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THE DIAGNOSTIC VALUE OF VAGINAL SMEARS IN CARCINOMA OF THE UTERUS*

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THE death rate from carcinoma of the female genital tract is approximately 32,000 per year in the United States and of this figure, four-fifths, or 26,000 deaths per year, may be said to be due to cancer of the uterus. This rate has remained practically constant during the past twenty-five years.[1]

One of the factors probably responsible for this rather discouraging situation is the fact that, despite the progress in methods of treatment, no significant improvement has been achieved in the diagnosis of malignant growths of the female genital tract, more particularly in their early stages. Indeed, it seems very likely that until enough is known about the etiology of cancer to make it possible to place efficient prophylactic weapons in physicians' hands, no radical change in the picture can be expected unless the introduction of new methods makes possible an early diagnosis of the disease.

Early diagnosis and treatment yield a high percentage of cures in both carcinoma of the fundus and of the cervix. The present difficulty in accomplishing an early diagnosis lies in the fact that we must depend largely upon the subjective symptoms of the disease to bring the patient to the physician, and by the time the patient becomes sufficiently aware

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of discomforts to seek help, the disease is far advanced. Even when the patient is seen early, the technique for making a positive diagnosis is not simple, as it involves a biopsy followed by the procedures necessary for microscopic examination, all of which are time consuming and relatively expensive. If by any chance a simple, inexpensive method of diagnosis could be evolved which could be applied to large numbers of women in the cancer-bearing period of life, we would be in a position to discover the disease in its incipiency much more frequently than is now possible. It is our purpose to present in this preliminary report the results of experimentation with such a method of diagnosis which appears to have great possibilities and which has been in use at the New York Hospital for the past eighteen months.

One of us (Papanicolaou) has studied the normal and abnormal variations in the vaginal smear in women and in animals for many years. Through these studies,[2, 3] which have been conducted since 1923 at the Woman's Hospital, at the New York Hospital, and at the Memorial Hospital, he has become aware of the fact that carcinoma of the fundus and carcinoma of the cervix are to some extent exfoliative lesions, in the sense that cells at the free surface of the growth tend to be dislodged and subsequently find their way into the vagina. Furthermore, a technique for collecting the cellular debris, smearing it upon glass slides, and staining it has been perfected, so that the various components may be studied. The method is so simple and inexpensive that it may be applied to large numbers of women.

METHOD OF TAKING THE VAGINAL SMEAR

A slightly curved glass pipette, 6 inches in length, 0.5 cm. in diameter, with a rounded tip and a small opening, and equipped with a strong rubber bulb for producing suction is used (Fig. 1). The patient is placed upon an examining table in the lithotomy position. The labia are separated and the rubber bulb compressed. The glass pipette is introduced into the posterior fornix of the vagina. The pressure on the bulb is then released and the suction produced serves to aspirate vaginal fluid with its cellular content into the glass tube. While aspiration is in progress, the tip of the tube is moved from one side of the fornix to the other so that all parts are sampled. The pipette is then withdrawn, and the vaginal material is spread upon the surface of a clean microscope slide with a sudden pressure on the bulb. Further spreading with the convex side of the pipette is advisable when the amount of fluid is abundant, as in cases where there is considerable bleeding. Very thick smears are not well penetrated by the fixing fluids and cannot be uniformly stained.

The slides are immediately plunged into a solution of equal parts of 95 per cent alcohol and ether. Drying of the smears should be carefully avoided as it results in the loss of the sharp outlines of the cells and in a change in their staining reactions. The fixation does not require more than a few minutes, but smears may be kept in the alcohol-ether solution over a long period of time and may be shipped in bottles. It is recommended that the slides not be kept in the fixative for more than a week or two as the cells slowly tend to lose their normal staining reaction. If ether is not available, plain alcohol (95 per cent) can be used. For shipping slides, a square bottle 1¾ by 1¾ by 4½ inches may be used (Fig. 1). Such a bottle may hold as many as 7 or 8 slides. An ordinary paper clip attached to each or every other one of the slides (Fig. 1) prevents the smears from rubbing against one another.

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

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

METHOD OF STAINING VAGINAL SMEARS

Detailed instructions for staining vaginal smears are given by Papanicolaou in a recent article.[4] For general gynecologic diagnosis, stains DF20 or DF32 give satisfactory results. Slides are first stained in hematoxylin. A good nuclear staining is very essential for the recognition of malignant changes.

