THE CYTOLOGIC ASPECTS OF BASAL CELL CARCINOMA*

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In reviewing cytologic studies of dermatoses (1–21), it is apparent that no particular attention has been paid to the morphologic peculiarities of the different cellular components.

Since 1958 most of the specimens taken for biopsy study in our Section of Dermatology, have been studied with reference to cellular components. In this way we have become familiar with the cytomorphologic aspects of a variety of cutaneous diseases, including inflammatory dermatoses and tumors.

During our studies of smears from tissues with basal cell carcinoma, a number of poorly understood facts required us to compare our results obtained from smears with results obtained by histologic examination, and to compare the results of the use of histochemical methods with both technics. We used the Giemsa stain because the usual methods of histologic staining did not reveal as much cellular detail as this study required.

METHOD

From among the total number of cutaneous diseases studied, we took 892 smears from 156 basal cell carcinomas. These constitute the basis of this study. Several smears were taken from each lesion.

A surgical specimen was obtained from the suspect lesion. Each specimen was then evenly divided. One half was used for routine histologic study and the other half was devoted to cytologic examination.

Sometimes. when soft-tissue lesions were at hand, the specimen for cytologic study was obtained with a small curet, an action which in most instances provided enough material to permit preparation of a satisfactory smear. When the latter method is employed in the presence of ulcerated or crusted lesions, care must be exercised to take tissues at deeper levels, so that a representative area of the lesion will be obtained.

Before imprinting of the tissue on the slide is started, it is advisable to remove all fatty tissues from the specimen with forceps and scissors, except when the suspected lesion involves the hypodermis. In the latter instance the lesion must be carefully delimited, so that any excess of fat is eliminated. Crusts and blood should be washed away with gauze soaked in isotonic solution of sodium chloride.

Details in the preparation of the tissue (or touch) imprint are very important. We have found that if these details are not properly followed, the number of cells obtained will be too small, and that adequate study of the cytologic aspects becomes difficult. When soft specimens are at hand the whole material is crushed between two slides on a flat surface. The section of tissue will adhere to one of the slides. In this manner, as digital compression is exerted and as successive tissue imprints are made in near-by areas, it is possible to cover most of the surface of the slide with an abundant number of cells which produces an even extension comparable to that of the blood smears used in hematologic studies. Experience will govern the amount of digital pressure to be applied to the upper slide. Excessive compression will bring about many artifacts, and too little compression will leave an acellular imprint. Harder specimens must be teased apart with fine scissors or a razor blade previous to the imprinting procedure. If the section of tissue is too slippery, blotting paper can be used on one of the slides. The procedure is carried out in the same manner, but only one slide is smeared at a time.

Satisfactory fixation is obtained by immersion of the slide in methyl alcohol for 5 minutes or by exposure to dry heat. Then the slides are covered with a solution containing 2 drops of Giemsa dye per milliliter of distilled water, and this solution remains on the slide for 40 minutes. Other staining technics have been used when necessary. They include dopa,[22] thyrosinase,[23] PAS,[23] silver carbonate,[24] Schmorl,[25] Lillie,[25] Masson-Fontana[25] and Ehrlich,[26] all being used alone or in combination with Giemsa stain. The Papanicolau,[23] Sudan,[24] and hematoxylin and eosin technics[24] also were used. Most of the slides studied cytologically were compared with the slides studied histologically. To obtain comparable results, we used the same histochemical technics on the slides used for histologic examination.

RESULTS

In the smears of basal cell carcinomas we have encountered the following types of cells: epithelial cells, melanocytes, unusual cellular elements which we designated "clear-nucleus cells" and cells of the dermal infiltrate. We shall describe the different peculiarities of these components as they appeared in the smears.

Epithelial Cells.—We have classified recog-

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nizable epithelial cells of the smears of these tumors in the following manner.

1. Small Cells.—These are elements which have a naked nucleus and chromatin which is closely packed and is round shaped or somewhat elongated. The size of these elements is approximately twice that of an erythrocyte (fig. 1). The outline of these cells is marked by deformity, indentation and lobulation, and these qualities are best seen in the aggregates, or "grapes", of clumped cells. Frequently it is possible to find groups of these cells which have superimposed nuclei. The transparent cytoplasm is very pale blue, and is visible in isolated elements against the uniformly stained background of the smear, and has a cuboid or quadrangular shape (fig. 2). The morphologic aspects of these small cells are similar to those of the epithelial cells of the basal and lowest strata of the histologic epidermis. It is possible to confuse these cells with lymphocytes, but lymphocytes are less deformed and possess a chromatin which appears to be denser as stained with Giemsa stain. The nucleoli are not recognizable.

