Experimental Carcinoma of the Cervix: A Comparative Cytologic and Histologic Study*

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Attempts to produce carcinoma of the uterine cervix experimentally began shortly after the discovery of the carcinogenic properties of crude tar. The earliest experiments consisted of the intravaginal application of crude tar by injection or implantation in the vaginal vault, cervix, and lower uterine segment. Although squamous metaplasia was frequently produced, invasive carcinoma developed in only a few cases (3, 5, 8, 25). The tumors were slow-growing, failed to metastasize, and often regressed even during continued application of tar.

With the stimulatory effect of estrogenic hormones upon the growth and development of female genital tissues an established fact, studies on the possible carcinogenic effect of various estrogens were reported by several workers (15-17, 28). A small number of lesions closely resembling early epidermoid carcinoma were observed in the cervices of monkeys that had received estrone parenterally and whose cervices had been traumatized (21). It is interesting to note that the epithelial metaplasia produced with estrone alone regressed following discontinuation of the hormone treatment; however, some of the animals which received estrogen together with trauma to the cervix developed invasive carcinomas. A small number of cervical carcinomas were also observed in mice whose neck and back had been painted with 1,2,5,6-dibenzanthracene while they simultaneously received injections of estrone. The results of this experiment would seem to indicate that estrogenic hormones possess some co-carcinogenic effect by sensitizing the uterine cervix to the carcinogenic substance even though the latter was applied at a distant site (24, 25). However, owing to the small number of animals which developed malignant lesions, it is difficult to draw any valid conclusions from these experiments. The prolonged subcutaneous administration of estrogens for periods of over 1 year produced cervical carcinomas in various strains of mice; one of these metastasized widely (10). However, the percentage of tumor yield was low, and the mortality of the mice from mammary carcinomas and pyometra was high. This experiment was further complicated by the fact that the development of mammary carcinomas occurred long before the appearance of uterine carcinomas. This often necessitated surgical removal of the mammary lesions to prevent death of the mice.

Experiments on the possible etiologic role of human smegma in cervical cancer were reported by Fishman et al. (7); no tumors in mice were found even after 16 months of intravaginal painting with smegma.

Purified carcinogenic substances applied directly to the uterine cervix resulted in a considerably higher yield of epidermoid carcinomas than occurred following either the application of crude tar or the injection of estrogens. The epithelium of the vaginal vault and cervix of the mouse responded to carcinogenic hydrocarbons in a manner similar to the epithelium of the skin, although the tumor yield from the vagina and cervix was significantly lower. This may have been owing to the fact that application of carcinogenic substances to the uterine cervix was technically more difficult, or that the cervical and endometrial secretion diluted or removed the applied carcinogen so rapidly that it became much less effective. Von Haam and Menzies (14) were able to produce carcinoma of the cervix in a high percentage of CSH mice by intravaginal painting with 3,4-benzpyrene by means of a wire loop applicator which permitted accurate placement of the carcinogen to the uterine cervix and vaginal fornices. Murphy (20) solved the problem of accurate placement by inserting a string saturated with methylcholanthrene into the cervical canal under direct visualization and was also successful in producing a considerable number of cervical carcinomas. The significance of these results is greatly increased by the fact that spontaneous uterine tumors in mice are extremely rare, num-
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bering less than 0.06 per cent according to reports by Slye, Holmes, and Wells (27). Reports on the experimental production of carcinomas of the uterine cervix are summarized in Table 1.

Von Haam and Menzies were able to demonstrate malignant cells in the vaginal smears of laboratory animals some time before the gross appearance of tumors and pointed out the great advantages of this procedure for studies in exfoliative cytology. Because decreased cellular adhesiveness is present in neoplastic tissues, the rate of cellular exfoliation is increased, and the vagina becomes a "cell sac" providing a steady supply of malignant cells. Since it was felt that this method would lend itself well to a comparative study of the histologic and cytologic changes which occur during the development of the neoplastic process, the present series of experiments was undertaken.