DESCRIPTION OF SMEARS SHOWING SQUAMOUS CARCINOMA OF THE CERVIX

Cervical malignancy, in our experience, is revealed in vaginal smears by the appearance of characteristic cells. These are, we think, derived from the superficial layers of the tumor which undergo continual desquamation. These cells show great variety of form and size, much greater

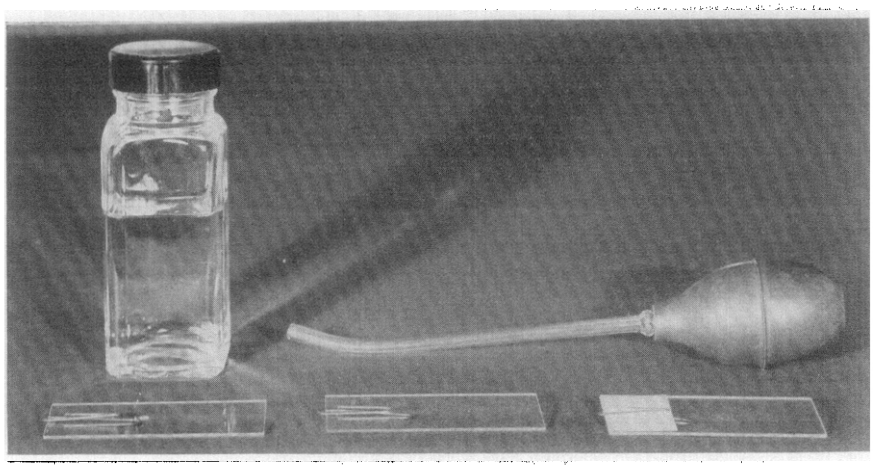

Fig. 1.—Pipette and bottle used for preparing vaginal smears.

than that seen in sections of the tumor. Their distinctive features lie in their structural abnormalities. They do not fall into the categories of any of the cell types found in the vaginal fluid of normal women or of women having benign tumors or other pathologic lesions of the uterus.

It is well to bear in mind that the isolated cells or at the most, groups of a few cells as seen in any smear preparation do not possess the orienting characteristics seen by the microscopist in tissue preparations of carcinomatous tissues, and that, therefore, a more intimate and critical knowledge of cytology is required for a correct interpretation. It is necessary, therefore, to enter into a detailed account of the characteristic appearance of the abnormal cells found in the vaginal smears of women harboring carcinoma of the fundus or cervix of the uterus.

The most characteristic feature of the abnormal cells is the atypical form and structure of their nuclei. These often are very large, far surpassing normal size (Figs. 3 and 4; compare with Fig. 2). The chromatin frequently shows a characteristic distribution in the form of conspicuous granules and of one or more small nucleoli (Figs. 3 and 4).

Some nuclei are in a stage resembling prophase as if they were undergoing changes preparatory to a mitotic division. Actual mitotic figures are very rarely seen. This is probably because of the fact that the epithelial cells found in the vaginal fluid are dead desquamated cells. The nuclei are often hyperchromatic and stain intensely (Figs. 2 and 3). Others

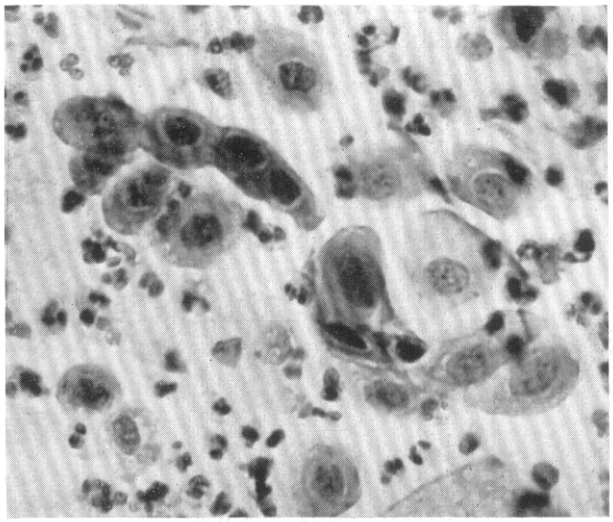

Fig. 2.—Group of normal basal cells. Menopause. ×800.

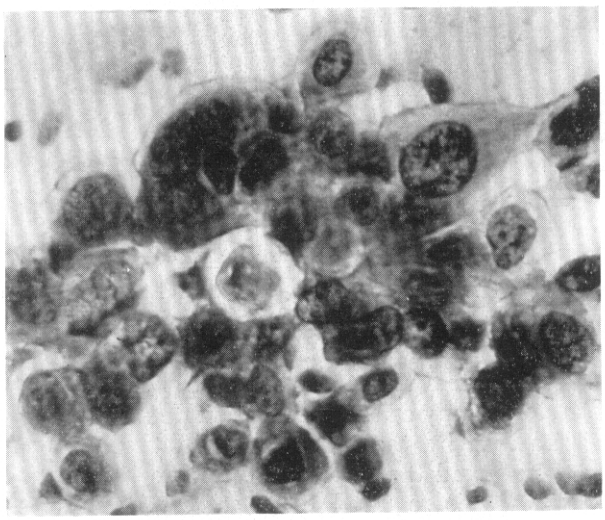

Fig. 3.—Group of abnormal cells. Carcinoma of cervix. ×800.

become very dense and lose their fine structure, whereupon they stain very deeply (Fig. 4). Small pyknotic nuclei as well as nuclei showing various degenerative changes are frequent. Some nuclei show indentations or irregular bulging. It is not unusual to find cells with more than one nucleus (Figs. 3, 4, and 5). This is probably the result of abnormal fragmentations or of amitotic divisions.