2. Blue Cells.—The most characteristic feature of the blue cells is the cytoplasm, which has a widely variable outline; frequently it is thorn-like or fusiform, but it also can be rhomboid, piriform, spawn, heart or racket-shaped. In other cells this outline may be rather ill-defined, but in all these cases the cytoplasm stained pale or intensely blue (fig. 3). The size of blue cells is variable, not only according to the lesion at hand, but also within the same smear and also within the same field. Usually the size of this cell is smaller than that of the melanocytes and "clear-nucleus cells" which will be described. The oval or elongated nucleus possesses a compact chromatin, and in some cases the cell is binucleated. The nucleoli are not visible. In addition, there are elements devoid of cytoplasm which are recognizable by the nuclear structure.

The morphologic aspects of blue cells are somewhat different from those of the epithelial cells found in squamous cell carcinoma. Usually these cells are smaller and have a denser nucleus. The morphologic characteristics resemble closely those of the elements found in smears from foreign body granulomas of hair origin, sacrococcygeal cysts or other lesions associated with abundant formation of new hair or destruction of hair. For this reason we designated these cells "pilar cells".

3. Cellular Syncytia.—These constitute a very unusual formation which has been found in a reduced number of smears. The characteristics are so striking that they warrant detailed description.

This formation has been found in smears containing an abundant number of blue cells. The formation was visible even under objectives of the lowest power. The size can be enormous, in some instances so great as to occupy the complete immersion field (fig. 4). It resembles a foreign-body giant cell of extraordinary volume. These cellular syncytia are oval or round, and are sharply delimited, with a very apparent cytoplasm which stains evenly blue in the initial elements. In more advanced stages the blue is more intense, and in some places it has a slightly gray cast, and an angular outline or irregularities. The syncytia contain a variable number of nuclei between three to a dozen and the distribution is irregular. The nuclei are round-shaped or oval, and contain dense and pasty chromatin. The nucleoli can be seen.

It is difficult to determine in the smears if these formations are the products of fusion of some cells or if they are the results of multiplication of the nuclei of one cell. The tendency toward angulation of some parts of the cytoplasm would support the first possibility. The morphologic aspects of those syncytia with a small number of cells resemble closely those of the "blue cells" above described. It is therefore possible that they could be derivatives of these cells.

In some cases a splitting can be seen between the different nuclei of these syncytia, or a cell can be discerned which is beginning to separate from the extensive cytoplasm, forming an element which has a clearer and somewhat areolar-like protoplasm and denser and shriveled nuclei, characteristics of keratinized cells (fig. 5). This fact would support the view that these syncytia correspond to the image of the clear "keratinization center" which it is possible to find in the histologic components of many basal cell carcinomas.

We have found analogous syncytial elements in smears of some squamous cell carcinomas of the scalp and also in smears of sacrococcygeal cysts, foreign-body granulomas of hair origin and calcified cysts of the face.

4. Eosinophilic Cells.—These cells are not characteristic of basal cell carcinoma alone, since they are found also in squamous cell carci-