MATERIALS AND METHODS

One hundred and ten C3H mice, 2–3 months of age, were used for the experiments; 80 animals were painted intravaginally twice weekly with a 1 per cent solution of 3,4-benzpyrene in acetone. Thirty animals, which served as controls, were painted intravaginally twice weekly with acetone. Painting was accomplished by means of cotton-tipped bacteriologic wire loops which were bent slightly to fit the curve of the vagina.

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SUMMARY OF EXPERIMENTAL PRODUCTION OF CARCINOMAS OF THE UTERINE CERVIX</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year and reference</th>
<th>No., species and strain of experimental animals</th>
<th>Carcinogen</th>
<th>Mode of application</th>
<th>Induction time (wks.)</th>
<th>No. tumors</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1926 (25)</td>
<td>White rat, No.?</td>
<td>Crude oil</td>
<td>I.V., skin</td>
<td>24</td>
<td>1</td>
<td>Precancer</td>
</tr>
<tr>
<td>1929 (5)</td>
<td>White mice, No.?</td>
<td>Crude tar in xylol</td>
<td>I.V.</td>
<td>13</td>
<td>5</td>
<td>Precancer</td>
</tr>
<tr>
<td>1938 (17)</td>
<td>Macaque rhesus monkeys</td>
<td>Estrone and cervical trauma</td>
<td>S.C.</td>
<td>10</td>
<td>1</td>
<td>Precancer</td>
</tr>
<tr>
<td>1955 (5)</td>
<td>White mice, No.?</td>
<td>Crude tar</td>
<td>I.V.</td>
<td>22</td>
<td>1</td>
<td>Epidermoid carcinoma</td>
</tr>
<tr>
<td>1958 (18)</td>
<td>40 mice</td>
<td>Estrone</td>
<td>S.C.</td>
<td>11–29</td>
<td>1</td>
<td>Epidermoid carcinoma</td>
</tr>
<tr>
<td>1958 (18)</td>
<td>1 mouse &quot;Old Buffalo&quot;</td>
<td>Estrone</td>
<td>S.C.</td>
<td>104</td>
<td>1</td>
<td>Carcinoma-like lesions</td>
</tr>
<tr>
<td>1958 (20)</td>
<td>27 mice</td>
<td>1,2,5,6-dibenzanthracene, Estrone</td>
<td>Skin-painted</td>
<td>28</td>
<td>5</td>
<td>Epidermoid carcinoma</td>
</tr>
<tr>
<td>1957 (91)</td>
<td>75 mice</td>
<td>1,2,5,6-dibenzanthracene, Estrone</td>
<td>Skin-painted</td>
<td>26–40</td>
<td>5</td>
<td>Epidermoid carcinoma</td>
</tr>
<tr>
<td>1958 (94)</td>
<td>254 mice—A, C57, D, CSH, CBA, new and old Buffalo</td>
<td>Estrone</td>
<td>S.C.</td>
<td>06</td>
<td>26</td>
<td>Precancer</td>
</tr>
<tr>
<td>1958 (6)</td>
<td>100 mice, CSH</td>
<td>Estradiol and Estrone benzoate</td>
<td>S.C.</td>
<td>38–52</td>
<td>19</td>
<td>1 metas. epiderm. ca.</td>
</tr>
<tr>
<td>1958 (14)</td>
<td>254 mice—A, C57, D, new and old Buffalo, CSH, CBA</td>
<td>Estrone benzoate</td>
<td>S.C.</td>
<td>4–50</td>
<td>0</td>
<td>Metaplasia</td>
</tr>
<tr>
<td>1940 (15)</td>
<td>170 white mice</td>
<td>Coal tar</td>
<td>Tar I.V.</td>
<td>30–35</td>
<td>1</td>
<td>Epidermoid carcinoma</td>
</tr>
<tr>
<td>1941 (1)</td>
<td>44 mice—C57, CBA</td>
<td>Estradiol benzoate</td>
<td>S.C.</td>
<td>52</td>
<td>25</td>
<td>Precancer</td>
</tr>
<tr>
<td>1942 (7)</td>
<td>10 mice—A</td>
<td>3,4-Benzpyrene</td>
<td>I.V.</td>
<td>29–56</td>
<td>10</td>
<td>Epidermoid carcinoma</td>
</tr>
<tr>
<td>1944 (4)</td>
<td>18 mice—CBA, C57, AKA</td>
<td>Estradiol benzoate</td>
<td>S.C.</td>
<td>10–28</td>
<td>9</td>
<td>Precancer</td>
</tr>
<tr>
<td>1948 (18)</td>
<td>55 mice—CSH</td>
<td>Estrogens and androgen</td>
<td>S.C.</td>
<td>29</td>
<td>13</td>
<td>Precancer</td>
</tr>
<tr>
<td>1955 (20)</td>
<td>BALB mice, No.?</td>
<td>Estradiol sensitized colloid particles</td>
<td>S.C.</td>
<td>64</td>
<td>38</td>
<td>Precancer</td>
</tr>
<tr>
<td>1958 (10)</td>
<td>80 mice—CSH</td>
<td>3,4-Benzpyrene</td>
<td>I.V.</td>
<td>16–40</td>
<td>31</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>1955 (16)</td>
<td>74 mice—A, C57</td>
<td>Methylcholanthrene-saturated string</td>
<td>I.C.</td>
<td>30–40</td>
<td>27</td>
<td>Invasive epidermoid carcinoma</td>
</tr>
</tbody>
</table>

