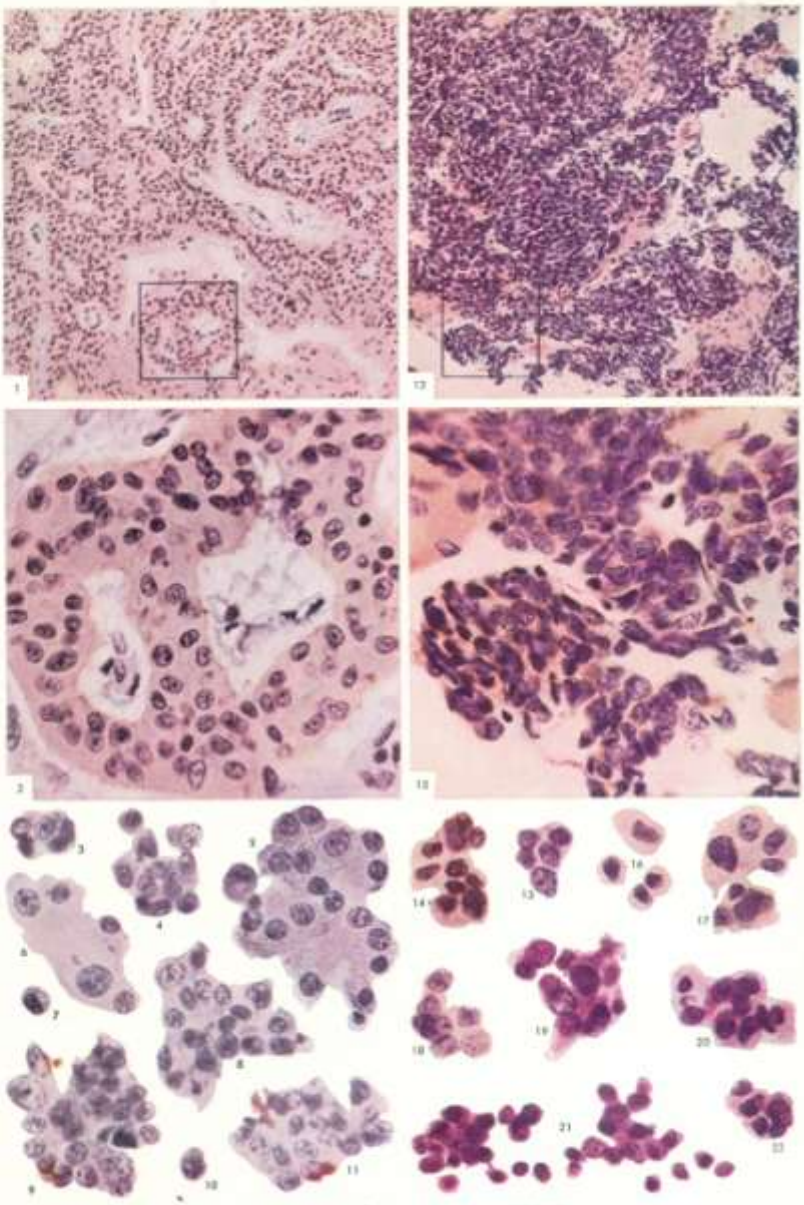
3–11. Clusters of tumor cells found in direct smears from the aspirated tissue. x 600. The pattern of the cell clusters suggests a neoplastic growth. The individual cells show some atypia such as moderate anisokaryosis, which is evident also in the section of the tumor (No. 2), but they show no distinctly malignant characteristics. The relative uniformity of the cells and the presence of an adequate amount of cytoplasm in the better-preserved clusters (No. 5) indicate the benign nature of the cells (compare Nos. 1–11 with Nos. 12–22). However, the chromatin pattern of the nuclei denotes some proliferative activity. Only a few cells, as in Nos. 4, 5 and 9, show eccentric nuclei and a cuboidal or low columnar form, in contrast to the exfoliated adenoma cells illustrated in C V, 20–21, which have a distinctly columnar form.

12–22. Carcinoma of the bronchus. Male. Age 59.

12–13. Section of the tumor biopsy. x 150 and x 600. The structural pattern and the cytology of this tumor are characteristic of an anaplastic carcinoma (oat cell type). The cells are small and their nuclei intensely hyperchromatic.

14–22. Exfoliated malignant cells. Sputum (Nos. 14–18, 20 and 22) and bronchial washings (Nos. 19 and 21). x 600. The small size of the tumor cells as seen in the section is reflected in that of the exfoliated cells. In some cells the cytoplasm is better preserved (Nos. 14, 16, 17 and 20) than in others in which it has been almost totally lost (Nos. 15, 18, 19, 21 and 22). Cytolysis is a rather frequent occurrence in cells exfoliating from this type of tumor. The nuclei tend to be very hyperchromatic and vary greatly in size and form. Many show degeneration and pyknosis. The exfoliated cells appear rather characteristically in dense clusters with single cells scattered around them, as in No. 21. Cells from this type of tumor are more frequently seen in bronchial washings, where, owing to their small size, they may often be misinterpreted as clusters of reserve cells of the bronchial mucosa. (See C V, 9–12.)

NOTE: Nos. 3–11: Shorr's stain. Nos. 14–22: OG–EA series.



SERIES CP Pathology-Cytology

PLATE II

Respiratory System

Lung carcinomas, metastatic and primary

CP II
DESCRIPTION AND DISCUSSION

Lung carcinomas, metastatic and primary. Photomicrographs.

1–8. Carcinoma in the lung metastatic from the pancreas. Female. Age 70.

1–2. Section through the metastatic lesion of the lung. x 150 and x 600. Metastatic adenocarcinoma cells forming cords and acini are shown in a nest surrounded by fibrous tissue.

3–8. Exfoliated malignant cells. Sputum (Nos. 3, 4, 6 and 7) and bronchial washings (Nos. 5 and 8). x 600. Nos. 3, 4, 6 and 7 represent clusters of malignant cells found in sputum smears. They resemble closely some of the cells shown in the section (No. 2). The differences in color are due to the use of different staining procedures (see page 58). The cell clusters show features suggestive of malignancy, such as crowding, disorderly grouping and anisokaryosis. Hyperchromasia, pyknosis and irregularity in the form of some nuclei are also evident. The two cells illustrated in Nos. 5 and 8 are suggestive of an adenocarcinoma, as indicated by their apparently columnar form and the malignant appearance of the nuclei (compare with C V, 25 and 33). The pattern of the clusters also suggests a malignant neoplasm, possibly a metastatic adenocarcinoma (compare with C V, 3).

