THE CYTOLOGIC FEATURES OF CARCINOMAS AS STUDIED BY DIRECT SMEARS *

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During the past 20 years the method of diagnosing cancer by visual examination of a single cell or a group of isolated cells has received more and more attention.[1-5] The method is based upon the assumption that morphologic as well as functional differences distinguish cancerous from noncancerous cells. The efficacy of the cytologic approach to the problem of tumor diagnosis is not yet proved. Its limitations and advantages are in part unknown, and only by controlled examination of large groups of tumors and normal tissues and experimentation with different methods will they be established. Therefore, this study was undertaken.

Almost since the advent of Virchow's cellular pathology attempts have been made to identify the characteristics of the cancer cell. The older work in this field was comprehensively reviewed by Quensel [4,6,7] (1928) with particular emphasis upon the cytologic features of malignant cells in exudates. In the recrudescence of interest in this method of diagnosis, MacCarty [2,8-10] with his group [11-14] in the United States and Dudgeon [1,15,16] with others [17-19] in England have been leaders in their respective countries. The development of the technic of needle biopsy of tumors [20-26] and the work of hematologists in the study of bone marrow,[27-31] lymph nodes,[32-39] and spleen [40] have all lent further impetus in this direction. Particular recognition must be extended to Papanicolaou [3,41,42] and others,[43-54] who in recent years have cultivated the method of single cell diagnosis to a point where it has found widespread practical application, particularly in examination of body excretions.

Despite this mass of evidence, the value of cytologic diagnosis of tumors is not widely acknowledged. The greatest skepticism is found among pathologists, and not without reason. Although there is a great difference between Borst's [55] statement, "Die Geschwulstzelle, auch die bösartige, hat weder in morphologischer, noch in chemischer, noch in irgend einer anderen Hinsicht etwas absolut Charakteristisches an sich," and that of MacCallum,[56] "No doubt, in time we shall have a reliable morphological criterion by which we may say definitely that an isolated cell is a cancer-cell or a normal cell," both indicate that the problem of single cell diagnosis of cancer is still to be settled. Rea-

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sons for this skepticism are found in the small number of tumors dealt with in most of the reports, the great variety of tumors within the small groups, the diversity of methods used, and often the inadequate number of photomicrographs, limiting comparison of results.

Detailed descriptions of cytologic characteristics, such as have been reported in bone marrow studies [31] and more recently in investigations of lymph nodes [36,39] and spleens,[40] have not been recorded for malignant tumors. As a consequence, cytologic descriptions of tumors are limited to generalities such as anisocytosis, peculiar structure of chromatin and hyperchromasia of nucleus, size of nucleoli, or emphasis upon a detail (Quensel,[4,6,7] MacCarty [2,8-10]) which may have statistical significance but is of little help in the interpretation of a given case.

In contrast is the work of Papanicolaou[3] and his followers.[45,46,48-50,52,54] Utilizing a standardized technic and concentrating upon the cells of body excretions (vaginal, bronchial, etc.), they have demonstrated the usefulness of cytologic diagnosis of carcinomas.

From the above it appears that the most fruitful approach to this problem would be the examination of a large number of tumors, using a standardized technic and cataloging the changes which would make it possible to differentiate malignant from nonmalignant cells, regardless of origin. Such careful studies might eventually reveal differences permitting further classification, *i.e.*, carcinoma, sarcoma, or perhaps within a certain group even a detailed diagnosis, as is possible today with histologic methods (adenocarcinoma, squamous cell carcinoma, etc.).

It is in this manner that the present study was carried out. The tumors examined were limited to histologically proved carcinomas from various sites, and search for answers to the following questions guided the investigation:

1. What are the cytologic characteristics of carcinomas studied by means of the smear technic? Or, rephrased, is it possible to distinguish between histologically recognized carcinoma and noncancerous tissue, using the cytologic method?

2. Is there a cell or cells which characterize carcinomas, and are such cells always absent in noncancerous conditions?

3. Is there any relationship between the organ from which the tumor arises and the type of carcinoma cells? That is, do the cells of a carcinoma of the breast differ from those of a carcinoma of the stomach or skin?

4. Is there any relationship between the structure of a given carcinoma (adenocarcinoma, squamous cell carcinoma) and its cytologic features? In other words, are the cells which make possible the cyto-

logic diagnosis of carcinoma the same in an adenocarcinoma as in a squamous cell carcinoma?

5. Is the cytologic picture (smear) of a metastatic tumor the same as that of the primary tumor?

Technic

The ideal method is to prepare smears of cells immediately upon removal of the tissue by the surgeon. Unfortunately, this was not possible in the majority of cases. Thus, the first problem was to determine what cellular changes occur following removal of tissue from the body. Since Rohr and Hafter [30] have shown that the changes in bone marrow after death are progressive, all of the tissues used in this study were refrigerated (4.0° to 5.0° C.) as soon as possible in order to retard these autolytic changes. To determine what changes the cells undergo at this temperature and what medium might best preserve them, fresh specimens were placed in four test tubes: (1) Fresh tissue in formalin, (2) fresh tissue in 0.85 per cent solution of sodium chloride, (3) fresh tissue in human plasma, and (4) fresh tissue untreated. All of the tubes were refrigerated (4.0° to 5.0° C.) and their contents examined in smears at intervals of 1 to 2, 6, 12, 24, 48, and 72 hours. It was observed that cells placed in formalin showed great changes in their staining reactions in less than 1 hour; those areas to which formalin had not penetrated (center of specimen) showed no tinctorial change. The cells in a physiologic solution of sodium chloride revealed remarkable changes within the first 12 hours—a fact noted many years ago by Forkner.[32] The nuclei became swollen, the chromatin structure assumed an irregular, wide-meshed, net-like appearance, and the nucleoli became more visible. Finally the cell disintegrated. Cells suspended in human plasma, or untreated, showed almost no changes in staining reaction, cell form, or shape up to 24 hours at refrigerator temperatures. After this, they usually changed slowly, but in a manner different from the cells suspended in physiologic solution. Because of this experience all tissues which could not be examined immediately were placed under refrigeration in human plasma or untreated.

The second problem was to discover which method would best preserve the cell form and size, and produce an even distribution of cells in the preparation. The usual methods for making smears described in the literature [1,31,36] and also touch preparations proved unsatisfactory for two reasons: (1) The cells were unevenly distributed and often were elongated in the direction of smearing; (2) tissues rich in mucus or fat (*e.g.*, mucosa of the gastro-intestinal tract, breast tissue) gave smears very poor in cells; the same was true of tumors which

contained much fibrous tissue. These methods, however, gave good results in material obtained from lymph nodes, spleen, and squamous cell carcinoma. Very poor results were obtained with the crush method [20,23,25] of forceful spreading of tissue between two slides; too many clumps of cells were present to permit uniform staining or to preserve the shape of the cells.

The following method was found to be most satisfactory with all organs and tumors. A fragment of tissue, not larger than 2 to 4 mm. in diameter, was placed on a slide and covered with 3 or 4 drops of pooled human plasma. Using two needles, the tissue was teased apart until the plasma was clouded by the suspended cells. One drop was transferred to a coverslip and then covered with a second coverslip, producing an even distribution of the cell suspension. The coverslips were then pulled apart and dried. This method is similar to that used by some hematologists [57] in the preparation of blood smears.

The third problem was the selection of a stain. It was desired to preserve the cells as well as possible and, at the same time, to differentiate clearly the various cellular structures. Most of the methods described in the literature, judging from illustrations and descriptions, did not seem to give these desired results. Usually the chromatin of the nucleus was clumped so as to prevent recognition of the more delicate structures (Martin and Ellis' [22] method with hematoxylin and eosin, Dudgeon and Barrett's [1] method with Schaudinn's solution and subsequent treatment with hematoxylin, iodine, and eosin). It was found impractical to use the more sensitive method of supravital staining described by Sabin [58] or Quensel's [4] method for examination of tumor cells, since in both cases it is necessary to use very fresh material, which was not always possible in this work. Two methods appeared to be better adapted to my purposes: The various combinations of Romanowsky stains currently in use in hematology,[31,57] which show clearly the details of cells; and Papanicolaou's [3] method, which has proved so popular in recent years in the examination of tumor cells of different body excretions (vaginal fluid, sputum, urine, gastric juice). Therefore, in this study several preparations of each specimen were stained, some with a combination of May-Grünwald-Giemsa's stain, others with Wilson's * stain, and still others by Papanicolaou's method (hematoxylin-EA_{25}-OG_6). Of the two hematologic methods, eventually only Wilson's stain, because of its simplicity, was retained, although both methods gave equally good results. The material stained by Papanicolaou's method was prepared upon slides, according to his directions.[3] In comparing the two methods (of Wilson and of Papanic-

* Phosphate buffered Wright's stain.

olaou), the details, particularly of the nucleus (chromatin structure), are more clearly differentiated by Wilson's stain, although Papanicolaou's method was sometimes more valuable for studying cell outlines. Specimens containing much fat, mucus, or colloid were likewise better stained by Papanicolaou's technic. Wilson's stain, on the other hand, had the advantage of bringing into greater contrast the nucleus (violet) and nucleolus (blue), as well as nucleus (violet) and cytoplasm (light blue to dark gray-blue). With Papanicolaou's method most of the tumor cell cytoplasm was stained green, the nucleus grayish violet. The nucleolus, although readily visible, appeared only as another variation of the same gray-violet; the chromatin was more distorted than when Wilson's stain was used. Both methods have been used to complement each other in almost every case.

Material

In accordance with the questions stated in the introduction, two classes of material were examined: Histologically proved carcinomas, and normal and noncancerous (inflammatory, benign tumor, etc.) tissues from various sites.

A total of 188 cases have been examined; 78 in the first group (carcinoma), and 110 in the second (control) group, noncancerous. From the 188 cases, 268 organs or tissues have been examined; 178 were noncancerous, 90 fell into the carcinoma group. They have been individually classified. For instance, if in a case of carcinoma of the cardia of the stomach, the tumor, the grossly unchanged mucosa of the fundus, and a mesenteric lymph node were examined, each area was separately classified as noncancerous or carcinomatous according to its histologic findings. In the same way two or more organs or tissues from a case without a carcinoma were individually examined and classified.

The noncancerous tissues are listed in Table I, and the carcinomas, according to the organ from which they were taken, in Table II. It must be emphasized that the classification in Tables I and II is based not upon cytologic, but upon histologic, examination. In this manner each organ or tissue was appropriately cataloged as either noncancerous or carcinomatous.

