Histopathogenesis of Carcinoma in situ of the Uterine Cervix *

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Cancer of the cervix is amenable to treatment provided it is diagnosed at the pre-invasive stage, and it is therefore imperative to be able to identify cases of symptomless cervical cancer, which invariably presents no visible alteration in the appearance of the mucous membrane. Fortunately, this is possible by study of vaginal smears for exfoliated cells.

In view of the ease with which carcinoma of the uterine cervix can be induced in mice and of its similarity to analogous lesions in women, it was thought that study of early cytological and morphological changes in cervical carcinoma of the mouse—especially at the in situ stage—might prove valuable for biological and therapeutic studies of human cervical carcinoma.

This paper reports on study of the progressive epithelial changes in the mouse cervix by means of exfoliated cells in vaginal smears and histological examination of the cervical epithelium after intravaginal painting with 3,4-benzpyrene. The authors consider that basal cell hyperplasia and the early grades of dysplasia are reversible if the carcinogenic agent is withdrawn, but that the lesion is likely to be invasive and progressive once the presence of large numbers of squamoid cells indicates that the dysplastic changes involve the superficial layers.

Cancer of the cervix is quantitatively the most important tumour in Indian women. It constituted 29.6% of all malignancies examined in the laboratory of Surgical Pathology, S.N. Medical College, Agra, during the period 1950-60. This is a much higher incidence than that reported from other countries (Khanolkar, 1948-50). This tumour presents two other distinctive features in India: (a) the younger age-group of patients (Gault, 1951) as compared with patients in England (Rewall, 1957) and the USA (Sadugor & Palmer, 1948), and (b) its greater malignancy.

Fortunately it is amenable to treatment by surgery and radiation, provided it is diagnosed early enough, preferably at the stage of pre-invasion. Patients treated at this stage by both radiation and surgery have a 100% survival rate after 5 years' follow-up (TeLinde, 1952; Kottmeier, 1953). This is in marked contrast to the very low survival rate among persons treated by both surgery and radiation during the advanced invasive stage (Kottmeier, 1953; Lewis, 1956). It is thus imperative to diagnose symptomless cervical carcinoma, which invariably presents no visible alteration in the appearance of the cervical mucous membrane. Study of vaginal smears for exfoliated cells affords this possibility (Meigs et al., 1943; Papanicolaou & Traut, 1943; Wahi & Jain, 1950). To be of real use, such study should make possible the diagnosis of cell changes in exfoliated cells from cases of pre-invasive cervical carcinoma.

For the better understanding of the various earlier phases of carcinogenesis and the resulting
morphological changes in the uterine cervical carcinoma, it is essential that the natural history of the disease should be studied in an experimental animal. Exfoliative cytology offers a distinct advantage in the study of the pathogenesis of the disease processes affecting epithelial tissues in experimental animals.

Experimentally induced carcinoma of the uterine cervix in specially bred mice shows a striking biological and pathological resemblance to human cervical carcinoma (Scarpelli & von Haam, 1957). In view of the ease with which carcinoma of the uterine cervix can be induced in mice and its similarity to analogous lesions in women, it is probable that study of early cytological and morphological changes in cervical carcinoma, specially at the in situ stage, may prove of considerable value in biological and therapeutic studies of human cervical carcinoma.

Thus a detailed cytohistological and quantitative cytographic study is presented of experimentally induced carcinoma in situ of the mouse cervix.

**METHODS AND MATERIAL**

Specially bred female virgin mice 2-3 months of age were painted intravaginally twice weekly with 1% solution of 3,4-benzpyrene in acetone. This method was preferred to the string technique, as we aimed at studying the detailed cytohistological alterations in the maximum number of animals at the carcinoma in situ stage. The duration of this phase appears to depend on the potency of the carcinogen as well as on the dosage and method of application. Control animals were similarly treated with acetone alone. Vaginal smears were made prior to each painting and stained by Papanicolaou’s method. The animals were sacrificed at varying intervals depending on the cytological interpretations. The uterus and the vagina were removed en bloc and step-serial sections stained for histological changes in the cervical and vaginal epithelium.

Quantitative cytographic studies were also carried out during the various stages of experimental carcinogenesis in order to find out whether any significant alteration occurred in the distribution of various types of cells.

**OBSERVATIONS AND COMMENTS**

Exfoliated cells in the vaginal smear revealed distinct morphological changes during the evolution of carcinogen-induced intra-epithelial carcinoma of the mouse cervix. These changes in the exfoliated cells closely resembled the morphological changes in the tissue of origin. On the basis of cytohistological alterations, five distinct lesions could be recognized:

(a) Acute inflammation,
(b) Basal cell hyperplasia,
(c) Epithelial dysplasia,
(d) Carcinoma in situ,
(e) Invasive carcinoma.