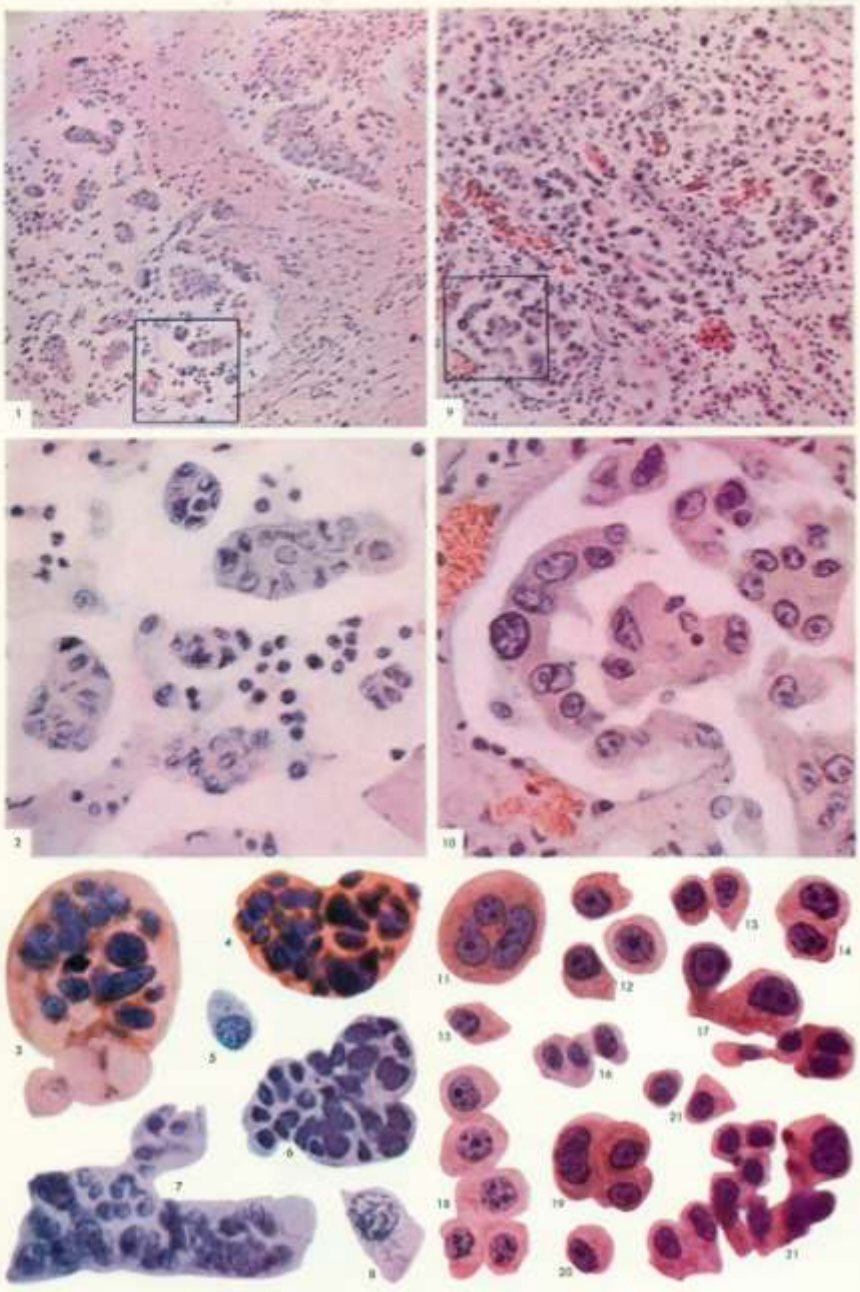
9–21. Alveolar cell (terminal bronchiolar) carcinoma of the lung. Female. Age 54.

9–10. Section of the lung through the area of the malignant lesion. x 150 and x 600. The pattern of the tumor is shown in No. 9. Note in No. 10 the enlargement and hyperchromasia of some nuclei, the binucleation, and the relatively prominent nucleoli.

11–21. Exfoliated malignant cells. Sputum. x 600. The exfoliated cells vary considerably in size from a small undifferentiated type, as in the left cell of No. 21, to the large multinucleated cell of No. 11. Multinucleation is rather frequently noted in cells exfoliated from this type of tumor, although its presence in other types of malignant neoplasms is not uncommon. Another multinucleated cell is shown in No. 19 and a binucleated one in No. 21. Some cells have a cuboidal or low columnar form (Nos. 12, 15, 16 and 21), whereas in others the glandular type of the cells is indicated by the eccentricity of the nucleus (No. 20). The hyperchromasia of the nuclei and the prominence of the nucleoli are plainly seen in this case.

(See also C III, 4 and C V, 24.)

NOTE: Nos. 3, 4 and 11–21: OC–EA series. Nos. 5–8: Glycogen series (procedure emphasizing basophilia).



SERIES CP Pathology-Cytology

PLATE III

Respiratory System

Bronchogenic carcinoma and malignant lymphoma (reticulum cell type)

CP III
DESCRIPTION AND DISCUSSION

Bronchogenic carcinoma and malignant lymphoma (reticulum cell type). Photomicrographs.

1–15. Bronchogenic epidermoid carcinoma. Male. Age 67.

1–2. Section of the tumor. x 150 and x 600. Note infiltration and areas filled with keratinized and necrotic tumor cells.

3–15. Exfoliated malignant cells. Sputum. x 600. Exfoliated acidophilic or orangeophilic epidermoid cells as shown in Nos. 3–6 and Nos. 8–14 are characteristic for this type of tumor. Some of the cells are extremely elongated (No. 4). The nuclei have irregular forms and exhibit various degenerative changes. Some are pyknotic, while others show fading which may ultimately result in the appearance of anucleated forms known as "ghost" cells (No. 12). Concentric cell arrangement as seen in early stages of pearl formation is also noticeable (No. 6). Some of the undifferentiated cells of the tumor are shown in No. 15.