Results

It is not intended to describe in this paper the cytologic features of noncancerous organs or tissues in smear preparations, except in so far as it may be necessary to elucidate the differences between noncancerous processes and carcinoma. On the other hand, it is necessary to describe the cytologic characteristics of those tissues which have been classified histologically as carcinoma.

The over-all cytologic picture of the carcinoma smears shows certain characteristics which occur repeatedly. These characteristics, which may be subdivided, suggest a possible cytologic classification. Five types could be distinguished among the smears of 90 carcinomas: The squamous cell type, the columnar cell type, the round cell type, the undifferentiated cell type, and the oat cell type.

Carcinoma Smears of Squamous Cell Type (Fig. 1)

In carcinoma smears of squamous cell type, squamous cells were present in varying numbers, just as in smears of normal skin or mucous membranes (Fig. 6); most of the cells, however, were atypical, varying in size, shape, and color. Instead of the usual polygonal form with

TABLE I

Tissues Histologically Classified as Noncancerous

Organ (tissue)	Number of specimens examined
Esophagus	2
Stomach (corpus and fundus, 8; pylorus, 2)	10
Duodenum	1
Jejunum	2
Ileum (no lesion, 1; inflammation, 2)	3
Colon	9
Rectum	5
Diverticulosis of intestine	1
Polyp of colon	1
Bronchus	1
Lung (no lesion, 1; lung abscess, 3)	4
Uterus (endometrium, 22; cervix (cervicitis), 26)	48
Ovary	9
Breast (chronic mastitis, 1; granulomatous lesion, 1; cystic hyperplasia, 2; fibrosing adenomatosis, 2; comedo-adenoma, 1; intracanalicular fibro-adenoma, 3; adenofibroma, 1)	11
Testis	2
Benign prostatic hypertrophy	4
Kidney (pyelonephrosis)	1
Bladder	1
Submandibular gland	1
Mixed tumor of parotid	1
Gallbladder (no lesion, 1; cholecystitis, 5)	6
Thyroid (cystic nodular goiter, 1; nontoxic adenoma, 1; lymphadenoid goiter, 1; hyperplasia and hypertrophy, 2; fetal fibro-adenoma, 1; nodular colloid goiter, 1; embryonal adenoma, 1; nodular goiter with fetal adenoma 1; colloid goiter,2; nodular thyroid, 1; nodular goiter with hyperplasia and colloid nodules, 1)	13
Lymph node (no lesion, chronic inflammation, Boeck's sarcoid)	16
Spleen	7
Skin	2
Striated muscle	1
Fibromyoma uteri	3
Sinusitis maxillaris	1
Nonspecific inflammation	6
Epiglottis	1
Lipoma	2
Papilloma of abdominal wall	1
Vagina	1
Giant cell epulis	1
Total	178

a dense, dark, almost pyknotic nucleus and abundant cytoplasm which stains light pink or bluish, the cells often were elongated and stained darker blue, but were still pale; the nucleus sometimes was larger than is normal, and the chromatin might be net-like. These cells were readily recognized as variations from the normal squamous cell. Figures 7 and 8 depict a group of cells which showed a close relationship to the

TABLE II

Neoplasms Histologically Classified as Carcinoma Listed by Location

Location	Number of specimens examined
Lip	3
Mouth	2
Esophagus	1
Stomach	9
Colon	7
Rectum	8
Larynx	1
Lung	4
Mediastinum	1
Uterus (endometrium, 3; cervix uteri, 6; infiltrating from ovary, 1)	10
Ovary (primary, 4; metastatic, 1)	5
Vulva	2
Breast	12
Pancreas	1
Thyroid	1
Kidney	1
Bladder	1
Peritoneum (metastatic)	2
Neck	1
Lymph node (metastatic)	13
Abdominal wall (metastatic)	1
Chest wall	1
Skin	3
Total	90

squamous cells described above. Their cytoplasm was bluish, sometimes blue, almost always well outlined; rarely, one or two borders merged gradually with the background. The size of the cell and its shape were more or less the same as those of the previously mentioned cells; sometimes they were slightly larger. Elongated forms and spindle-like types were more common than in the former. The nucleus, however, was always larger, varying from the size of a large lymphocyte (8 to 10 μ) to 15 μ or more in diameter. When the nucleus was large, it formed the greater portion of the cell, so that the cytoplasm was relatively sparse. The nucleus, as a rule, was well delineated and round, rarely slightly oval. The chromatin formed a regular, medium-sized meshwork. It contained one nucleolus, rarely two or three, each 2 or 3 μ in diameter. Nucleoli were usually round and blue or bluish; exceptionally they were dark blue and slightly irregular. This cell is designated a "nucleolated squamous cell." In some instances cells were

seen which resembled those found in the deeper layers of normal epithelium (basal cells, according to Papanicolaou [3]). They usually appeared in groups; single cells were seen rarely. These cells are henceforth termed "malignant epithelial cells" (Figs. 9 and 10). They varied in size (10 to 20 μ), and were rhomboid or polygonal. The cytoplasm was dark blue and rather sparse; it was not very sharply outlined, but the border was still visible. Sometimes keratin formation, as described by some authors,[26] was seen in the cytoplasm in the form of red granules, small rods, or dust. The main mass of the cell usually was constituted by the nucleus, which was oval to round and usually slightly irregular; large forms predominated. The chromatin was granular, irregular, rather coarse, amorphous, and often was condensed in the form of a ring at the periphery of the nucleus. The nucleoli were multiple (one to four), dark blue, irregular, poorly outlined, and measured 1 to 3 μ or more in diameter. Another cell seen in smears of this type was a giant cell (Fig. 13), which always appeared singly. Exceptionally as many as three or four per low-power field might be seen. The sparse cytoplasm, which stained dark blue, usually was present only at the poles of the nucleus, disappearing gradually without sharp borders at the periphery. Sometimes the cell contained small cytoplasmic vacuoles. The nucleus was large (20 to 40 μ) and usually irregular because of many indentations. It possessed numerous nucleoli, which were blue; but, because of the presence of small granules of dense chromatin which produced a turbid appearance, the nucleoli often were difficult to see. A fibroblast-like cell (Figs. 11 and 12) was frequently seen in these smears. It usually was larger than true fibroblasts. The cytoplasm was darker, often dark gray-blue, poorly outlined and usually fringed at its margin. The nucleus was long, oval, and narrow; the chromatin was net-like, its strands sometimes thick and irregular; the nucleoli were light blue to blue. The term "pseudofibroblast" will be used to identify this cell. These cells varied in size and sometimes showed transitional forms to the above-mentioned giant cells and nucleolated squamous cells; on the other hand, it frequently was difficult to distinguish them from fibroblasts. Such cells have been described by Papanicolaou [3] and others in vaginal smears of carcinoma of the cervix. Comparing the smears with the histologic preparations led to the opinion that they probably are not fibroblasts. It is more probable that these spindle cells are derivatives of epithelium. In connection with this it should be mentioned that in experimental tumor transplantation occasionally a carcinoma will show sarcoma-like changes, that is, the appearance of spindle cells. The explanation (Ewing [59]) is that the spindle cells represent derivatives of epithelium, rather than a change in the character of the tumor (carcinoma to sar-

coma). Besides cells of the four types mentioned above, in carcinoma smears of squamous cell type were found erythrocytes, leukocytes, fibrocytes, and fibroblasts. (For their description see Tischendorf.[39])

One or another cell form, as described above, may predominate in the smear. Basically, however, the smears show a very similar appearance. Smears of this type were found in cases of carcinoma of the lip, mouth, esophagus, larynx, bronchus, cervix uteri, vulva, kidney, skin, and in some metastatic carcinomas in lymph nodes. In one case of cervical "carcinoma *in situ*," only such cells as are illustrated in Figure 10 (malignant epithelial cells) were seen.

Carcinoma Smears of Columnar Cell Type (Fig. 2)

The columnar cell (Figs. 14 and 15) in all its variations characterized this type. In its usual form it measured approximately 6 by 15 μ, possessed a round to oval nucleus, and light blue to blue cytoplasm with well demarcated outlines. Sometimes red, dust-like granules were visible at one end of the cytoplasm. Among these cells were others which had larger nuclei. These sometimes were irregular; their chromatin was arranged in strands of varying thickness. Often small, irregular nucleoli were seen. The cytoplasm of these cells was poorly defined and dark grayish blue. Another cell similar in many respects to those just described was found only in carcinomas (Fig. 16). It, too, was more or less columnar, showed the same variations as the above described cells, with the addition that usually two or three irregular, dark blue nucleoli were present. Where only one nucleolus was found, it was large and round. The nucleoli might occasionally be pale but retained all the other properties described. This cell was named the "malignant columnar cell." The third cell type, seen only in carcinomas, was the "undifferentiated cell" * (Fig. 19) with no cytoplasm or with a little cytoplasm in the form of a narrow, perinuclear, light grayish zone. The nucleus measured 12 to 20 μ in diameter. It usually was round and irregular with net-like chromatin, the strands of which were rather thick, sometimes forming wide meshes. At other times the chromatin was more granular in appearance. The nucleoli were large, pale blue, irregular, and often multiple. Large round cells were seen also in smears of this type (Fig. 17). They usually were round and relatively well delineated. The nucleus usually was about 12 to 18 μ in diameter, round, and predominantly regular. The chromatin did not form strands in most of these cells, but usually was amorphous in appearance and irregularly distributed, producing darker and lighter areas. The nucleoli were round, dark blue, and often masked by the dense chromatin, so that their borders were not clearly visible.

*The term "undifferentiated cell" is one of convenience. Whether it is cytogenetically less differentiated than the other cells is not known.

In other cases in which the chromatin formed an irregular, coarse net, the nucleoli were seen readily and were round, large (3 to 8 μ), and blue. The cytoplasm was bluish or blue and well preserved in many of these cells; in some, however, its margins faded gradually into the background. These cells, examined under low power, frequently resembled plasma cells, at first glance. The large, round cells might achieve considerable size so that they formed giant cells (Fig. 18) comparable in magnitude to those seen in the squamous cell smear. However, the cytoplasm of these cells, in contrast to that of the giant cells of the squamous cell smear, surrounded the nucleus and was not localized largely at the poles. This appearance suggested that the cells were derived from the large round cells. Giant cells, as illustrated in Figure 13 and described in connection with smears of the squamous cell type, were seen also in the smears of the columnar type. The same was true of the pseudofibroblast (Fig. 12). Both of these forms were rare in smears of this type, as compared to those of the squamous cell type. In addition to the above-mentioned cells, leukocytes, erythrocytes, fibroblasts, fibrocytes, and macrophages were present.

