Methylcholanthrene-Induced Carcinoma of the Mouse Cervix: An Electron Microscope Study

G. Randolph Schrod and Charles D. Foreman

(Department of Pathology, University of Louisville School of Medicine, Louisville, Kentucky)

SUMMARY

Fine structural alterations in the squamous epithelium of mouse cervix following application of the carcinogen 20-methylcholanthrene have been studied with the electron microscope. Only minor nuclear changes that are not seen with the light microscope were demonstrated. Focal dissolution of the basement membrane which separates the squamous epithelium from the stroma, and projections of epithelial cytoplasm into the stroma characterize the stage of early invasive carcinoma.

Dense intramitochondrial bodies were noted in basal epithelial cells of treated animals before the stage of hyperplasia, but not in control animals. The possible significance of this mitochondrial change is discussed briefly.

METHODS AND MATERIALS

Virgin female C3H mice, obtained from Jackson Laboratory, Bar Harbor, Maine, were maintained on routine laboratory food; six animals were used as controls. Beginning at the age of 11 weeks, in the remaining animals the cervix was painted tri-weekly with a 1% solution of 20-methylcholanthrene in acetone. Two animals were sacrificed at weekly intervals from age 15 weeks (after 12 paintings) to age 30 weeks (after 58 paintings). In addition, 2 animals were sacrificed 7 weeks after the 58th painting and 2 animals 10 weeks after the 58th painting.

Under ethyl ether anesthesia the abdomen was opened and the uterus and upper vagina excised and placed in cold buffered sucrose osmium (8). The cervix was rapidly dissected from the adjacent tissue and cut into small blocks. Fixation was continued at 2°—4°C for 1 hr. Following dehydration in graded alcohols the tissue was embedded in Epon 812 according to the method of Luft (26) or in 8:1 butyl-methyl methacrylate with the addition of 5% benzoyl peroxide. The methacrylate was polymerized at 37°C under ultraviolet light.

Thick (1.0—1.5 μ) sections stained with hematoxylin and eosin or toluidine blue were examined with the light microscope. Thin (0.05—0.06 μ) sections stained with lead hydroxide were examined with a Siemens Elmiskop I electron microscope.

In the past few years a number of reports on the fine structure of epidermoid carcinoma of the uterine cervix have been published. Most of these reports have described spontaneous carcinoma of the human cervix (2, 3, 7, 15—17, 20, 21, 27, 28, 35); a few have discussed chemically induced carcinomas of the mouse cervix (12, 13, 31, 34).

In general, it has been demonstrated that the fine structure of malignant squamous epithelial cells differs quantitatively from normal squamous epithelial cells but not qualitatively. It is of interest that viral-like bodies have been noted at times in experimentally induced cervical carcinoma in mice (13, 34).

The carcinogen 20-methylcholanthrene has been used in this laboratory during the past few years to induce epidermoid carcinomas in the cervix of C3H mice. In addition to a light microscopic study of the cervical lesions as they progressed to carcinoma, determinations of nuclear mass, water content, and nuclear deoxyribonucleic acid (DNA) were made at various stages of cancer induction in an attempt to determine at which point irreversibility of the lesion begins (4, 5, 10, 11).

When 20-methylcholanthrene is applied tri-weekly to the mouse cervix a sequence of changes in the epithelium occurs with regularity. After 14 applications inflammation is present but there is no cellular atypia. Basal cell hyperplasia is usually present after 28 applications and dysplasia after 36 to 40 paintings. It takes on the average 54 applications for mice of the C3H strain to develop invasive carcinoma. A stage similar to in situ carcinoma as described in the human cervix is usually not recognized.

Furthermore, when the animals are treated only 14 times and then followed by sequential cervical smears, dysplasia frequently occurs but usually regresses. When the cervix is painted only 28 times and then followed by cervical smears, dysplasia occurs and invasive carcinoma usually follows.

It is the purpose of this paper to describe certain fine structural changes in the mouse cervix following treatment with 20-methylcholanthrene.

