HISTOLOGICAL AND AUTORADIOGRAPHICAL STUDIES ON INTESTINAL TUMORS OF RAT INDUCED BY ORAL ADMINISTRATION OF N,N'-2,7-FLUORENYLENEBISACETAMIDE

(Plates XCIV-XCVII)

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Synopsis

Intestinal tumors were induced in 96 (51%) out of 188 Buffalo rats which were fed basal commercial diet containing 0.025% N,N'-2,7-fluorenylenebiscacetamide. About 50% of the tumors was found in the distal part of the ileum and the proximal part of the colon. Average number of tumors per rat was 3.8 in both sexes and most of the tumors was less than 1 cm in diameter. A gross and histological classification of the induced tumors is proposed. Histological atypism was most prominent in umbilicated type and metastasis to regional lymph nodes was found in 3 cases (12.5%) of this type.

At one hour after intraperitoneal injections of tritiated thymidine, distribution pattern of labeled cells in the intestinal mucosa was entirely different between non-neoplastic and neoplastic tissues. In the latter, the labeled cells were distributed diffusely in the whole neoplastic glands, while in the former they were found exclusively in the proliferative zone of glandular epithelia in the mucosa. Population density of the labeled cells in the proliferative zone exceeded that of neoplastic tissues.

INTRODUCTION

N-2-Fluorenylaceticamide is known as a carcinogen with multtarget potentiality. The main target organ of this compound for male rats is the liver and for female, the mammary gland. Morris, Stewart, et al. reported that one of its derivatives, N,N'-2,7-fluorenylenebiscacetamide (2,7-FAA), has a carcinogenic potency also for the stomach and intestine of Buffalo rats. In a series of experiments with this carcinogen, with nearly 100 cases of intestinal tumor at hand, the authors tried to analyze the process of tumorigenesis in the intestine.

MATERIALS AND METHODS

Buffalo rats of both sexes were used in the present study. They were offsprings of the rats delivered from Dr. H. P. Morris in 1964. Grouped by sex and by age, they were placed in metal cages and basal commercial diet (CE-2, CLEA Japan Inc., Tokyo) containing 0.025% 2,7-FAA (Tokyo Kasei Co.) was given continuously from 1 to 3 months old till the end of the experiment. Most of the rats were killed under ether anesthesia when their death appeared to be imminent.

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All effective rats, surviving more than 100 days after the start of 2,7-FAA feeding, were autopsied and, after macroscopical examinations, all major organs were fixed in 10% Formalin. Special cares were taken for the stomach and intestines. They were separated from the mesentery, extended, and fixed with pins on a wooden board. The length of the entire intestine was measured and after opening of the intestinal canals, site, number, size, and shape of intestinal tumors were recorded. Histological specimens were prepared by routine procedures and were stained mainly by Hematoxylin and Eosin.

Twenty-eight animals, which were fed the same dose of 2,7-FAA for 27 weeks, were injected with 1.0 μCi of tritiated thymidine (5 μCi/mM) per g body weight intraperitoneally. The animals were sacrificed 1 hr after the injection, and pieces of the intestine including tumors were fixed in 10% Formalin and embedded in paraffin. The thin sections (3 μ) were coated with nuclear track emulsion NTB-2 (Eastman Kodak Co., U.S. A.) by the dipping method and developed with D-19 (Eastman Kodak Co., U.S.A.) after 2 weeks of exposure. The labeled cells were counted microscopically on autoradiographic histological specimens. In order to avoid error, the countings were made independently by three investigators.

**RESULTS**

Tumors were induced in several organs. Incidence of tumors is summarised in Fig. 1. Main target organ for male rats was the liver (91.1%) and for the females, the mammary gland (70.3%) but the sex difference was larger in the former than in the latter. Lung tumors, most of them being benign adenoma, were slightly more frequent in the males, while leukemia was found a little more in females.

Intestinal tumors were found in 96 (51.1%) out of 188 effective rats surviving more than 100 days after start of the continuous feeding of 2,7-FAA. Incidence of the tumor was slightly higher in males (56.5%) than in females (44.9%) (Fig. 1).