Both undifferentiated and differentiated epidermoid cells are comparable to those seen in the section (No. 2). The difference in the color of the cytoplasm is due to the use of different staining procedures (see page 58). No. 7 shows a small cell adjacent to a histiocyte. The latter can be identified by the ingested dust particles.

(See also C III, 8–9, 13–16; C IV; and C V, 13.)

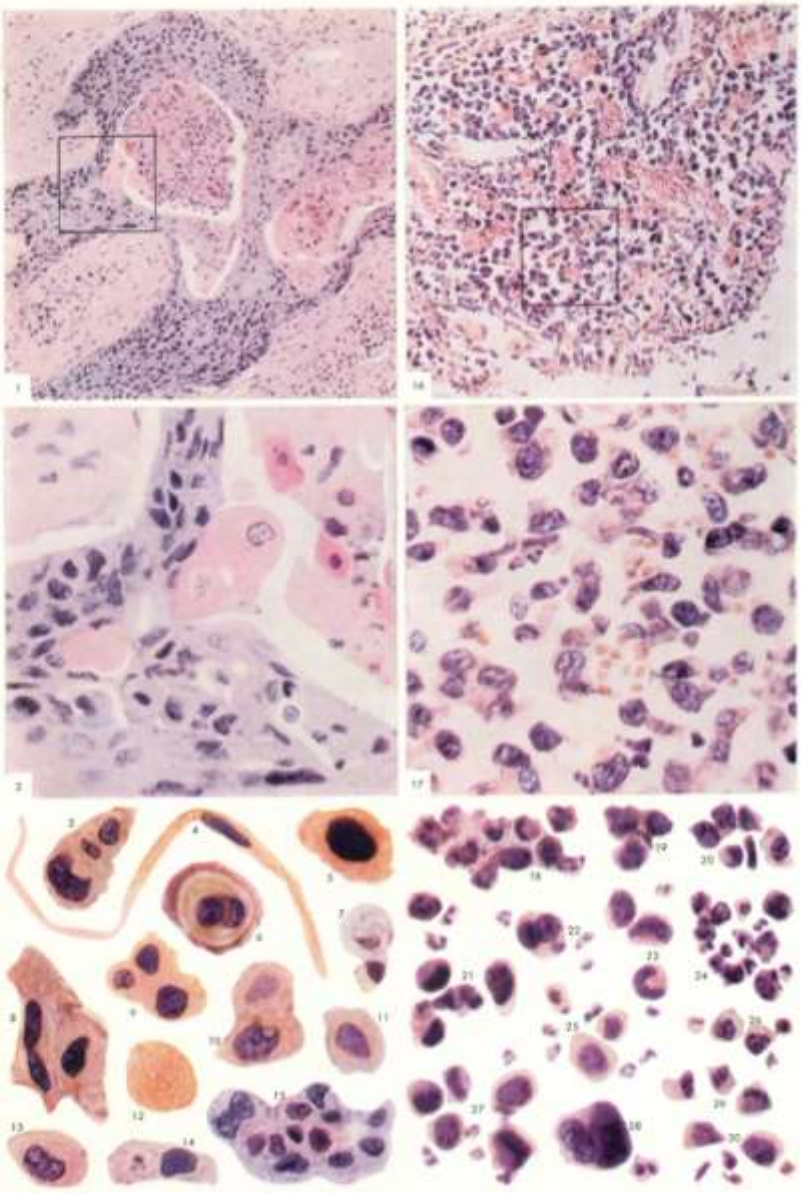
16–30. Malignant lymphoma (reticulum cell sarcoma) with lung involvement. Male. Age 30. (Same case as C V, 27.)

16–17. Section of the lung through an area of the tumor. x 150 and x 600. The malignant cells have practically replaced the bronchial mucosa. A few columnar epithelial cells can be seen in the lower part of the section in No. 1. Cellular and nuclear details are shown in section in No. 2.

18–30. Malignant cells. Sputum. x 600. The exfoliated cells compare well with those of the section (No. 2), although they exhibit a greater variety in size and form. The cytology, in general, is consistent with that of a reticulum cell sarcoma. Some of the cells are bi- or multinucleated (Nos. 23, 28). Most cells appear singly or in small irregular groups. The nuclei show hyperchromasia, pyknosis, and other degenerative changes. Many polymorphonuclear leucocytes may be seen scattered among the tumor cells.

(See also C II, 9 and C V, 27–30.)

NOTE: Nos. 3–15 and 18–30: OG–EA series.