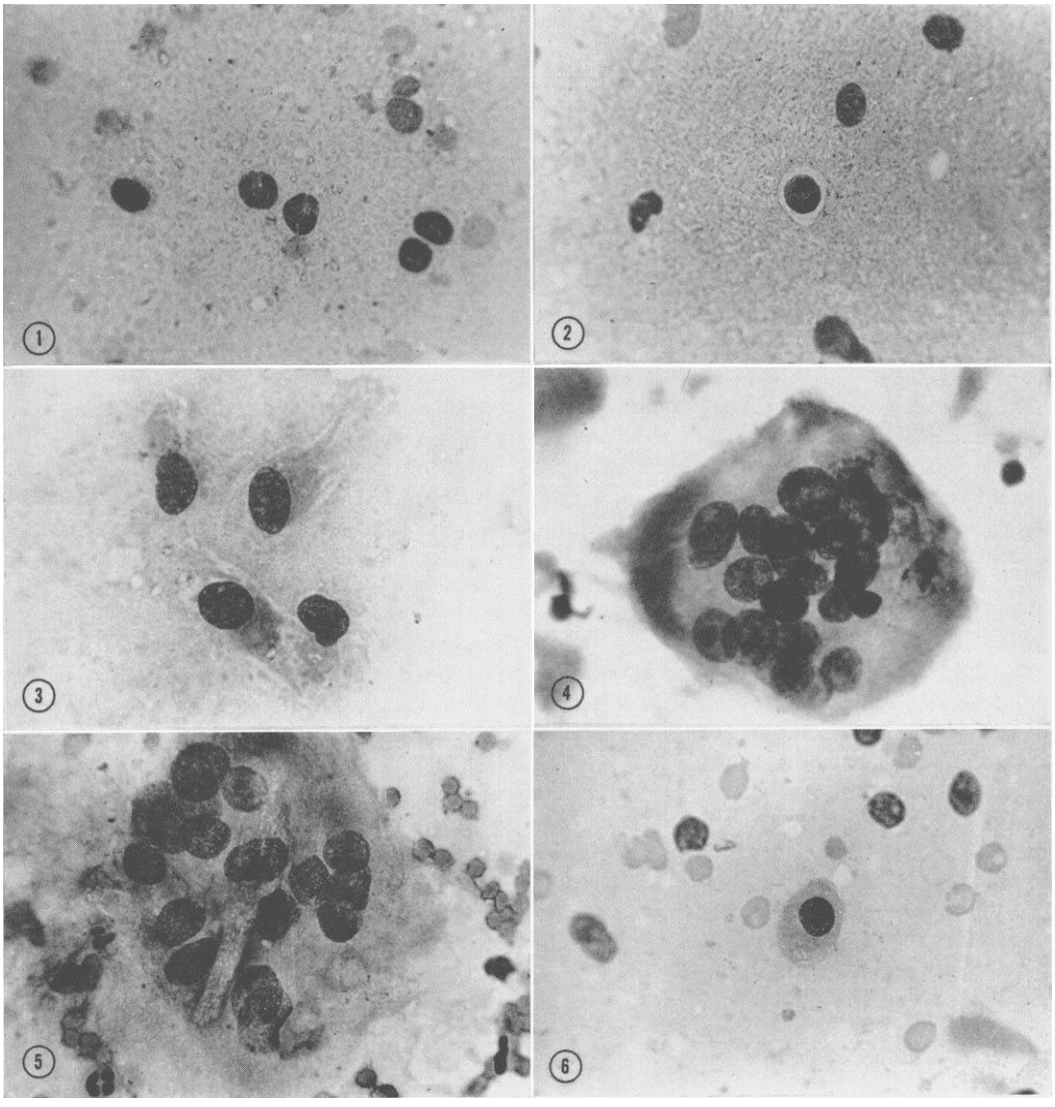


FIG. 1. Group of small cells. Note the dense and closely packed chromatin. Giemsa stain. × 459
FIG. 2. The transparent cytoplasm of one small cell is visible here against the granular background. Giemsa stain. × 459.
FIG. 3. "Blue cells" showing the thorny and angulated cytoplasm, characteristic of these elements. Giemsa stain. × 459.
FIG. 4. Cellular syncytia. Note the enormous amount of nuclei, the evenly stained protoplasm and the sharp outline which tends slightly to the angulation. Giemsa stain. × 459.
FIG. 5. Enormous cellular syncytia. In some parts of the cytoplasm there is an areolar aspect, presumably keratinized. Giemsa stain. × 459.
Fig. 6. Eosinophilic cell in the center of the field. Small cells surrounding it. Giemsa stain. × 459

noma. Eosinophilic cells are approximately two or three times the size of an erythrocyte. The most typical feature is the round and intensely eosinophilic cytoplasm, evenly colored or sometimes slightly granular. The nucleus has a round or oval outline, and stains intensely violet with Giemsa stain (fig. 6). In many elements the nucleus is pyknotic or may even be reduced to a dot. In some cases the nucleus is surrounded by a blue background questionably denoting keratinization. We have judged these cells to be dyskeratotic elements.

It is difficult to distinguish these cells from mastocytes, which possess a protoplasm with

reddish metachromasia that could be confused with the protoplasm of the eosinophilic cell. Mastocytes usually have coarser granulations, but when they are closely packed the cytoplasm may assume a uniform appearance.

Melanocytes.—The most striking finding in smears of these tumors undoubtedly was the great number of melanocytes in slides of many basal cell carcinomas which, on clinical or histologic grounds, gave no indication of such an abundance of melanocytes, even when special histologic technics for melanin were used. In the smears the melanocytes were easily recognizable, and the cells could be identified with Giemsa stain, even under the low-power objective.

