MORPHOLOGY OF THE MALIGNANT SQUAMOUS CELL

A Study of Six Thousand Cells Derived from Squamous Cell Carcinomas of the Uterine Cervix*

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The study of cellular structure has been employed extensively in investigating the female genital tract. As early as 1847, according to Papanicolaou,[1] Pouchet examined human vaginal smears, although this work now is of only historic significance. Our knowledge of the estrus cycle in animals is, in part, the result of cell studies made by many investigators, including Stockard and Papanicolaou,[2] Long and Evans,[3] Allen,[4] Selle,[5] Murphey,[6] and Corner.[7] Similar studies on the cellular elements in the human vaginal smear by Lehmann,[8] Papanicolaou,[9] King,[10] Ramírez,[11] and Moser[12] were concerned primarily with the physiologic process, although the use of the vaginal smear as a diagnostic aid was considered also. Thus, for at least 30 years, the study of exfoliated cells in vaginal smears has provided a useful experimental method for investigating the female genital tract, but only more recently has it been employed effectively as a means of detecting carcinoma.

A morphologic study of cells detached from tissues by physical means was used by early workers seeking a rapid method for microscopic diagnosis. Dudgeon and Patrick[13] reported on the use of wet-film preparations made by scraping the tissue with a scalpel. Employing a similar technic, Wrigley[14] studied lesions of the female genital tract and was able to identify uterine neoplasms readily by this means. Dudgeon and Barrett[15] further investigated the use of wet-film preparations in a detailed study and correctly detected 462 of 469 malignant tumors, including those of uterine origin. The interest in cytologic examination as a diagnostic procedure declined with the general acceptance of the frozen section technic.

During the course of his studies on the exfoliated cells in human vaginal smears, Papanicolaou[16] identified abnormal cells existing in the presence of carcinoma, and in 1943 Papanicolaou and Traut[17] published their monograph on the detection of uterine carcinoma by cytologic methods. Interest in the study of exfoliated cells was revived and the procedure was applied to other anatomical sites.[18] As a result,

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cytologic methods have provided a simple and useful approach to the early recognition of carcinoma.

The purpose of this investigation is to record in detail the morphologic features of neoplastic cells derived from squamous cell carcinoma involving the uterine cervix and to determine if possible the significance of these features in relation to the histopathologic findings.

Material and Methods

The specimens used in this investigation were chosen from over 20,000 cases accumulated by the Cytology Laboratory of this institution. The selection of specimens was based entirely on the abundance of malignant tumor cells present, although the ultimate distribution of cases was similar to that encountered in actual practice, with the less anaplastic neoplasms being more common. In many instances the cases utilized were those in which the cytologic interpretation was of material value in establishing the diagnosis.

The cytologic preparations were obtained either by scraping the uterine cervix or by aspirating the contents of the cervical canal. A tongue blade or the Ayre wooden spatula was used to scrape the cervical portio vaginalis and to spread the material over the surface of a glass slide. Cervical aspiration was obtained by means of a glass pipette and the secretion was expressed onto the surface of a glass slide. By applying a second glass slide and drawing the two apart, the apposing surfaces of both slides presented an even distribution of the material.

The tissue spreads were immediately immersed in a fixing solution composed of equal parts of 95 per cent alcohol and ether, and later stained by the technic described by Papanicolaou.[19] In general the EA36 modification was employed; however, the EA50* also was used in a few cases. Additional staining technics were utilized as will be indicated later in the discussion.

A minimum of 100 cells with morphologic features warranting classification as malignant tumor cells were studied in each of 60 cases so that in all over 6,000 cells were evaluated. All cases were examined by both investigators, each of whom studied 50 cells in order to check the validity of the findings and to assure a thorough coverage of the specimens. While this method of examination was used throughout the study, the findings in general were in close agreement despite the somewhat indefinite criteria which could be applied. The cellular features in each slide were recorded and measurements were obtained by means of an ocular micrometer and by the use of planimetry.

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In order to ascertain whether there was any correlation between the cytologic findings and the degree of anaplasia as determined by histopathologic examination, the tissue sections were reviewed and classified. Those cases of carcinoma *in situ* involving both surface epithelium and glandular spaces but without more definite evidence of invasion in the sections available were classified as group I, while group II included those cases in which there was, in addition, equivocal evidence of invasion. Group III included those cases in which there was invasive carcinoma arranged in well defined cell cords, while in group IV the process was more diffuse and cell cords were less prominent.

On histopathologic study the distribution of the cases was as follows: group I, 23 cases; group II, 8 cases; group III, 16 cases; and group IV, 13 cases. With the exception of the cases believed to be carcinoma *in situ,* the histopathologic classification was also a general index of the cellular differentiation of the neoplasm. Well differentiated tumors were common in group II, partially differentiated tumors in group III, and poorly differentiated carcinomas in group IV.

Cellular Morphology

Cellular Forms

A varied cellular configuration was encountered in this study. Of the 6,000 cells examined, 39.2 per cent were considered as being oval, 23 per cent were polyhedral, 20.2 per cent were more or less rounded, and 11.3 per cent were classified as irregular in outline. A total of 3.9 per cent were recognized as elongated forms, and only 0.7 per cent represented the so-called tadpole cells. There were 104 isolated nuclear forms surrounded by little or no discernible cytoplasm, accounting for 1.7 per cent of the cells examined.

