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# High Prevalence of Caecal Dysplasia in Wistar/Furth Substrain Rats*)

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## ABSTRACT

Offspring of spontaneous colon tumor prone rats (WF/O) bred in our laboratory developed tumors in a very low incidence (less than 0.2%), but about half of these rats had dysplastic epithelium with frequent erosion in the caecum. In nine animals (4.4%) submucosal glands of the caecum were observed. These dysplasia accompanied inflammatory cell reactions and regional lymph nodes were enlarged and packed with many plasma cells. Hybrid of WF/O rats and other inbred Long-Evans strain (LE) rats, having no dysplastic lesion of the caecum, had caecal dysplasia in a low incidence (10%). These data suggest that genetic factor(s) play an important role in the occurrence of caecal dysplasia in WF/O rats. These changes may be a precondition to the spontaneous occurrence of tumors in the proximal portion of the ascending colon with the aid of promoting factor(s).

## INTRODUCTION

Spontaneous occurrence of colon tumors has been rarely reported in rats[1-4,6,7]. All reported tumors occurred in the proximal part of the ascending colon in relatively young animals and had the same macroscopic and microscopic features. There was a diffuse thickening of the bowel wall with or without surface ulceration. Histologically, they were diagosed as well or moderately differentiated adenocarcinoma[1-4,6-9]. Local lymph node metastases were sometimes recorded[3,8]. Attempts to transplant these tumors have all failed[7]. However, Miyamoto[8-10] in Osaka University has reported a high incidence and continuous occurrence of colon carcinoma in highly inbred Wistar/Furth substrain (WF/O) rats. These colon carcinoma-prone rat were kindly provided us by Dr. Miyamoto and kept under strict brother-sister mating in our laboratory and more than one thousand WF/O rats were obtained. Among them only two rats developed spontaneous colon carcinoma in the ascending portion of the colon, which is a very low incidence compared to that reported by Miyamoto[8-10]. To elucidate the mechanism of this apparently low incidence of spontaneous colon tumor in our laboratory, alimentary tracts of WF/O rats, other inbred Long-Evans (LE) rats and hybrid of these two strains of rats were examined histologically.

## MATERIALS AND METHODS

*Animals*

Colon carcinoma-prone Wistar/Furth substrain (WF/O) rats which were kindly provided us by Dr. Miyamoto (Osaka University, Osaka) in 1978 and 1980 were maintained by brother-sister mating in our laboratory. Long-Evans strain (LE) rats which were provided us by the courtesy of Dr. T. Yoshida (National Institute of Genetics, Mishima) in 1979 were also maintained by brother-sister mating in our laboratory. Females of LE rats and males of WF/O rats were mated and hybrid ($F_1$) rats thus obtained were also examined. All rats used in this experiment were weaned 4 to 6 weeks after birth and were housed in plastic

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cages, 3–4 rats per cage, in an air-conditioned room at 24±2°C. They were fed a commercial pelleted diet MF (Oriental Yeast Co., Ltd., Tokyo) and given tap water *ad libitum*.

*Experimentel design*

A total of 206 WF/O rats, 35 LE rats and 29 $F_1$ rats which were untreated controls for another experiment or used only for mating and were sacrificed between January 1980 and March 1982, were included in this study. These WF/O rats were the 2nd to 7th filial offspring of spontaneous colon tumor prone rats. About half of WF/O rats were followed for more than 12 months. Most of the LE rats and $F_1$ rats were killed at about 7 months after birth. After complete autopsy, all parts of the alimentary tract from the esophagus to the descending portion of the colon were removed for histological examination in the early phase of this experiment. Since it was elucidated that pathological changes were confined to the caecum and the ascending part cf the colon, these parts were examined microscopically in the later part of the experiment.

*Histological studies*

Longitudinal sections were obtained from the mesenteric side of the ascending colon and caecum. When there were any macroscopic changes such as erosions, these parts were studied histologically. They were fixed in 10% neutral formaldehyde and embedded in paraffin, cut 4 μm in thickness and stained with hematoxylin and eosin (HE). Selected tissues were stained by periodic acid-Schiff (PAS) reaction, high iron diamine-Alcian blue (pH 2.5) (HID-AB), Azan-Mallory and Giemsa.