SERIES DP Pathology-Cytology

PLATE I

Digestive System

Carcinoma of the esophagus and carcinoma of the stomach

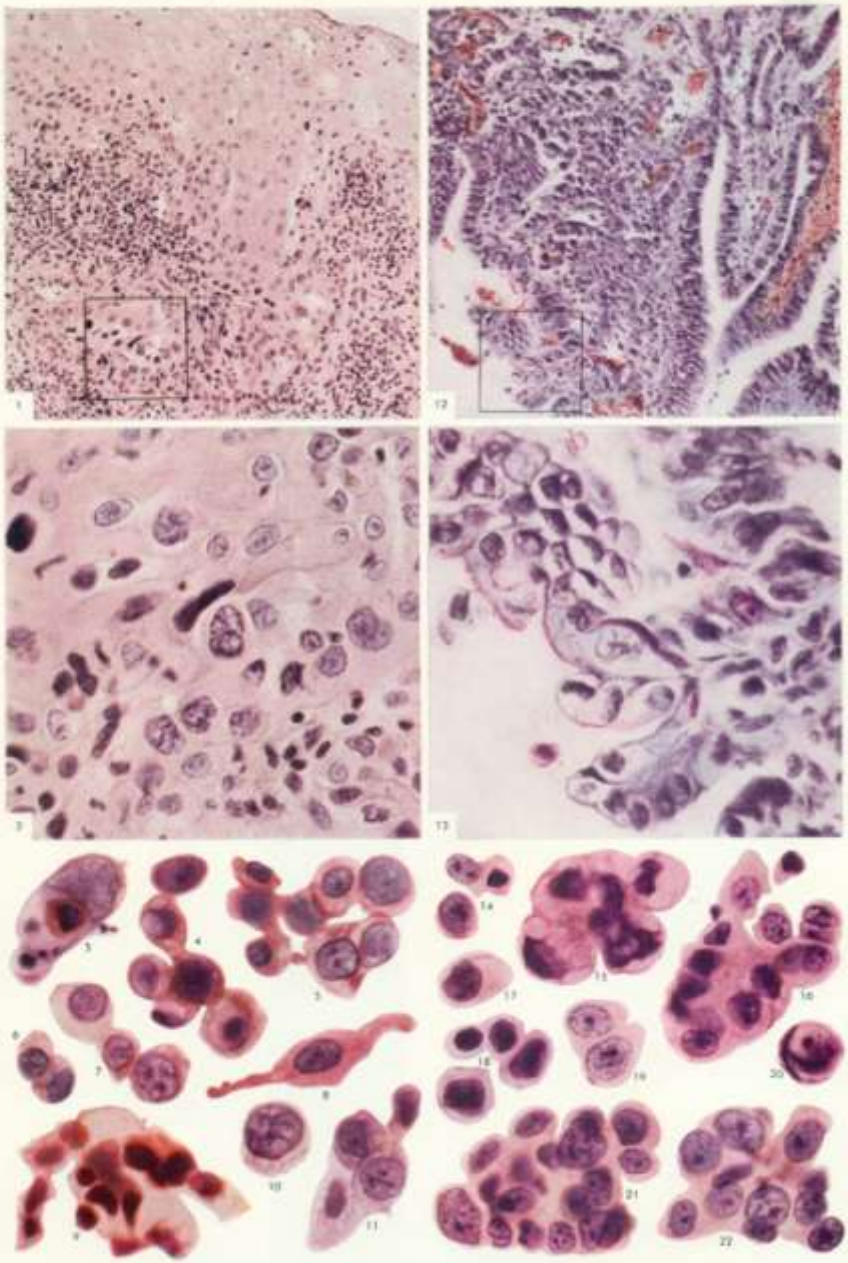
DP I
DESCRIPTION AND DISCUSSION

Carcinoma of the esophagus and carcinoma of the stomach. Photomicrographs.

1–11. Carcinoma of the esophagus. Male. Age 77.
1–2. Section of a biopsy of the esophagus in the area of the malignant lesion. x 150 and x 600. Well-differentiated epidermoid carcinoma infiltrating the esophageal wall.
3–11. Exfoliated malignant cells. Esophageal aspirate. x 600. The epidermoid type of the exfoliated cells is apparent, although only a few cells show marked differentiation (No. 8). Round cells with a large nucleus and relatively sparse cytoplasm (Nos. 5, 7, 10) are often seen in smears from esophageal washings in cases of carcinoma of this organ. (Compare No. 5 with D IV, 14.) Vacuolation is present, though usually moderate. The nuclei tend to be hyperchromatic. A disproportionately enlarged nucleus in a large cell showing engulfment of a smaller cell is illustrated in No. 3. The nucleoli vary greatly in number and size. Degeneration, pyknosis, and fading of the nuclei are shown in No. 9. (See also: D III, 6; D IV, 14–18).

12–22. Carcinoma of the stomach. Male. Age 67.
12–13. Section of the gastric carcinoma. x 150 and x 600. Area of the tumor showing an adenomatous structure with papilloid projections.
14–22. Exfoliated malignant cells. Gastric balloon specimen. x 600. The adenocarcinoma pattern is clearly indicated in No. 15 by the sharp delineation of the cluster, the pronounced vacuolation of its cells and the eccentricity of the nuclei. Nos. 15 and 16 give the impression of detached papillary projections like the one shown in the lower part of the section (No. 2). Most of the cells are of moderate size. The nuclei show considerable variation in size and form. Hyperchromasia, pyknosis, and other degenerative changes are evident. Engulfment with a cup-like peripheral nucleus in the outer cell is shown in No. 20. Some relatively large nucleoli, a few stained red, may be seen in Nos. 19 and 22.

NOTE: Nos. 3–11 and 14–22: OG–EA series.



SERIES DP Pathology-Cytology

PLATE II

Digestive System

Carcinoma of the pancreas and carcinoma of the rectum

DP II
DESCRIPTION AND DISCUSSION

Carcinoma of the pancreas and carcinoma of the rectum. Photomicrographs.

1–13. Carcinoma of the pancreas with generalized metastases. Female. Age 63. (Same case as D II, 14 and 15 and D V, 9.)

1–2. Section of the tumor infiltrating omental fat. x 150 and x 600. The tendency to gland formation is indicated in No. 2.