genital canal. The cotton-tipped wire loops were carefully placed in the vagina, which was stretched open by dorsal flexion of the tail, and inserted until firm resistance was felt; the cervix was then painted with several rotary motions and the wire loop removed. The animals were kept in groups of five in an air-conditioned room and fed Purina Dog Checkers supplemented by daily addition of fresh bread and carrots.

Smears were prepared previous to each painting period by injecting a small quantity of saline into the vagina followed by immediate aspiration. The smears were fixed immediately and stained by the method of Papainosou (25). In addition, smears were prepared for the following cytochemical reactions: Feulgen’s reaction for the determination of deoxyribonucleic acid (5); methyl green-pyronine stain of Brachet for the determination of ribonucleic and deoxyribonucleic acid (3); the PAS reaction of McManus for the detection of glycogen (15); and Gomori’s reaction for the detection of alkaline phosphatase (11); and Gomori’s reaction for the demonstration of phosphatidase (10). Smears for cytochemical studies were fixed in an alcohol-ether mixture except for those used for the phosphomimase and alkaline phosphatase reactions, which were fixed in ice-cold acetone. The standard histochemical procedures were modified for application to vaginal smears as follows: Smears prepared for the Feulgen reaction were subjected to a HCl hydrolysis at 60°C for 12 minutes. The substrate used for the phosphomimase reaction was a 0.1 M solution of the ammonium salt of p-chlorophenyldiamidophosphoric acid commercially available from Bayer-Leverkusen, Germany. The reaction was modified by shortening the incubation time to 18 hours. Rinsing with distilled water and acetate buffer was limited to 15 minutes. The above-cited cytochemical reactions were performed once weekly. Smears stained with the Papanicolaou method were examined for the degree of esters, the presence of leukocytes and erythrocytes, and the appearance of atypical and malignant epithelial cells.

As soon as abnormal cells appeared in sufficient number to be considered diagnostically significant, the animals were sacrificed, the uterus and vagina removed, and serial sections prepared for microscopic examination. Seven groups of animals were sacrificed during a period from 18 to 40 weeks after the beginning of the experiment; this permitted a comparison between early and late cytologic and histologic changes produced by the carcinogen. By means of differential counts, quantitative “cytograms” were constructed; although they do not reveal the complete estrous cycles of the animals, they permitted us to follow the cytologic pattern observed during the period of experimental carcinogenesis.

