Experimental Carcinoma of the Colon*†
ALEXANDER HORAVA AND EMMERICH VON HAAM
(Department of Pathology, Ohio State University, Columbus, Ohio)

Although spontaneous malignant neoplasms of the large intestine are rare in rodents, the susceptibility of the colon to chemical as well as physical carcinogens has been proved by various experimental means. The chemical carcinogens used for this purpose include 20-methylcholanthrene (2, 14), 2-amino- and 2-acetylaminofluorene (8), 2-acetoaminofluorene (6), alcohol (8), and benzidine (16). The physical carcinogens reportedly successful are total-body irradiation by x-rays (5), feeding of radioactive yttrium (9, 10), and whole-body fast neutron irradiation (19). Except in the rats fed with radioactive yttrium, the incidence of carcinomas of the large intestine in animals exposed to the above-mentioned carcinogens was invariably low, ranging from 1 to 15 per cent of all animals in each reported experiment. Technical difficulties in delivering appropriate doses of carcinogen to the large intestine appear to be the main obstacle in experimental work.

Our present experimental work dealt with two problems: We tried to increase the production of malignant tumors in the colons of rats by using a modification of the method described by Murphy (12) and implanting a string impregnated with the carcinogen through an appendicostomy into the colon. We also tried to follow the pathogenesis of colonic neoplasms by cytological studies of fecal smears with the established cytological criteria used for the diagnosis of ulcerative and neoplastic lesions in the human colon (1, 4, 7, 11, 15, 17).

MATERIALS AND METHODS

Animals.—Seventy-two adult male albino Wistar rats (CFW strain, supplied by Carworth Farms, Inc., New City, N.Y.) of an average body weight of 170–180 gm. were kept in individual cages and fed Purina Dog Chow pellets and tap water ad libitum. Sixty-two animals carried carcinogen-impregnated strings, while the remaining ten rats served as controls and carried plain cotton strings.

Preparation of carcinogen-impregnated string.—20-Methylcholanthrene crystals were heated in a jar to the melting point. A cotton string 17 cm. in length was then immersed in it for several minutes, leaving approximately 15 mm. of the end of the string not impregnated by the carcinogen. This end was later fixed to the distal portion of the appendix to avoid contact of the carcinogen with the connective tissues at the operative site. The carcinogen was then allowed to solidify at room temperature on the string. Each string contained approximately 350 mg. of 20-methylcholanthrene.

Surgical procedure.—A laparotomy and appendicostomy were performed under deep ether anesthesia. The folded carcinogen-soaked string was then inserted through the appendicostomy opening into the lumen of the ceco-appendix. The nonimpregnated end of the string was fixed by double surgical silk sutures to the appendicostomy site, and the wound was closed by a serosa-to-serosa closure. The laparotomy incision was sutured with double surgical silk. Within 24–48 hours the string in the ceco-appendix unfolded, and the distal portion was carried to the anus by the bowel contractions. Excess string protruding through the anal orifice was cut off. Post-operative complications were rare and consisted of a few peri-appendiceal abscesses and enterocutaneous fistulas.

Preparation of cytological material.—The semifixed fecal bolus of rats is usually covered with a thin layer of mucus which contains many well preserved epithelial cells. The freshly defecated bolus was gently rolled over a clean microscopic slide, care being taken not to break the bolus in order to avoid a massive contamination of the smear by fecal material. Smears were prepared from each animal at weekly intervals during the entire duration of the experiment. The smears were stained with Papanicolaou's technic and occasionally by the mucicarmine stain.