All cases showed neoplastic cells which were classified as round (Fig. 1) or oval (Fig. 2). The highest incidence of either round or oval cells was seen in those tumors which were classified in group III on histopathologic examination. Polyhedral forms (Fig. 3) were more numerous in cells derived from carcinoma *in situ* and were less prominent in the more anaplastic neoplasms. Irregular cell forms (Fig. 4) were infrequent in the presence of neoplasms classified in group III and were more numerous in tumors classified in group IV. Thus the form of the neoplastic cells was of little significance, since varied configurations were observed in the cells from a given neoplasm.

The elongated cells (Figs. 7 and 8) identified in this study have been recognized by Papanicolaou and Traut[17] and other authors under a somewhat varied nomenclature, including streamer cells, fiber cells,

and pseudofibroblasts. The last is objectionable since neither the cell body nor the nuclear structure resembles that of the fibroblast. The cell form is characterized by an elongated cell body with well defined cytoplasmic boundary. While variable in size, it may attain several hundred microns in length, although smaller forms are more common. A total of 238 cells of this type were identified. They were more numerous in the presence of neoplasms classified in groups III or IV, and were common in group I as well. The highest incidence recorded in 100 cells was 15 per cent.

The term tadpole has been introduced by Papanicolaou and Traut[17] to describe an unusual cell form (Fig. 9) found in the presence of carcinoma. The cell body is characterized by a more or less rounded portion from which there extrudes a narrow, sometimes tapering, cytoplasmic process. Undue importance has been attached to this cell form which is uncommon in the presence of carcinoma, only 40 being identified in this series. The tadpole cells were more numerous in the presence of carcinomas classified as grade III and a maximum incidence of 7 per cent was found.

A more detailed subclassification of the cell forms seen in the presence of carcinoma seems unwarranted. The cellular pleomorphism so common in carcinoma studied by tissue sections is equally apparent in cell preparations, where the aberrant forms are frequently exaggerated. The method employed in making the tissue spreads contributes to the cellular pleomorphism and many cell bodies show varying degrees of elongation in a plane parallel to that of the spreading instrument. The distortion resulting in the tissue spreads is of some aid in the recognition of neoplastic cells, although it involves few cellular elements.

Cell Size

An exact measurement of size is difficult to establish when dealing with structures of irregular configuration, and cell area cannot be accurately computed from measurements of the greatest cellular dimensions. A more reliable determination of area can be obtained by the use of planimetry and the results of such a study will be reported at a later date.

The observations made in the course of this investigation do not permit detailed conclusions as to the size of the cell forms seen. In general the neoplastic cells varied only moderately in size. The variation encountered in those cells derived from more anaplastic carcinomas was not significantly different from that existing in the cells

from incipient carcinoma. This was not in agreement with our previous impression of a greater uniformity in the size of malignant squamous cells encountered in carcinoma *in situ*. The validity of these observations can be determined only by employing more accurate means of measurement.

The malignant tumor cells arising in neoplasms which had been irradiated showed a more marked variation in cell size, although such cases were few. This variation was not as marked as is observed in specimens from vaginal fluid.

Cell Membrane

The cytosome is limited by a membrane which can be resolved into an inner plasma membrane and an outer true membrane. The plasma membrane is usually not visible with the microscope but can be demonstrated by other means. It is concerned with cell permeability. A more resistant external membrane is well developed in plant cells where it serves a protective function. In animal cells, however, it is less prominent and more variable in nature.

A well defined peripheral limiting membrane was uncommon in the neoplastic cells of this series (Fig. 1). A well defined, regular cytoplasmic boundary accentuated in some cells by the suggestion of greater density in the peripheral cytoplasm was more commonly observed, with 55.9 per cent of the 6,000 cells studied showing this feature. An indefinite and less well defined cytoplasmic boundary (Fig. 4) was present in 42.4 per cent of the cells classified and in 1.7 per cent there were only isolated nuclear forms with little or no discernible cytoplasm (Fig. 4).

A poorly defined cell membrane has been considered by some authors as an important criterion for the recognition of the malignant tumor cell. Since the outer membrane is ill defined in many normal cells of the organism, the poor definition of this structure in many cells interpreted to be derived from carcinoma is not unusual.

The cells occurring in the presence of less anaplastic carcinoma as determined by histopathologic examination showed a somewhat higher incidence of well defined cell borders, and an equal predominance of ill defined cell boundaries was observed in cells derived from the more anaplastic carcinomas in group IV. This general statement as to the definition of the cytoplasmic boundary cannot be utilized to predict the degree of anaplasia present in the underlying neoplasm. The 100 cells studied from patients with carcinoma *in situ* frequently showed

well defined cytoplasmic borders; however, with similar histopathologic findings, the cell boundaries in fewer cases were consistently poorly defined.

The Cytoplasm

Of the 6,000 cells examined, 66.4 per cent had basophilic cytoplasm; in 30.9 per cent an acidophilia was observed; and in 2.7 per cent the staining reaction of the cytoplasm was considered to be indefinite. Neoplastic cells derived from the less anaplastic carcinomas showed a higher incidence of cytoplasmic basophilia, while 45 per cent of the cells showing an acidophilic cytoplasm were from neoplasms classified histopathologically as group IV.

Cytoplasmic vacuolization was noted in 2.2 per cent of the cells examined (Fig. 5). Of these, 69.6 per cent occurred in cells derived from carcinoma *in situ*. The vacuoles were either single or multiple and only rarely were they of such size as to displace the nucleus. The superficial location of carcinoma *in situ* and the poor nutrition in the cell masses probably account for the high incidence of degenerative changes such as vacuolization.

Cytoplasmic vacuolization was noted in 2.2 per cent of the cells examined and existed with equal frequency in the cells arising from tumors in each of the four histopathologic groups.