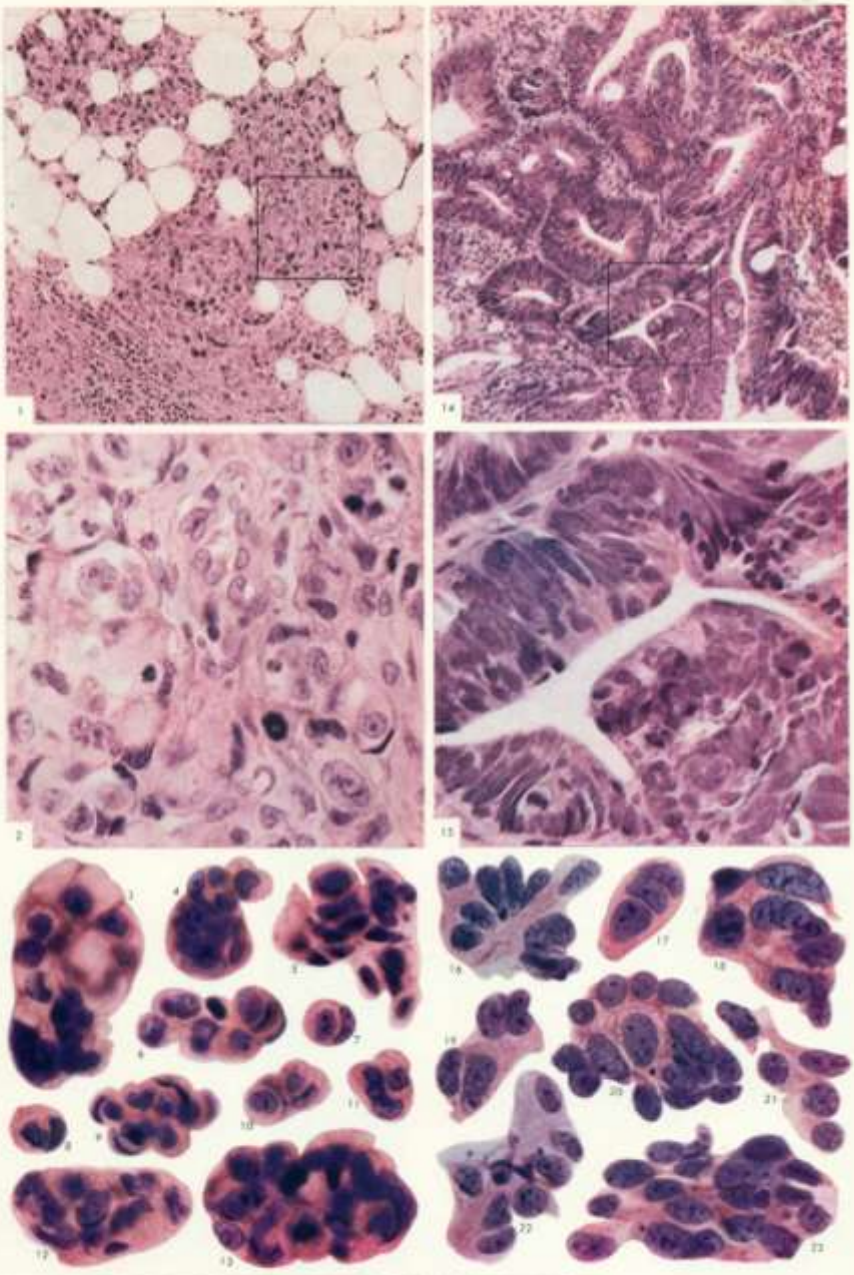
3–13. Exfoliated malignant cells. Duodenal drainage. x 600. The malignant character of the cells is indicated by the many nuclear and cellular abnormalities. The glandular type of the tumor is revealed by the vacuolation of the cells and the configuration of the clusters into well-circumscribed units. The nuclei are comparable in size with those of the section, but are more deeply stained and show degeneration and pyknosis. There are several cup-like nuclei (Nos. 6–9) which compare with similarly shaped nuclei in the section (No. 2).

14–23. Adenocarcinoma of the rectum. Female. Age 65.

14–15. Section of the tumor. x 150 and x 600. Note the many neoplastic glands lined with columnar cells and the presence of mitotic figures.

16–23. Exfoliated malignant cells. Rectal washings. x 600. The cells show distinctly malignant features and are grouped in irregular clusters. Some vacuolation is evident. The nuclei are hyperchromatic and tend to have an elongated or oval form, which is fairly characteristic for this type of tumor. Multinucleation may be seen in No. 18 and engulfment in No. 17. Multiple nucleoli or karyosomes are clearly shown. One nucleus in early prophase transformation is present in the upper left of No. 16.

NOTE: Nos. 3–13 and 16–23: OC–EA series.



SERIES EP Pathology-Cytology

PLATE I

Exudates

Carcinoma of the lung secondary to the pleura, pleural mesothelioma, and carcinoma of the gall bladder secondary to the peritoneum

EP I
DESCRIPTION AND DISCUSSION

Carcinoma of the lung secondary to the pleura, pleural mesothelioma, and carcinoma of the gall bladder secondary to the peritoneum. Photomicrographs.

1–11. Papillary adenocarcinoma of the lung, possibly of alveolar cell origin. Female. Age 63.

1. Section of the primary tumor. x 600. Tumor cells and cell clusters are seen lying apparently free within a cavity lined with a single layer of cells of the same type. The pattern is consistent with that of a terminal bronchiolar or alveolar cell carcinoma.

2–11. Exfoliated malignant cells. Pleural fluid. x 600. The sharply outlined clusters, the marked cytoplasmic vacuolation, and the eccentricity of some nuclei suggest a glandular cell type. The configuration of the clusters gives the impression of a papillary growth. The nuclei are more hyperchromatic and more variable in size than those of the corresponding cells in section No. 1 (see page 58). Engulfment of one cell by another with distortion of the nuclei into cup-like forms is shown in Nos. 6, 9 and 11.
(See also E II, 26.)

12–29. Pleural mesothelioma. Male. Age 34. (Courtesy of Dr. Albert Rubenstone and Dr. David Meranze of the Albert Einstein Medical Center, Southern Division, Philadelphia.)

12. Representative section of the pleural tumor. x 600. The cells are loosely grouped. Some nuclei show rather prominent nucleoli.

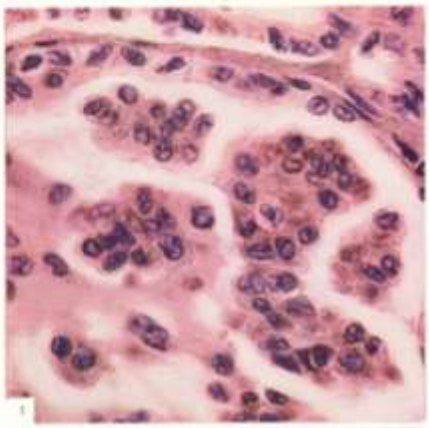
13–29. Exfoliated malignant cells. Pleural fluid. x 600. The smears in this case contained many single cells and relatively few clusters. The malignant nature of the cells, particularly the nuclear atypia, is shown more distinctly in the exfoliated cells than in the cells of the section (No. 12). The marked variation in the size and pattern of the cells and their nuclei contrasts with the relative uniformity of the cells in the section. Some cells contain single large vacuoles. In some of these vacuolated cells the nuclei are pushed toward the periphery and assume a cup-like form (Nos. 17, 19, 28). Bi- or multinucleation and engulfment are also prevalent (Nos. 17–19, 22 and 24–26). Some nuclei have rather prominent nucleoli (Nos. 14, 16, 17, 21, 25 and 27). A structural peculiarity of the nucleus often, though not exclusively, seen in malignant cells is the concentration of the chromatin in one half of the nucleus, as in the upper nucleus of No. 22. An abnormal mitotic figure is seen in No. 23 (compare with E I, 7 and 11). The appearance of the cells suggests a mesothelial origin (compare with E I, 4, 7 and 10 and E II, 1 and 2).