The melanocyte of the basal cell carcinoma is widely variable as to size and shape; it may be very large or small, may be pigmented or achromic and may have abundant or scanty protoplasm. The morphologic description will be given below, in the remarks pertaining to cytologic study.

1. Typical Melanocyte.—The nucleus is the most characteristic part of this cell. The size of the nucleus ranges from two to fifteen times that of the erythrocyte. As a rule it is oval or kidney-shaped, with no indentation, and it has remarkable morphologic uniformity. The chromatin, arranged in a very fine network, is dust-like in some areas. A uniform, pale blue nucleolus often is seen within the chromatin mesh. The size and shape of the cytoplasm of the melanocyte vary infinitely. In some instances long dendrites are present, in which case the shape may resemble that of an octopus, star or spoon. In other instances the dendrites are bipolar, ameboid, with blunt ends, or are absent.

Several types of cytoplasm have been observed in melanocytes of basal-cell carcinomas: one is a uniform type of cytoplasm which stains pale blue or gray with Giemsa stain; another is a colorless cytoplasm which appears well defined against the stained background. One of the most frequently encountered types of cytoplasm is foam-like. In some smears most of the melanocytes would belong in the latter category. The area surrounding the nucleus best exhibits the foam-like quality. In that area the network is less dense and the areolar spaces are wider. The foam-like quality also is to be seen in the peripheral dendrites which appear as a pale tree against a darker background. The foam-like appearance apparently is produced by minute vacuoles of different size, always clearly limited by a cytoplasmic membrane.

Melanin granules appear as India-ink dots under Giemsa stain. They may be absent or very sparse or may fill the cytoplasm almost completely. They may be spread evenly or may be found only at the outermost periphery. The size ranges from that of a very fine dust to that of coarse granules almost equal to the size of the granules found in melanophores. In some melanocytes the melanin granules are attached only on the distal pole of the dendrites. In others the melanin granules are seen filling the spaces of the cytoplasmic mesh or are noted to be limited to some areas. Other granules, which might be premelanin, are present in some melanocytes. They stain gray-blue with Giemsa stain. In some instances the cytoplasm has no granules, but possesses a smokelike hue.

The melanocytes in the smears yielded the same histochemical reactions as those of the melanin-producing cells in the histologic sections. When dopa stain was used after the method of Iijima and Watanabe,[22] with incubation periods of 3 to 6 hours, the cytoplasm of the dendritic cells became smoky or brown, a change which in some cases made the foam-like quality even more evident (fig. 7). The nucleus appeared to be unstained. The change was best disclosed when the combination of dopa and Giemsa stains was employed. Tyrosine incubation and counterstaining with Giemsa stain produced no changes in all smears thus treated. When silver stains (silver carbonate or Masson-Fontana) were used, it was possible to demonstrate the argentaffin nature of the cytoplasmic granules. In some cells the silver-blackened aggregates were so fine that only in the periphery of the cytoplasm could be visualized. In others all the cytoplasm appeared to be heavily stained.

The melanic nature of these granules also was demonstrated by the characteristic changes present in these cells in smears treated with Schmorl and Lillie stains. Decoloration of the melanic granules was more difficult in the smears than in the histologic specimens. When solutions of potassium permanganate or hydrogen peroxide were used, decoloration could be obtained only incompletely in smears if 48 to 72 hours was allowed to elapse for the process.

The cytologic aspects of basal cell carcinomas

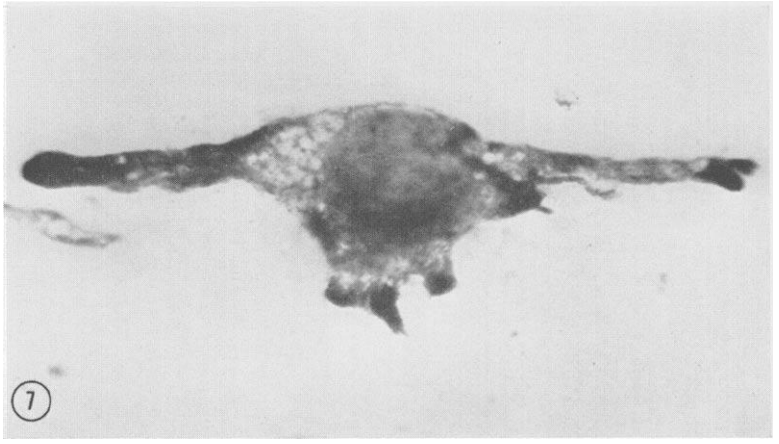


FIG. 7. Melanocyte showing the characteristic areolar aspect of cytoplasm. The pigmentation is more pronounced in the poles of dendrites. Dopa stain. × 977.

include some very characteristic morphologic pictures of melanocytes appearing repeatedly and uniformly in many smears. We have classified these types in the following manner.