The cytoplasm also shows abnormal changes. It is often dense and hyperchromatic, particularly in the cells of the basal type (Figs. 3, 4, and 5). Such cells may appear either singly or in compact dark-staining clusters. Their form and size vary greatly. Some of the basal cells assume elongate, spindlelike, triangular, or amoeboid forms (Fig. 6).

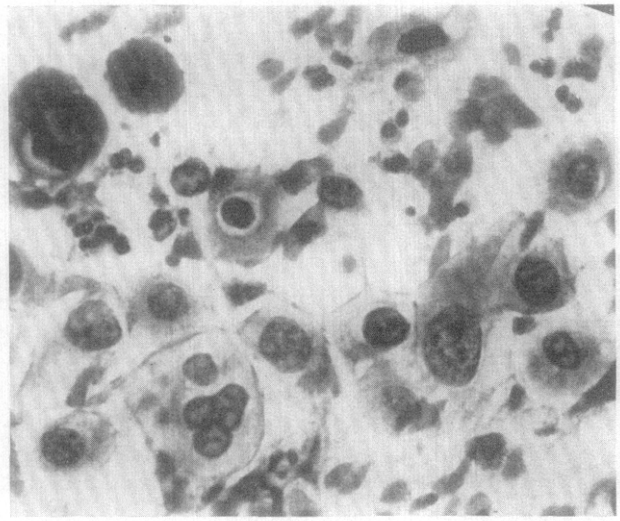

Fig. 4.—Abnormal cells. Carcinoma of cervix. ×800.

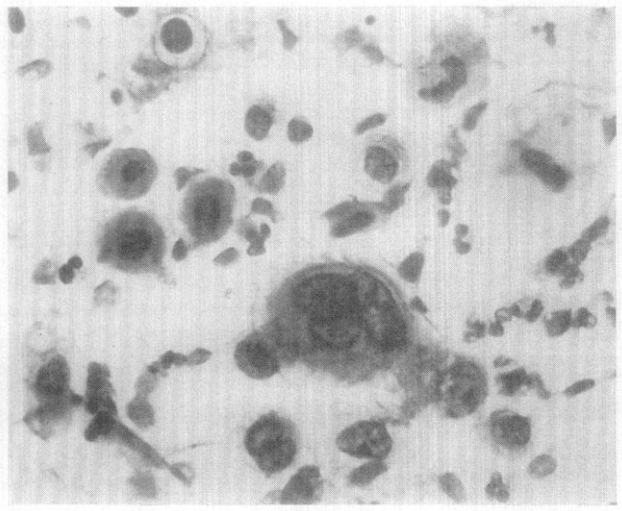

Fig. 5.—Abnormal cells. Carcinoma of cervix. ×800.

Basal cornified cells are not uncommon. Vacuolization of the cytoplasm is a characteristic feature (Fig. 7). The vacuoles may be empty or they may contain leucocytes, erythrocytes, cellular debris, or some pink-staining fluid. Sometimes the vacuoles occupy one side of the cell, while the dense cytoplasm and the nucleus are concentrated on the other side.

A commonly found, very characteristic cell type is an extremely elongated one resembling a smooth muscle fiber. Long fibrous cells of this type are modified epithelial cells and appear either isolated or in groups (Figs. 8 and 9). Another deformed cell type which is often seen is one having the form of a tadpole, with a spherical head containing

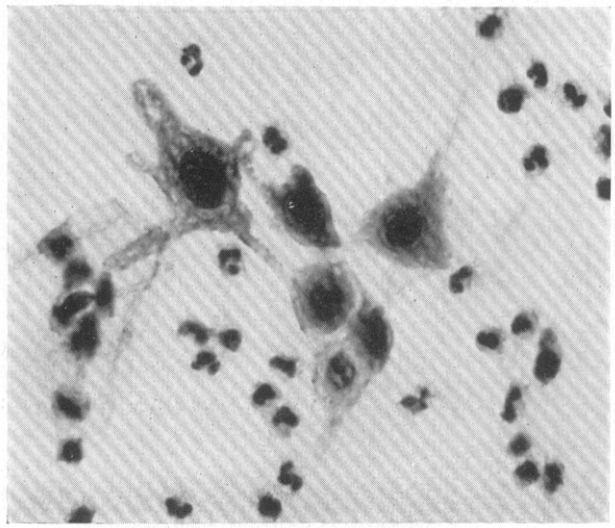

Fig. 6.—Group of aberrant basal cells. Carcinoma of cervix. ×800.