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OBSERVATIONS

Although they have been described previously (1, 2, 16, 23, 24), certain fine structural features of the basal layers of normal squamous epithelium of mouse cervix deserve mention. Most striking is the markedly irregular contour of the cell membrane of basal cells (Fig. 1). There is a correspondingly irregular contour of the basement membrane. The nuclei of basal cells are occasionally irregular in outline, but deep nuclear clefts are unusual. Nucleoli are prominent. Cristae are most often oriented transversely to the long axis of the mitochondria. The endoplasmic reticulum is not prominent; most appears to be of the rough surfaced variety. The majority of ribonucleoprotein particles are unrelated to membranes. Golgi zones are small.

The first fine structural alteration in the cervical epithelium of the experimental animals was seen after 16 paintings. In some of the mitochondria of the basal cells dense homogeneous bodies were present (Fig. 2). These bodies varied in size from 0.1—0.4 μ. They were found in the epithelial cells of subsequently sacrificed animals up to but not including the stage of invasive carcinoma. Frequently a single mitochondrion contained several bodies (Fig. 3).

With progression of the lesions, infoldings of the nuclear membranes were more prominent and numerous. The nucleoli were generally larger than those of normal epitelium and were frequently multiple. Significant widening of the intercellular spaces of the squamous epithelium was evident after 29 or more paintings with the carcinogen. With widening there was a corresponding increase in the number of villous projections of the epithelial cell surface into these spaces (Fig. 4).

At the stage of dysplasia (36—40 paintings) degenerating epithelial cells were frequently present. These cells contained fragmented nuclei, myelin figures, and numerous lysosomes. Less numerous but structurally similar cytoplasmic inclusions were found in other epithelial cells with no evidence of nuclear degeneration (Fig. 4).

Straightening of the basement membrane, initially focal, was first noted in the cervical epithelium of animals painted 43 times with methylcholanthrene (Fig. 5). In more advanced lesions a straight appearance of the basement membrane was rather constant. Early invasion was characterized by focal dissolution of the basement membrane and projections of rather clear cytoplasm of the basal cells into the stroma. In an appropriate field, membrane-enclosed cytoplasmic structures were seen in the subepithelial area of the stroma, presumably representing invasive blebs of epithelial cell cytoplasm (Fig. 6).

At the stage of invasive carcinoma single epithelial cells and nests of epithelial cells were interspersed among stromal cells (Fig. 7). Many of the malignant invasive epithelial cells were huge and contained large nuclei. Nucleoli were generally multiple and sometimes bizarre in contour. The cytoplasm of these cells contained few mitochondria. Numerous ribosomes were present and the cisternae of the endoplasmic reticulum were prominent (Fig. 8).

DISCUSSION

The major alterations noted in squamous epithelium of the mouse cervix treated with methylcholanthrene include enlargement of the intercellular spaces, prominent cytoplasmic villous projections, infoldings of the nuclear membrane, increase in the number of nucleoli, and straightening of the normally irregular contour of the basement membrane. Similar alterations in the fine structure of cervical squamous epithelium have been described in human cancers and in experimental animals, with benzpyrene used as the carcinogen. The changes in mouse cervical epithelium at the stage of early invasion are similar to those described by Frei in squamous carcinomas of the skin of Swiss mice induced with benzanthracene and croton oil (18).

The appearance of dense intramitochondrial bodies in the epithelial cells was the most interesting finding in this study. We have seen morphologically identical structures in the hyperplasia of mouse cervical epithelium induced by podophyllin, which is not a carcinogen (G. R. Schrodt, unpublished data), but not in untreated cervixes. Mitochondrial bodies of similar appearance have been seen in hyperplasia of mouse epidermis induced by croton oil (19, 29), in epidermis treated with certain carcinogens (29, 32), in epidermis of vitamin A deficient mice (33), and in the small intestine of mice which were fed 20-methylcholanthrene (6). Mitochondrial bodies have been noted in normal colonic epithelial cells (14), uterine epithelium (25), mucoid cells of gastric mucosa (22), and interstitial cells of the testis (9). These larger mitochondrial bodies are to be distinguished from small mitochondrial granules which are seen in a variety of cells and regarded as divalent cation-binding sites (30).