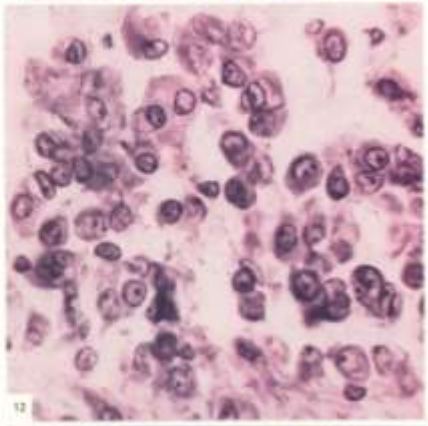
30–41. Carcinoma of the gall bladder metastatic to peritoneum, adrenals, and thoracic and right axillary lymph nodes. Female. Age 63.

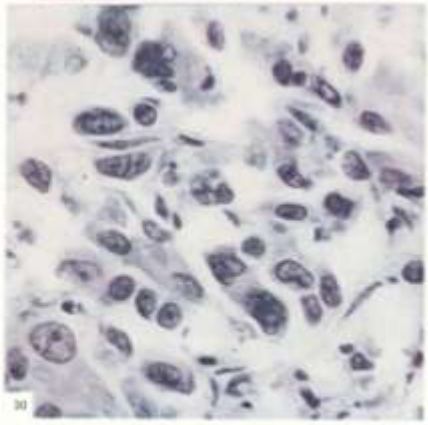
30. Representative section of the malignant lesion. x 600. This section of the tumor illustrates the predominant cell type; however the pattern cannot be well discerned in the limited area shown at this high magnification.

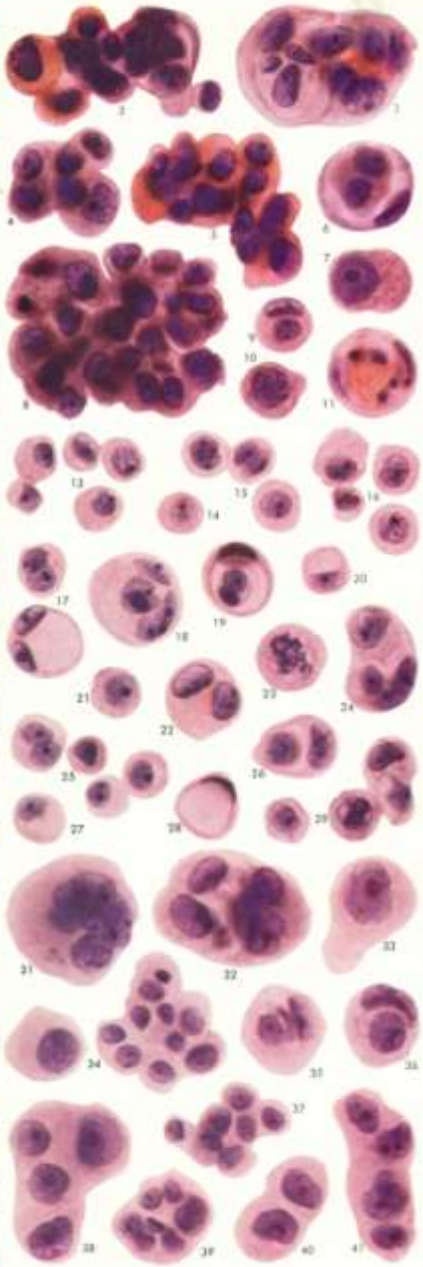
31–41. Exfoliated malignant cells. Pleural fluid. x 600. The malignant nature of the cells is obvious. The morphologic characteristics of the cells and their nuclei are consistent with the presence of carcinoma but are not indicative of their origin and type. There is marked variation in the size of the cells and the nuclei, multinucleation (Nos. 31, 32), and hyperchromasia. There is also engulfment (No. 36) and concentration of the chromatin to one side of the nucleus (No. 38 and others).

NOTE: Nos. 2–5, 8 and 11: Glycogen series. Nos. 6, 7, 9, 10, 13–29 and 31–41: OG–EA series.









SERIES EP Pathology-Cytology PLATE II

Exudates

Carcinomas of the breast, stomach, and ovary secondary to the pleura or peritoneum

EP II
DESCRIPTION AND DISCUSSION

Carcinomas of the breast, stomach, and ovary secondary to the pleura or peritoneum. Photomicrographs.

1–10. Carcinoma of the breast, recurrent. Male. Age 71.

1. Section of the primary breast tumor. x 600. This section was chosen to illustrate the cytology of the tumor rather than its pattern.

2–10. Exfoliated malignant cells. Pleural fluid. x 600. The grouping of the cells and the configuration of the clusters are characteristic of an adenocarcinoma. There is marked vacuolation of the cells and formation of cup-shaped nuclei (Nos. 3, 5, 6, 9 and 10) apparently related to the high degree of engulfment seen in these clusters. An indication of engulfment may be noted also in the section (No. 1). The extensive engulfment and pronounced vacuolation of exfoliated cells found in nipple discharge from cases of adenocarcinomas of the breast are shown in F I, 10; F II, 13, 14, 16, 17, 19 and 27; and FP I, 14, 16 and 23. It should, however, be noted that marked vacuolation may also be observed in benign lesions such as papillomas of the breast (see FP I, 3 and 10 and F I, 7; also in F II, 3–6, a case diagnosed as atypical papillomatosis, borderline malignancy). Such extreme vacuolation is also seen in cells exfoliated from other malignant neoplasms, chiefly adenocarcinomas of various organs (see pages 15 and 16). In this case, the nuclei of the exfoliated cells exhibit structural characteristics which correspond to those of the section (No. 1) but show slightly greater variation in size and form. Relatively large nucleoli are seen in some exfoliated cells (Nos. 4, 5, 7 and 8) and in a few cells of the section (No. 1).

(See also E II, 5–6 and 12–14.)

11–22. Carcinoma of the stomach with multiple metastases. Male. Age 72.

11. Representative section of the primary gastric tumor. x 600. No typical acinar structure can be recognized in this section, but the glandular origin of the tumor is indicated by the predominant cell type and the cytoplasmic vacuolation. Engulfment and signet ring formation may also be noted.

12–22. Exfoliated malignant cells. Peritoneal fluid. x 600. The grouping pattern of the exfoliated cell clusters is typical of an adenocarcinoma. Engulfment and vacuolation with displacement of the nuclei toward the periphery of the cells are quite prominent (Nos. 12, 13, 15, and 18). Engulfment with four cells telescoped into one another is shown in No. 19. A corresponding structure can also be seen in the upper part of the section (No. 11). The malignant nature of the cells is evident in both the exfoliated cells and those of the corresponding section (No. 11).