**Acute inflammation**

This was reflected in the vaginal smears by an abundance of polymorphonuclear leucocytes, many of which showed clumping around necrotic epithelial cells, whereas few epithelial cells showed evidence of endophagocytosis (Fig. 1). The cervical and vaginal epithelium showed the formation of micro-abscesses (Fig. 2). In a few animals the subepithelial tissue showed focal and diffuse collection of polymorphonuclear leucocytes with some evidence of oedema and congested blood vessels. Focal acute ulceration of the squamous mucosa, as observed by Scarpelli & von Haam (1960), was not seen.

This stage lasted for about two to three weeks and subsequently the cervical and vaginal mucous membrane adapted to the action of biweekly carcinogen painting. Only the severity of the inflammatory exudative reaction and its persistence for two to three weeks clearly differentiated it from metaestrus in the mouse. The oestrus cycle continued normally during this phase except for the continued presence of polymorphonuclear leucocytes in the vaginal smears and the cervical and vaginal mucosa.

**Basal cell hyperplasia**

No definite cytological pattern was encountered in the majority of these animals. The vaginal smears were essentially normal. A few animals, however, showed increased exfoliation of the normal basal, parabasal and atypical basal types of cells. The explanation for the normal cytological pattern in the majority of the animals showing basal cell hyperplasia is clear, as the hyperplastic basal cells are covered by mature benign superficial cells. On the other hand, the presence of increased numbers of basal, parabasal and atypical basal types of cells in a few animals was due to the presence of focal areas of ulceration of the overlying epithelium.
HISTOPATHOGENESIS OF CARCINOMA IN SITU OF THE UTERINE CERVIX

FIG. 1-5

1. Vaginal smear showing endophagocytosis. Papanicolaou's stain. × 600.
3. Basal cells, showing early dysplastic changes. Papanicolaou's stain. × 600.
4. Intermediate cells, showing early dysplastic changes and granulations. Papanicolaou's stain. × 600.
5. Basal cells, showing coarse Sudan-black-B-positive granules. × 600.
6. Groups of basal cells showing large cytoplasmic vacuolarations. Papanicolaou's stain. × 600.


FIG. 11-15

11. Group of basal cells showing perinuclear haloes. Papanicolaou's stain. × 450.
17. Advanced dysplasia in vagina with papillomatous hyperplasia. Haematoxylin-eosin stain. x 100.
18. Dysplasia in cervix with elongated spindle-shaped cells on the surface. Haematoxylin-eosin stain. x 100.
FIG. 21-25

21. Malignant basal cells showing finely granular chromatin pattern. Papanicolaou’s stain. × 800.
22. Malignant basal cells showing coarse chromatin pattern. Papanicolaou’s stain. × 800.
23. Malignant basal cells showing translucent chromatin pattern. Papanicolaou’s stain. × 600.
25. Carcinoma in situ of cervix showing stripping phenomenon. Haematoxylin-eosin stain. × 100.
FIG. 26
CYTOGRAM OF VAGINAL SMEAR OF NORMAL MOUSE

- Keratinized cells
- Luteinized cells
- Basal cells

WEEKS OF EXPERIMENT

FIG. 27
CYTOGRAM IN EPITHELIAL DYSPLASIA, SHOWING NORMAL CYCLIC CELLULAR PATTERN

- Keratinized cells
- Luteinized cells
- Basal cells
- Atypical cells
- Precociously cornified cells

WEEKS OF EXPERIMENT
HISTOPATHOGENESIS OF CARCINOMA IN SITU OF THE UTERINE CERVIX

FIG. 28
CYTOGRAM IN CARCINOMA IN SITU, SHOWING INCREASE OF BASAL AND ATYPICAL CELLS WITH THE APPEARANCE OF MALIGNANT CELLS AND SLIGHT DECLINE IN KERATINIZED AND LUTEINIZED CELLS

FIG. 29
CYTOGRAM IN EARLY INVASIVE CARCINOMA, SHOWING PRACTICALLY THE SAME SITUATION AS IN CARCINOMA IN SITU.
FIG. 30
CYTOGRAM IN ADVANCED INVASIVE CARCINOMA, SHOWING SIGNIFICANT INCREASE IN MALIGNANT CELLS AND SHARP DECLINE IN KERATINIZED AND LUTEINIZED CELLS

