An Electron Microscopic Study of Carcinoma \textit{in Situ} and Invasive Carcinoma of the Cervix Uteri\(^*\)

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SUMMARY

A comparative study between normal cervical stratified squamous epithelium, squamous-cell carcinoma \textit{in situ}, and invasive squamous-cell carcinoma of the cervix has been carried out by electron microscopy. The nuclei of malignant tumor cells reveal the following notable alterations: increased nuclear membrane infoldings, increased density of chromatin granules just inside the nuclear membrane, and enlargement of nucleoli with more prominent differentiation between dense and finely granular areas. These changes are more prominent in invasive carcinoma than in carcinoma \textit{in situ}. In the cytoplasm, mitochondria are more numerous than in comparable normal cells, and they are enlarged and swollen. Glycogen is absent from invasive carcinoma cells but present as occasional small droplets in the cytoplasm of carcinoma \textit{in situ} cells.

Attachment plates are still present between carcinoma cells, but they are less numerous and not so regularly arranged as in normal epithelium. Also, there is an absence of the usual dense cytoplasmic plaque at the attachment plates in some carcinoma cells. Cytoplasmic villous projections are present at the cell membrane of carcinoma cells, while they are not found in normal cervical epithelium.

The basement membrane is found to be complete and intact in carcinoma \textit{in situ}, but it is straighter than normal. In invasive carcinoma, the basement membrane is usually absent but may be present in imperfect form. Where the basement membrane is absent, small protrusions of carcinoma cell cytoplasm in the adjacent stroma can be seen, apparently representing the ultrastructural aspects of invasion by malignant neoplastic cells.

The results of an electron microscopic and histochemical study of the epithelium of the normal cervix has recently been reported (3). The present material represents an extension of this investigation, including a study of carcinoma \textit{in situ} and invasive squamous-cell carcinoma of the cervix, undertaken in a manner similar to that utilized in the study of normal cervical epithelium. Albertini \textit{et al.} (1) and Vogel and Glatthaar (15) have recently reported some observations on the electron microscopy of cervical carcinoma. However, much is still to be learned about the ultrastructure of these lesions. In particular, the changes in carcinoma \textit{in situ} and invasive carcinoma require further comparative study and correlation. Additional study of the fine structure of malignant cells in general is especially needed for a further understanding of the biology of malignant neoplasia. In addition to nuclear and cytoplasmic changes in malignant cells, the status of basement membrane, the relationship and attachment of tumor cells to one another, and the ultrastructural aspects of neoplastic cell infiltration are some of the features of malignant tumors that may be clarified by electron microscopic study.

MATERIALS AND METHODS

Invasive squamous-cell cervical carcinomas from six patients and tissue from carcinoma \textit{in situ} of the cervix in five patients were studied.

Blocks of tissue from the cervix obtained by biopsy and from hysterectomy specimens were fixed for 2 hours in a chrome-osmium solution adjusted to pH 7.4 (8). All tissues studied were fixed immediately upon their removal from the...
patient. After fixation, the specimens for electron microscopic study were dehydrated in graded ethyl alcohol solutions, embedded in methacrylate, and sectioned on a Porter-Blum microtome at approximately 250A with the diamond knife (11). The sections were placed on celloidin-coated grids and examined with an RCA-EML microscope. Thick sections of plastic-embedded material were also prepared and stained with hematoxylin and eosin for correlation with electron micrographs.

Sections of tissue were taken on either side of and immediately adjacent to the specimen removed for electron microscopic study and were processed for paraffin sections for additional histological study and primary identification of the lesions.

RESULTS

In accordance with the findings of light microscopy (Figs. 1, 2) electron microscopic study showed enlargement and variability of size of nuclei in invasive and in situ carcinoma of the cervix. The fine structure of the cells in the in situ and invasive lesions was essentially similar, differing mainly in degree of alteration from the normal.