(See also D II, 4 and 5.)

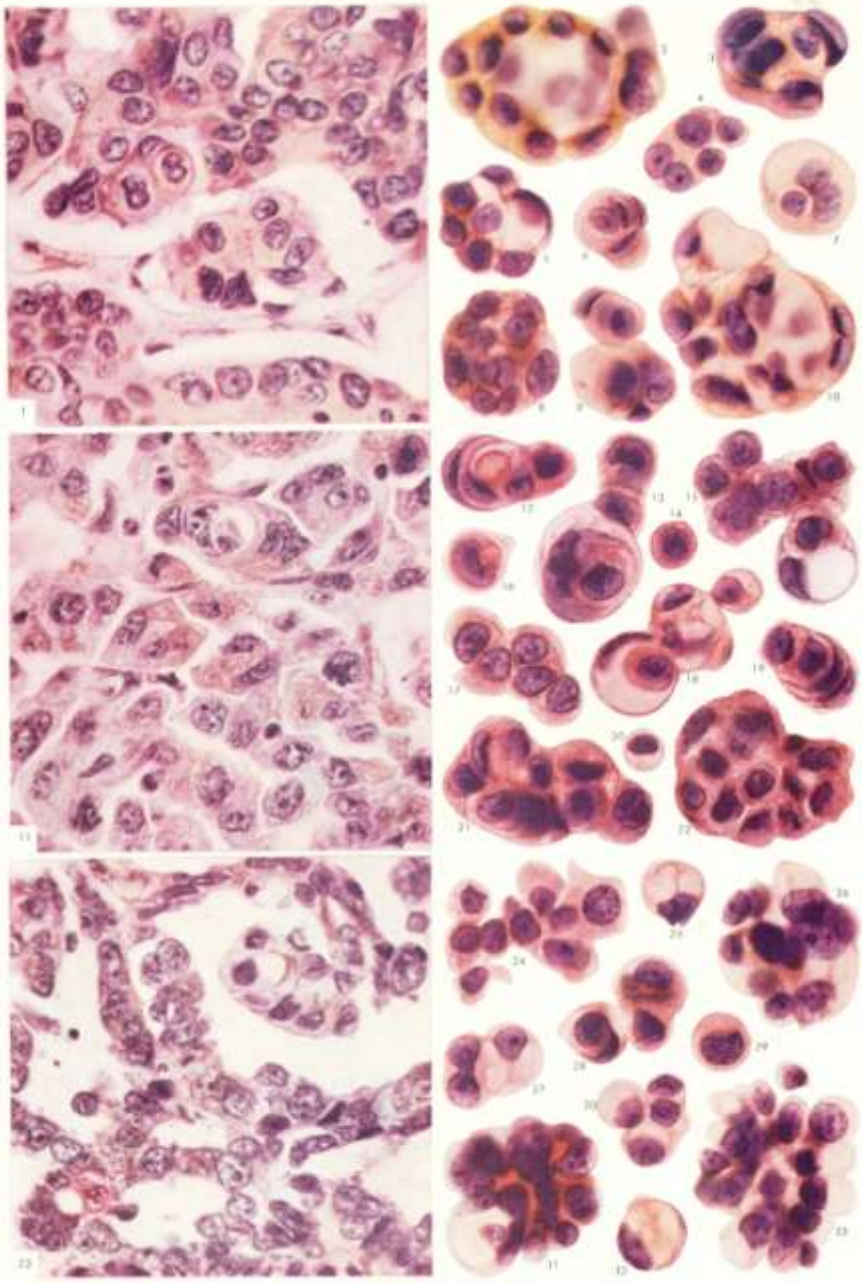
23–33. Cystadenocarcinoma of the left ovary with multiple metastases. Female. Age 54.

23. Section of the primary ovarian tumor. x 600. The tumor consists of strands and papillary projections of malignant cells with interspaces containing fluid and debris. Vacuolation of some cells may be noted.

24–33. Exfoliated malignant cells. Pleural fluid. x 600. The adenomatous type of the tumor is reflected in the pattern of the exfoliated cell clusters. As in the other two cases illustrated in this plate, there is pronounced vacuolation (Nos. 25–27 and 30–33); although the exfoliative cytology in each of the three cases has its own distinctive characteristics. The nuclear abnormalities denote the malignant nature of the cells. The nuclei of some exfoliated cells (Nos. 26 and 27) and of a few cells in the section (No. 23) show relatively large nucleoli. One of the two cells of No. 27 has a dumbbell-shaped nucleus with symmetrically placed nucleoli giving the impression of a cell dividing amitotically.

(See also A X, 12; A XII, 9–12; E I, 24 and 25; E II, 7–9; and AP I, 12, 14, 17 and 18.)

NOTE: Nos. 2–10, 12–22 and 24–33: OG–EA series.



SERIES FP Pathology-Cytology

PLATE I

Breast

Benign papilloma of the breast and carcinoma of the breast

FP I
DESCRIPTION AND DISCUSSION

Benign papilloma of the breast and carcinoma of the breast. Photomicrographs.

1–11. Intraductal papilloma. Female. Age 54. (Same case as F I, 7.) Primary diagnosis by smears.

1–2. Section of the mammary gland in the area of the tumor. x 150 and x 600. Part of the tumor is shown within a dilated duct. The papillary projections are clearly outlined. The cells have a fairly uniform structure and show no malignant features.