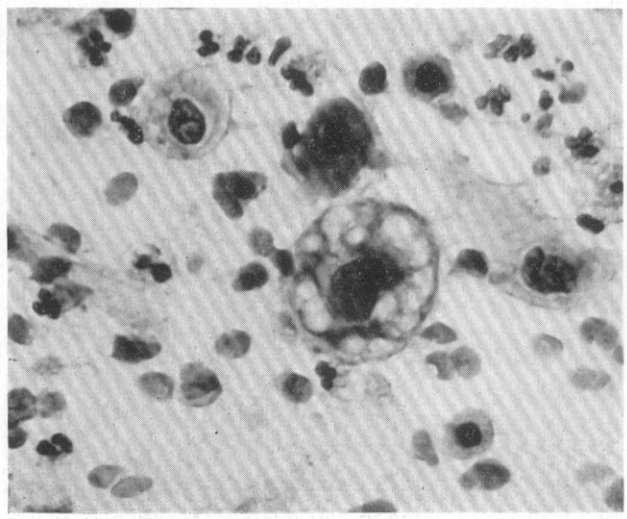

Fig. 7.—Abnormal vacuolated cell. Carcinoma of cervix. ×800.

the nucleus and a tail-like prolongation (Fig. 9). The bulging of the heavier part which contains the nucleus is sometimes placed more centrally, causing a narrowing of the cell at both ends. Other cells attain very large sizes and acquire the most unusual forms.

These "aberrant" cell types are numerous only in advanced cases of malignancy. They are relatively rare in the early stages of the disease and a thorough search of several slides is often necessary before their presence can be established. For this reason a negative diagnosis should always be made with extreme caution. Considering that even

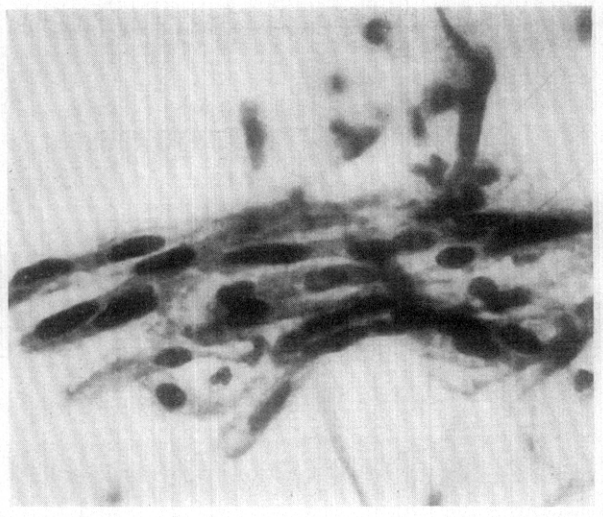

Fig. 8.—Abnormal epithelial cells showing extreme elongation ("pseudofibrous" type). Carcinoma of cervix. ×800.

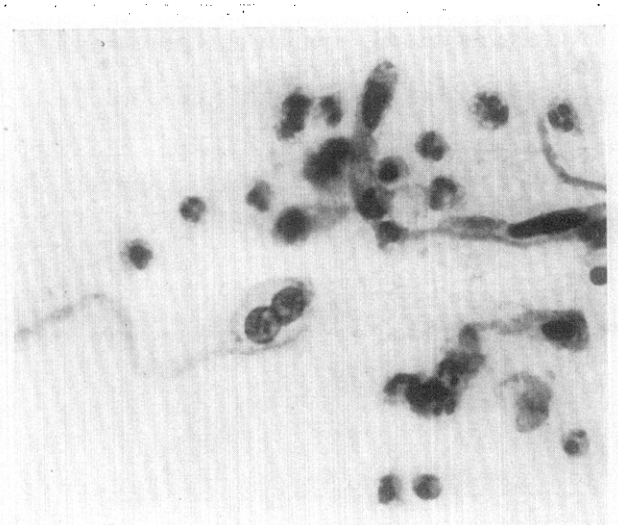

Fig. 9.—"Tadpole" cell with two nuclei, and other elongated cells. Carcinoma of cervix. ×800.

in the advanced cases the number of cells derived from the tumor forms only a small part of the total number of desquamated cells present within the vagina, it is natural to find the normal epithelial cells always in the majority. The large squamous superficial cells usually predominate in younger women, while the basal cells appear in larger num-

bers after the menopause. The relative number of these cells varies greatly. The number and the form of the cornified cells also show considerable variability. In some cases there is an excess of cornified cells, indicating a hyperestrin condition. This was observed more frequently