Many of the nuclei of the malignant cells revealed marked irregularity of contour due to deep clefts and infoldings of the nuclear membrane (Fig. 3). This was considerably more marked in invasive as compared with in situ carcinoma. The nuclear infoldings extended for variable distances, and an appreciation of their extent requires a consideration of the fact that the two-dimensional effect failed to show in any one nucleus the full extent of this process. Narrow extensions of cytoplasm were commonly seen extending into these clefts, and in some instances the islands of cytoplasm appeared to be isolated within the nucleus. The nuclear membrane itself showed the usual double profile and the presence of nuclear pores, which were seen in like manner in normal cells. In almost all nuclei of invasive carcinoma cells there was found a zone of increased density of granules located just within the nuclear membrane. Similarly, zones of dense, granular nuclear material extended inward along the infoldings of the nuclear membrane. Some of these dense areas, deeper within the nucleus, were sectioned at a plane which did not include the infolded nuclear membrane. Such dense chromatin areas within the nucleus were apparently identical with the deeply stained nuclear bodies seen in light microscopy, which have been called pseudonucleoli (15). In carcinoma in situ lesser degrees of condensation of fine chromatin granules at the nuclear membranes were found. The chromatin granules themselves were of normal size, but they were more densely arranged in the nuclei of carcinoma cells than in normal cells.

The nucleoli in these malignant tumor cells were larger than normal and sometimes multiple. A more abundant, dense nucleolar component was noted, while maplike areas of a less dense, almost homogenous material were also seen within the nucleoli (5). These latter areas were similar in density to the fine nuclear granules, and in many areas, by fortuitous section, they were found continuous with nucleoplasm (Fig. 4). A striking feature of these nucleoli was their frequent location at the base of a nuclear cleft. Appropriate sections showed the nucleolus to lie almost in contact with the crypt of an infolding of the nuclear membrane (Fig. 5). A zone of cytoplasm could usually be seen within the cleft, extending into the nucleolar area. Dense aggregates of chromatin granules were also found surrounding the nucleoli in the same manner that they were approximated to the nuclear membrane and its infoldings.

In the cytoplasm of cells of invasive and in situ carcinoma, certain changes were also noted when they were compared with normal cervical epithelium. Mitochondria appeared to be increased in number, and they were somewhat larger. Cristae were less well formed, frequently being incomplete, and the matrix of the mitochondria appeared swollen and vacuolated. RNP granules were more numerous and were densely arranged throughout the tumor cells, but the individual granules were not measurably enlarged. There was a greater tendency for them to occur in small clusters, however, in carcinoma cells. No well formed endoplasmic reticulum was noted in the cytoplasm of cervical carcinoma cells, but neither was the endoplasmic reticulum prominent in normal cervical epithelium. It is notable that in the cells of carcinoma in situ occasionally small droplets of glycogen formation were visible (Fig. 6), while in invasive carcinoma cells no glycogen formation has been observed.

The cell membranes of invasive and noninvasive carcinoma were the seat of certain alterations. The attachment plates were still present in both, but they did not occur as regularly as in normal epithelium (Figs. 7, 8). Occasionally, there were seen major segments of the cell membrane where attachment plates were completely absent, whereas in normal cervical epithelium they were regularly placed about every one to two micra. The structure of the attachment plates in carcinoma cells was quite similar to that found in nor-
clefts of attachment plates were not as prominent in carcinoma cells as in normal epithelium, and dense cytoplasmic plaques on either side of the sometimes in carcinoma they were entirely absent (Fig. 9). More striking than these changes was the occurrence of numerous, delicate villous-like cytoplasmic projections on the surface of carcinoma stratified squamous epithelium. However, the occurrence of numerous, delicate villous-like cytoplasmic projections on the surface of carcinoma cells. They were present in both invasive (Fig. 8) and in situ carcinoma cells (Fig. 10) but were much more marked in the invasive tumors. These delicate projections extended into the intercellular spaces, where they remained free from attachment. Usually from three to five were present in the area between two attachment plates, and a few projections actually arose from the sides of the plates.

Intercellular spaces between carcinoma cells were increased in size. There was a considerable amount of finely granular material in these spaces, and occasionally they contained small lipide droplets, mitochondria, and other cellular debris. Frequently, remnants of dead tumor cells were surrounded by enlarged intercellular spaces (Fig. 11). This was observed especially in invasive, but to a lesser degree also in noninvasive, carcinoma. Also, in some intercellular spaces of invasive carcinoma, collagen fibrils were observed (Fig. 12). They were not encountered, however, in the noninvasive carcinomas.