The cells identified in this study were more commonly isolated, and only 14.3 per cent were in 140 syncytial masses or tissue fragments. The latter were variable in size and were equally distributed throughout the four categories of neoplasm.

Concentrically arranged cell aggregates resembling epithelial pearls were identified in 5 instances and their distribution in the tumors was not significant. Such structures rarely occur in cellular preparations obtained from the uterine cervix and are of little significance unless their cellular constituents show definitely abnormal changes. The aggregate shown in Figure 6 was identified in the presence of squamous cell carcinoma but its cellular components are not unusual.

The Nucleus

Nuclear Forms

The configuration of the nucleus in the normal functioning and growing cells is related to the shape of the cytosome. Oval, round, and polyhedral cells have oval or round nuclear forms; and in cylindric or fusiform cells the nucleus is more elongated.

An oval nuclear form (Figs. 2, 12, 13, and 14) was present in 74.5 per cent of the cells in this series, while a round nucleus (Figs. 1, 16, and 17) was seen in 15.8 per cent of the malignant tumor cells. Irreg-

ular nuclear form (Fig. 10) was observed in 9.7 per cent of the cells. The incidence of oval and round shapes is more or less in proportion to the number of round, oval, or polyhedral cells identified. The nuclear forms with a more irregular outline, however, were not necessarily observed in cells classified as irregular but occurred in many different cell forms. The irregular nuclei included polypoid and lobate shapes and others characterized only by the term bizarre. A marked elongation of the nucleus was observed in the fiber cells of unusual length, and the nuclear outline in the so-called tadpole cell was in some instances a miniature replica of the cell shape.

Multiple nuclei (Fig. 15) were observed in 1.9 per cent of the cells examined and occurred with equal frequency in the cells arising from tumors in each of the four histopathologic groups.

Absolute evidence of mitotic division in the malignant tumor cells in this series was limited in extent. One cell was seen in metaphase (Fig. 11) and two additional cells were observed in anaphase. The location of two of these cells in syncytial masses offered proof of their epithelial origin, while the third mitotic figure was noted in a cell whose cytoplasm merged with that of an adjoining cell of neoplastic origin.

There were many additional cells whose nuclear patterns suggested an earlier phase of indirect cell division. In Figure 19 the nuclear structure is typical of that encountered in prophase. The nuclear membrane of this cell is ill defined and nucleoli are discernible. In many other malignant tumor cells the distribution of the chromonemata was similar to that observed in early prophase. In these cells, however, the nucleoli were not apparent. An impending mitotic division might easily explain the distribution of the nuclear chromatin in many of the cells encountered in this study.

*Nuclear Size**

The cells derived from normal stratified squamous epithelium show a marked variation in their nuclear size. The large polyhedral cells of superficial origin possess relatively small and sometimes pyknotic nuclei whose areas are frequently less than one-sixtieth of that of the cells, while smaller and more deeply lying cells in the parabasal zone are characterized by nuclei which occupy from one-third to one-fifth of the cell areas. This relationship between nuclear and cytoplasmic area can be expressed in the nuclear-cytoplasmic ratio which is useful in the recognition of the malignant tumor cell.

Many cells derived from the so-called atrophic cervical epithelium show nuclear-cytoplasmic ratios ranging from 1:2 to 1:5 as deter-

* Planimetry studies were conducted by Dr. D. G. Johnston. A more complete report on cytoplasmic-nuclear ratios in the cytologic diagnosis of cancer will appear elsewhere.

mined by planimetry. This is notable since many of the cells derived from carcinoma *in situ* show comparable ratios ranging from 1:2 to 1:4, although ratios greater than 1:2 also are encountered. In the presence of invasive carcinoma the neoplastic cells frequently show a nuclear-cytoplasmic ratio which is greater than 1:2 although many cells show ratios ranging from 1:2 to 1:3.

It is apparent that alterations in the nuclear-cytoplasmic ratio may be significant in some malignant tumor cells; however, there is a considerable variation. Isolated cells derived from apparently normal epithelium similarly may possess a markedly altered nuclear-cytoplasmic ratio; thus, even a marked change is not necessarily indicative of the neoplastic cell.

Nuclear Structures

The normal fixed and stained nucleus in the interphasic or metabolic period shows several basic component parts. These are: the nuclear membrane; the chromonemata, which contain the chromatin; chromocenters or karyosomes; the nucleolus; and the spaces occupied by the nuclear sap or karyolymph.[20]

The Nuclear Membrane

The nuclear membrane appears as a well defined structure, which according to Demerec[21] does not have the power of repair after rupture and collapses with the loss of karyolymph. It is observed in the interphasic nucleus and disappears during mitotic division.

The cells in this study were characterized by a nuclear membrane which was frequently accentuated (Fig. 14) and in some instances wrinkled (Figs. 3 and 18). The latter finding occurred in 20.9 per cent of the 6,000 cells examined and was observed in 71 per cent of the neoplastic cells derived from incipient carcinoma. It was less frequent in the cells arising from more anaplastic tumors. The frequency of this alteration of the nuclear membrane in cells derived from carcinoma *in situ* has previously been noted by one of us.[22] It is most likely the result of retrogressive changes in the cell and may be associated with intranuclear or intracytoplasmic vacuoles.

Chromonemata

The chromonemata appear as fine, irregular threads in the normal interphasic nucleus and contain a substance known as matrix; collectively these constitute the chromosomes.

A detailed study of the nuclear chromatin architecture apparent in the cells of this series resulted in a classification based on the pre-

dominant pattern encountered. The following categories were identified:

1. A uniformly finely granular pattern in which there were fine, distinct clumps of uniform size and staining reaction (Fig. 12).