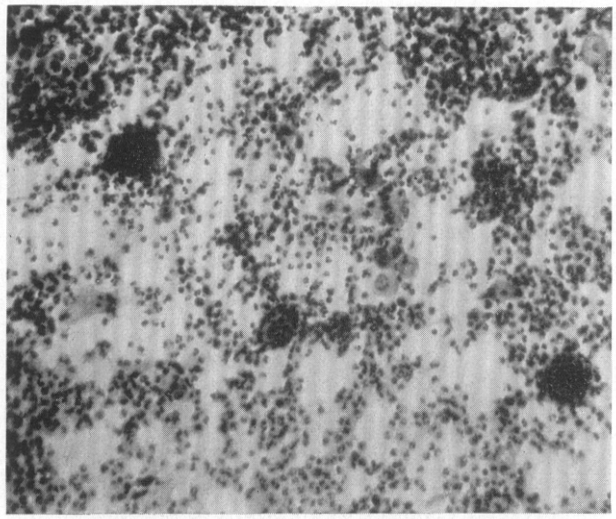

Fig. 10.—Extreme leucocytosis with clusters of polymorphonuclears. Carcinoma of cervix. ×220.

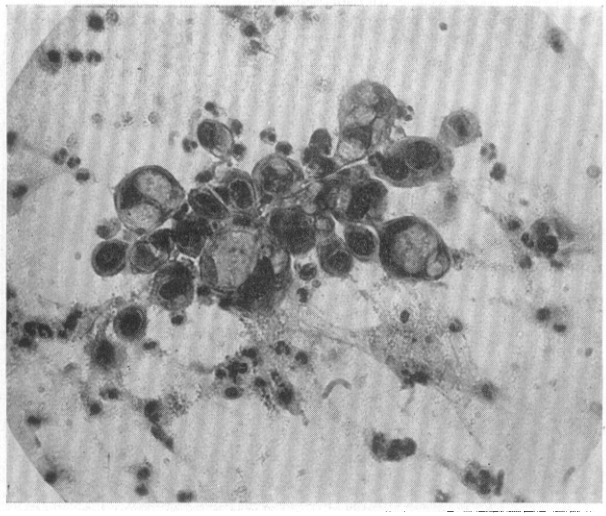

Fig. 11.—Group of abnormal histiocytes, some with very large vacuoles. Carcinoma of cervix.

in the early stages of malignancy. Cornification is sometimes due to the coexistence of other pathologic conditions like vaginitis or chronic cervicitis, or to the presence of trichomonas which is not uncommon in cases of cervical malignancy.

Blood elements are quite conspicuous in the vaginal smear of cervical malignancy. Erythrocytes are generally found in large numbers. Many

show degeneration and have lost their hemoglobin. Fibrination is very pronounced. The complete absence of blood is so rare even in the early stages that it may be considered in favor of a negative diagnosis. The leucocytes are as a rule very numerous, more particularly in the advanced cases (Fig. 10). The polymorphonuclears are often seen in

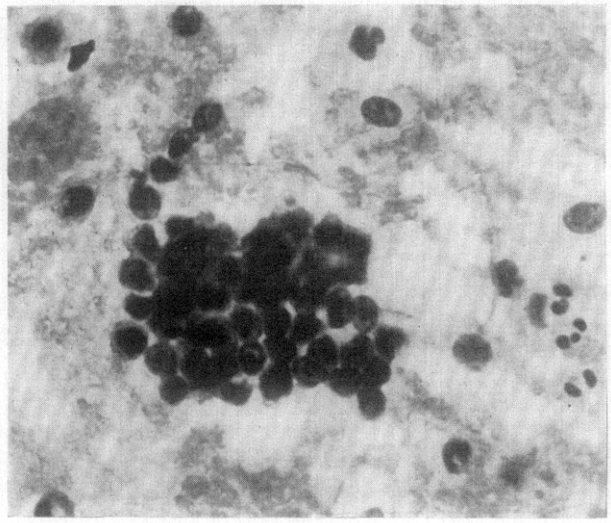

Fig. 12.—Cluster of normal endometrial cells. Menstruation. ×800.