Carcinoma smears of the columnar cell type have been found in carcinomas of the stomach, colon, rectum, and ovary, and in some metastatic carcinomas of lymph nodes.

Carcinoma Smears of Round Cell Type (Fig. 3)

In some cases classified as carcinoma of round cell type, large, round cells (Figs. 17 and 18) composed the entire smear; in other cases some columnar cells (Fig. 16) were present. The impression, in the latter event, might be that of a smear of the columnar type with predominance of large, round cells. In other instances no columnar cells could be seen. In addition, a few undifferentiated cells might be visible.

Smears of this type have been seen chiefly from carcinomas of the cardia of the stomach and in some carcinomas of the breast, lung, pancreas, bladder, and uterus. This type was seen twice in smears of metastatic carcinoma.

Carcinoma Smears of Undifferentiated Cell Type (Fig. 4)

What has been said of smears of the round cell type may also be repeated in a description of the undifferentiated cell type, with the difference that the predominating cell was that previously described as the "undifferentiated cell" (Fig. 19). Smears which revealed such a picture have been obtained from carcinomas of the stomach, colon, rectum, ovary, and breast.

Carcinoma Smears of Oat Cell Type (Fig. 5)

The word "oat" is used for this type because the characterizing cells resembled those found in the sputum of patients with carcinoma of the respiratory tract and described as oat cells. Two forms of small cells were seen in this type. One represented cells which could not be differentiated from those of normal organs or tissues. The nucleus was the size of that of a small to large lymphocyte with little variation in size and shape; it was round to oval. The chromatin usually was dense, sometimes granular and irregularly distributed. Nucleoli were very rare. No cytoplasm was present, or, if present, it appeared only as a narrow bluish zone about the nucleus. The cells usually appeared in large sheets and might be compared to the type which Rohr and Hegglin [29] have designated "kleinzelliger Carcinom Typ" in bone marrow. There appears to be justification for their opinion that a diagnosis may not be made on the basis of a single cell, but only from cells which occur in the form of sheets. Whether the latter is true in every case is, in my opinion, open to question, because in some smears of chronically inflamed spleens and lymph nodes similar cells have been found, often in small groups.

The second small cell is that described in the literature [16] as the oat cell of bronchial carcinoma. It was similar to the first small cell, except that it possessed a definite but narrow rim of blue cytoplasm. The cytoplasm formed a short bipolar process. This cell likewise appeared frequently in sheets, but might sometimes be recognized when isolated. Finally it should be mentioned that, just as in the smears of the first two types, other cells were present: erythrocytes, leukocytes, fibroblasts, fibrocytes, and macrophages.

Smears of this type were seen in one case of carcinoma of the thyroid, in a case of pulmonary carcinoma, and in almost half of the proved metastatic carcinomas in lymph nodes.

Table III presents the different types of smears and the histologic classification of the carcinomas from which the smears were made.

Discussion

1. *What are the cytologic characteristics of carcinomas studied by means of the smear technic? Is it possible to distinguish between histologically recognized carcinoma and noncancerous tissue using the cytologic method of study?*

The most widespread and accepted method of cancer diagnosis—histologic study of tissue—is based upon the recognition of several changes in structure, many of which are cytologic: increase in the number of cells, marked variation in their size and shape differing from

the original cell type, increase of the chromatin mass, large nucleoli, multiple and abnormal mitotic figures, loss of polarity, and infiltration of the surrounding tissue (Aschoff,[60] Ewing,[59] MacCallum [56]). All may be studied by cytologic methods except the last, which is often considered the most important. Since cytologic technics preserve the components of a cell in greater detail than the methods of histology, vari-

TABLE III

Types of Carcinoma Smears with Corresponding Histologic Classification

Type of smear	Histologic classification
Squamous cell type, 29	Squamous cell carcinoma (lip, 3; mouth, 2; esophagus, 1; larynx, 1; lung, 1; cervix uteri, 4; vulva, 2; lymph nodes, 5; skin, 2) Carcinoma (bronchogenic, 2; kidney, 1; neck, 1; cervix uteri (*in situ*), 1) Tissue with degenerating neoplastic(?) cells (skin, 1) Benign* (chronic cervicitis, 1; cervix with no lesion, 1)
Columnar cell type, 22	Adenocarcinoma (stomach, 4; colon, 5; rectum, 6; endometrium, 1; uterus, 1; ovary, 2; lymph node, 2) Malignant adenoma (uterus, 1)
Round cell type, 13	Adenocarcinoma (stomach, 3; cervix uteri, 1; endometrium, 1; lymph node, 1) Carcinoma (lung, 1; pancreas, 1; bladder, 1; mediastinum, 1; duct cell of breast, 1; canalicular of breast, 1; medullary of breast, 1)
Undifferentiated cell type, 14	Adenocarcinoma (stomach, 2; rectum, 1; colon, 1; peritoneum, 2; abdominal wall, 1; ovary, 3) Carcinoma (rectum, 1; chest wall, 1; scirrhous of breast, 1; medullary of breast, 1)
Oat cell type, 9	Adenocarcinoma (thyroid, 1) Carcinoma (colon, 1; lymph node, 5) Benign* (nodular hyperplasia and fibrosing adenomatosis of breast, 1; cystic hyperplasia of breast, 1)
No finding, 4	Carcinoma (canalicular of breast, 2; scirrhous of breast, 2)

*These cases will receive further comment.

ous cytologists from time to time have selected one or more of the above characteristics as their criteria for cancerous changes.

The opinion popular among older authors (literature by Quensel [7]) was that cancerous cells are larger than normal cells, and that the ratio of nucleus to cytoplasm is changed in favor of the former. This observation also has been made more recently,[3,38] but the change is considered suggestive, rather than characteristic, of malignancy. My smears showed that the majority of carcinomas do have larger cells than those of the tissue from which they arose. It appeared impossible, however, to make a diagnosis of malignancy on the basis of the size of a cell or the ratio of nucleus to cytoplasm.

Quensel [4,6,7] studied malignant cells in body fluids using the method of supravital staining. He stated that the nucleolus is of diagnostic importance. In his cases of carcinoma the nucleoli varied in diameter from 3 to 10 μ; exceptionally they were smaller, 1.5 to 2 μ. In contrast, the nucleoli of endothelial cells (control) measured 1 to 1.5 μ, very occasionally 2 to 3 μ. He showed that the size of the nucleolus in carcinoma is independent of that of its nucleus. This is indicated by the ratio, nucleolus:nucleus, which is 0.20 to 0.60 in carcinoma, as compared to 0.14 to 0.20 or less in endothelial or other cells. Further characteristics of malignancy, which Quensel considered less important, are giant vacuoles (larger than 40 μ) and irregular sheet formation with partial or total overlapping of nuclei, as compared to other cells in body fluids in which the vacuoles are smaller and the cells form "plaques." The change in the size of nucleoli was not found in sarcomas. Quensel's results were confirmed by Zadek and Karp [61] and Zadek,[62,63] who studied tumors of pleura, peritoneum, lung, and urogenital tract. MacCarty and his co-workers,[2,8-10,13] using a different method, arrived at similar results showing that the ratio of the nucleolar to nuclear surface, estimated as an average from a large number of cells (25 to 100), was always greater in malignant processes than in any other lesions of a particular organ (tissue). This ratio varied from 1:5 to 1:17 in malignancy and from 1:13 to 1:45 in control cases; they considered this ratio to be of diagnostic value. Rohr and Hegglin [29] showed that in the bone marrow only a certain number of carcinomas possess the cell characteristics which Quensel ascribed to them. Other carcinoma cells may have small nucleoli or even none. Thus, while it may be that a large nucleolus or a certain ratio of the nuclear diameter (surface) to that of the nucleolus may be characteristic of carcinoma cells in body fluids, it is not necessarily true for carcinoma cells in general. Similar observations have been made on my material. Quensel and MacCarty's observations seem applicable to a large number of my carcinomas (stomach, breast, and endometrium). Many others, however (lung, thyroid, and some of the breast carcinomas) did not fit into this scheme. The cells of metastatic tumors often had only small nucleoli or none.

Irregularity of cell shape and size, changes in the structure of chromatin, and atypical staining reactions of cytoplasm have been stressed by almost every author who has studied malignant tumors cytologically, either directly from the tumor or in excretions and body fluids. These changes have not been considered diagnostic, but only suggestive of malignancy. Similar changes can be seen in inflammatory lesions as well as in benign tumors, usually, however, to a lesser extent. The

same opinion may be expressed concerning irregular sheet formation with overlapping of nuclei. While this phenomenon is common in carcinoma, it can be seen also in other processes (inflammation). In summary, therefore, it may be said that many cellular changes suggest malignancy but are neither sufficiently constant nor sufficiently prominent to establish their diagnostic significance. When seen in a smear, they should, however, awaken the suspicion of the observer.

These suggestive changes as seen in my material are summarized in Table IV. As may be seen in this table, which is based on the study of smears from 90 carcinomas, there were certain cellular changes suggestive of a process which may not be benign. The size of the individual *cell* in carcinoma smears was, in general, larger than that in noncancerous processes; this was especially noticeable when the cells were compared with those of the tissue from which the carcinoma arose. Variation in size and shape might often be very marked, although some tumors showed a relatively uniform cell picture (instances of carcinoma of bronchus, carcinoma of thyroid). The cells were individually disposed or were present in sheets. When in the latter form, the nuclei were partially superimposed and polarity was lost to a greater or lesser degree, as compared with noncancerous processes, in which the "plaques" consisted of a single layer of cells, the nuclei of which were regularly arranged. There were, however, noncancerous processes which might show the same phenomena as carcinoma, but to a lesser extent. The adhesion of cells to form groups of various sizes and shapes was much more commonly seen in noncancerous epithelium than in carcinomas, in which most of the cells usually lay separated from each other.

The *cytoplasm* of the cells in carcinoma smears was either scanty or poorly outlined, merging gradually with the background. Few of the carcinomas possessed cells which were relatively rich in cytoplasm (nucleolated squamous cells, Fig. 7; some of the malignant columnar cells, Fig. 16). The cytoplasm varied from light grayish pink to blue and often dark gray-blue. Noncancerous cells usually were light blue to blue and, in addition, had more abundant and better outlined cytoplasm.