2. Nevoid Melanocyte or Micromelanocyte.—This type of melanocyte is the only one found in a large number of these tumors. The nucleus is perfectly oval, with or without a nucleolus; the appearance is identical in all elements, and monomorphism also is characteristic. The cytoplasm may be rounded or cuboidal, with pseudopods or with short, thick dendrites sometimes appearing only on one side (fig. 8). In some elements the melanin is so diffused that the cytoplasm stains with a hue between gray or blue, producing images which it is difficult to interpret. Occasionally, the melanin appears to have accumulated on one side of the nucleus, forming a triangular cap. The melanin granules usually found in the micromelanocyte are coarser than those found in the larger melanocyte. The term, "nevoid melanocyte", is used here because of the close resemblance between this type of melanocyte and the one found in smears of the intradermal nevi.

3. Macromelanocyte.—The macromelanocyte is found in large numbers in some basal cell carcinomas. The size is so large that sometimes it is not possible to visualize all the cytoplasm of a macromelanocyte in a single field under the high-power objective. The nucleus may exhibit indentations and may appear to be folded or kidney-shaped. The nucleolus sometimes is double. The chromatin is typical of that of melanocytes.

4. Achromic Melanocyte.—In some smears the achromic melanocyte is found in large numbers. It is easily identified by the dendrites and morphologic aspects of the nucleus. As a rule the cytoplasm appears to be colorless, in sharp contrast with the stained background (fig. 9). In other instances the cytoplasm is gray, blue or foam-like (fig. 10).

Clear-Nucleus Cells.—In most smears of basal cell carcinomas we have found a variable number of characteristic cellular elements which we have called "clear-nucleus cells". These may be extraordinarily abundant in some smears in which they constitute 60 to 80 per cent of all cells, or they may be found only in isolated instances in a few fields in other smears. The nuclei, always devoid of cytoplasm, have characteristics identical to those of the melanocytes previously described. The similarity is striking when both types of cells are seen in the same field. Clear-nucleus cells have the same oval-shaped outline, the same disposition of chromatin mesh and analogous morphologic aspects of the nucleoli (fig. 11). In other fields the chromatin is seen to be somewhat denser or it may appear in a more kidney-shaped form or a rather lobulated form. Even the size of the nucleus of clear-nucleus cells is proportionate to the size of the nucleus of melanocytes, being small in the smears in which we found micromelanocytes and larger in smears in which macromelanocytes pre-