RESULTS

On the basis of our histopathologic studies, three distinct lesions could be differentiated: (a) dysplasia of the epithelium of the cervical and vaginal mucosa; (b) noninvasive carcinoma (carcinoma in situ); and (c) invasive carcinoma. Epithelial dysplasia was found in six animals sacrificed at an early stage of the experiment, noninvasive carcinoma in 21 animals, and invasive carcinoma in fifteen animals (Table 2). In fourteen animals the pathologic changes were restricted to the uterine cervix (Fig. 1), in ten animals the lesion was present in the vaginal wall (Fig. 2), and in eighteen animals it was impossible to determine whether the primary site was the uterine cervix or the vaginal mucosa (Fig. 3). Cervical dysplasia appeared during a period of 12–28 weeks after the painting had been started, carcinoma in situ during a period of 16–40 weeks, invasive carcinoma during a period of 21–40 weeks. None of the control animals painted with acetone developed any gross or microscopic lesions of carcinoma in situ or invasive carcinoma, although evidence of vaginitis with some epithelial dysplasia could be demonstrated in some.

Our cytologic studies revealed definite nuclear and cytoplasmic changes in the exfoliated cells during the development of cervical dysplasia and carcinoma. Certain cellular changes preceded by a considerable time the appearance of neoplasms and therefore might be classified as precancerous changes. Other changes were demonstrable only in the presence of either carcinoma in situ or invasive cancer and therefore were considered as changes typical of malignant cells.

**TABLE 2**

<table>
<thead>
<tr>
<th>TYPE OF LESION</th>
<th>Vagina</th>
<th>Cervix</th>
<th>Both</th>
<th>TOTAL</th>
<th>WEEKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial dysplasia</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>12-28</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>21</td>
<td>16-40</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>2</td>
<td>4</td>
<td>9</td>
<td>15</td>
<td>21-40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10</td>
<td>14</td>
<td>18</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

Epithelial dysplasia.—The histological concept of cervical dysplasia as defined by Reagan and Moore (26) consists of a disturbance in the regular differentiation and keratinization process of the basal-cell layer with considerable “disorderly” arrangements of the cells but without evidence of increased proliferation. In addition, we observed some loss in the polarity of basal cells, and cells undergoing necrosis became intensely acidophilic and attracted small intra-epithelial accumulations of leukocytes (micro-abscesses) (Fig. 4). Cells exfoliated from this lesion had pale, large or multiple nuclei, cytoplasmic granules, vacuoles, and precocious cornification (Figs. 5–7). These cells were considered “atypical” and were not present during an ordinary inflammatory process. They were also present in animals with invasive carcinoma but were not considered malignant.

Carcinoma in situ.—Carcinoma in situ, or intraepithelial carcinoma, showed a marked proliferation of basal and parabasal cells within widened epithelial boundaries but within intact basal membranes (Fig. 8). The nuclei of these cells were enlarged and hyperchromatic, there was an increased number of mitotic figures, and one had a definite
impression of “cell crowding” (Fig. 9). Because of
the latter phenomenon, the atypical appearance of
individual cells was sometimes not so impressive in
the histologic preparation as in epithelial dysplasia.
Cells exfoliated from this lesion were character-
ized by large nuclei with definite change in the
nuclear-plasmatic ratio, having a coarse chromatin
pattern, multiple and atypical nucleoli, and in
observed in our animals was mostly of the well dif-
ferentiated squamous-cell type (Fig. 10). The is-
lands of cancer cells showed numerous pearly
bodies and were usually surrounded by a very se-
vere inflammatory reaction. Only in a small group
of animals did we obtain an undifferentiated,
highly anaplastic type of carcinoma with small
pyknotic cells and no evidence of keratinization