Distal part of the small intestine (terminal ileum) and proximal part of the large intestine (ascending colon) were the frequent site of tumor formation. Frequency of the tumors in these two parts was about 50% of all intestinal tumors developed. In other parts, incidence of tumor was far less, even though slightly higher in the jejunum than in the ileum and the colon (Fig. 2).

Average number of tumor per rat was almost the same (3.8) in both sexes but frequency of the number of the rat with a single intestinal tumor was higher in females than in males (Table I).

Size of the tumors was mostly within 1.0 cm and only 10% in males and 4% in females had tumors over 1.0 cm in maximum diameter. In males, average diameter of the tumors was slightly larger in accordance with the duration of 2,7-FAA feeding, but such a tendency was not noticed in females (Table II). Average number of tumors in a rat increased with the period of 2,7-FAA feeding in both sexes (Table II). The tumor detected earliest at autopsy was 128 days and the latest was 301 days.

Intestinal tumors induced were classified by their gross appearance into the following 5 types; hemispheric, plateau, polypoid, polyp, and umbilicated (Photos 1 to 5). Tumors of pedunculated polyp type were most frequent in the terminal ileum and they often caused sudden death of the host animals due to irreparable invagination into the cecum.
INTESTINAL TUMOR INDUCED BY 2,7-FAA

Fig. 1. Frequency of tumor in organs

[Bar chart showing frequency of tumor in different organs, with bars for male and female, and categories such as liver, mammary gland, lung, intestine, and leukemia.]

Fig. 2. Frequency of tumor in each section of the intestines

[Bar chart showing frequency of tumor at different distances along the intestine, with bars for male and female.]

(Length of small intestine was translated as 120 cm and length of large intestine as 15 cm)

Otherwise, no significant correlation was observed between gross appearance of the tumors and their site of origin.

Histologically, most tumors had features similar to adenoma, in spite of severe intramural growth. Destructive or infiltrative growth indicating obvious malignant nature was not found in this experiment.
Based on the grade of cellular and structural atypia, the tumors were classified histologically into three groups of slight, moderate, and severe atypia (Photos 6 to 8). Most tumors were composed of neoplastic tubules with slight or moderate atypia, but in tumors of umbilicated type, severe atypia similar in feature to that of well-differentiated adenocarcinoma was not infrequently observed (Table III). Subserosal invasion of neoplastic tubules was most frequent and most prominent as well in this type (Table IV).

Metastasis to the regional lymph nodes was detected histologically in 3 out of 25 cases of umbilicated type, in which tumors were located in the proximal colon and had a maximum diameter of 1.7 cm (Photo 12).

The Paneth granules were not infrequently seen in neoplastic tubules, especially in those with slight atypia, but not visible in neoplastic tissues with severe atypia (Table V).

One hour after intraperitoneal injection of tritiated thymidine, labeled cells were found regularly in proliferative zone of normal intestinal mucosa, while such regular distribution was not found in neoplastic lesions and labeled cells were distributed diffusely in the tumor-composing neoplastic tubules (Photos 9 to 11). Average index of the labeled cells was 27.8% in neoplastic tubules, which is slightly higher than that (18.5%) of whole non-neoplastic tubules, but lower than that (41.1%) of non-neoplastic tubules when it was compared to the proliferative zone (Fig. 3). In neoplastic tubules, no significant difference in distribution of the labeled cells was found between protruding and deeply invading lesions. The labeled cells were often encountered in neoplastic epithelia with Paneth's granules (Photos 13 and 14).
INTESTINAL TUMOR INDUCED BY 2,7-FAA

Table III. Gross Type and Histological Atypia of Induced Tumors

<table>
<thead>
<tr>
<th>Gross type</th>
<th>slight</th>
<th>Frequency of atypia (%)</th>
<th></th>
<th></th>
<th>unclassified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>moderate</td>
<td>severe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemispheric</td>
<td>65.8 (104)</td>
<td>31.6 (50)</td>
<td>1.3 (2)</td>
<td></td>
<td>1.3 (2)</td>
</tr>
<tr>
<td>Plateau</td>
<td>75.6 (31)</td>
<td>22.0 (9)</td>
<td>0</td>
<td></td>
<td>2.4 (1)</td>
</tr>
<tr>
<td>Polypoid</td>
<td>76.9 (30)</td>
<td>15.4 (6)</td>
<td>2.6 (1)</td>
<td>5.1 (2)</td>
<td></td>
</tr>
<tr>
<td>Polyp</td>
<td>86.9 (73)</td>
<td>13.0 (11)</td>
<td>0</td>
<td>1.1 (1)</td>
<td></td>
</tr>
<tr>
<td>Umbilicated</td>
<td>32.0 (8)</td>
<td>52.0 (13)</td>
<td>8.0 (2)</td>
<td>8.0 (2)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>85.7 (6)</td>
<td>0</td>
<td>0</td>
<td>14.3 (1)</td>
<td></td>
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</tbody>
</table>