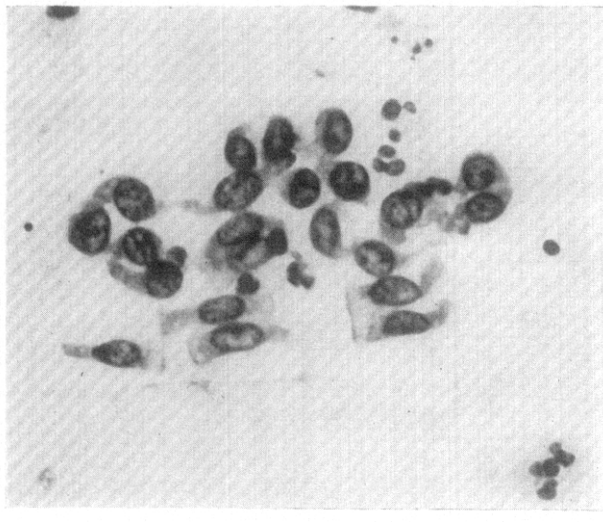

Fig. 13.—Abnormal cell group. Adenocarcinoma of fundus. ×800.

groups which are very characteristic. Degenerating forms are not infrequent. Eosinophiles which are rarely found in normal smears are more frequently seen in malignancy, though in small numbers. Leucocytes in general have a somewhat limited diagnostic value because their number undergoes great fluctuations under different conditions.

It is also affected by the presence of certain bacterial forms. The bacterial flora in cervical malignancies is usually rich and consists of various types with cocci and diplococci predominating. Trichomonas and chronic inflammatory infections may cause considerable leucocytosis.

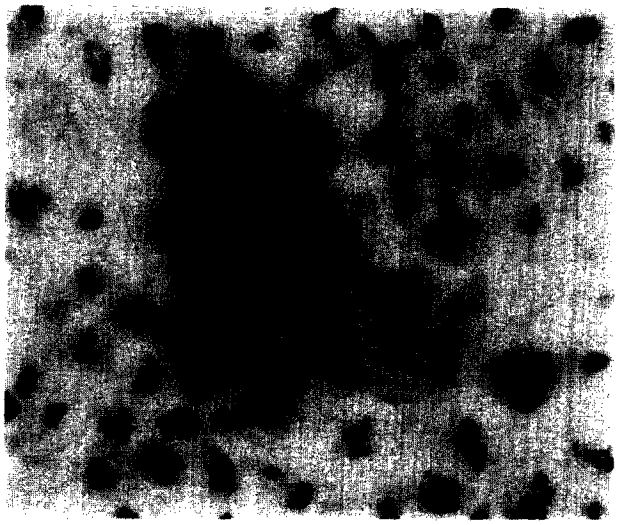

Fig. 14.—Abnormal cell group. Adenocarcinoma of fundus. ×800.

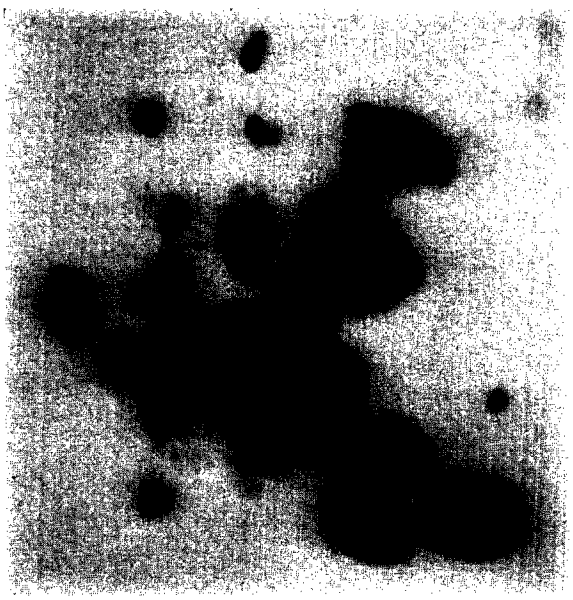

Fig. 15.—Abnormal cell groups. Adenocarcinoma of fundus. ×800.

The histiocytes deserve special consideration. They are invariably present in carcinoma smears and usually display a high phagocytic activity. They phagocytize dead leucocytes, erythrocytes, and other cellular debris. They appear single or in groups. Their size varies greatly. The larger ones reach the size of small epithelial cells with which they

may often be confused. Their cytoplasm is vacuolated. Some of the vacuoles are large and seem to persist after digestion of engulfed elements (Fig. 11). Their nuclei tend to grow and to become hyperchromatic. Histiocytes with more than one nucleus may be seen. A good many undergo degeneration. The cytoplasm and nucleus both become pyknotic. After ingestion of erythrocytes, the cytoplasm often becomes acidophilic and stains red with eosin. The number of degenerating histiocytes appears to be relatively high.