The same morphologic variations which were described in carcinoma cells, in general, can be said to characterize the *nucleus*. It was usually larger than that of the control group and sometimes attained giant dimensions (Figs. 13 and 18). It was round to slightly oval, often irregular, in contrast to the nucleus of noncancerous tissue, which was more regular. Anisonucleosis and poikilonucleosis were almost always present in the cytologic picture of carcinoma, rarely in the control

TABLE IV

Changes Suggestive of Malignancy Seen in the Smears of 90 Carcinomas as Compared with Those of 178 Control Tissues

	Findings based on examination of single cells		Findings based on examination of groups of cells	
	Noncancerous tissue	Carcinoma	Noncancerous tissue	Carcinoma
Cell	Regular in size and form; color light gray to blue	Irregular (anisocytosis and poikilocytosis); larger than the original tissue cell; color often dark gray-blue	Uniform appearance; in rows or "plaques"	Anisocytosis, poikilocytosis; in clumps and large sheets
Cytoplasm	Usually well outlined; moderate to abundant in amount	Poorly outlined; rarely well limited; often nuclei without cytoplasm; commonly little cytoplasm		
Nucleus	Round, oval, kidney-shaped; usually less than 15 μ in diameter	Round or oval, often irregular; usually 15 μ or more	Nuclei show regular arrangement	Nuclei cover each other and show loss of polarity; anisonucleosis and poikilonucleosis
Chromatin	Dense, clumped, or net-like, but regular	Irregular, coarse, net-like; irregular, granular; dusty; chromatin often forms a clearly visible ring at the border of the nucleus		
Nucleolus	None, or small to medium size, regular in shape; light blue or violet; rarely dark	Medium size to large or giant, often irregular; light blue to dark grayish blue		
Ratio of cytoplasm: nucleus	In favor of cytoplasm	Usually in favor of nucleus		
Ratio of nucleolus: nucleus		High		

group. When, therefore, a high degree of irregularity was present in a noncancerous process and a low degree in a carcinoma, they were indistinguishable on this basis.

The *chromatin* in the control group was dense and clumped; if net-like, it was regular and delicate. In carcinomas the chromatin was irregular, was composed of coarse strands, and formed a net-like structure. This was sometimes small-meshed, and at other times showed large, irregular meshes; both forms might be present in the same smear. In still other cases the chromatin formed a grid of coarse strands or consisted of irregular, coarse granules; it might have a dense, irregular, amorphous appearance. At times the chromatin was condensed in the form of a ring at the periphery of the nucleus.

The *nucleolus,* considered by many authors to be one of the most suggestive or even diagnostic indices of malignancy, particularly in carcinoma, was not visible in the majority of the cells of the control group. When present, it usually was not larger than 1 to 2 μ in diameter. It was regular and stained light blue or the same color as the chromatin. Exceptions, however, were found: in some of my specimens, identified histologically as progestational endometrium, the nucleoli were large and sometimes stained dark blue; the same was noted in some examples of atrophic endometrium, in tubular epithelium of a case of chronic pyelonephritis, and in the mucosal cells of a bladder with carcinoma. The smear from the last mentioned was obtained from an area reported histologically as showing "no lesion." The nucleolus of carcinoma cells assumed very peculiar forms (Text-Fig. 1). It might

Text-Figure 1. Appearance of nucleoli in malignant cells.

attain giant proportions up to the size of a small lymphocyte (6 μ) and occupy most of the nuclear mass (c). Nucleoli might be multiple, and were then usually irregular (a, d). The color might be light blue (a, b, c), as seen in noncancerous material, or dark grayish blue (d, e, f). Sometimes the outlines were indistinct and the nucleolar substance merged into the surrounding chromatin (f). At other times the nucleoli might be partially hidden in the mass of chromatin (giant cells, large round cells).

The *nucleus-cytoplasm ratio,* in my smear material, has often aroused the suspicion that carcinoma might be present, namely, when the mass of the nucleus was obviously larger in proportion to cytoplasmic mass than in the control cells. However, as already indicated, this cannot be considered a rule. The same may be said of the ratio of the nucleolar surface and/or diameter to that of the *nucleus,* which in carcinoma is usually much greater than in the control group. Zadek [61,62] stated that the nucleolus-nucleus ratio is 1:4 to 1:20 in carcinoma cells in body fluids, compared to 1:25 to 1:100 in endothelial cells. MacCarty claimed that by his method the ratio is 1:5 to 1:17 in malignant processes, as against 1:13 to 1:45 in noncancerous processes. Since my method (smear) is different from that of Zadek (supravital stain) and of MacCarty (sections of fresh tissue), it is impossible to confirm or deny their conclusions.

Many of the changes mentioned above are seen also in histologic sections and are constantly utilized by pathologists. The smear technic permits better preservation of cytologic details and therefore more exact observation of minute structures. It must again be stressed that all of the features described above are suggestive, rather than diagnostic, of carcinoma. Extreme variations, however, may eventually prove to be diagnostic.

2. *Is there a cell, or are there cells, which characterize carcinomas, and are they always absent in noncancerous conditions?*

If malignant tumors express their abnormality not only in tissue structure (irregularity, infiltration), but also in cell structure, there arise two possibilities of recognizing cancer by its isolated cells: All of the cells of a tumor may have morphologic characteristics indicative of malignancy; during the development of a malignant tumor some of the cells may possess or acquire morphologic peculiarities as a consequence of abnormal function. It is these "peculiar" cells which would appear to be of importance in the recognition of cancer.

Smears of five different types, as previously described, were found in my material of 90 carcinomas, which were obtained from various organs and parts of the body and represented diverse histologic categories (adenocarcinoma, squamous cell carcinoma, comedo-carcinoma). A number of atypical cells were seen which were not found in smears of the control group,* consisting of 178 specimens from normal organs, inflamed tissues, and benign tumors. The atypical cells, listed below, were constantly present in the carcinoma smears of appropriate type:

(a) The nucleolated squamous cell (Figs. 7 and 8)

* Four exceptions to this statement are described in succeeding paragraphs.

(b) The malignant giant cell (Fig. 13)
(c) The malignant epithelial cell resembling basal cells (Figs. 9 and 10)
(d) The pseudofibroblast (Figs. 11 and 12)
(e) The malignant columnar cell (Fig. 16)
(f) The undifferentiated cell (Fig. 19)
(g) The large round cell (Figs. 17 and 18)
(h) The oat cell (Fig. 5), only if in large sheets

These cells have been described above; the names have been selected on the basis of the most impressive feature of each. Cell *d* (pseudofibroblast) is not always distinguishable from fibroblasts; cell *h* (oat cell) is usually recognizable only when in sheets.

In some of the types of smears one of the above listed cells may predominate, or may be so numerous as to give the impression of being the only form present. This is usually the case in smears of the undifferentiated and round cell types. In other smears more than one kind of atypical cell was seen, as in the squamous cell type (nucleolated squamous cell, pseudofibroblast, giant cell), and in the columnar cell type (malignant columnar cell, large round cell, undifferentiated cell). Besides these atypical cells, in the majority of smears the proper cells of the organ in which the carcinoma was situated were seen also. In many cases it seemed possible to trace transitional forms from the atypical cells seen only in carcinoma smears to the epithelial cells of the control group. It should again be stressed that the atypical forms (*a* to *h*) are not considered to be the only malignant cells, since in smears of histologically proved metastases cells were seen which could not be distinguished from those found in inflammatory or other noncancerous processes. Another example of this observation is the smear of the oat cell type, in which often no cells were present which could not be found in the control group, and only the abnormal location and arrangement of the cells made probable the diagnosis of carcinoma. The assumption that a pathologic process may manifest itself in function only, without morphologic expression, would explain this finding.

Because previous workers [2,4,61] frequently have sought a common denominator among the structures of cells obtained from malignant tumors, an attempt was made to find such factors in atypical cells (*a* to *h*) (size of nucleolus, giant vacuoles, ratio of nucleolus to nucleus). None had general application. The cells (*a* to *h*) as units differ from the cells of the control group, but not in their parts. The possibility may not be abandoned, however, that in some tumors one detail of the cell may be sufficiently constant and characteristic to

establish its diagnostic value. Quensel [4] and others [2,61] made such claims for the nucleolus of carcinoma cells in body fluids. The general application of such a rule does not seem to me to be justified.

For the 188 cases examined, there was agreement between the histologic diagnosis and the conclusion reached from the examination of smears in 179 cases. In the remaining 9 cases the conclusion reached from smears was apparently falsely positive in 5 instances and falsely negative in 4. These 9 cases are summarized in the following paragraphs.

A. Apparently False Positives

1. Case 33 was a 35-year-old female considered clinically to have a carcinoma (stage I) of the cervix. Histologic examination did not confirm the diagnosis, but showed a chronic cervicitis. The smear revealed a squamous cell carcinomatous type.

2. Case 37 was a 34-year-old female with a clinical diagnosis of menorrhagia. Sections removed from the corpus uteri showed progestational endometrium; from the cervix, no lesions. Smears of the cervix revealed some nucleolated squamous cells with a more grayish violet cytoplasm than is usually seen in this cell form. No other cells of types *a* to *h* were seen.

3. Case 172 was a 66-year-old male with a clinical diagnosis of carcinoma of the face (temporal area). The pathologist reported: "Here and there in the dermis are isolated atypical cells with hyperchromatic nuclei that possibly are degenerating neoplastic elements, but of this we cannot be certain." The smear showed cells of characteristic squamous cell carcinoma type.

4. Case 185 was a 26-year-old female with a questionable clinical diagnosis of carcinoma of the breast. Sections taken for biopsy showed cystic hyperplasia of the breast; the smear showed the oat cell type.

5. Case 188 was a 20-year-old female with the clinical diagnosis of questionable benign tumor of the breast. Sections taken for biopsy showed lobular hyperplasia and fibrosing adenomatosis of the breast. The smears showed again the oat cell type and some giant cells, as illustrated in Figure 13.

B. Apparently False Negatives

1. Case 78 was a 46-year-old female. The clinical diagnosis was carcinoma of the breast; the histologic diagnosis was canalicular carcinoma of breast. The smear made from tissue in the neighborhood of the removed tumor could not be evaluated because of changes due to the antiseptic agent used in that area. Another smear taken from the periphery of the breast was negative for cells *a* to *h*.