Figures in parentheses indicate the number of tumors.

Table IV. Gross Type and Grade of Invasion of Induced Tumors

<table>
<thead>
<tr>
<th>Gross type</th>
<th>mucosa</th>
<th>Frequency of invasion (%)</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>submucosa</td>
<td>muscularis propria</td>
<td>serosa</td>
<td>unclassified</td>
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<tr>
<td>Hemispheric</td>
<td>19.6 (31)</td>
<td>34.8 (55)</td>
<td>25.3 (40)</td>
<td>20.3 (32)</td>
<td>0</td>
</tr>
<tr>
<td>Plateau</td>
<td>26.8 (11)</td>
<td>46.3 (19)</td>
<td>19.5 (8)</td>
<td>7.3 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Polypoid</td>
<td>30.8 (12)</td>
<td>41.0 (16)</td>
<td>28.2 (11)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Polyp</td>
<td>59.5 (50)</td>
<td>36.9 (31)</td>
<td>2.4 (2)</td>
<td>1.2 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Umbilicated</td>
<td>0</td>
<td>8.0 (2)</td>
<td>20.0 (5)</td>
<td>72.0 (18)</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>28.6 (2)</td>
<td>0</td>
<td>0</td>
<td>57.1 (4)</td>
<td>14.3 (1)</td>
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</tbody>
</table>

Figures in parentheses indicate the number of tumors.

Table V. Grade of Atypia of Neoplastic Tubules and Incidence of Cells with Paneth Granule

<table>
<thead>
<tr>
<th>Grade of atypia</th>
<th>-</th>
<th>±</th>
<th>+</th>
<th>++</th>
<th>unknown</th>
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</thead>
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<tr>
<td>Slight</td>
<td>27.8 (70)</td>
<td>10.3 (26)</td>
<td>19.4 (49)</td>
<td>28.2 (71)</td>
<td>14.3 (36)</td>
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<tr>
<td>Moderate</td>
<td>50.6 (45)</td>
<td>14.6 (13)</td>
<td>11.2 (10)</td>
<td>11.2 (10)</td>
<td>12.4 (11)</td>
</tr>
<tr>
<td>Severe</td>
<td>100.0 (5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unclassified</td>
<td>75.0 (6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25.0 (2)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate the number of tumors.

Fig. 3. Ratio of $^3$H-thymidine-labeled epithelia of intestinal mucosa in rats given 2,7-FAA

![Graph showing the ratio of $^3$H-thymidine-labeled epithelia in normal and neoplastic tubules](image)
For analysis of tumorigenesis of the intestine, the rate of labeled cells was counted in various parts of normal intestinal mucosa. The rate was slightly higher in cells of the jejunum than in the ileum (Fig. 4). No close correlation, therefore, was found between the rate of labeled cells and frequency of tumors in various parts of the intestine. In proliferative zone of non-neoplastic intestinal mucosa, the rate of labeled cells was slightly higher in rats given 2,7-FAA than in non-treated ones.

**DISCUSSION**

Experimental induction of intestinal tumor has been reported by several investigators using various kinds of chemical compounds; Wilson, DeEd, and Cox\(^{15}\) with 2-FAA, Stewart\(^{13,14}\) and Morris\(^{4,5}\) with 2,7-FAA, Laqueur\(^3\) with cycasin, Evans\(^2\) with bracken, Spjut et al.\(^9,11\) with 2,3-dimethyl-4-aminobiphenyl, Druckrey\(^1\) with 1,2-dimethylhydrazine, and Schoental\(^10\) with N-ethyl-N-nitrosourea. Histological features of the induced intestinal tumors reported by these investigators are mostly well-differentiated tumors and few of them showed histological changes suggesting highly differentiated adenocarcinoma.