FIG. 31
AVERAGE DISTRIBUTION OF CELLS IN NORMAL MOUSE CERVIX AND THOSE SEEN IN FOUR TYPES OF CERVICAL LESIONS
Some of these cells showed significant cytoplasmic alterations consisting of increased basophilia and fine to coarse granulation giving an intensely basophilic staining reaction (Fig. 3 and 4). A few of these stained positively with Sudan black B stain (Fig. 5). Histochemically, some of these granules have been reported to be strongly positive for the disulfide group, which is one of the chief constituents of keratin. Studies on the ultrafine structures of these granules show that they may represent altered mitochondria in which electron microscopy revealed the deposit of a dense, structureless material (Scarpelli, 1961). Many of these cells contained large cytoplasmic vacuoles which were negative for glycogen and fats (Fig. 6). The exact nature of cytoplasmic granulation and vacuolation is still a matter of dispute, although most workers consider them to be a degenerative process (Scarpelli, 1961).

The atypical basal type of cells were characterized by altered shape, cellular enlargement, nuclear enlargement, normal N/C ratio, decreased density of chromatin and bi- and multi-nucleation (Fig. 7).

Tissue sections of basal cell hyperplasia showed proliferation of the basal layer of cells to 4-6 layers’ thickness. There was a minimal loss of polarity of the basal cells. Only rare mitotic figures were observed. The rest of the mucosal epithelium appeared morphologically normal (Fig. 8 and 9).

Epithelial dysplasia

In addition to the cytological alterations observed in a few cases of basal cell hyperplasia, epithelial dysplasia was characterized by the presence of precociously cornified cells of the basal type, perinuclear haloes and a few to a fair number of abnormal squamoid forms of cells.

The precociously cornified cells showed intense eosinophilia. Frequently these cells were associated with damaged nuclei (Fig. 10). The basal cells with hyperchromatic nuclei and finely granular chromatin exfoliated during dysplasia in experimentally induced lesions bear a striking resemblance to the cells exfoliated in human cervical dysplasia. Similar observations have been reported by Lapid & Goldberger (1951) and Reagan et al. (1953).

The so-called “perinuclear haloes” were commonly encountered during epithelial dysplasia (Fig. 11). They are also seen in association with trichomanad infestation and in neoplastic processes. Their nature is still disputable; and it has been suggested that they may be a result of cell maturational shrinkage of the nucleus (Brux & Dupronte, 1961), or artefacts, or membrane and cytoplasm defects on the surface of the cell either induced by some mechanical factor (Sagiorgouli, 1959) or representing an intra-nuclear clear area (De Girolami, 1961). Ayre (1949) considered these as important evidence corroborating the theory of the pre-cancer cell complex.

The abnormal squamoid forms of cells arise from an epithelial lesion which may be benign, suspicious or malignant. By themselves alone they cannot be used to give a definite diagnosis. These are, however, not seen in normal epithelium.

The abnormal squamoid forms encountered during epithelial dysplasia consisted of spindle, fibre, snake and tadpole forms. These cells differed from the normal only in their aberrant shape. We interpret the cells with normal-looking nuclei as atypical non-malignant forms.

The fibre cells and snake cells may be defined as abnormal epithelial cells with an extremely elongated form and with an elongated and pyknotic nucleus (Fig. 12, 13). These cells may be of either ectocervical or endocervical origin and represent markedly aberrant forms. Their staining reaction depends largely on the extent of their cytoplasmic differentiation. Delicate longitudinal fibril can be demonstrated in the cytoplasm of the more elongated forms and the caudate forms. This is probably a remnant of the fibrillar apparatus which is now believed to be related to the process of keratinization. The length of the cells is usually 5-20 times their width. Their outlines are well-defined. The nucleus is usually single, small, contracted and pyknotic and has an elongated form. It is situated in the middle of the cell in close contact with the lateral cell borders. Both cells and the nuclei are usually tapered at their ends. The nuclei take up a deep stain with haematoxylin. They invariably present a dense chromatinic appearance, thus obscuring the structural details.

Other types of abnormal squamoid forms encountered during epithelial dysplasia are the spindle cells and tadpole cells (Fig. 14, 15 and 16). Spindle cells are smaller forms of fibre cells, whereas the tadpole cells have the nucleus in the broad head end of the cell. The proportion of spindle cells was only 1%-5% during epithelial dysplasia. The presence of any significant number of spindle-shaped cells in the vaginal fluid is grounds for suspicion of a neoplastic process.