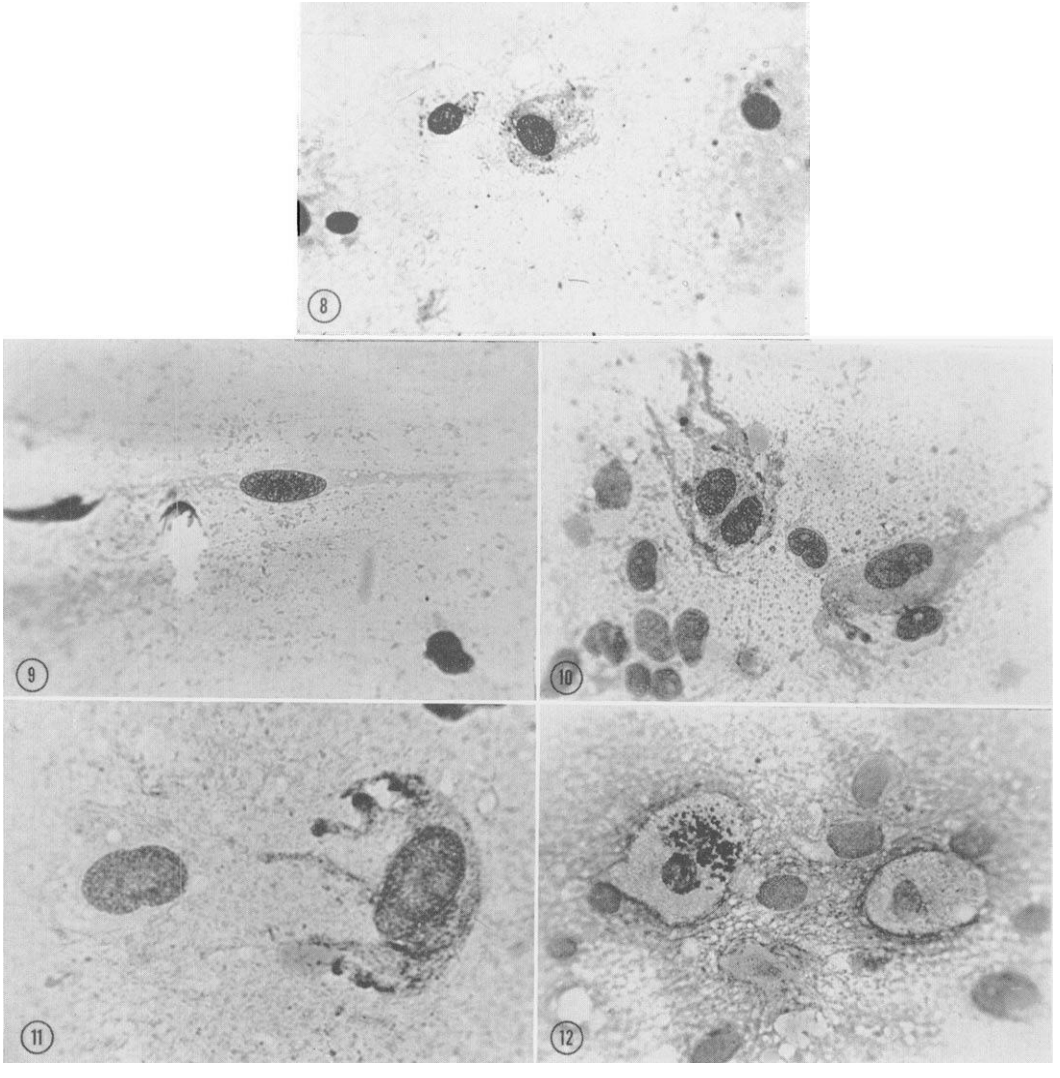


FIG. 8. Nevoid melanocyte. Its nucleus perfectly oval is smaller than those of typical melanocyte. Dendrites are short and blunt. In the same field there are also "clear-nucleus cells" and another melanocyte almost without cytoplasm. Giemsa stain. × 459.

FIG. 9. Achromic bipolar melanocyte. The protoplasm is also vacuolate. Giemsa stain. × 459

FIG. 10. Achromic melanocytes stained smoke-hue with the Giemsa. In the same field there are also some "clear-nucleus cells". Giemsa stain. × 459.

FIG. 11. Clear-nucleus cell and melanocyte. This picture shows the extraordinary similitude between both cells. Giemsa stain. × 918.

FIG. 12. Two chromatophores, one of them almost without melanin. Note the foamy aspect of cytoplasm. Giemsa stain. × 459.

dominated. In some sections stained with Giemsa stain or (with much better results) with the combination of dopa and Giemsa stains, it seemed to be possible to discern a sequence from the clear, acytoplasmic nucleus of these cells to a ramifying and pigmented melanocyte. In the smears containing abundant clear-nucleus cells the second-named combination of stains clearly showed many nondendritic cells with ring-rimmed, dopa-positive cytoplasm and also many instances of beginning formations of dendrites imperceptible under Giemsa stain alone.

The possible relationship between both

types of cells (in the sense that "clear-nucleus cells" could be related to melanocytes) is a question which remains unanswered. In favor of the validity of such a relationship is the fact that in smears of pigmented nevi we also found cells without cytoplasm and with clear nuclei contiguous to fully develop melanocytes. The "clear-nucleus cells" also are very similar to elements found mixed with the melanocytes detected by means of our technic in smears of normal hair bulbs.

Cells From the Inflammatory Infiltrate.—It would be superfluous to describe the morphologic aspects of inflammatory cells more or less abundant in these tumors. The lymphocytes, plasma cells, fibroblasts and histiocytes are well-known elements. In the smears of cutaneous lesions there are, however, some peculiarities. For instance, plasma cells at times are binucleated or atypical, without the characteristic cartwheel distribution of the chromatin. Histiocytes easily can be confused with "clear-nucleus cells". Fibroblasts are recognizable in the smears almost only by the ovoid or elongated nuclei with dense chromatin distributed in a mosaic-like or striped pattern, with clearer triangular or parallel lines through the nuclei. Endothelial cells seen in the smears present a very typical picture.