RESULTS

The animals were killed at varying intervals, and the large intestine was studied histologically by the usual methods. Twenty animals were killed in the 14th week after the insertion of the methylcholanthrene-impregnated string. All animals showed at the autopsy multiple small and large ulcers in the ceco-appendix. No gross tumors were identifiable at that time. The strings were found disintegrated and reduced to a short stump protruding into the lumen of the appendix. New strings impregnated with the same material were therefore inserted into the ceco-appendices of the remaining 42 experimental rats. Plain cotton strings were reinserted into the ten control animals. Eight months later, all animals were killed.
and autopsies performed. Two animals exposed to the carcinogen showed extensive ulceration in the ceco-appendix with fixation of this organ to the surrounding structures by thick fibrous bands. Seventeen other animals showed focal induration of the wall by gray homogeneous tissue and irregularly shaped punched-out defects of the overlying mucosa (Fig. 1). All animals displayed more or less extensive focal ulceration within the ceco-appendix. Two polypoid lesions measuring 4 mm. in greatest diameter were found in the cecum of two carcinogen-exposed rats. Examination of the entire colon revealed all lesions limited to the ceco-appendix, where the carcinogen-impregnated string was probably in closest contact with the mucosa. None of the control animals showed ulceration of the cecum.

Histopathological examination of the colons of the animals killed in the 14th week of the experiment revealed focal ulcerations of the ceco-appendiceal mucosa and occasionally small ulcers in the ascending colon in all cases. No significant lesions were found in the lower portions of the large intestine. The mucosa adjacent to the edges of the ulcers showed evidence of hypersecretion of mucus as revealed by mucicarmine and PAS stains. In six animals mucosal glands lined by irregularly shaped cells with a moderate degree of cellular pleomorphism (Fig. 2a) were found. These changes were classified as epithelial dysplasia and were found either in the areas of epithelial hyperplasia or at the edges of the ulcers. Occasionally, small groups of atypical columnar epithelial cells were found in the submucosa and muscularis. Two animals showed dysplastic glands containing foci of pleomorphic columnar cells with loss of polarity and with prominent nuclei containing prominent clumps of chromatin (Fig. 2b). The cytoplasm of these cells was basophilic. There was a marked shift in nuclear-cytoplasmic ratio in favor of nuclear size. There was no evidence of invasion, and therefore these lesions were classified as intra-epithelial carcinomas (carcinoma in situ). All carcinogen-exposed animals showed marked chronic inflammatory infiltrates in the submucosa characterized by a conspicuous number of eosinophilic polymorphonuclear leukocytes.

Eleven months after the insertion of the carcinogen-impregnated strings, microscopic examination revealed invasive carcinoma of either adenomatous or mucoid type in nineteen animals (Fig. 3). The invasion of normal tissues by the tumors was limited to the submucosa in four animals, to the wall of the ceco-appendix in twelve, and extended into the mesocecal fibroadipose tissue in three animals (Fig. 4). No malignant cells or tissues were identified within the lymph nodes examined. Additional intra-epithelial carcinomas were identified in the cecum of seven animals, and epithelial dysplasia in seven other rats. The remaining nine rats showed on microscopic examination more or less extensive ulcers with some focal epithelial dysplasia. No tumors were found in the colons of animals where the carcinogen-impregnated string was surrounded by a large fecolith. Microscopic sections of the two polypoid lesions revealed only chronic granulation tissue covered by hyperplastic mucosa. The lower portions of the large intestines from the experimental animals did not show any significant pathological changes. The colons of the control animals displayed only slight inflammatory changes around the appendicostomy site without any epithelial changes. Our results are summarized in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Weeks of Exposure to Carcinogen</th>
<th>Inflammation only</th>
<th>Type and Distribution of Lesions</th>
<th>Invasive Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>No.</td>
<td>Per cent</td>
<td>No.</td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>42</td>
<td>48</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Totals: 62</td>
<td>21</td>
<td>34</td>
<td>13</td>
</tr>
</tbody>
</table>