The spindle form and other aberrant forms of cells are evidence of abnormal maturation of the
cell, the abnormality affecting the superficial layers primarily. Cytochemical studies of spindle-shaped squamoid cells done by Baschann (1958) clearly support the positive proof of the presence of pre-keratin and keratin in the cytoplasm of these cells.

Tissue sections during epithelial dysplasia showed a gross disturbance in the differentiation and keratinization of the basal cell layer with disorderly arrangement of the cells. The basal layer of cells showed loss of polarity in many areas. During the earlier stage mitotic figures or cell-crowding were minimal. Focal intra-epithelial micro-abscesses were commonly encountered.

In advanced epithelial dysplasia there was extensive cellular proliferation with papillomatous overgrowth in some places (Fig. 17). The superficial layers of cells, however, showed normal stratification and keratin formation with the presence of elongated spindle-shaped cells on the surface (Fig. 18). Few of the cells were in mitosis. This picture of cervical dysplasia bears a striking similarity to dysplastic lesions in human cervices.

Carcinoma in situ and invasive carcinoma

It is felt that on the basis of the purely cytological criteria of the exfoliated cells a definite differentiation between carcinoma in situ and invasive carcinoma cannot be reached. A similar view has been pronounced by Scarpelli & von Haam (1960) and Reagan & Hamonic (1956).

The morphology of exfoliated cells from cervical carcinoma in situ is extremely diverse, and can be differentiated from invasive carcinoma cells and atypical cells in conditions other than cancer solely by comparison. However, certain important cell types and quantitative differences appeared to exist between epithelial dysplasia, carcinoma in situ and invasive carcinoma which were considered significant. The number of basal and atypical cells observed in carcinoma in situ was higher than in epithelial dysplasia. This may be interpreted as evidence of greater maturation in dysplasia than in carcinoma in situ. The number of isodiometric cells decreases as the epithelium progresses from carcinoma in situ to invasive carcinoma. A striking feature at the earlier stage is the relative absence of the malignant abnormal squamoid forms of cells so characteristic of invasive cervical carcinoma. The normal cell population shows a significant disorder in invasive carcinoma in contrast to carcinoma in situ.

The important cell type observed in carcinoma in situ of the mouse cervix was the malignant basal type of cell, which presented a marked variation in size with large nuclei, increased N/C ratio and multiple macronucleoli. These cells were either single or in loose groups (Fig. 19 and 20). The chromatin pattern of these cells presented either a particular type of fine granularity which is never found in an invasive cancer cell (Fig. 21), or coarse chromatin (Fig. 22), or a translucent chromatinic pattern (Fig. 23). These cells closely resemble the malignant basal cells described in human cervical carcinoma in situ lesions by Neiburgs & Pund (1949).

The cytoplasmic changes were usually similar to, though less pronounced than, those during epithelial dysplasia, except that cytological features of atypical keratinization characterized by the appearance of small cells with deep-orange-coloured homogenous cytoplasm and pyknotic nucleus were found in fair number.

It has been noted above that at this stage there was a relative absence of the abnormal squamoid forms of cells. This observation is in close conformity with the cytological changes observed in human cervical carcinoma in situ lesions. Contrary to what was stressed by Stoll (1958), we did not find any significant number of lunar-shaped spindle cells in cases of carcinoma in situ. In general, the smears from carcinoma in situ lesions had a clear background with normal cornification of the superficial cells.

Our histological criteria of carcinoma in situ include the involvement of the entire depth of the mucosal epithelium, presenting irregular loss of mucosal epithelium and an irregular loss of polarity, with the superficial layers as disorderly as their deeper counterparts but within an intact basement membrane (Fig. 24).

The altered epithelial pattern is characterized by complete loss of cell maturation, hypercellularity with cell overcrowding and cellular atypias involving the entire mucosal epithelium. Mitotic figures are increased in number and occasionally extend to the superficial layers. Growth or extension of carcinoma in situ into the endocervical glands or along the endometrial epithelium is not taken as invasion, although this was a very rare feature in our experimental carcinoma in situ cases. This histological picture closely resembles the pre-invasive carcinoma of human cervical lesions. This observation differs somewhat from that put forward by Scarpelli & von Haam (1960), who have observed considerable
surface keratinization in some of their carcinoma in situ lesions. These types of lesions appeared to us as dysplastic lesions which showed complete regression of epithelial changes on cytohistological correlation if left for a sufficient length of period.