DESCRIPTION OF SMEARS SHOWING ADENOCARCINOMA OF FUNDUS

The general characteristics of the vaginal smear of adenocarcinoma of the fundus are in many ways similar to those of carcinoma of the cervix. The prevailing types of "aberrant" cells are, however, of different origin. They are derived from the endometrium, and are carried

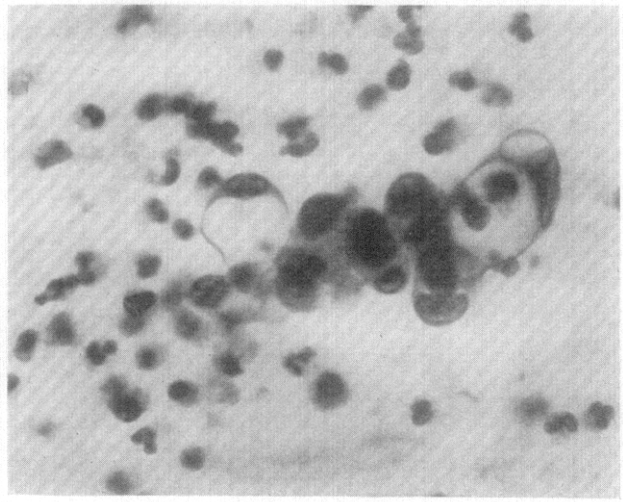

Fig. 16.—Abnormal cell group including some large histiocytes. Adenocarcinoma of fundus. ×800.

into the vagina by the uterine bloody discharge. They do not show the high variability displayed by the modified squamous epithelial cells found in cervical carcinomas. They are considerably smaller and their variations in size and form are more limited.

The most frequently found cell types have a cuboidal, columnar, or spindlelike form and appear singly or in dark-staining clusters, which can be easily spotted with low power (Fig. 13; compare with Fig. 12). Some cells become elongated, or rounded, and show some resemblance to cells found in carcinomas of the cervix. Sometimes the cells are so densely grouped together that their outlines become very indistinct (Fig. 14). Vacuolated cells are not infrequent. A very characteristic cell type is one that has a large vacuole which is often filled with dead leucocytes or with cell debris. Such cells are evidently of histiocytic nature and perform a phagocytic action. They appear single, grouped, or intermixed with the uterine cells (Figs. 16 and 17).

The nuclei tend to be hyperchromatic and show marked variations in size (Figs. 14 to 17). Some are considerably enlarged (Fig. 15). They generally have an oval or rounded shape, and their chromatin is distributed in the form of conspicuous dark-staining granules. The cells are sometimes plasmolyzed and the nuclei are set free. Such nuclei appear single or in irregular clusters and are very characteristic.

The blood cells and the histiocytes show the same reactions as in carcinomas of the cervix. The histiocytes are, as a rule, numerous and display a high phagocytic activity.

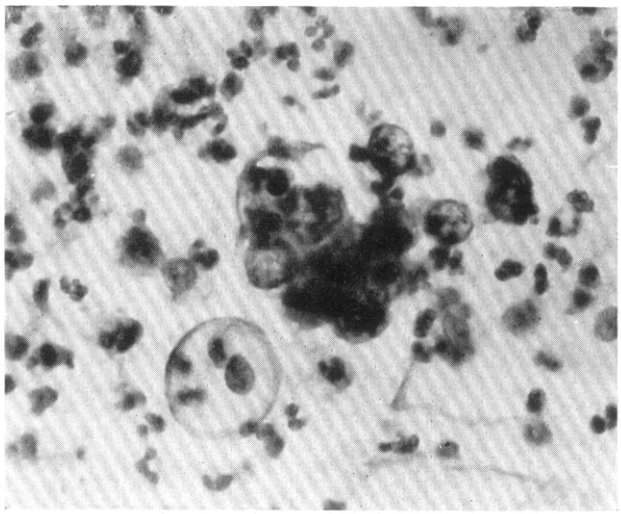

Fig. 17.—Abnormal cell group including one large histiocyte. Adenocarcinoma of fundus. ×800.

SMEAR REACTIONS IN CARCINOMAS OF OTHER ORGANS OF THE FEMALE GENITAL TRACT

Carcinomas of the vagina or vulva are characterized by large, abnormal basal and superficial squamous cells, some of which are cornified. Unusually large cornified cells with highly aberrant forms and enlarged or fragmented nuclei are particularly frequent. In malignant tumors of the ovary, the findings have not been consistent. In some cases the vaginal smears showed no deviations from the normal. In others, some atypical cells have been noticed, the nature of which is somewhat obscure. It is very likely that such cells are not derived from the ovarian tumor, but from possible metastatic lesions of the uterus. The number of the cases studied up to the present is too small to permit definite conclusions.