2. A uniformly finely granular pattern with irregular clumps (Fig. 13) similar to the first noted basic pattern but with, in addition, larger aggregates scattered at random throughout the nucleus.

3. A uniformly finely granular pattern with strands (Fig. 14).

4. A uniformly finely granular pattern with irregular peripheral clumps (Fig. 14).

5. A uniform, but coarsely granular, pattern (Fig. 19).

6. An irregular coarsely granular pattern.

7. A translucent nuclear mass in which no definite granules, clumps, or strands were visible but which transmitted light.

8. An opaque, nuclear mass which did not transmit light (Fig. 10).

This somewhat general classification was employed in order to determine the most common nuclear pattern seen in the malignant squamous cell. A basic granular pattern was most commonly observed in this series. A uniform fine granularity with irregular clumping characterized the nucleus in 41.1 per cent of the cells studied, and a uniformly finely granular pattern was seen in 32.8 per cent of the cells. In 9.4 per cent of the cells the nucleus was classified as translucent and in 5.3 per cent an opaque nuclear mass was identified. The remaining nuclear patterns were less numerous and collectively represented 11.4 per cent of the 6,000 cells studied.

The relative incidence of the various nuclear patterns was the same in cells derived from tumors which were considered more anaplastic on histopathologic examination as in cells arising in less anaplastic lesions. A uniform distribution of each of the nuclear patterns was seen throughout the four groups established by examination of tissues and there was no significant statistical variation.

The varied nuclear architecture was in some instances investigated by cytochemical means. The most important nuclear component chemically is a nucleoprotein composed of simple proteins and nucleic acid, which is largely situated in the chromatin. The desoxyribonucleic acid content of the nucleus can be studied by means of the nuclear reaction of Feulgen, as shown by Feulgen and Rossenbeck[23] and later by Stowell.[24] When the Feulgen reaction was applied to the nuclei showing an opaque or translucent mass (Fig. 16), the desoxyribonucleic acid was apparent in granular or linear distribution (Fig. 17). The material accounting for the translucency or opacity was, perhaps, pro-

tein in nature. Quantitative measurement of the desoxyribonucleic acid in the nucleus in several cases revealed a significant increase in this component. This was true in the various nuclear patterns encountered. Further study by cytochemical means will be necessary to validate these observations, although Caspersson[25] has demonstrated that the nucleic acid content of the cell varies during mitosis, indicating that it plays an essential rôle in cell division.

It is these cytochemical features which are of importance in the production of the hyperchromatism which has been so long described as a feature of the malignant tumor cell. This, however, is not alone due to an actual increase in desoxyribonucleic acid but depends on the state of protein metabolism and other factors, study of which was beyond the limits of this investigation.

The Nucleolus and Karyosome

A well defined body, the nucleolus, is frequently present within the nucleus of the cytosome. Morphologically, in the fixed and stained preparation the nucleolus is sharply defined, usually rounded, homogeneous, and in general acidophilic. This is the true nucleolus, which histopathologically contains a ribonucleic acid, probably in the form of a nucleoprotein, according to Caspersson and Schultz.[26] Nucleolus-associated chromatin is frequently encountered. The latter obscures the presence of the underlying nucleolus and may rarely simulate the appearance of the so-called false nucleoli. These are aggregates of chromatin termed karyosomes or chromatin nucleoli which are similar histochemically to the chromatin filaments of the nucleus, being composed of histone, non-histone protein, and desoxyribonucleic acid, according to Mirsky and Pollister.[27]

Absolute histochemical identification of the true nucleolus is impossible without appropriate staining technics. However, a general idea as to the size and the incidence of this structure can be gained from the preparations in this series employing morphologic criteria alone.

In general, the cells interpreted to be derived from malignant neoplasms did not show prominent nucleoli when morphologic criteria were employed. While observed in a total of 16 cases, their incidence was significant in only 3 cases. Of 100 cells studied in each of these cases, 16, 89, and 95 revealed definite macronucleoli, while in the remaining 13 cases the greatest incidence was 3 in 100 cells. Thus the macronucleolus was a significant finding in only 3 cases and was unrecognized in the remaining 44 cases.

The macronucleoli were single or multiple (Fig. 4), and located in either central or eccentric positions. While usually rounded, they

were frequently irregular in outline and rarely presented bizarre forms similar to those noted by Hauptmann.[28] They measured approximately 4 to 5 μ in maximum dimension, thus justifying the designation macronucleoli. The exaggerated size of the structure, which far exceeds that seen in tissue sections, is probably due to distortion resulting from the method of preparing the tissue spreads.

Since some authors consider the nuclear-nucleolar area ratio of importance in the recognition of the neoplastic cell, these values were computed for the 3 cases showing more numerous macronucleoli. By means of an ocular micrometer the greatest and least dimensions of the nucleus and nucleolus were averaged and this value was assumed to be the diameter of a round mass of comparable size. A total of 300 cells were examined and in these the average nuclear-nucleolar area ratio was 25:1. Naidu,[29] in computing the nuclear-nucleolar area ratio of malignant tumor cells from lesions of the uterine cervix by more exact methods, arrived at a ratio of 26:1. The similarity in ratios suggests that the distortion produced by making the tissue spreads involves the nucleus and nucleolus to a comparable degree.