In our series, we did not observe in any significant numbers the focal carcinoma in situ cervical lesion reported by Scarpelli & von Haam (1960) in a small percentage of their experimental animals. In practically all our carcinoma in situ lesions the diffuse type of pattern was observed. The extensive, diffuse involvement of the cervical or vaginal mucosa by the carcinoma in situ lesion is evidence in favour of a multicentric pattern of origin. This also lends support to the contention of Willis (1944) that the carcinogenic stimulus acts on large groups of cells and extensive areas of epithelium rather than on a single cell.

A few of our carcinoma in situ lesions showed a tendency to separation from the underlying stroma—the so-called "stripping phenomenon" (Fig. 25). Similar observations have been reported by Scarpelli & von Haam (1960) in experimental carcinogenesis of the uterine cervix and by Reagan and associates (1955) in pre-invasive cervical carcinoma in humans. This phenomenon is considered to be due to a progressive loss of tonofibrils during carcinogenesis. However, the demonstration of tonofibrils in well-differentiated tumours suggests a relation between cellular differentiation and tonofibril formation.

Cytophographic studies

On the basis of qualitative cytophographic studies alone it was not possible to differentiate categorically between non-invasive and invasive carcinoma. However, interesting differences were found to exist between epithelial dysplasia, carcinoma in situ and invasive carcinoma when these were studied by weekly differential cell counts. There was no significant cytophological difference between basal cell hyperplasia and epithelial dysplasia. In carcinoma in situ the number of atypical cells and basal cells increased along with the appearance of malignant cells. Cornified and luteinized cells showed a slight decrease. In advanced invasive carcinoma there was observed a significant increase in the number of malignant cells along with a sharp decline of cornified and luteinized cells, reflecting probably a disordered hormonal state of the body.

Fig. 26, 27, 28, 29, 30 represent the cytophograms of normal mouse, and those obtained during epithelial dysplasia, carcinoma in situ, early and advanced invasive carcinoma, respectively. The average distribution of normal cells and of those seen in four types of cervical mouse lesions is shown in Fig. 31.

CONCLUSIONS

Investigation of the progressive epithelial changes in the mouse cervix in experimental carcinogenesis by study of exfoliated cells in vaginal smears and histological examination of the cervical epithelium has helped to understand the histopathogenesis of carcinoma in situ of the cervix.

The lesions seem to progress through the stages of acute inflammation, basal cell hyperplasia, different grades of dysplasias and finally to carcinoma in situ. Though distinct cell types are detected at different stages, depending mainly on the layer of the epithelium and the degree of involvement, yet no distinctive and constant pattern can be said to evolve. However, the carcinoma in situ stage is characterized by the presence of malignant basaltype cells, increased atypical basal and parabasal cells and comparative scarcity of abnormal squamoid cells. With the occurrence of the invasive stage the number of squamoid cells increases significantly.

Basal cell hyperplasia and the early grades of dysplasia are considered reversible if the carcinogenic agent is withdrawn. However, once the dysplastic changes involve the superficial layers, as shown by the presence of squamoid cells in large numbers, the lesion is likely to be invasive and progressive.

RÉSUMÉ

Le cancer du col de l'utérus peut être justiciable d'un traitement s'il est diagnostiqué avant la phase d'invasion. Les études faites sur la sousir aident à comprendre les phénomènes histopathologiques du cancer in situ chez la femme. Les auteurs décrivent les changements cytophographiques et morphologiques que permet de déceler l'examen des cellules exfoliées dans les frottis vaginaux de la sousir affectée d'un cancer du col, provoqué par bagnéonnage au benzpyrène-3,4. Les lésions semblent passer par les stades suivants: inflammation aiguë, hyperplasie basale, divers stades de dysplasie, et finalement le cancer in situ. Bien qu'à divers stades on puisse reconnaître certains types cellulaires, en relation essentiellement avec la couche épithéliale affectée, on ne peut décrire le déroulement d'un
processus précis et régulier. On peut affirmer cependant que le cancer in situ est caractérisé par la présence de cellules basales malignes, par un nombre accru de cellules basales et parabasales atypiques, et par une rareté relative de cellules squameuses anormales. Dès le début de la phase d'invasion, le nombre de cellules squameuses s'accroît notablement. L'hyperplasie basale et les premiers stades de la dysplasie sont considérés comme réversibles si l'agent cancérogène est supprimé. Toutefois, lorsque les manifestations dysplasiques affectent les couches superficielles, ce qui se traduit par la présence d'un grand nombre de cellules squameuses, il y a des risques que le cancer progresse et devienne envahissant.

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