TABLE 8
CYTOLOGICAL-HISTOLOGICAL CORRELATION

<table>
<thead>
<tr>
<th>Week of</th>
<th>ANIMALS WITH LESIONS</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cer-</td>
<td>Dyspla-</td>
<td>Hyper-</td>
<td>Carci-</td>
<td>Invasive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>vitis</td>
<td>sia</td>
<td>plasia</td>
<td>noma</td>
<td>carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>Cornif.</td>
<td>Lutein</td>
<td>cells</td>
<td>cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cells</td>
<td>cells</td>
<td>cells</td>
<td>(per cent)</td>
<td>(per cent)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>70-75</td>
<td>20-25</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65</td>
<td>50</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>55-40</td>
<td>25-30</td>
<td>30-45</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>40</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>10-70</td>
<td>15-30</td>
<td>10-70</td>
<td>6-10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>3</td>
<td>65-75</td>
<td>15-30</td>
<td>5-10</td>
<td>8-17</td>
<td>2-4</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>65-85</td>
<td>15-30</td>
<td>5-15</td>
<td>11-12</td>
<td>3-17</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>4</td>
<td>60-65</td>
<td>15-30</td>
<td>5-20</td>
<td>19-38</td>
<td>4-9</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>1</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>75</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60-65</td>
<td>25-80</td>
<td>3-15</td>
<td>15-20</td>
<td>15-45</td>
<td>15-45</td>
<td></td>
</tr>
</tbody>
</table>

some cases quite bizarre shapes (Figs. 12–15). The
cytoplasmic changes were usually similar to those
found in cells with epithelial dysplasia, but the

process of atypical keratinization was found much
more frequently, and the appearance of small cells
with a deep orange color of the cytoplasm was
quite characteristic.

Invasive carcinoma.—The invasive carcinoma

(Fig. 11). Exfoliated cells from this lesion were not
much different from those found in animals with
intra-epithelial carcinoma, with the exception that
they appeared in larger numbers. This seemed to
us added proof, if such is still needed, that intra-
epithelial carcinoma should be considered a malig-
nant and not a premalignant lesion.

Table 3 represents a correlation between the
histologic type of lesion and the cytologic findings
in vaginal smears immediately before the animals
were sacrificed. The number of atypical cells in the
vaginal smears varied from 4 to 46 per cent in ani-
mals with epithelial dysplasia, from 8 to 38 per
cent in animals with carcinoma in situ, and from
26 to 64 per cent in animals with invasive carci-

Table 1.—The different average distribution of normal,
 atypical, and malignant epithelial cells in the four types of
histopathological lesions. These are based on vaginal smears
taken immediately before the animals were sacrificed.

* Fifteen per cent malignant cells were found in one animal
with epithelial dysplasia.

TYPE OF LESION

Chart 1.—The different average distribution of normal,
 atypical, and malignant epithelial cells in the four types of
histopathological lesions. These are based on vaginal smears
taken immediately before the animals were sacrificed.

* Fifteen per cent malignant cells were found in one animal
with epithelial dysplasia.


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the sharp cytologic difference between epithelial dysplasia and carcinoma in situ.

On the basis of our weekly differential counts, other interesting differences were found to exist between dysplasia, carcinoma in situ, and invasive carcinoma. Smears from animals with dysplasia and carcinoma in situ showed no significant changes in the normal epithelial cell population, while in animals with advanced invasive tumors a marked increase in basal cells existed. This is shown in Chart 2, which represents the cytogram of animal 70, which was sacrificed 40 weeks after the beginning of the experiment with a large fungating carcinoma of the cervix; malignant cells had been demonstrated constantly for the last 12 weeks. The cyclic nature of the cellular pattern was markedly altered at about the 24th week, and luteinized cells were almost absent for the last 10 weeks.

In most of the animals with epithelial dysplasia or noninvasive carcinoma of the cervix, the cyclic cellular pattern was only slightly disturbed, and luteinized cells could be found in considerable numbers at set intervals. In animals with invasive carcinoma, keratinized and luteinized cells decreased sharply, and basal cells of the parabasal and intermediate types appeared to predominate. Red blood cells were generally absent during the stages of dysplasia and noninvasive carcinoma. They were always a prominent feature in animals with invasive carcinoma.

The results of the various cytochemical reactions are presented in Table 4. Deoxyribonucleic acid seemed increased in large and atypical cells, but this increase was not uniform and never so pronounced as in the leukocytes of the exudate.