In the present experiment, also, no invasive adenocarcinoma was found on histological examination and almost all the tumors showed a feature similar to adenoma with severe heterotopic growth but, in tumors of umbilicated type, suspicious malignant lesions were encountered not infrequently. We tentatively diagnosed them as "adenoma with severe atypia," although metastases were found in the regional lymph nodes. From histological point of view, borderline change between benign and malignant neoplasm is a matter of much discussion but presence of metastases might be considered as one of the criteria for malignancy. If this criterion was acceptable, at least 3 cases in this experiment might be called a highly differentiated adenocarcinoma. At present, however, all the neoplastic lesions would better be called "intestinal tumor."
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The reason why the tumors occurred preferably in the terminal ileum is not known. One of the authors (T.N.) reported previously, under an almost the same experimental condition, that majority of the tumors were found in the upper part of the jejunum. The different result between these two experiments is obscure but it might be due to such factors as the kind of carcinogen used, alteration of gene composition in Buffalo rat, content of basal diet, or other unknown intrinsic or extrinsic factors.

Interpretation of different pattern of the distribution of labeled cells between non-neoplastic and neoplastic tissues might have essential importance for understanding the nature of tumors induced. The results obtained in the present experiment strongly suggest that the kinetics of cell renewal may essentially be changed in neoplastic tissues from normal ones. Proliferative zone or germinal layer is not visible in neoplastic tissues, and this is distinctly different from normal intestinal mucosa. The whole cells composing the tumor are capable of DNA synthesis and seem to lack in normal cell maturation. It is assumed from these findings that the neoplastic tissues might have no system of cell renewal as in the case of normal mucosa and, for this reason, they might overgrow gradually upon non-neoplastic mucosa. From autoradiographic study, Springer et al. expressed the change of early stage of intestinal carcinoma induced by 1,2-dimethylhydrazine as “characterisiert durch eine Entdifferenzierung und Proliferationssteigerung.”

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REFERENCES


EXPLANATION OF PLATES XCIV~XCVII

Photo 1. Hemispheric type. Neoplastic tissues proliferated chiefly in submucosa and protruded the mucosa. ×10.

Photo 2. Plateau type. Hyperchromatic tubules proliferated upward with broad basis. ×20.

Photo 3. Polypoid type. This tumor has the form of a polyp but muscularis mucosae is not fused together, as in the case of polyp type. ×10.

Photo 4. Polyp type. The tumor is in the form of a pedunculated polyp. Muscularis mucosae is fused in the center of tumor. ×10.

Photo 5. Umbilicated type. Neoplastic tissues proliferated mainly in the serosa forming a large nodule. ×10.
Photo 6. Neoplastic tubules with slight atypia. Hyperchromatic nuclei of columnar epithelia lacking in goblet cells are seen in the center of this photo. ×100.

Photo 7. Neoplastic tubules with moderate atypia. Structure of the proliferating neoplastic tubules is more irregular than that in Photo 6. ×100.

Photo 8. Neoplastic tubules with severe atypia. Pattern of the proliferating neoplastic tissues is quite similar to that of adenocarcinoma. ×40.

Photo 9. Autoradiograph of the lesion of non-neoplastic and neoplastic intestinal mucosa. Labeled cells are seen regularly in the basal layer (proliferating zone) of the non-neoplastic mucosa (left half). They are distributed diffusely in the neoplastic tubules (right half). ×40.

Photo 10. Autoradiograph of neoplastic tubules with moderate atypia. Labeled cells are seen evenly throughout atypical columnar epithelia. ×100.

Photo 11. Autoradiograph of neoplastic tubules with severe atypia. Labeled cells are seen in neoplastic tubules, which are proliferating heavily toward serosa. ×40.

Photo 12. Metastasis to regional lymph node. Lymph node in right half is occupied completely by well-differentiated adenocarcinoma. Outward growth of umbilicated tumor is seen in the left half. ×10.

Photos 13 and 14. Autoradiographs of neoplastic tubules containing the Paneth granules. Labeled cells are visible in neoplastic epithelia with abundant Paneth granules. ×400 and ×250.