The large size of the nucleolus in many neoplastic cells has been noted by MacCarty[30] and other investigators as well. The infrequency of this finding in cells interpreted to be of neoplastic origin in this study deserves some comment. The staining technics employed were not ideal for detection of the nucleolus and yet in many cells the structure was clearly seen. The translucency or opacity of the nuclear mass could easily hide the nucleolus in some cells; however, other nuclei in which a finely granular chromatin pattern was observed similarly failed to show nucleoli. A marked increase in nucleolar size is apparent during intensive growth and occurs with cytoplasmic protein synthesis as stated by Caspersson.[25] Since many of the specimens in this series are from patients with histopathologic evidence of carcinoma *in situ*, the cells might be less likely to exhibit features common to cells of more malignant types.

The 3 cases showing numerous nucleoli are of interest since each specimen was obtained from a patient undergoing irradiation therapy. Although this has been noted previously in our material, it is by no means a constant finding after irradiation. Graham[31] has stated that the nucleolus is usually not apparent in neoplastic cells showing irradiation effect. There was only limited evidence of irradiation effect in either the neoplastic cells or non-neoplastic cells in these cases. In 2 cases there was clinical evidence of progression or recurrence.

The specimen showing the highest incidence of nucleoli was obtained from a patient with poorly differentiated squamous cell carcinoma.

However, there is insufficient evidence to permit any correlation between the frequency of nucleoli and the degree of anaplasia seen in the histopathologic material.

Summary and Conclusions

The more significant morphologic characteristics of the cells in this series are seen in the nucleus. These are by no means specific changes encountered in neoplastic cells alone, but are manifestations of altered cellular metabolism, of an unlimited growth potential, and in some cells of degeneration. The nuclear features in their relative order of importance are: structural changes in the chromonemata; altered nuclear-cytoplasmic ratio; hyperchromatism; variations in nuclear size and shape; and, when present, a macronucleolus.

The most common basic nuclear pattern encountered was a diffusely granular chromatin. This was in some instances associated with strands or with larger aggregates of variable size and shape. When the Feulgen technic was employed, these patterns and their variants were seen even in those cells which showed an opaque or translucent nuclear mass with routine staining procedures. The nuclear translucency or opacity cannot be explained on the existing cytochemical evidence. However, there is reason to believe that they represent retrogressive changes and may be the result of proteolytic activity.

A significant alteration in the nuclear-cytoplasmic ratio may be present in cells interpreted to be derived from carcinoma, but many cells derived from carcinoma *in situ* show a ratio within the range of that observed in atrophic parabasal cells during the menopause. In addition, isolated cells from an apparently normal cervical epithelium may show a nuclear-cytoplasmic ratio greater than 1:2. A high nuclear-cytoplasmic ratio is by no means common to all neoplastic cells.

Nuclear hyperchromatism was seen in many cells during the course of this study and is due to cytochemical changes. Quantitative changes in this respect may be difficult to evaluate and the relative intensity of the stain employed is variable. Drying of the cells frequently changes the staining reaction so that hyperchromia cannot be appreciated. The fundamental nuclear pattern is much more reliable in the recognition of the neoplastic cell than is the degree of hyperchromatism.

Tissue spreads commonly show a marked variation in the size and shape of nuclear forms in malignant tumor cells. However, this is not true of all carcinomas. This feature is perhaps overstressed in the literature and is not applicable to all neoplasms.

The macronucleolus can be a useful criterion when it exists. Many of the tumors in this series, however, did not show prominent nucleoli and their absence in cells obviously of neoplastic origin is not explained.

Cellular characteristics in addition to those pertaining to the nucleus are of significance in the recognition of malignant squamous cells but are somewhat less important. Abnormal cellular forms and variations in size and shape are seen; however, these are less common than nuclear changes.

The identification of malignant squamous cells in tissue spreads depends on a detailed study of the cellular components. Those specimens containing numerous cells with many of the features described are readily recognized as being derived from carcinoma; however, greater experience is required to evaluate specimens with limited changes in a few cells. A definite quantitative factor is involved in the interpretation of such specimens. This factor applies to both the changes encountered in the individual cells and to the number of cells present showing such changes. This can best be learned by experience. In general, a cell of undetermined type should possess at least two characteristics of the malignant tumor cell before being recognized as such. When these various cellular criteria are intelligently employed an accurate cytologic examination is possible. However, such interpretation should be based on the specimen in general rather than on an isolated cell.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 15

FIG. 1. An isolated spherical malignant tumor cell showing a well defined cell membrane. × 600.