DISCUSSION

It will be seen from the foregoing descriptions that the interpretation of the smear depends upon an intimate knowledge of the cytologic characteristics of the vaginal fluid. It may also be inferred that this is a somewhat more difficult matter than the problem presented by the diagnosis of carcinomatous tissues. However, it is safe to say that the

requirements are not above the powers of any trained cytologist or pathologist after these have had adequate training and sufficient experience. From our experience it should be said that in general gynecologic diagnosis, the vaginal smear technique presents greater difficulties than those presented by some of the conditions in which it has been widely used, such as the evaluation of estrogenic effect upon the vaginal epithelium.[5]

During the past two years we have collected and studied many hundreds of vaginal smears from normal women and those suffering from gynecologic disease, and we feel that cells pathognomonic of cervical and fundal carcinoma can be definitely recognized. We are not yet in a position to offer a statistical proof of the reliability of this method of diagnosis, but we can say that in our experience it yields a high percentage of correct diagnoses when checked by tissue biopsies. There is evidence that a positive diagnosis may also be obtained in some cases of early disease. The simplicity of the method, the lack of inconvenience to the patient during its application and the possibility of obtaining daily information over a long period of time makes it very useful in following the progress of the disease after operative procedures or x-ray treatments.

In presenting this method of diagnosis at this time, we hope that it may prove to be a dependable means whereby the principal malignant diseases of the uterus can be recognized; and further that because of its simplicity, it may eventually be applied widely so that the incipient phases of the disease may come more promptly within the range of our modern modes of treatment which have been proved highly effective in early carcinoma. In conclusion, it may be well to reiterate that whereas the method makes the material for examination easily and frequently obtainable at low cost, the interpretation of the smear requires the services of a careful and discriminating cytologist who has had experience in this field. Few persons can be depended upon for this work at the present time; however, if the method proves to be worthy of further development, as we expect it to be, then it will be possible in a relatively short time to provide the necessary facilities for instruction.

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DISCUSSION

DR. JOSEPH HINSEY (by invitation).—This problem is one that is different from tissue pathology. In tissue pathology we have not only the cytology of the cells involved, but also the orientation of those cells in the tissues. Here we are dealing primarily with the cytology of cells and are faced with problems which are like those of the hematologist who makes his examination of the blood smear.

The interpretations presented here have been possible only through the very broad and long study which Dr. Papanicolaou has made of the cytology of these cells in the female reproductive tract, combined with the knowledge of gynecologic pathology which Dr. Traut possesses.

DR. FRANK R. SMITH.—Any one who has seen a roentgen ray picture of a woman who has recently been curetted and then had lipiodol injected into the uterus and sees the Christmas tree effect of the dissemination of the lipiodol, would be impressed with the dangers of curettage as a method of diagnosis in carcinoma of the body of the uterus. Any method that will eliminate that obvious danger must necessarily be encouraged. At Memorial Hospital we have realized that danger for some time. We have emphasized the fact that the term curettage should be avoided, and we prefer to say that we obtain a biopsy with the curette. The advantage of having a curette or some other instrument to palpate the interior of the uterus is nevertheless not to be overlooked.

DR. HOWARD C. TAYLOR, JR.—This problem has to be met in three steps of which Dr. Traut and Dr. Papanicolaou's presentation is the first. They have succeeded in demonstrating that when certain types of cells are found, cancer is present in the cervix or the fundus of the uterus. That in itself may have considerable practical importance, because in patients in whom there is vaginal bleeding and in whom you suspect cancer of the endocervix or the corpus, you may find these cells. When the test is positive, you may almost assume that cancer is present above the external os.

The next step is the more difficult one. That is to demonstrate that when these cells are not present in the vaginal smear, cancer is not present in the cervix or the endometrium. In other words the value of negative findings in the smear must also be established from a practical standpoint which is an even more important point to establish than the first one.

The third step is to organize medical practice so that these tests may become a routine. It is worth while here to recall the experience which we have had with the Schiller test. This was a practical test, widely written about, supported by prominent gynecologists. Yet I do not think there is any gynecologic clinic which is practicing this test routinely at the present time.

DR. I. C. RUBIN.—Many years ago I had the privilege of showing several cases from the Schottländer laboratory of the von Rosthorn Clinic, Vienna, of incipient carcinoma of the cervix which were only pin point in size and were found in cases of prolapse. The diagnosis was doubted by a number of investigators. Since then the findings in the cases originally presented have been corroborated by Cullen and others, and the slides shown by Drs. Papanicolaou and Traut present the same histologic features.

The question arises as to the extent that this simple vaginal smear method will help us in diagnosing, not the obvious lesions of the cervix, but the deeply-seated carcinoma of the fundus and possibly those rare cases of Fallopian tube carcinoma from which may be shed and discarded some of these malignant cells.

The great value, besides diagnosing fundal carcinoma, is that one can avoid a traumatic curettage. A traumatic biopsy of the cervix may possibly also be avoided. If this proves diagnostic in a large number of cases of the hidden type of carcinoma and of the early, hitherto unrecognizable carcinoma of the cervix by the usual means at our disposal, including the colposcope, then we have made a great advance in the diagnostic armamentarium in this field.