2. Case 103 was a 45-year-old female with the questionable clinical diagnosis of carcinoma of the breast. The sections showed comedo-carcinoma of breast. The smear taken from the neighborhood of the tumor was negative for cells *a* to *h*.

3. Case 127 was a 43-year-old female. The clinical diagnosis was breast tumor; the histologic diagnosis, scirrhous carcinoma. The smear was negative.

4. Case 215 was a 57-year-old female. The clinical diagnosis was carcinoma of the breast; the histologic diagnosis, scirrhous carcinoma. The smear taken from the depth of the tumor showed only different forms of epithelial and connective tissue cells.

In the group of apparently false positive diagnoses, cases 33 and 172 presented the characteristic squamous cell type, despite the absence of carcinoma in the histologic preparations. I cannot explain this dis-

crepancy. Some investigators [3,53] using Papanicolaou's method on vaginal smears have noted false positives similar to mine. The correctness of one or the other finding can be determined only by observation of the patient's future course. Cases 37, 185, and 188 showed only a few cells of types *a* to *h*.

In the second group of apparently false negative diagnoses, the failure to detect tumor cells in the smears from cases 78 and 103 is not significant, since the tissue was taken from the neighborhood of the tumor rather than from the tumor itself. Although the possibility of failure in these cases was realized, the smear was taken nevertheless, since in some cases tumor cells were detected far from the neoplastic center and from locations which the pathologist reported as containing "no lesion" (Fig. 20). Cases 127 and 215 must be considered failures. It is of interest that most instances of disagreement between histologic diagnosis and cytologic findings occurred in breast tissue (6 of 9). A possible reason, at least for the negative cytologic findings, is the large amount of fat present, which makes it more difficult to obtain satisfactory smears.

In summary, specimens from 268 regions were examined from the 188 cases. In 9 instances the cytologic and histologic findings were in apparent disagreement. One or another form of atypical cell (*a* to *h*) was found in the smears of 86 of 90 histologically recognized carcinomas. Usually more than one cell form appeared in the individual smear. Similar atypical cells have been found also in 4 cases classified histologically as noncancerous. In the smears of 174 of 178 specimens histologically classified as noncancerous (no lesion, inflammation, benign tumor) the search for cells *a* to *h* gave negative results.

3. *Is there any relationship between the organ from which a tumor arises and the type of carcinoma cells? That is, do the cells of a carcinoma of the breast differ from those of a carcinoma of the stomach or the skin?*

This material indicates that the organ within which a carcinoma arises exerts little influence upon the type of smear from that carcinoma. However, as has already been shown (Table III), smears of certain types are seen more frequently in certain organs than in others. This is probably dependent upon the type of epithelium which exists in that particular organ. Thus, the squamous cell type is met commonly in neoplasms of organs which normally possess squamous epithelium; the columnar cell type predominates in neoplasms of the mucosa of the gastro-intestinal tract. This is shown in Table V.

4. *Is there any relationship between the structure of a given carcinoma (adenocarcinoma, squamous cell carcinoma) and its cytologic*

character in smears? Are the cells which make possible the cytologic recognition of carcinoma the same in an adenocarcinoma as in a squamous cell carcinoma?

Table VI shows the relationship between the histologic structure of carcinomas and the types of smears obtained from them.

As may be seen, the type of the smear is related to the histologic

TABLE V

Relationship Between Site of Carcinoma and Type of Smear

Organ	Type of smear					No finding	Total
	Squamous cell	Columnar cell	Round cell	Undifferentiated cell	Oat cell		
Lip	3						3
Mouth	2						2
Esophagus	1						1
Stomach		4	3	2			9
Colon		5		1	1		7
Rectum		6		2			8
Larynx	1						1
Lung	3		1				4
Mediastinum			1				1
Uterus	5(2)*	3	2				10(2)
Ovary		2		3			5
Vulva	2						2
Breast			4	4	(2)	4	12(2)
Pancreas			1				1
Thyroid					1		1
Kidney	1						1
Bladder			1				1
Peritoneum				2			2
Neck	1						1
Lymph node	5	2	1		5		13
Abdominal wall				1			1
Chest wall				1			1
Skin	3						3
Total	27(2)	22	14	16	7(2)	4	90(4)

*Numbers in parentheses indicate negative histologic findings.

classification. All of the squamous cell carcinomas yielded smears of squamous cell type. The adenocarcinomas, however, are distributed among the columnar, round, undifferentiated, and oat cell types with predominance of the first three. The group classified histologically only as "carcinoma" produced smears of different types, since it embraced cases of carcinoma whose histologic appearance did not permit a more detailed classification. The special forms of breast tumors (duct carcinoma and canalicular carcinoma) fall into the round cell and undifferentiated types.

5. *Is the cytologic picture (type of smear) of a metastatic tumor the same as that of the primary tumor?*

It may be seen (Table VII) that of 17 examples of metastatic carci-

noma, 6 retained the original pattern in the metastases, 5 changed into the oat cell type of smear, whereas for the remaining 6 no conclusions could be drawn because the primary carcinoma was not available for study. As may be noted, when a change occurred in the metastases, it was always to the oat cell type, regardless of the type represented by the primary tumor. This change was seen only in metastases in lymph nodes. The results, although too few in number to justify conclusions,

TABLE VI

Relationship Between Histologic Diagnosis and Type of Smear

Histologic diagnosis	Type of smear					No finding	Total
	Squamous cell	Columnar cell	Round cell	Undifferentiated cell	Oat cell		
Squamous cell carcinoma	20						20
Adenocarcinoma		21	7	10	3		41
Adenoma, malignant		1					1
Carcinoma (without classification)	7		4	4	4		19
Duct cell carcinoma			1				1
Canalicular carcinoma			1			2	3
Medullary carcinoma			1	1			2
Scirrhous carcinoma				1		2	3
Benign	(2)*				(2)		(4)
Total	27(2)	22	14	16	7(2)	4	90(4)

*Numbers in parentheses indicate negative histologic findings.

TABLE VII

Comparison of Cytologic Findings in Primary Carcinomas and Their Metastases

Organ	Primary tumor		Metastases	
	Histologic diagnosis	Type of smear	Organ	Type of smear
Mouth	Squamous cell carcinoma		Lymph node	Squamous
Stomach	Adenocarcinoma	Undifferentiated	Lymph node	Oat cell
Stomach	Adenocarcinoma	Columnar	Lymph node	Columnar
Stomach	Adenocarcinoma	Round	Mediastinum	Round
Colon	Adenocarcinoma	Columnar	Lymph node	Columnar
Colon	Adenocarcinoma		Ovary	Columnar
Rectum	Adenocarcinoma	Columnar	Lymph node	Oat cell
Bronchus	Carcinoma	Squamous	Lymph node (1)	Oat cell
Bronchus	Carcinoma	Squamous	Lymph node (2)	Oat cell
Lung	Carcinoma	Round	Lymph node	Round
Ovary	Adenocarcinoma		Uterus	Columnar
Ovary	Adenocarcinoma	Undifferentiated	Peritoneum	Undifferentiated
Breast	Carcinoma	Undifferentiated	Lymph node	Oat cell
Skin	Carcinoma		Lymph node	Squamous
Neck	Squamous	Squamous	Lymph node	Squamous
Scalp	Squamous		Lymph node	Squamous
			Lymph node	Squamous

have been discussed because of the relative lack of attention given to this finding in the literature (Naidu [13]).

Conclusions

Ninety carcinomas were studied cytologically, utilizing both Wilson's and Papanicolaou's stains. The smears were made directly from the tumors and were compared to similar preparations from 178 noncancerous tissues.

Eight cell types were recognized as characteristic of carcinoma and were found in 86 of the 90 histologically proved cancers. Similar cells were found in only 5 of the 178 noncancerous controls.

A larger number of cases must be studied before the validity of other cytologic expressions of carcinoma may be considered as proved.

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References

1. Dudgeon, L. S., and Barrett, N. R. The examination of fresh tissues by the wet film method. *Brit. J. Surg.*, 1934-35, **22**, 4–22.
2. MacCarty, W. C. Identification of the cancer cell. *J. A. M. A.*, 1936, **107**, 844–845.
3. Papanicolaou, G. N., and Traut, H. F. Diagnosis of Uterine Cancer by the Vaginal Smear. The Commonwealth Fund, New York, 1943, 46 pp.
4. Quensel, U. Zytologische Untersuchungen von Ergüssen der Brust- und Bauchhöhlen mit besonderer Berücksichtigung der karzinomatösen Exsudate. *Acta med. Scandinav.*, 1928, **68**, 458–501.
5. Stenius, F. Zur Bewertung des epithelialen Harnsedimentes für die Diagnose der Blasengeschwülste. *Acta chir. Scandinav.*, 1924, **58**, 240–255.
6. Quensel, U. Zur Frage der Zytodiagnostik der Ergüsse seröser Höhlen. *Acta med. Scandinav.*, 1928, **68**, 427–457.
7. Quensel, U. Zytologische Untersuchungen von Ergüssen der Brust- und Bauchhöhlen mit besonderer Berücksichtigung der karzinomatösen Exsudate. *Acta med. Scandinav.*, 1928, suppl. **23**, 1–107.
8. MacCarty, W. C. The malignant cell. *J. Cancer Research*, 1929, **13**, 167–172.
9. MacCarty, W. C., and Haumeder, E. Has the cancer cell any differential characteristics? *Am. J. Cancer*, 1934, **20**, 403–407.
10. MacCarty, W. C. Further observations on the macronucleolus of cancer. *Am. J. Cancer*, 1937, **31**, 104–106.
11. von Haam, E., and Alexander, H. G. Cytological studies of malignant tumors. *Am. J. Clin. Path.*, 1936, **6**, 394–414.
12. Mulholland, S. W. A study of prostatic secretion and its relation to malignancy. *Proc. Staff Meet., Mayo Clin.*, 1931, **6**, 733–735.
13. Naidu, V. R. The value of enlarged nucleoli in the diagnosis of malignancy. *Proc. Staff Meet., Mayo Clin.*, 1935, **10**, 356–362.
14. Strohl, E. L. Cytologic study of a suprarenal tumor. *Proc. Staff Meet., Mayo Clin.*, 1935, **10**, 353–355.
15. Dudgeon, L. S., and Patrick, C. V. A new method for the rapid microscopical diagnosis of tumours. *Brit. J. Surg.*, 1927, **15**, 250–261.
16. Dudgeon, L. S., and Wrigley, C. H. On the demonstration of particles of malignant growth in the sputum by means of the wet-film method. *J. Laryng. & Otol.*, 1935, **50**, 752–763.