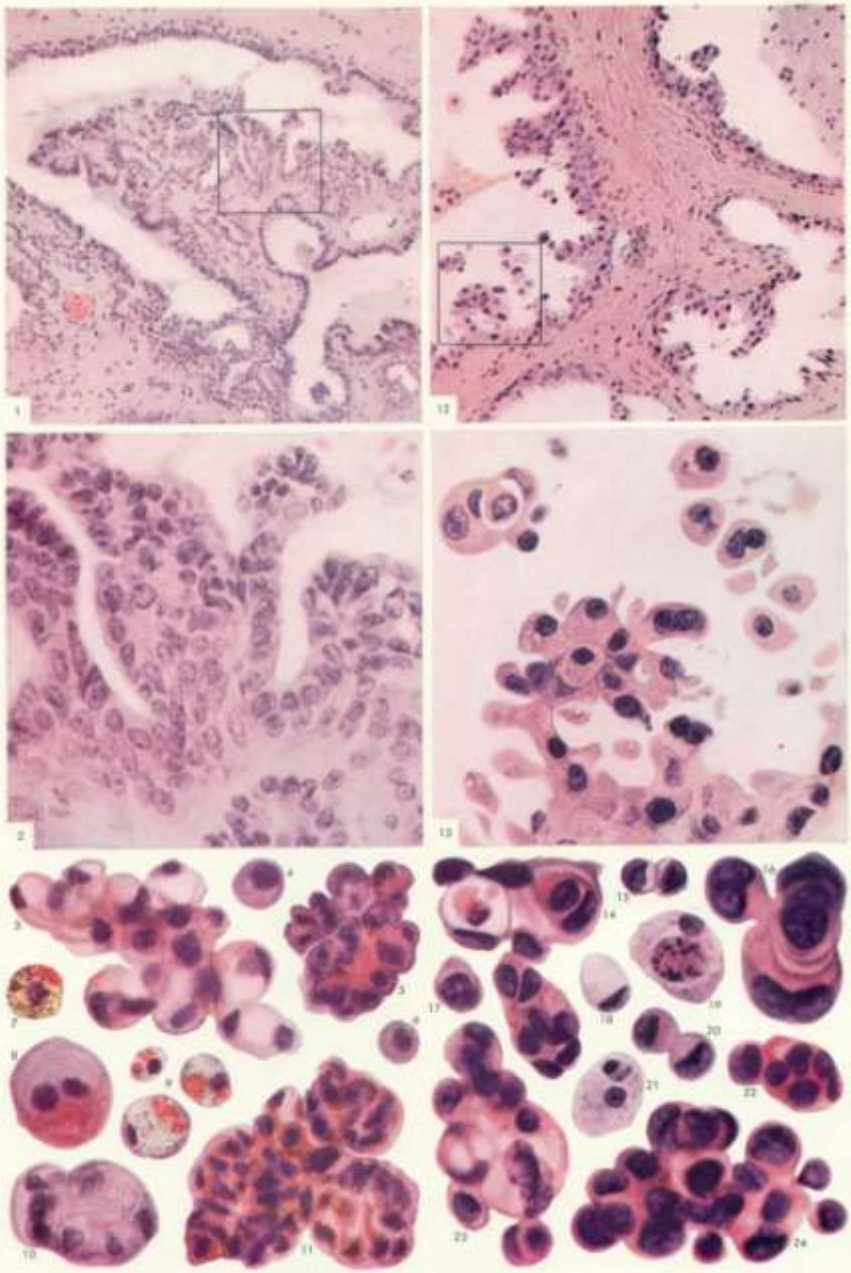
3–11. Exfoliated benign neoplastic cells and foam cells. Breast secretion smear. x 600. The clusters in 3, 5, 10 and 11 are obviously small tissue fragments exfoliated from the tips of some of the papillary projections of the tumor. There is considerable variation in the size of the cells and their nuclei. The cluster in No. 11 consists of cells which differ only slightly from the small undifferentiated duct cells of the mammary gland. Clusters Nos. 5 and 10 show enlargement and differentiation of their cells resulting in a cytologic pattern more typical of papilloma (compare with F I, 6 and F II, 3). The cells of No. 3 exhibit very pronounced vacuolation which may be observed in cases of papilloma as well as of carcinoma (compare with F I, 7; F II, 4–6, 17 and 19; and FP I, 23). The deep staining of the nuclei, which is partly due to degeneration and pyknosis, conveys a false impression of hyperchromasia, but the uniformity in size and structure of the cells and their nuclei and their orderly arrangement within each of the clusters reveals their benign nature (compare with Nos. 14–24). The cells in Nos. 4 and 6 show paranuclear vacuolation and a heavy peripheral cytoplasmic rim, changes which are frequently seen in cells exfoliated from papillomas. The cell of No. 8 is an atypical binucleated cell. Nos. 7 and 9 illustrate foam cells with ingested erythrocytes. The cell of No. 7 also contains many hemosiderin granules (compare with F I, 3). The nuclei of the foam cells are often pyknotic and eccentrically located.

12–24. Comedo carcinoma of the right breast. No infiltration seen. Female. Age 44. Primary diagnosis by smears. (Courtesy of the Strang Prevention Clinic, Memorial Center, New York.)

12–13. Section of the mammary gland showing dilated ducts lined with malignant cells. x 150 and x 600. The detachment of cells and cell clusters from the tips of the papilloid projections within the ducts is clearly demonstrated. The malignant nature of the cells can be readily appreciated by a comparison with the benign cells of Nos. 1 and 2.

14–24. Exfoliated malignant cells and foam cells. Breast secretion smear. x 600. The prolific exfoliation of cells with conspicuous malignant characteristics in this case of an early non-infiltrating carcinoma is noteworthy, as it affords good evidence that exfoliation of cells from carcinomas of the breast begins at a very early stage, prior to invasion and to lymph node involvement. The malignant cells illustrated here exhibit many of the most valid cytologic criteria of malignancy. There is marked nuclear enlargement and hyperchromasia. The arrangement of the cells within clusters is disorderly and the cell borders are largely obliterated (Nos. 22 and 24 and cluster between 17 and 18). Engulfment and telescoping of cells with peripheral displacement of the nuclei of the outer cells are prominently pictured (Nos. 14, 16, 23 and 24). Pronounced vacuolation is shown in No. 23. The cells of Nos. 15, 17, 18 and 20 illustrate smaller, less differentiated cells resembling some of the cells of the section (No. 13). Foam cells displaying phagocytic action by the ingestion of necrotic cells are shown in Nos. 19 and 21. An evaluation of the essential structural differences between cells exfoliated from benign and malignant lesions of the breast can be best appreciated by a comparison of the cells illustrated in Nos. 14–24 with those of Nos. 3–11. (Compare also F II, 2 with F II, 11 and 28.)

NOTE: Nos. 3, 4 and 8: Glycogen series. Nos. 5–7, 9–11 and 14–24: OG–EA series.



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Numerals preceded by a letter (a 1, a 2, etc.) refer to pages in the Plates section.
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