Notwithstanding the foregoing, two types of inflammatory cells are particularly interesting in connection with the manifold aspects which can appear in smears of these lesions.

1. The Chromatophore or Melanophore.—This type of inflammatory cell is found in extreme abundance in smears of some basal cell carcinoma but may be few in others. This particular cell is, however, an almost constant accompaniment of the cytologic characteristics of these tumors.

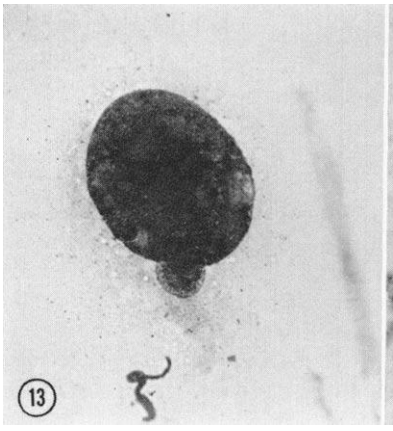
In most cases the chromatophore or melanophage appears with a small nucleus and is slightly larger than an erythrocyte. It is ovoid or ellipsoid, and has chromatin which is disposed in a lax network. The nucleus is surrounded by a transparent and circular protoplasm. Coarse granules of melanin in widely variable quantities are conspicuous in the cytoplasm; they range from very few to so many that they may almost completely obscure the nucleus, appearing in such an instance as black spots of considerable size. At times the cytoplasm is visible only because of this outline formed by the melanin granules disposed in the outermost periphery of the melanophage. The melanophage can attain great volume. In smears it is possible to see that what appears in histologic sections to be melanin depots of the dermis are, in reality, aggregates of voluminous melanophages completely filled with melanin.

In some smears the cytoplasm of these cells is not transparent, but has a foam-like appearance, as if it contained not only melanin but also lipoidic droplets (fig. 12). Nevertheless, this foamy aspect is different from that of the melanocyte, in which vesiculation is lighter, clearer and more nearly delimited. In order to detect a possible lipomelanic macrophagy these smears were treated with Sudan-black, with positive results.

At times these foamy chromatophores are binuclear or plurinuclear, producing, in the latter case, the characteristic picture of a macrophage giant cell. Perhaps vesiculation of the chromatophores is one reason for frequent misinterpretation because of the resemblance of this picture to that of some glandular or secreting elements.

2. Mastocytes.—Mastocytes are described herein because they present manifold aspects which can engender possible confusions. They may be found in great numbers in some smears of these tumors. The size of mastocytes is somewhat variable. The nucleus possesses a dense chromatin which it is difficult to stain with Giemsa stain. The cytoplasm is filled with characteristic cytoplasmic granules and the outline of the cytoplasm may be spherical, ovoid or elongated. Sometimes this outline is dendritic, with expanding processes very similar to those of melanocytes, but nevertheless, with shorter and blunt ends. Frequently, in some fields, the stain is seen to act incompletely on the granulations, so that they become violet. This factor may give rise to additional confusion with melanocytes.

Atypical and Nonconstant Elements.—The cellular anaplastic features frequently found in many other tumors are rarely recognizable in smears of basal cell carcinoma. In some smears of basal cell carcinoma which histologically showed anisocytosis and monstrosities, it is possible to observe voluminous nuclei, irregularly shaped, with protrusions and multiple or giant nucleoli similar to those found in the presence of Bowen's disease or carcinoma *in situ* (fig. 13). It is also possible to find degenerative