### Table 4

**SUMMARY OF CYTOCHEMICAL STUDIES**

<table>
<thead>
<tr>
<th>Reaction or stain</th>
<th>Substance tested for</th>
<th>Atypical cells</th>
<th>Malignant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feulgen Methyl green-pyronine PAS Phosphatase reaction of Gomori</td>
<td>DNA RNA Glycogen Phosphomimidase</td>
<td>Increased Increased No change Increased</td>
<td>Increased Increased No change Markedly increased</td>
</tr>
<tr>
<td></td>
<td>Alkaline</td>
<td>Equivocal</td>
<td>Equivocal</td>
</tr>
</tbody>
</table>

(Figs. 20, 21). Ribonucleic acid was also increased in the cytoplasm and the nucleoli of malignant cells. However, the staining technic with methyl green-pyronine proved difficult, and reliable results
could not be obtained. The glycogen content of the malignant cells seemed not to be increased as compared with normal cells, nor was the alkaline phosphatase reaction significantly different. The phosphamidase reaction was strongly positive in many atypical and malignant cells, but again the results were not consistent. In some instances the reaction was present in the cytoplasm (Fig. 22), while in other malignant cells the reaction was more pronounced in the nucleus (Fig. 23) or was entirely absent (Fig. 24). The reaction depended greatly on the technic employed and for this reason demanded a very exact standardization of the technical method. While in our experience cells from normal animals did not give a positive reaction, we feel that only a comparatively small number of exfoliated malignant cells were phosphamidase-positive.

DISCUSSION

The pathogenesis of 3,4-benzpyrene-induced cervical and vaginal carcinoma in C3H mice seemed to be characterized by definite cytologic and histologic changes. The development of carcinoma appeared to progress from epithelial dysplasia to carcinoma in situ and finally to invasive carcinoma. Qualitative and quantitative changes in the cell pattern exhibited by exfoliated cells paralleled the histologic changes during the development of neoplasia. These cytologic changes can be classified into the groups of precancerous and malignant changes. Precancerous changes, with the exception of precocious cornification, preceded the appearance of neoplasia by a considerable period of time and were also found occasionally in cells from control animals painted with acetone alone. The impairment of normal growth and maturation of vaginal and cervical epithelium and the appearance of precociously cornified basal cells suggested to us some close relationship. The malignant changes were primarily characterized by alteration of the nucleus, although variations in size and shape of cells were most marked in animals with invasive carcinoma. Nuclear pyknosis, precociously cornified basal cells, leukocytes, and red blood cells were found so constantly in animals with invasive or noninvasive carcinoma that they could be spoken of as representing a “malignant pattern,” although none of these changes by itself was diagnostic of a malignant process. This observation is identical to one made by von Haam (13) during the examination of a large number of vaginal smears in women.

It is interesting that the normal cyclic cellular pattern of the animals continued during the first two stages of neoplastic development and was only disturbed in animals with invasive carcinoma. This must be interpreted as an effect of the disease upon the hormonal health of the animals and not as a failure of the remaining vaginal epithelium to respond to estrogens. Epithelial dysplasia preceded the appearance of noninvasive carcinoma by a considerable length of time. The fact that it was only very occasionally observed in our control animals would suggest that it may belong to the group of precancerous lesions, although additional experiments are needed to support this theory.

Our cytochemical studies were disappointing in that no clear-cut difference among normal, atypical, and malignant cells could be established. Many obviously malignant cells gave a strong reaction for ribonucleic and deoxyribonucleic acid and were phosphamidase-positive. The increase in the deoxyribonucleic acid content of malignant nuclei is probably related to the polyploidy so characteristic of cancer cells. However, it was also felt that many malignant cells did not give these reactions, and for this reason we cannot consider any of these reactions as significant or possessing much diagnostic value. We feel, however, that the equivocal results we have obtained may be attributed in part at least to the length of time the exfoliated cells had been in the vaginal pool. The importance of this factor becomes quite evident when one considers that all intracellular substances are subject to denaturation. Therefore the varying intensities of reaction seen in cells of the same type may be a reflection of cellular hypoxia, malnutrition, senescence, trauma, or death following exfoliation. More definitive investigations with regard to cyto-

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**Fig. 1.** Mouse uterus with advanced carcinoma of the cervix. The distended urinary bladder is secondary to urethral obstruction. ×2.