In noninvasive carcinoma the basement membrane was clearly present and continuous (Fig. 13). The membrane was characterized by a straightness of course, whereas in the normal it was markedly folded, following the contour of foot processes of the basal cells (Fig. 14). In invading carcinoma of the cervix the basement membrane was variable. Usually it was completely missing. In a few instances, however, a complete basement membrane could be seen around a deep and obviously invasive nest of carcinoma cells. More often, small isolated segments of basement membrane material were observed around the invading cell group (Fig. 4), and where it was present it formed a sharp demarcation line between cytoplasm of tumor cells and adjacent collagen fibrils. When it was absent, however, pseudopod-like extensions of cytoplasm of tumor cells might be seen among adjacent collagen fibers (Figs. 4 and 15). In some instances whole tumor cells were seen lying in the stroma, surrounded on all or several sides by collagen fibrils. Numerous dense, narrow cytoplasmic masses were sometimes found near a main nest of neoplastic cells but separated from the nest by collagen fibrils. By the density of RNP granules, these cytoplasmic masses were determined to be distant extensions of the tumor cells, failing to show, at that plane of section, the connection to the cell body from which they arose.

**DISCUSSION**

There are certain quantitative differences in the fine structure of the tumor cells in in situ carcinoma of the cervix as compared with invasive carcinoma. The main points of difference are that in invasive carcinoma the nuclei are more deeply and irregularly clefted; the nuclear fine granules show greater condensation at the nuclear membrane; finger-like formations at the cytoplasmic membrane are more pronounced; and the interface of the malignant cells of invasive carcinoma with adjacent stroma is broken by cytoplasmic protrusions into the connective tissue spaces at sites where basement membrane is missing. Further studies of additional examples of noninvasive and invasive carcinoma are required, however, before these differences can be evaluated definitively as significant criteria to distinguish invasive from noninvasive carcinoma.

Although it has been stated (4) that the study of the ultrastructure of nuclei in malignant cells has not revealed any outstanding differences from the nuclei of non-neoplastic cells, in this study several variations from comparable normal cells have been described. These are believed to have a significant bearing upon the growth characteristics of malignant cells. One of the variations from normal is the infolding and cleft formation of the nuclear membrane. These have been noted previously (9) in malignant tumor cells. They are especially marked in the cells of invasive cervical carcinoma but occur to some extent also in noninvasive cervical carcinoma. We have also observed marked infolding of the nuclear membrane in the malignant cells of a mouse leukemia (L1210) and in several cases of human leukemia. It is probable that alterations of nuclear structure in malignant tumor cells may be related to their increased production of DNA protein (6). Foldings and clefts of the nuclear and cytoplasmic membrane could result in increased nuclear and cytoplasmic interface, thus providing an increased supply to the nuclear material of precursors of DNA protein. The increased density of chromatin granules in the zone just within the nuclear membrane, and extending inward into the nucleus along the course of the membrane infoldings, might be interpreted as the site of formation of new DNA protein from materials passing from cytoplasm into the nucleus.

1. Unpublished data.
Hypertrophy of the nucleolus and multiple nucleoli are well known to occur in malignant tumor cells. The inner structure of the nucleolus can be studied in detail with electron microscopy, and the relationship of the nucleolus to the nuclear membrane can be demonstrated in many nuclei. The nucleolus reveals two distinctive components, differing in electron density. There is a strong suggestion that the less compact portion is continuous and may be identical with the fine granules of the nucleus. The nucleolus might therefore be suspected of playing a role in DNA protein formation. The presence of dense aggregates of fine nuclear granules around the nucleolus further supports such a role. The close relation of nucleolus to the crypts of nuclear membrane infoldings and to the accompanying band of cytoplasm would provide a means of supply of precursor materials to a site of increased synthesis of DNA protein. By this means, the well known process of nucleolar hypertrophy and duplication in malignant cells may be correlated with increased synthesis of DNA protein within the nucleus and the resulting nuclear enlargement.