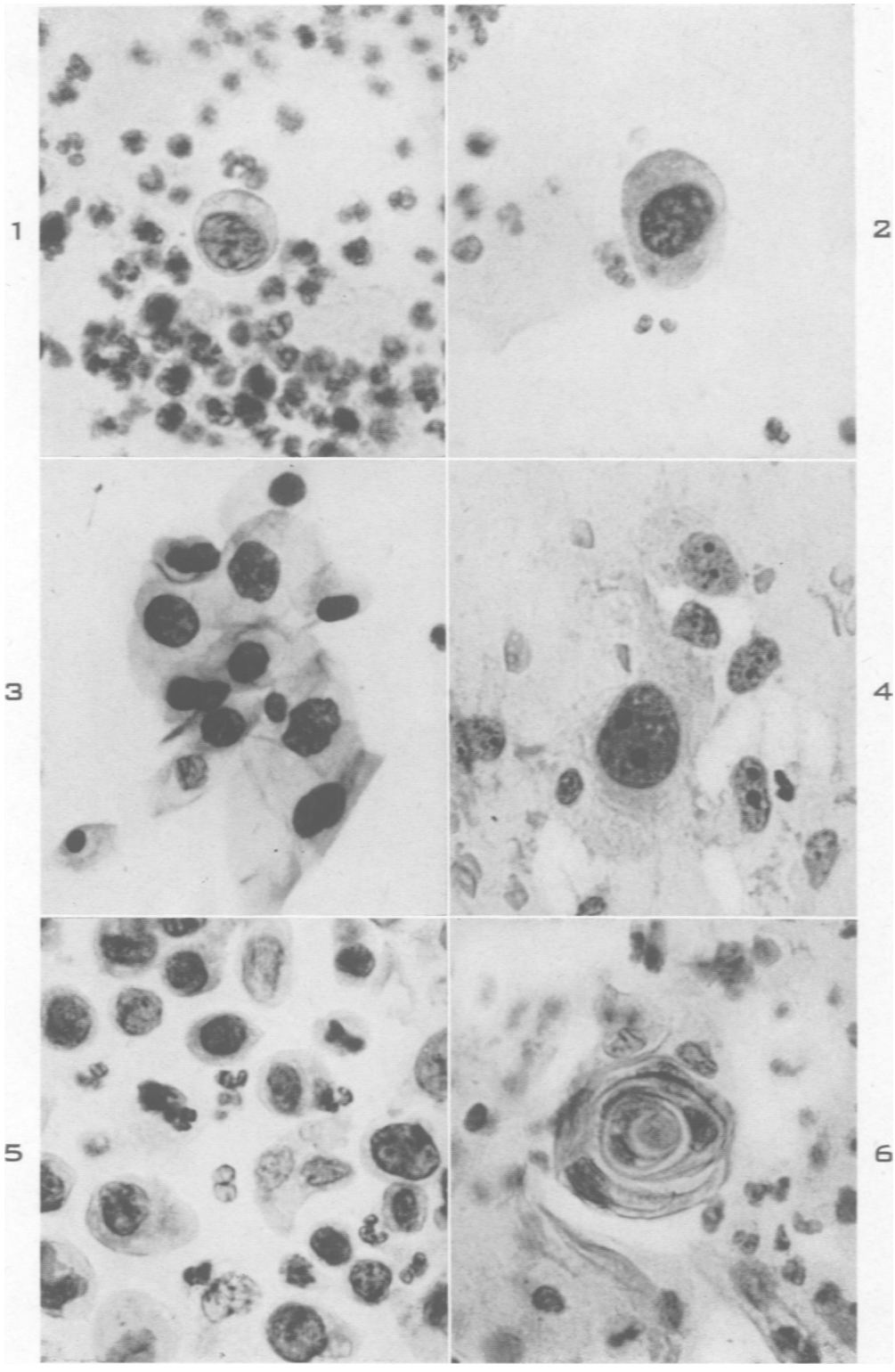
FIG. 2. An isolated oval malignant tumor cell. × 600.

FIG. 3. A group of polyhedral cells derived from carcinoma *in situ*. The nuclei are variable in size and shape and one nucleus shows prominent wrinkling of the limiting membrane. × 600.

FIG. 4. A large irregular neoplastic cell characterized by an ill defined cytoplasmic boundary, alteration in the nuclear-cytoplasmic ratio, granular chromatin, and prominent nucleoli. Small cell forms also are seen, several of which show little or no cytoplasm. × 600.

FIG. 5. Cells derived from carcinoma *in situ* showing variation in nuclear size and shape. The nuclear membranes show limited wrinkling and nuclear degeneration is evident. Cytoplasmic vacuolization is present in several cells. × 600.

FIG. 6. An epithelial pearl whose component cells are not sufficiently altered to warrant classification as neoplastic cells. × 600.



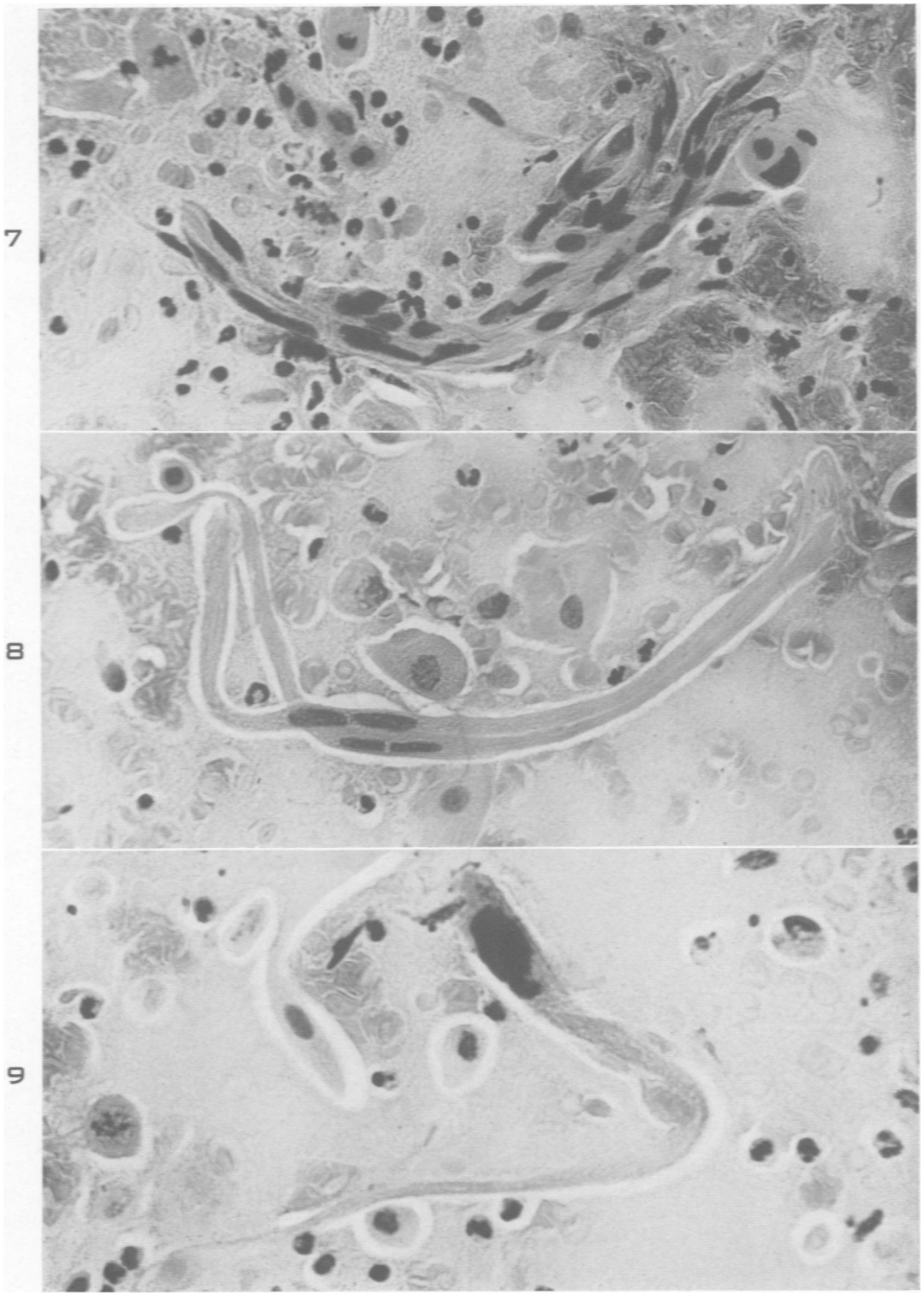
Reagan and Moore The Malignant Squamous Cell