**Fig. 2.** Mouse uterus with a carcinoma of the vaginal wall. ×3.

**Fig. 3.** C3H mouse with an extensive cervical carcinoma filling the entire pelvis. ×585.

**Fig. 4.** Cervical dysplasia. There is a disorderly arrangement of both basal and parabasal cells with intra-epithelial micro-abscesses. ×125.

**Fig. 5.** Cervical dysplasia. Vaginal smear. Parabasal cell with two nuclei. ×1250.

**Fig. 6.** Cervical dysplasia. Vaginal smear. Basal cell containing cytoplasmic granules. ×1250.

**Fig. 7.** Cervical dysplasia. Vaginal smear. Basal cell containing cytoplasmic vacuoles. ×1250.

**Fig. 8.** Carcinoma in situ. A proliferation of intensely basophilic cells can be noted within an intact basement membrane. ×125.
Fig. 9.—Carcinoma in situ. High power photomicrograph showing hyperchromatism, mitotic figures and marked cellular crowding. ×475.

Fig. 10.—Well differentiated epidermoid carcinoma of the cervix. Marked keratinization with pearl formation is present. ×125.

Fig. 11.—Anaplastic epidermoid carcinoma of the cervix. This lesion is composed of pleomorphic cells arranged in dense sheets with no evidence of keratinization. ×125.

Fig. 12.—Vaginal smear. Malignant basal cells showing hyperchromatism, marked variation in size and indistinct cytoplasmic borders. ×1250.

Fig. 13.—Vaginal smear. Malignant basal cells showing macronucleoli. ×1250.

Fig. 14.—Vaginal smear. Malignant basal cells exhibiting hyperchromatism, pleomorphism, macronucleoli, perinuclear chromatin condensation, and mitosis. ×1250.

Fig. 15.—Vaginal smear. Multinucleated malignant basal cell. ×1250.
FIG. 16.—Vaginal smear. Malignant cell of the fiber cell type. × 875.

FIG. 17.—Vaginal smear. Malignant cell of the tadpole cell type. × 1250.

FIG. 18.—Vaginal smear. Cluster of elongated malignant cells (fiber cells). × 550.

FIG. 19.—Vaginal smear. Malignant basal cells exhibiting marked pyknosis (anaplastic carcinoma). × 1250.

FIG. 20.—Feulgen reaction. Normal cervical epithelium. × 675.

FIG. 21.—Feulgen reaction. Epidermoid carcinoma of the cervix. Distinct increase in nuclear size and nuclear content of deoxyribonucleic acid. × 675.

FIG. 22.—Phosphamidase reaction. Vaginal smear. Malignant basal cell exhibiting a strong cytoplasmic and no nuclear reaction. × 1250.

FIG. 23.—Phosphamidase reaction. Vaginal smear. Malignant basal cell showing a moderately positive cytoplasmic and strongly positive nuclear reaction. × 1250.

FIG. 24.—Phosphamidase reaction. Vaginal smear. Multinucleated malignant basal cell showing a negative reaction in cytoplasm and nucleus. × 1250.
chemical characteristics of malignant cells are now under way in our laboratory.

SUMMARY

1. The results of our experimental study indicate that the pathogenesis of 3,4-benzpyrene-induced carcinoma of the cervix and vagina in CSH mice is characterized by four definite stages: acute and chronic inflammation, epithelial dysplasia, noninvasive carcinoma, and invasive carcinoma.

2. On the basis of cytologic and histologic study it was established that a definite correlation existed between the histopathology and the cells exfoliated during these stages.

3. Atypical and malignant cells could be identified and distinguished among the exfoliated cells during the development of the neoplastic process. No differentiation could be made on the basis of exfoliated cells between noninvasive and invasive carcinoma.

4. The cyclic pattern of estrus in animals with dysplasia and carcinoma in situ was similar to that found in normal animals. It was definitely altered in animals with invasive carcinoma.

5. Cytochemical studies showed an increase in deoxyribonucleic acid and ribonucleic acid and a positive phosphamidase reaction in many but not all exfoliated malignant cells.

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Experimental Carcinoma of the Cervix: A Comparative Cytologic and Histologic Study

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