Alterations in cytoplasmic structure have not been completely evaluated in malignant tumor cells, although variations in function and differentiation have been described (9). Bernhard (4) refers to the variability of mitochondria in different malignant tumors. In the cells of carcinoma of the cervix, mitochondria appear to be increased in number. The significance of this is not apparent. Evidently, there is no correlation between the number of mitochondria and the formation of glycogen, since this product of functional differentiation is entirely missing in the carcinoma cells. The abnormal swelling and vacuolation of mitochondria, which were observed in cervical carcinoma cells, have been described in other malignant cells (4) and may prove to constitute a basic change in the cells of malignant neoplasms. However, in view of the known occurrence of mitochondrial swelling as the result of fixation artifact, and of mild cellular degeneration, it is difficult to interpret this change in malignant neoplastic cells.

It has been suggested that invasive properties of malignant cells may be related to a deficiency of intercellular connections (7) leading to decreased adhesiveness between the tumor cells. It is, therefore, of considerable interest to observe that the cells of invasive squamous-cell carcinoma of the cervix continue to form attachment plates, even though there is some deficiency in this form of structural differentiation. It is questionable whether the degree of this abnormality is sufficient to lead to a lack of cellular adhesiveness. Probably more closely associated with the invasive property is the behavior of malignant cells at their interface with connective tissue. The absence of complete delineation by a basement membrane may be of significance in accounting for the penetrating invasion of malignant tumor cells. The degree of resistance to invasion of neoplastic cells which is exerted by basement membrane is still uncertain, because of lack of detailed knowledge of its physical characteristics. However, since tumor cells do not extend between collagen fibrils when basement membrane is present, it appears probable that the membrane exerts a restricting influence on their outward extension and invasion. Furthermore, it is not unusual to see extensions of cytoplasm of the tumor cells for considerable distances into the spaces between collagen fibrils when basement membrane is absent. On the basis of this behavior of malignant tumor cells, the following mechanism of neoplastic cell invasion may be postulated: In the absence of basement membrane, tumor cells first extend pseudopod-like protrusions of cytoplasm into adjacent stroma; as the result of cell multiplication and increasing pressure within the cell nest, the entire cell body protrudes into the stroma, following its "pseudopod" through a-

Fig. 1.—Photomicrograph of squamous-cell carcinoma of cervix in situ. The squamous epithelium reveals an anaplastic appearance, but "basement membrane is intact." Hematoxylin and eosin stain. Mag. X380.

Fig. 2.—Photomicrograph of invasive squamous-cell carcinoma of cervix. Isolated groups of anaplastic tumor cells are seen, surrounded on all sides by stroma. Hematoxylin and eosin. Mag. X380.

Figures 3-15 are electron micrographs. A 1-μ marker is located in the lower left hand area of each picture.

Fig. 3.—A neoplastic cell of invasive carcinoma. A large nucleolus (NU) are apparent, both of which are located near nuclear membrane clefts. Condensations of nuclear fine granules (NG) inside the nuclear membrane and around the nucleolus can be seen. Magnification approximately X5400.

Fig. 4.—A cell of invasive carcinoma. A large nucleolus is observed. A dense material (D) is present, as well as a less dense, finely granular substance (G) which is continuous with the nucleoplasm. An isolated crypt of a nuclear cleft (X) is noted, seemingly within the nucleus. It is, however, surrounded by nuclear membrane around which a dense collection of fine nuclear granules can be seen. Such a collection of nuclear material would be visible as a small pseudonucleus in light microscopy. A small segment of incomplete basement membrane (BM) is observed between the stroma (S) and the carcinoma cell. Swollen and vacuolated mitochondria are observed in the cytoplasm. Magnification X5400.
FIG. 5.—A cell of invasive carcinoma. The nucleus contains several notches and infoldings (X) of the nuclear membrane. Some of the clefts extend in the direction of the nucleolus. Magnification X5400.

FIG. 6.—Portions of two cells in carcinoma in situ. The nuclear membrane (N) has shallow infoldings. A large nucleolus (NU) is present. There is a small droplet of glycogen (GL) in the narrow rim of cytoplasm. Magnification X5400.