17. Bamforth, J. The examination of the sputum and pleural fluid in the diagnosis of malignant disease of the lung. *Thorax*, 1946, **1**, 118–127.
18. Barrett, N. R. Examination of the sputum for malignant cells and particles of malignant growth. *J. Thoracic Surg.*, 1938-39, **8**, 169–183.
19. Gowar, F. J. S. Carcinoma of the lung; the value of sputum examination in diagnosis. *Brit. J. Surg.*, 1942-43, **30**, 193–200.
20. Coley, B. L., Sharp, G. S., and Ellis, E. B. Diagnosis of bone tumors by aspiration. *Am. J. Surg.*, 1931, **13**, 215–224.
21. Ferguson, R. S. Prostatic neoplasms; their diagnosis by needle puncture and aspiration. *Am. J. Surg.*, 1930, **9**, 507–511.
22. Martin, H. E., and Ellis, E. B. Biopsy by needle puncture and aspiration. *Ann. Surg.*, 1930, **92**, 169–181.
23. Martin, H. E., and Ellis, E. B. Aspiration biopsy. *Surg., Gynec. & Obst.*, 1934, **59**, 578–589.
24. Sharp, G. S. The diagnosis of primary carcinoma of the lung by aspiration. *Am. J. Cancer*, 1931, **15**, 863–870.
25. Stewart, F. W. The diagnosis of tumors by aspiration. *Am. J. Path.*, 1933, **9**, 801–812.
26. Tischendorf, W. Cytologische Tumordiagnostik an Organpunktaten. *Klin. Wchnschr.*, 1941, **20**, 398–401.
27. Fleischhacker, H., and Klima, R. Die diagnostische Bedeutung der Sternalpunktion bei Morbus Gaucher und bei Knochenmarksmetastasen. *München. med. Wchnschr.*, 1936, **83**, 2051–2055.
28. Markoff, N. Zur Frage der Knochenmetastasierung bösartiger Geschwülste. Ergebnisse der Sternalpunktion. *Deutsches Arch. f. klin. Med.*, 1938, **182**, 47–56.
29. Rohr, K., and Hegglin, R. Tumorzellen im Sternalpunktat. *Deutsches Arch. f. klin. Med.*, 1936, **179**, 61–79.
30. Rohr, K., and Hafter, E. Untersuchungen über postmortale Veränderungen des menschlichen Knochenmarks. *Folia haemat.*, 1937, **58**, 38–50.
31. Rohr, K. Das menschliche Knochenmark. G. Thieme, Leipzig, 1940, 286 pp.
32. Forkner, C. E. Material from lymph nodes of man, I. *Arch. Int. Med.*, 1927, **40**, 532–537.
33. Forkner, C. E. Material from lymph nodes of man, II. *Arch. Int. Med.*, 1927, **40**, 647–660.
34. Guthrie, C. G. Gland puncture as a diagnostic measure. *Bull. Johns Hopkins Hosp.*, 1921, **32**, 266–269.
35. Leitner, S. J. Die diagnostische Verwertbarkeit der Lymphdrüsenpunktion bei entzündlichen Lymphknotenaffektionen. *Acta med. Scandinav.*, 1940, **105**, 558–577.
36. Stahel, R. Diagnostische Drüsenpunktion. G. Thieme, Leipzig, 1939, 66 pp.
37. Tischendorf, W. Über die Verwertbarkeit von Lymphknotenpunktaten zur Differentialdiagnose der Lymphdrüsenerkrankungen. *Deutsches Arch. f. klin. Med.*, 1938-39, **183**, 448–466.
38. Tischendorf, W., and Frank, A. Tumorzellen im Knochenmark-, Lymphknoten- und Organpunktat. *Deutsches Arch. f. klin. Med.*, 1940, **186**, 534–547.
39. Tischendorf, W. Morphologisch-klinische Beobachtungen bei Erkrankungen des lymphatischen Gewebes. G. Thieme, Leipzig, 1942, 83 pp.
40. Moeschlin, S. Die Milzpunktion. B. Schwabe, Basel, 1947, 205 pp.
41. Papanicolaou, G. N., and Marshall, V. F. Urine sediment smears as a diagnostic procedure in cancers of the urinary tract. *Science*, 1945, **101**, 519–520.
42. Papanicolaou, G. N. Diagnostic value of exfoliated cells from cancerous tissues. *J. A. M. A.*, 1946, **131**, 372–378.

43. Ayre, J. E. A simple office test for uterine cancer diagnosis. *Canad. M. A. J.*, 1944, 51, 17–22.
44. Ayre, J. E., Bauld, W. A. G., and Kearns, P. J. Test case to show value of cervical cytology smear in uterine cancer diagnosis. *Am. J. Obst. & Gynec.*, 1945, 50, 102–105.
45. Ayre, J. E. Vaginal cell examination as a routine in diagnosis. *South M. J.*, 1946, 39, 847–852.
46. Daut, R. V., and McDonald, J. R. Diagnosis of malignant lesions of the urinary tract by means of microscopic examination of centrifuged urinary sediment. *Proc. Staff Meet., Mayo Clin.*, 1947, **22**, 382–386.
47. Fremont-Smith, M., Graham, R. M., and Meigs, J. V. Vaginal smears as an aid in the diagnosis of early carcinoma of the cervix. *New England J. Med.*, 1947, 237, 302–304.
48. Herbut, P. A., and Clerf, L. H. Bronchogenic carcinoma. *J. A. M. A.*, 1946, 130, 1006–1012.
49. Herbut, P. A., and Lubin, E. N. Cancer cells in prostatic secretion. *J. Urol.*, 1947, 57, 542–551.
50. Jones, C. A., Neustaedter, T., and MacKenzie, L. L. The value of vaginal smears in the diagnosis of early malignancy. *Am. J. Obst. & Gynec.*, 1945, 49, 159–168.
51. Ludden, T. E., and McDonald, J. R. Diagnosis of tumors of the kidney by cytologic investigation of urinary sediment. *Proc. Staff Meet., Mayo Clin.*, 1947, **22**, 386–390.
52. Meigs, J. V. The vaginal smear; practical applications in the diagnosis of cancer of the uterus. *J. A. M. A.*, 1947, 133, 75–78.
53. Seibels, R. E. Carcinoma of the cervix primarily diagnosed by the stained vaginal spread. *Am. J. Obst. & Gynec.*, 1947, 54, 343–346.
54. Woolner, L. B., and McDonald, J. R. Bronchogenic carcinoma; diagnosis by microscopic examination of sputum and bronchial secretions. *Proc. Staff Meet., Mayo Clin.*, 1947, **22**, 369–381.
55. Borst, M. Infektion und Geschwülste. *München. med. Wchnschr.*, 1928, 75, 11–13.
56. MacCallum, W. G. A Textbook of Pathology. W. B. Saunders Co., Philadelphia & London, 1928, ed. 4, 1177 pp.
57. Wintrobe, M. M. Clinical Hematology. Lea & Febiger, Philadelphia, 1946, 862 pp.
58. Sabin, F. R. Studies in living human blood-cells. *Bull. Johns Hopkins Hosp.*, 1923, 34, 277–288.
59. Ewing, J. Neoplastic Diseases. W. B. Saunders Co., Philadelphia & London, 1940, ed. 4, 1160 pp.
60. Aschoff, L. Pathologische Anatomie. G. Fischer, Jena, 1936.
61. Zadek, I., and Karp, H. Zytodiagnostik des Karzinoms aus Punktaten und Sekreten. *Deutsche med. Wchnschr.*, 1932, 58, 1043–1045.
62. Zadek, I. Die cytodiagnostischen Kennzeichen der Krebszellen. *Acta med. Scandinav.*, 1933, 80, 78–92.
63. Zadek, I. Cytodiagnostik beim abscedierten Lungenkrebs. *Deutsche Ztschr. f. Chir.*, 1936–37, 248, 583–589.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 197

FIG. 1. Case 148. Bronchogenic carcinoma; smear of squamous cell type. Wilson's stain. × 137.

FIG. 2. Case 52a. Adenocarcinoma of rectum; smear of columnar cell type. Wilson's stain. × 295.

FIG. 3. Case 21. Ductus cell carcinoma of mammary gland; smear of round cell type. Wilson's stain. × 137.

FIG. 4. Case 150. Adenocarcinoma of ovary; smear of undifferentiated cell type. Wilson's stain. × 295.