PLATE 16

FIG. 7. A group of elongated cells derived from squamous cell carcinoma. × 420.

FIG. 8. Unusually elongated cells. × 420.

FIG. 9. An example of tadpole cell. × 420.



Reagan and Moore The Malignant Squamous Cell

PLATE 17

FIG. 10. Cells derived from carcinoma *in situ* showing variation in nuclear size and shape. There is limited wrinkling of the nuclear membranes. Two cells show irregular and opaque nuclear forms. × 600.

FIG. 11. A cell in mitotic division (metaphase). The cytoplasm merges with that of the contiguous malignant tumor cell. × 600.

FIG. 12. The nuclei in these cells show a more or less uniform, finely granular pattern. × 600.

FIG. 13. An isolated cell whose nuclear pattern is considered finely granular with irregular clumping. × 600.

FIG. 14. Nuclei of variable size show a basic, finely granular chromatin pattern. The chromatin is also in strands and in isolated aggregates of larger size. One nucleus shows limited peripheral clumping of chromatin. × 600.

FIG. 15. Binucleate and trinucleate malignant tumor cells. × 600.

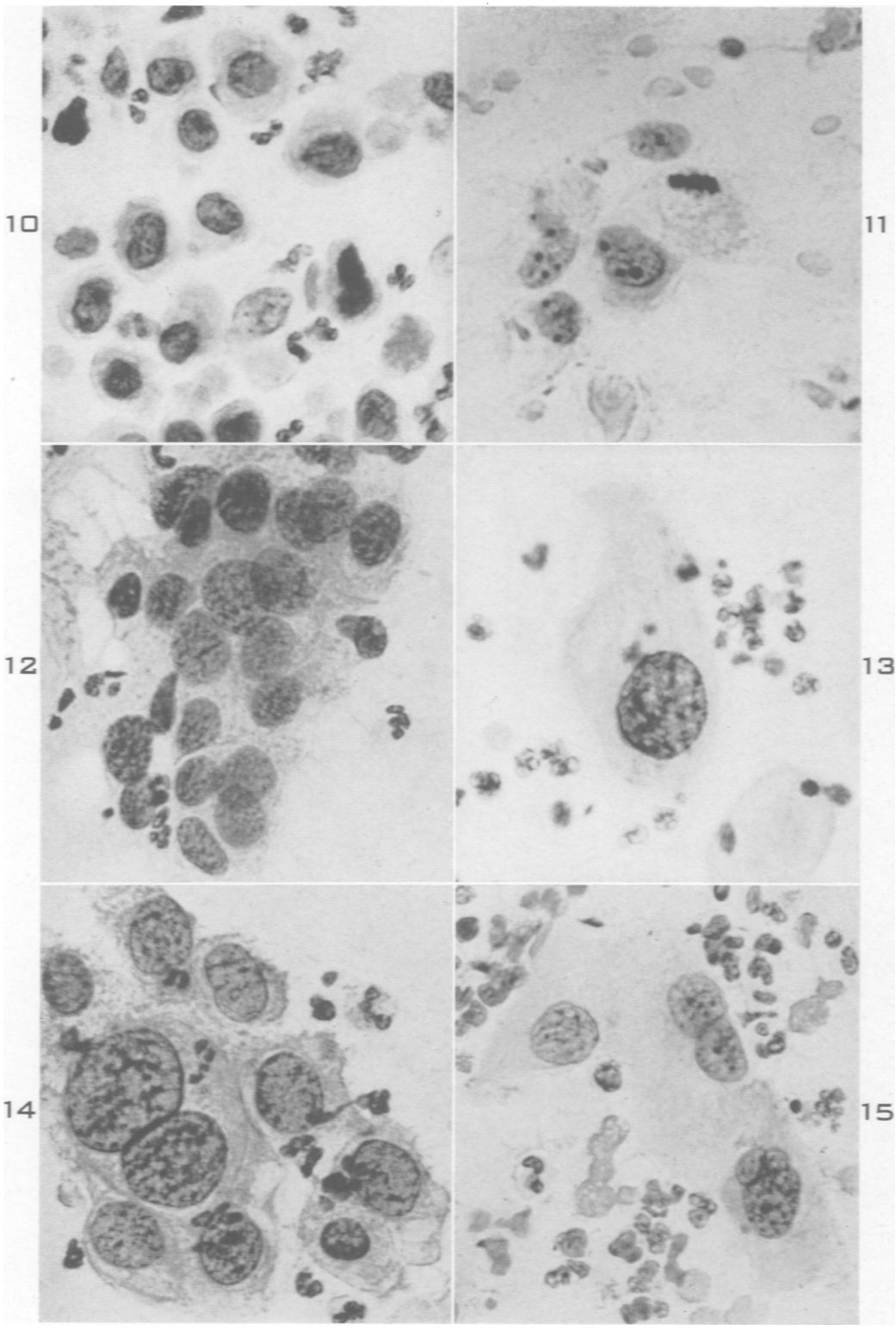


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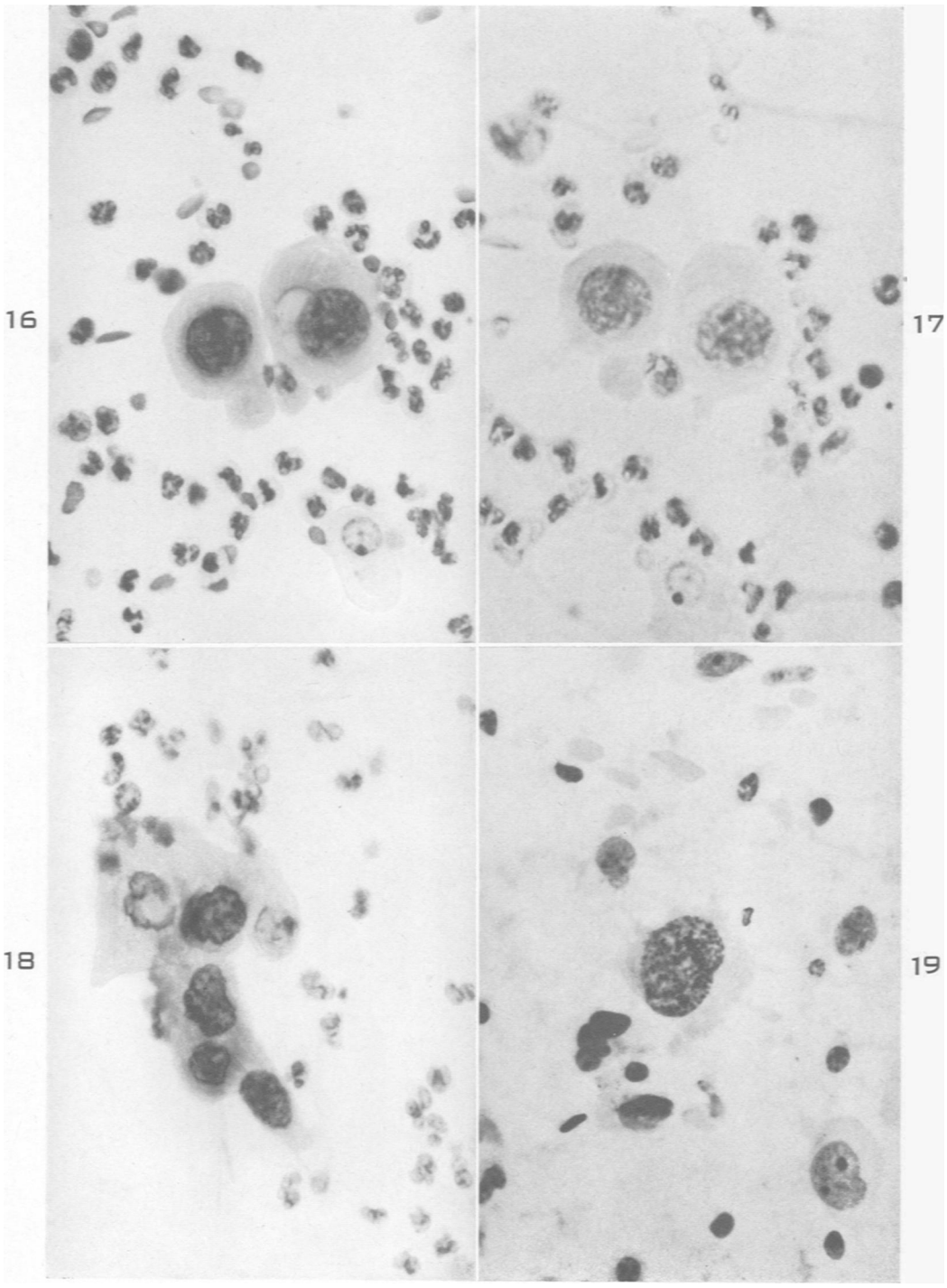
PLATE 18

FIG. 16. Two malignant tumor cells whose nuclei are somewhat translucent as stained with EA36. × 600.

FIG. 17. The cells shown in Figure 16 when stained with the Feulgen reaction to show the disposition of desoxyribonucleic acid. × 600.

FIG. 18. A group of cells showing marked wrinkling of the nuclear membranes. × 600.

FIG. 19. An isolated cell showing a nuclear pattern considered to be uniformly and coarsely granular. The disposition of the nuclear chromatin, the poorly defined nuclear membrane, and discernible nucleoli are suggestive of a prophase stage of mitosis. × 600.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