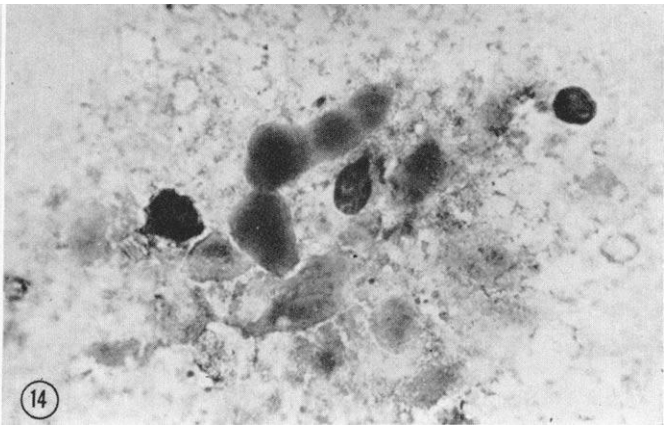


FIG. 13. Basal-cell carcinoma of Bowenoid type. Monstrous nucleus devoid of cytoplasm with a protrusion in one pole. The dense and granular chromatin is irregularly distributed. It has an extraordinary similitude to the Bowen cell as it appears in smears. Giemsa stain. × 536.
FIG. 14. Eosinophilic masses resembling cellular structures. Giemsa stain. × 536

faults as karyorrhexis, nuclear pyknosis, cellular "shadows" and a mucous aspect or vesiculation of the cytoplasm. Many of these figures are very difficult to classify, and can cause frequent misinterpretations.

In these smears epithelial cells which have become completely keratinized often have been found in the form of anuclear plates of quadrangular, pentagonal or triangular shape; they may be isolated or shriveled or aggregated in mass. It is also possible to observe keratinocytes which have a fusiform or rectangular outline and a protoplasma which is blue, yellow, light red or is completely transparent.

In the morphea-like basal cell carcinoma it is very difficult to classify correctly the cellular elements of tissues viewed in smears. The chromatin of all the cells is so closely packed that in most cases it is impossible to distinguish it.

Background of the Smears.—The acellular substance of the background of the smears in some cases has a granular aspect; in others it is rather mucoid or filamentous; in still others it stains uniformly. Naturally, cystic and cylindromatous carcinomas are those in which the amorphous substance is more abundant. In these smears are seen round masses of a pasty and intensely eosinophilic substance, sometimes so voluminous that they occupy the complete field under the oil-immersion objective. Some smaller masses appeared to possess a structure and distribution which resembled in some manner those of the degenerated cells (fig. 14). Some smears were literally filled with these formations. Frequently we have found these masses in basal cell carcinomas of the eyelids. The histologic appearance of these masses has been searched for unsuccessfully by us in the corresponding histologic preparations.

COMMENT

All histologic preparations of basal cell carcinoma exhibited in cytologic smears the types of cells we have described. The relative number of each type varied widely in different smears. We believe that this fact points to a common origin of all such tumors. Some of the observations in the present report seem to suggest the hypothesis of pilar derivation of basal cell carcinomas. The process of keratinization undergone by epidermal cells, with progressive vacuolization or transparency of the cytoplasm and pyknosis of the nucleus, has not been observed in smears of these carcinomas. Keratinization of individual cells of squamous cell carcinomas has been observed only in the "eosinophilic cells". On the other hand, we did find typical keratinizing multinuclear cells, with sudden keratinization of the cytoplasm, and these cells have been found also in smears of other processes with neoformation or destruction of hair. In the presence of the latter disturbances, a cell which very closely resembles the "blue cell" also has been observed.

These facts and the great number of melanocytes exhibiting active and possibly neoplastic

proliferation of the pigmentary system, as well as the presence of the "clear-nucleus cells" which we have described, mixed with melanocytes in smears of the normal hair bulb, seem to favor the theory of the "primary epithelial germ" origin of these tumors.

SUMMARY AND CONCLUSION

A simple method is described for obtaining smears from cutaneous diseases for cytologic purposes. With the aid of this technic 892 smears from 156 basal cell carcinomas were studied, with the following results.

1. We found in these tumors epithelial cells of three types: (1) elements devoid of cytoplasm, with closely packed chromatin which we have designated "small cells"; (2) cells possessing a characteristic blue cytoplasm—"blue cells"—and (3) dyskeratotic elements possessing an acidophilic cytoplasm—"eosinophilic cells". Also in this group were seen some rather unusual syncytial formations which possibly are the cytologic versions of the "keratinization centers" (keratin-cell complex) seen in histologic preparations of sections of these tumors.

2. Moreover, in many smears a variety of types of melanocytes were seen in an abundance unsuspected on clinical or histologic grounds.

3. Finally, we found a bizarre cellular element, the "clear-nucleus cell", devoid of cytoplasm and characterized by a structural chromatin pattern which resembles closely that of melanocytes.

The peculiarities of the dermal infiltrate in the smears also are described.

On the basis of this study, the authors believe that the melanocytic system possibly is an active factor in these tumors, playing the role of participant in the neoplastic proliferation.

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