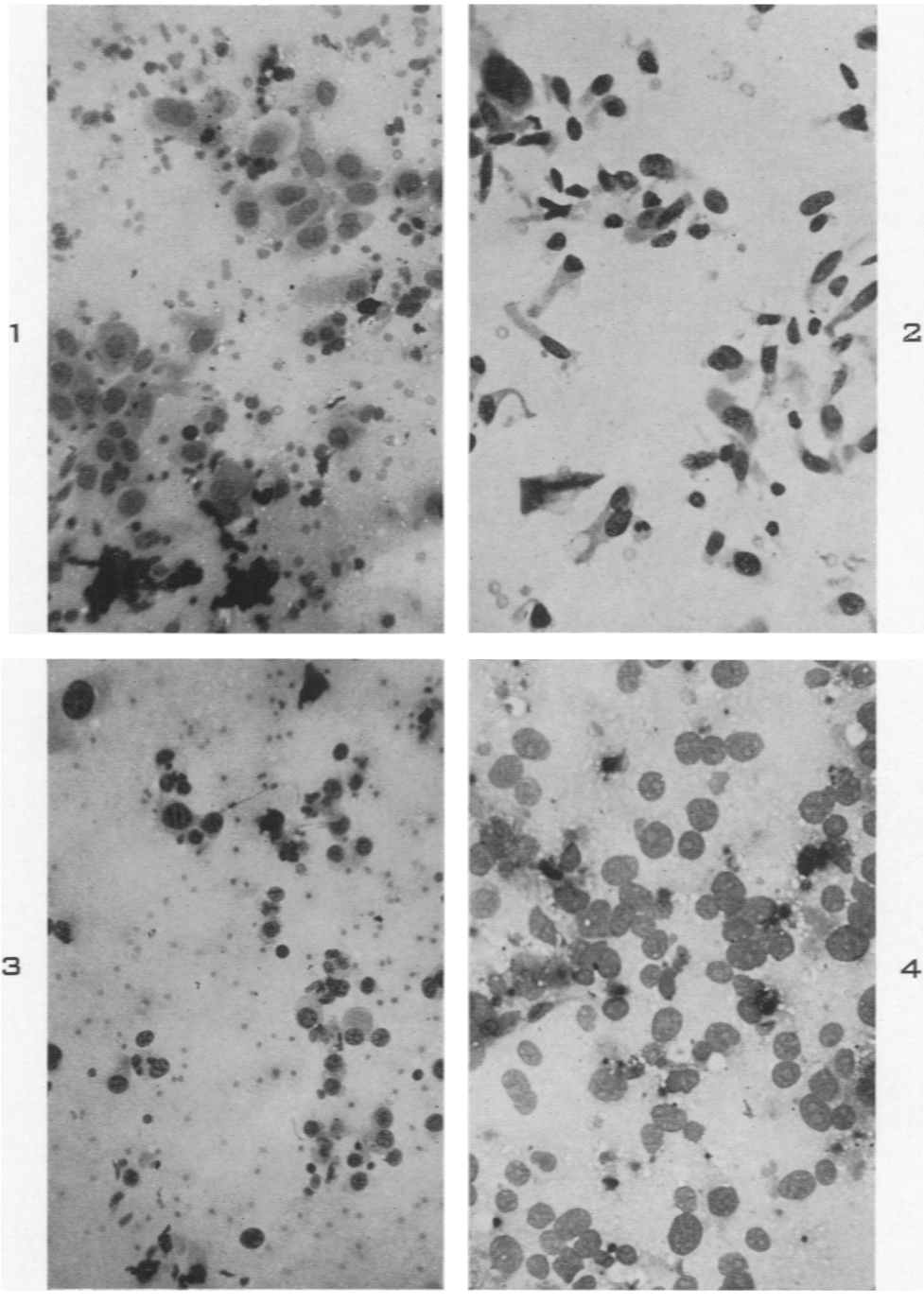
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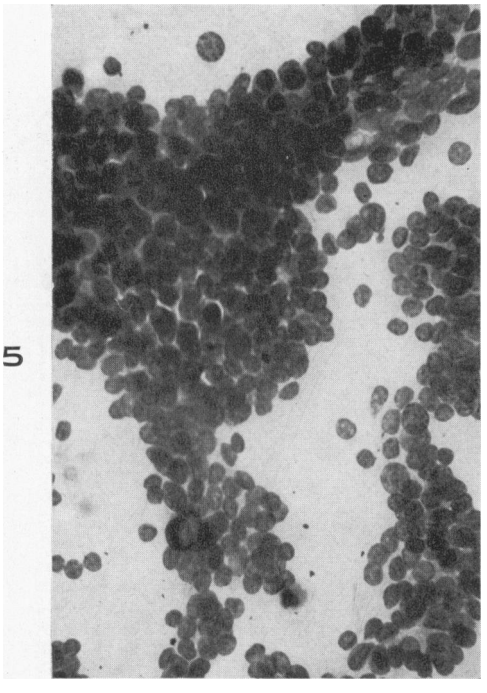
PLATE 198

FIG. 5. Case 110c. Adenocarcinoma of thyroid; smear of oat cell type. Wilson's stain. × 295.

FIG. 6. Case 75a. Chronic cervicitis; squamous cells. Wilson's stain. × 684.

FIG. 7. Case 111a. Squamous cell carcinoma of cervix uteri; nucleolated squamous cells. Wilson's stain. × 684.

FIG. 8. Case 148. Bronchogenic carcinoma; nucleolated squamous cells. Wilson's stain. × 684.



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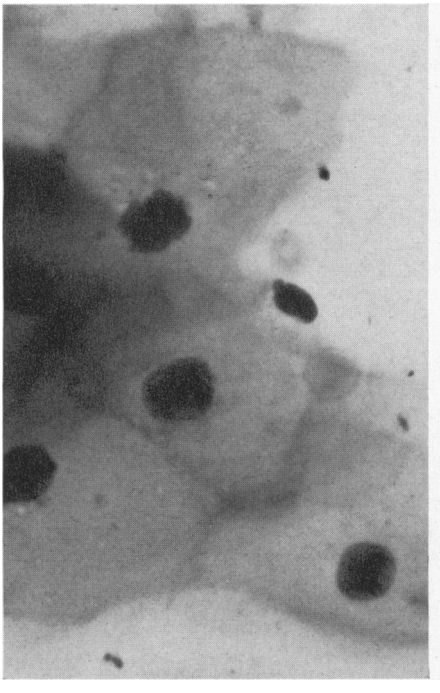

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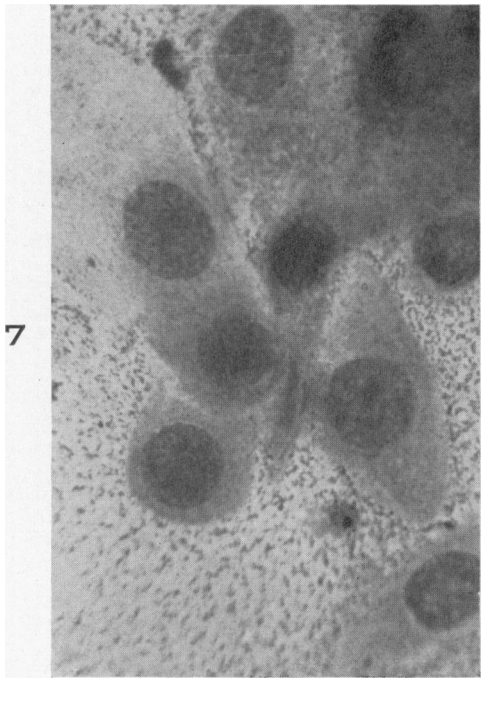

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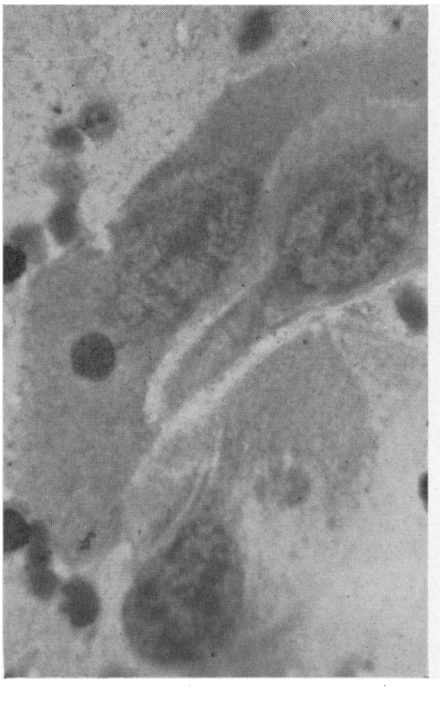

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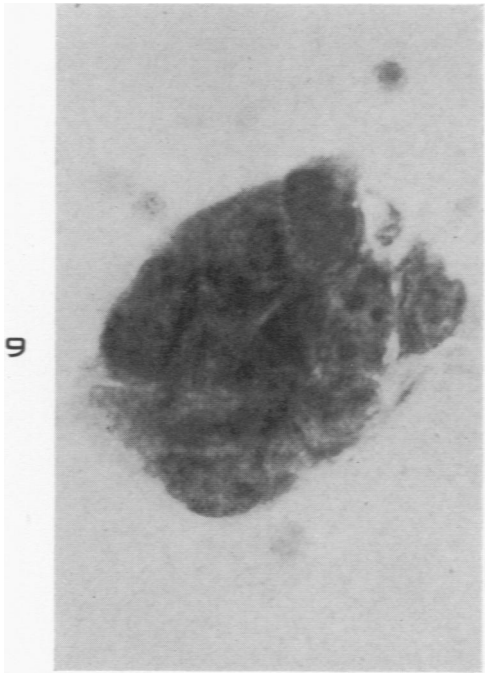
PLATE 199

FIG. 9. Case 58. Squamous cell carcinoma of vulva; malignant epithelial cells. Wilson's stain. × 684.

FIG. 10. Case 11. Carcinoma *in situ* of cervix uteri; malignant epithelial cells. Wilson's stain. × 684.

FIG. 11. Case 158b. Squamous cell carcinoma of esophagus; pseudofibroblasts. Wilson's stain. × 295.

FIG. 12. Case 111a. Squamous cell carcinoma of cervix uteri; pseudofibroblast between two other cells, one in mitosis. Wilson's stain. × 684.



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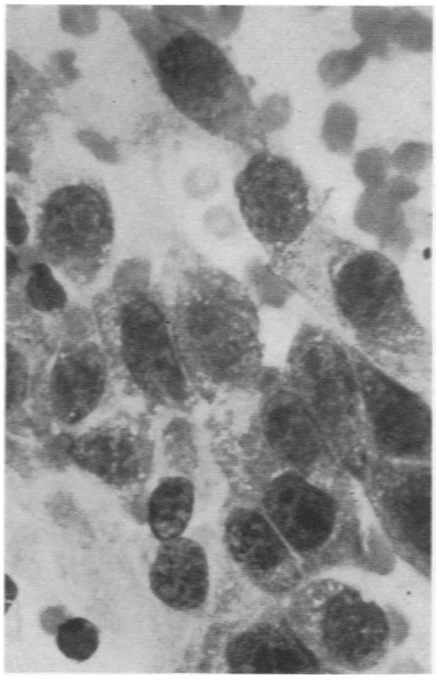

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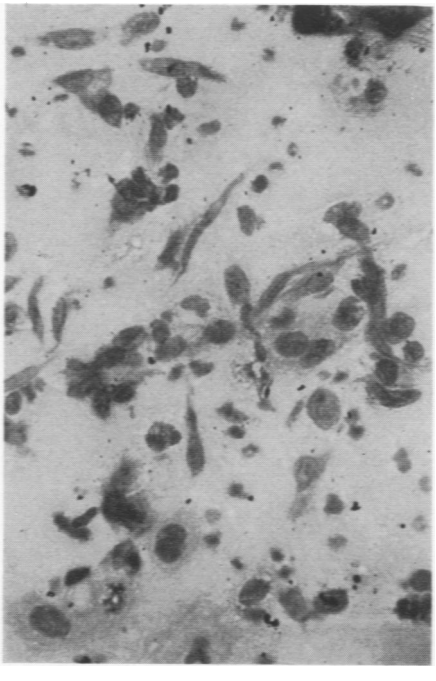