FIG. 7.—A cell of precornified zone of normal cervical stratified squamous epithelium. Small, dense mitochondria (M) which were verified in higher magnifications, and peri-nuclear glycogen (GL) are seen in the cytoplasm. A small, dense nucleolus (NU) is present within the nucleus. The attachment plates (AP) are regularly placed, and prominent, dense areas are seen on either side of the junction of the adjacent intercellular trabeculae. The cell membrane between adjacent attachment plates is smooth, and no cytoplasmic projections are seen. Magnification X5400.

FIG. 8.—Portion of a cell and intercellular space in invasive carcinoma. Attachment plates (AP) are present, but less regularly placed than normal. Large segments of cell membrane (CM) are devoid of attachment plates. Numerous cytoplasmic projections (P) are seen along the cell membrane, extending into the intercellular spaces (IS). Magnification X8000.
FIG. 9.—Portions of two cells in invasive carcinoma. Two attachment plates (AP) are noted between adjacent cells. In neither of these is there a dense plaque of cytoplasm, which is always present in the attachment plates of normal epithelium. Magnification ×18,000.

FIG. 10.—Portions of three cells in carcinoma in situ. The intercellular spaces (IS) between three adjacent cells are shown. Two attachment plates (AP) are present, and several cytoplasmic projections (P) are noted. These are less numerous and complex than those in invasive carcinoma. Magnification ×19,000.

FIG. 11.—Area in invasive carcinoma. A disintegrating cell (DC) is present, between adjacent viable cells. Its nuclear material (N) is dense and vacuolated. A body resembling a nucleolus (NU) is noted in the cytoplasm. Although the cell is apparently dead or dying, mitochondria (M) which were verified in higher magnification, cell membrane (CM), and attachment plates (AP) are still evident and seem to be intact. Magnification ×5400.

FIG. 12.—Portions of two cells in invasive carcinoma. Collagen fibrils (C) are seen in the intercellular spaces between adjacent neoplastic cells (A and B). Magnification ×9000.
Fig. 13.—Basal area of carcinoma in situ. The basement membrane (BM) is present and intact. The membrane is straight and smooth (compare with basement membrane in normal epithelium in Fig. 14). Magnification ×11,000.

Fig. 14.—Basal area of normal cervical epithelium. The basement membrane (BM) follows a tortuous course owing to foot processes (F) of the basal epithelial cells. Intercellular spaces (IS) are wide, and at this level the basal cells have narrow bodies. Magnification ×9000.

Fig. 15.—Portions of two cells in invasive carcinoma. At the junction of stroma (S) and carcinoma cells (A and B), the basement membrane is missing. At (X) a long cytoplasmic projection from the carcinoma cell can be traced into the stroma. Several lipid droplets (L) are found in the more distally placed cytoplasmic extension. Magnification ×9000.
boid movement; these cells then grow and divide in the new location, enlarging the original carcinoma cell group or setting up new and separate satellite foci of growth. By this means, collagen fibrils would be, and are so observed, isolated between the carcinoma cells of newly invading groups. Such an ameboid motility has been ascribed to malignant tumor cells by other observers (10).

These observations suggest that basement membrane is not a static material that is once formed and remains unaltered for an indefinite period. Instead, it must be assumed that, when the carcinoma cells have extended into a new area and have set up an additional or new junction line with collagenous tissue, a new basement membrane lamina is produced. This is probably derived from the connective tissue elements themselves. Thus, the demonstration of basement membrane around a cluster of tumor cells does not necessarily indicate that it is carcinoma in situ.

The cytoplasmic membrane of carcinoma cells exhibits an interesting alteration in the presence of villous-like processes which extend into the intercellular spaces. These are seen especially in invading carcinoma but also to some extent in carcinoma in situ. These projections are easily differentiated from attachment plates in that they are more delicate and do not intercommunicate to form intercellular bridges. Similar cytoplasmic projections from other types of malignant cells have been observed (14). These projections increase the surface area of contact between the tumor cells and their fluid environment, thus probably increasing the supply of nutritive materials to the rapidly growing cells. It is also possible that they may be related to the phenomenon of pinocytosis (12), through which fluid and particles are drawn into the cell at the cytoplasmic membrane. These cytoplasmic projections are probably somewhat mobile, and it is conceivable that they may be alternately extending and retracting in the living state.

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REFERENCES

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