Frei (19), in his study of the effects of croton oil on ear epidermis, suggested that the intramitochondrial bodies might be related to the formation of keratohyaline granules. Setälä et al. (32), in their study of the effects of locally applied carcinogens to mouse epidermis, noted that dense mitochondrial bodies were the most characteristic alteration found. Nakai et al. (29) found mitochondrial bodies in epidermal cells following treatment with croton oil as well as with 7,12-dimethylbenzanthracene. They concluded that the mitochondrial changes were associated with inflammatory or cytotoxic effects in the cell and not with carcinogenesis. Hökfelt and Nilsson (25) have suggested that these mitochondrial bodies might be a reaction of the mitochondria to an accelerated metabolism. The chemical nature of the mitochondrial bodies in our preparations and in the reports cited above have not been determined. It has been suggested that they may be lipid (9).

Dense intramitochondrial bodies in our preparations were first noted after 16 applications of 20-methylcholanthrene, i.e., shortly after inflammatory changes are seen but before hyperplasia occurs. Furthermore, Christopher (11) has shown that when methylcholanthrene is applied tri-weekly to mouse cervical epithelium, inflammatory changes are noted after 14 applications. If, at the end of 14 applications, the animals are followed by sequential cervical smears, dysplasia (as defined by the light
A fine structural change occurs in cervical epithelial cells painted with 20-methylcholanthrene before any significant change is noted with the light microscope, both under conditions which produce cancer and under those which do not produce cancer.

The significance of the mitochondrial bodies described in this report and in those of other investigators is at present a matter of conjecture. In our study the mitochondrial bodies at first glance appear to be a significant finding which predicts a sequence of changes eventually leading to invasive cancer. Their presence in reversible podophyllin-induced hyperplasia and in "reversible" methylcholanthrene lesions suggests that they may merely represent a non-specific cytotoxic effect, as proposed by Nakai (29), or evidence of increased metabolic activity, as suggested by Hökfelt and Nilsson (25).

REFERENCES


Fig. 1.—Epithelial stromal junction of untreated mouse cervix. Arrows point to basement membrane. Nucleus (N) has a fairly regular outline. Collagen fibrils can be noted in the stroma (S). $\times 33,000$. The insert is a light photomicrograph of a 1-$\mu$ section taken from the same block and stained with toluidine blue. $\times 500$.

Fig. 2.—Basal cervical epithelial cell of C3H mouse painted 16 times with methylcholanthrene. Note the mitochondria (m) containing dense bodies. Basement membrane (b). $\times 30,000$. The insert is a light photomicrograph of a 1-$\mu$ section from the same block. $\times 500$.

Fig. 3.—Part of a cervical epithelial cell. This animal had been painted 58 times with methylcholanthrene. Mitochondria with one or several intramitochondrial dense bodies are present. L, lipid body. $\times 57,000$.

Fig. 4.—Mouse cervical epithelium after 58 paintings with methylcholanthrene, demonstrating the widened intercellular spaces (i). Desmosomes (d) are still present. Degenerating cells contain fragmented nuclei (N). Mitochondria (m) with dense bodies are still present. No invasive cancer was seen in the section. $\times 17,400$. Insert is a light photomicrograph of a 1-$\mu$ section taken from the same block. $\times 500$. 

806
Fig. 5.—Basal epithelium of mouse cervix treated 58 times with methylcholanthrene. The basement membrane (b) is straight. Intercellular space (i) is widened. A mitochondrion (m) containing a dense body is present. × 24,000.

Fig. 6.—Early invasive carcinoma of mouse cervix. The basement membrane separating the epithelium from the stroma is absent for the most part. An occasional remnant (arrow) is seen. Clear blebs of epithelial cytoplasm are dispersed among collagen fibrils and are close to a small blood vessel (B). N, nucleus of epithelial cell; f, fibroblast. × 8000. The insert is a light photomicrograph of a 1-μ section taken from the same block. × 500.
FIG. 7.—Invasive squamous carcinoma of mouse cervix. No basement membrane separates the epithelial cells (e) from connective tissue cells (s). One of the epithelial cells contains a large nucleus (N) with a huge nucleolus; c, collagen fibrils. × 6500. The insert is a light photomicrograph of a 1-μ section showing invasive carcinoma. The section was not taken from the same block as the electron microscope section, but from another block from the same animal. × 300.

Fig. 8.—Part of 2 epithelial cells from an area of invasive cancer. Numerous ribonucleoprotein particles are dispersed in the cytoplasm. Cisternae of the endoplasmic reticulum (er) are prominent although still infrequently seen; i, dilated intercellular space. × 60,000.
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