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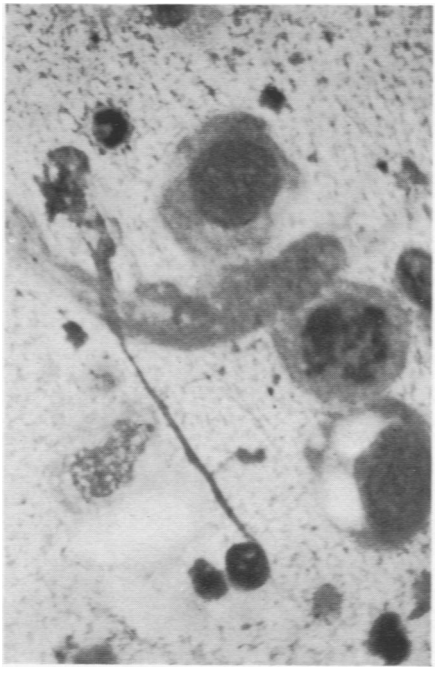

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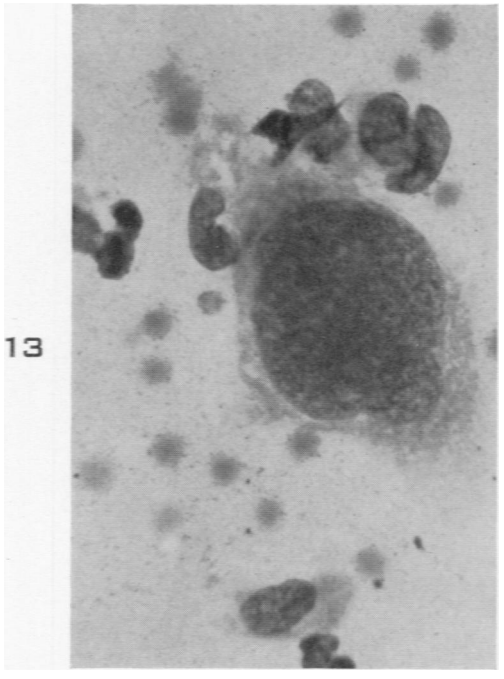
PLATE 200

FIG. 13. Case 119. Squamous cell carcinoma of lip; malignant giant cell. Wilson's stain. × 684.

FIG. 14. Case 24a. Chronic granulomatous inflammation of lung; columnar cells from bronchial epithelium. Wilson's stain. × 684.

FIG. 15. Case 140a. Chronic cervicitis and endocervicitis. columnar cells. Wilson's stain. × 684.

FIG. 16. Case 52a. Adenocarcinoma of rectum; malignant columnar cells. Wilson's stain. × 684.



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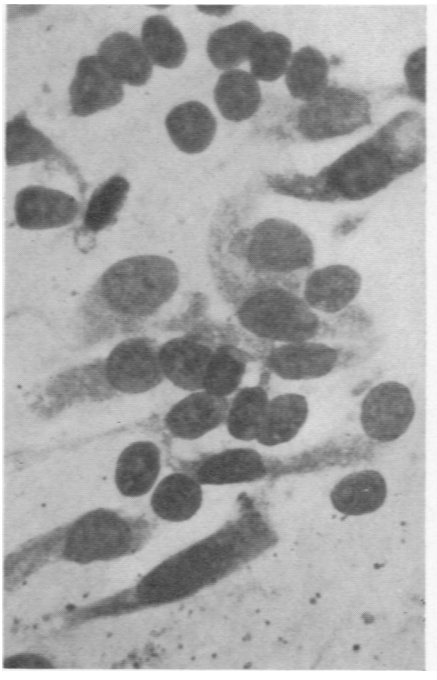

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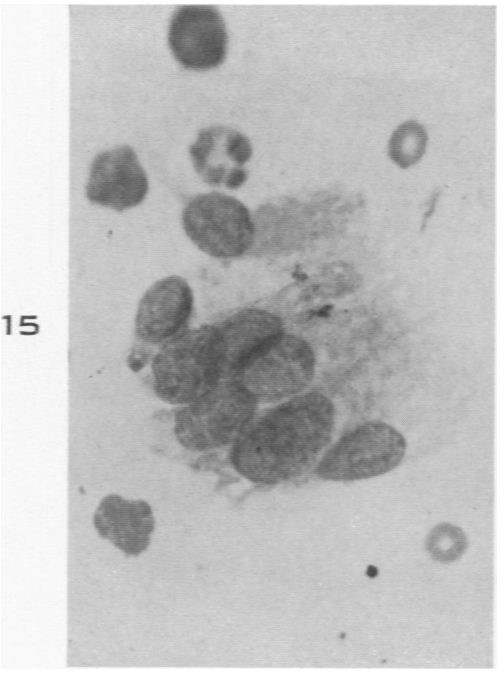

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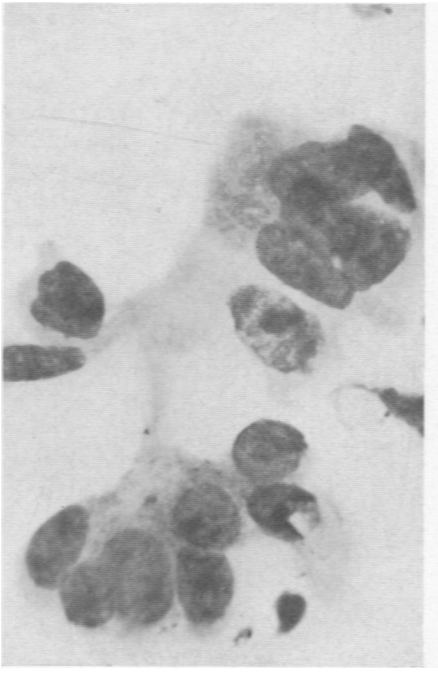

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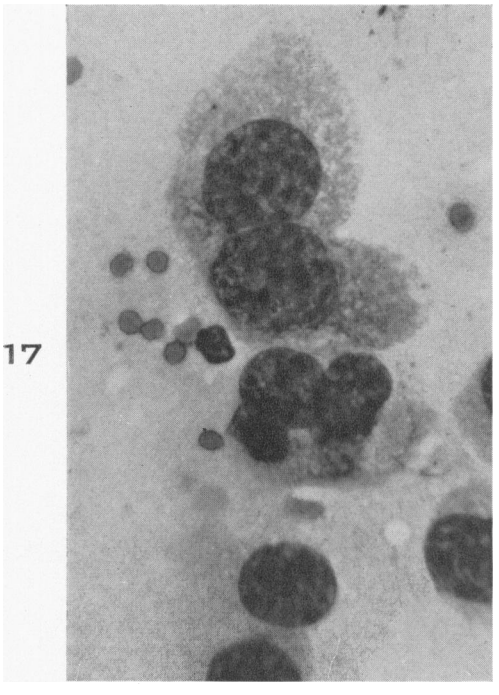
PLATE 201

FIG. 17. Case 21. Duct cell carcinoma of mammary gland; large round cells. Wilson's stain. × 684.

FIG. 18. Case 14a. Adenocarcinoma of stomach; large round cells (giant form). Wilson's stain. × 684.

FIG. 19. Case 150. Adenocarcinoma of ovary; undifferentiated cells. Wilson's stain. × 684.

FIG. 20. Case 15b. Adenocarcinoma of stomach; tumor cells in fundic mucosa, histologically classified as "no lesion." Wilson's stain. × 350.



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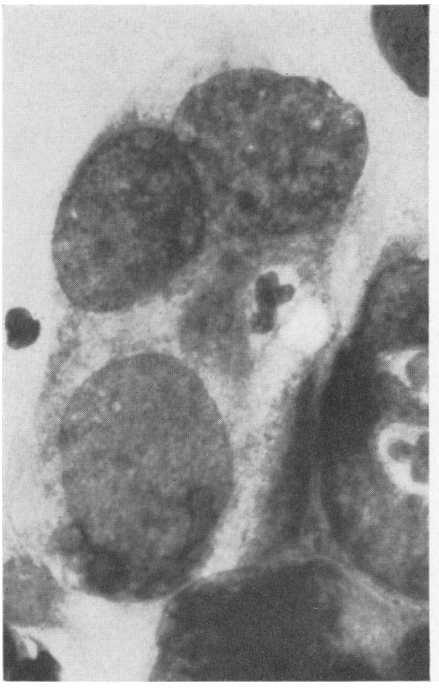

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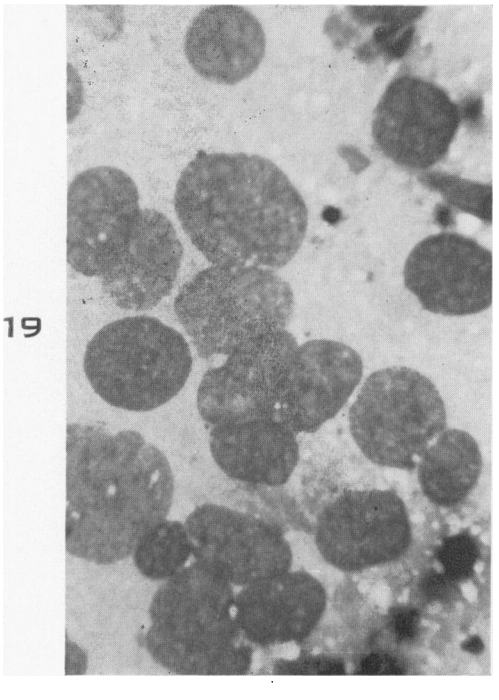

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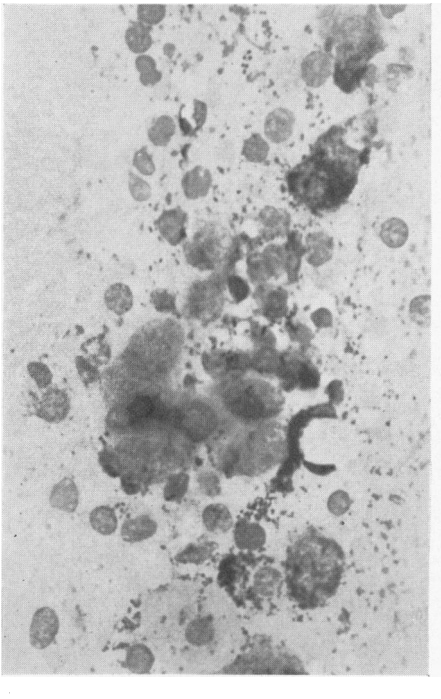

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