Cytological studies.—The analysis of fecal smears was greatly facilitated by a classification of all cellular elements into five morphological groups: (a) well preserved columnar cells either normal or pathologic (Fig. 5a); (b) desiccated columnar cells (Fig. 5b); (c) inflammatory cells of all types; (d) squamous cells from anal epithelium; (e) contaminating cellular elements (e.g., vegetable cells, pollen granules, etc.). During the course of the experiment, over 2300 fecal smears were prepared and analyzed. Well preserved columnar cells were found in about 40 per cent of the smears. The others showed only desiccated and distorted epithelial cells. Occasionally, they contained no epithelial cells at all but only bacterial masses, food particles, and other contaminants. The diagnostic
value of fecal smears in the rats was found to be limited to the smears containing well preserved epithelial cells from the mucous layer of a freshly defecated bolus. As in the cytodiagnostic classification of human and experimental smears from the uterine cervix, three categories were used for the classification of pathological changes: smears having predominantly inflammatory changes, those showing epithelial dysplasia, and smears suspected to reflect a malignant process in the large intestine.

Insertion of a plain or a carcinogen-impregnated string was followed by the appearance of a large number of polymorphonuclear leukocytes in the smears with many cellular fragments and prominent mucous shreds (Fig. 6). The inflammatory reaction persisted in the carcinogen-exposed animals, varying in intensity from time to time, while it disappeared in the control animals within 2 weeks after the insertion of the string. Columnar cells showing an increased nuclear-cytoplasmic ratio were first seen in smears from the experimental animals 2 weeks after insertion of the carcinogen-impregnated strings (Fig. 7). Fourteen weeks after the start of the experiment, prior to sacrifice of the first twenty animals, several smears were classified as suspicious for malignant cells. However, histological sections from these animals permitted only a diagnosis of epithelial dysplasia. The second insertion of carcinogen-impregnated strings was again followed by the appearance of acute inflammatory cells accompanied by a conspicuous number of dysplastic columnar cells. This was followed within 2–6 weeks by the appearance of cells which we classified as “malignant” by one or more of the following characteristics: large vesicular or irregularly shaped nuclei with prominent clumps of chromatin, or large, deeply basophilic nuclei, a scant cytoplasm, marked nuclear pleomorphism and an increased nuclear-cytoplasmic ratio (Fig. 8). It should be pointed out here that only exceptionally did we encounter a continuous exfoliation of malignant cells in successive smears from the same animal. Numerical data on the over-all accuracy of the cytodiagnosis in our experimental series are given in the “Discussion.” In rare instances, toward the end of the experiment, clumps of atypical cells could be identified in the smears. On two occasions only, when clumps of very atypical cells were seen in smears from diarrheic stools of animals with a palpable mass in the abdomen, a definite diagnosis of colonic carcinoma was made. All other smears were classified either as inflammatory, dysplastic, or suspicious changes. The histogenesis of experimental carcinoma of the ceco-appendix was fairly well reflected by the changes in the smears. The sequence from a chronic ulcerative process in the cecum to epithelial dysplasia, and later to carcinoma, could be easily followed by the fecal smear technic.

**DISCUSSION**

The experiment described here confirms the previous observations by others that the large intestine of the rat is susceptible to the induction of malignant epithelial tumors by local application of 20-methylcholanthrene. The high incidence of carcinomas in our experimental animals can be explained by the massive dose of the carcinogen remaining in contact with the mucosa for many weeks. Apparently, the chronic ulcerative process caused by the experimental procedure facilitated the development of carcinomas.

There are several technical pitfalls inherent in our experimental method. The operative procedure itself is rather time-consuming and difficult, since all precautions must be taken to avoid leakage of fecal material into the peritoneal cavity during and after surgery. The disintegration of the string within several weeks was unexpected, since a rather thick cotton string had been used. Another difficulty was the occasional impaction of feces around the folded string in the cecum, which resulted in the formation of a fecolith and prevented tumor development. The absence of connective tissue tumors (sarcomas) in our animals, as compared with the results of Berenblum et al. (2), may be explained by the fact that no solvent was used in our experiment and the carcinogen was in contact only with epithelial cells or with inflammatory granulation tissue.

While the cytological analysis of fecal smears proved of definite value for the observation of the pathological changes, they proved less useful for a specific diagnosis in any individual animal at each single instance of the smear preparation. The degree of epithelial changes was often overestimated in the first 14 weeks of the experiment,

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**Fig. 1.**—Gross photograph showing the ulcerated and indurated mass of a carcinoma of the ceco-appendix. X100.

**Fig. 2a.**—Hyperplastic gland with variation in size and shape of nuclei, classified as epithelial dysplasia. X300.

**Fig. 2b.**—Glandular epithelium with loss of secretory activity, marked nuclear pleomorphism, and loss of cellular polarity, classified as carcinoma in situ. X300.

**Fig. 3.**—Invasive adenocarcinoma of the cecum. X100.

**Fig. 4.**—Adenocarcinoma of the cecum invading the mesentery. X100.
FIG. 5a.—Fecal smear showing well preserved columnar cells. ×430.

FIG. 5b.—Fecal smear showing distorted and desiccated columnar cells. ×430.

FIG. 6.—Fecal smears showing degenerated and pyknotic epithelial cells with a large number of polymorphonuclear leukocytes, indicating an acute inflammatory lesion. ×430.

FIG. 7.—Marked variation in nuclear size as well as in nuclear-plasmic ratio of epithelial cells, characteristic of epithelial dysplasia. ×430.

FIG. 8.—Elongated pleomorphic cells with large, dark nuclei and prominent nucleoli, characteristic of adenocarcinoma cells. ×430.
and this error was partly due to the subjective tendency of the observer to designate every smear showing one or more bizarre cells as suspicious of malignancy. This experience led us to emphasize that in a fecal smear a single or several scattered cells with the usual morphological criteria of malignancy are not conclusive for a diagnosis of carcinoma of the large intestine. At most, these smears should be interpreted as "suspicious" of a malignant process, and a more detailed study is necessary to establish the diagnosis. A diagnosis of colonic carcinoma by the fecal smear alone could be made only in cases where clusters of malignant columnar cells were found.

At the termination of the experiment, prior to sacrifice of the animals, the lesions were cyto logically classified as follows: four animals with inflammatory changes only, five rats with epithelial dysplasia, 34 animals suspected of having colonic carcinoma, and two rats with the definite diagnosis of carcinoma of the large intestine. Histopathologic examination of the colons from these animals revealed: nine inflammatory lesions only, seven ceco-appendices with epithelial dysplasia, seven animals with carcinoma in situ, and nineteen carcinomas showing various degrees of invasion. Thus, we had eight false positive diagnoses, where smears were interpreted as suspicious of cancer but only epithelial dysplasia was found on histologic examination. The fact that in our experimental animals the development of cecal carcinomas was always accompanied by ulceration and epithelial dysplasia added to the diagnostic difficulties. Nevertheless, we feel that the fecal smears from our experimental animals proved to be of definite value in that it was thus made possible to suspect the presence of a malignant process in the rat colon. The definitive diagnosis of carcinoma of the colon could be accurately made only in animals which continuously exfoliated atypical cells in large numbers or in clusters.

SUMMARY

1. The insertion of 20-methylcholanthrene-impregnated cotton string through an appendicostomy into the ceco-appendix of 62 male albino rats elicited a chronic inflammatory process in this organ, which was accompanied in most instances by epithelial dysplasia. During the course of the experiment, nine intra-epithelial carcinomas and nineteen invasive carcinomas of the ceco-appendix were identified on histologic sections.

2. The development of these lesions was followed by weekly fecal smears which were classified into three pathologic categories: (a) inflammatory, (b) dysplastic, and (c) suspicious for malignancy. The individual fecal smear was found helpful for a diagnosis of inflammation accompanied or unaccompanied by epithelial dysplasia. The analysis of the series of successive smears from each individual animal permitted a reasonably accurate evaluation of the nature and stage of the pathologic process in the ceco-appendix of the experimental animals.

REFERENCES


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