## RESULTS

*Incidence of caecal dysplasia*

The incidence of caecal dysplasia is summarized in Table 1. As no sex difference could be observed, the data were combined for both sexes. Almost all the dysplasia were found in the caecum with only a few cases in the ascending colon. Moreover, when there was dysplasia in the ascending colon, dysplasia could be invariably found in the caecum. In WF/O rat the incidence of dysplastic epithelium was 41% between the age of 6 and 12 months, 51% between the age of 13 and 18 months, and 69% in age of 19 months and over. There was a gradual increase in incidence with age. In LE rats no caecal dysplasia was observed, but in $F_1$ rats the incidence of caecal dysplasia was 10%.

*Macroscopic and microscopic appearances of caecal dysplasia*

In typical cases the surface of the caecal mucosa was not smooth and tiny multiple erosions were observed. However, dysplasia were also found in regions where no remakable macroscopic changes could be seen.

In caecal dysplasia of WF/O and $F_1$ rats, there were some cellular atypias such as nuclear polymorphism and hyperchromatism, increased nucleo-cytoplasmic ratio, and disturbance of cellular polarity. Slight irregularity in the form and structure of the crypts and decrease in the number of goblet cells were observed (Figs. 1 and 2). Sometimes dysplastic epithelia of the caecum were located next to erosive lesions. Most of these dysplastic epithelia stained negative with PAS and HID-AB methods, but a few cases stained blue with the latter method indicating the presence of sialomucin[12]. In almost all cases many inffammatory cells (eosinophils, lymphocytes and plasma cells) were seen in the mucosa and submucosa. In nine

**Table 1.** Incidence of caecal dysplasia

| Strain of rat | Sex | Age of animals in month | | |
|---|---|---|---|---|
| | | 6–12 | 13–18 | 19–28 |
| WF/O | M | 28/68 (41%) | 16/36 (44%) | 10/12*(83%) |
| | F | 14/35 (40%) | 22/38*(58%) | 10/17*(59%) |
| LE | M | 0/16 | | |
| | F | 0/18 | 0/1 | |
| LE×WF/O | M | 2/14 (14%) | | |
| | F | 1/15 ( 7%) | | |

* Three animals in each group have submucosal glands.

**Fig. 1.** Microscopic appearance of a normal caecal mucosa of a WF/O rat sacrificed at the age of 8 months after birth. (Hematoxylin-eosin stain, ×260)

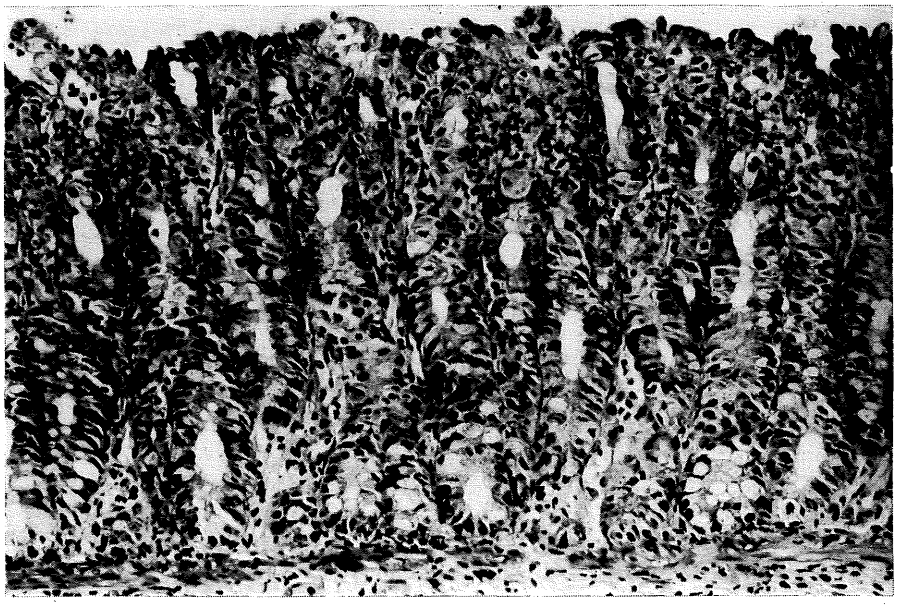

**Fig. 2.** Microscopic appearance of a dysplastic caecal mucosa of a WF/O rat sacrificed at 7 months after birth. Slight cell atypism and irregularity in the form and structure of the crypts are observed. There are many inflammatory cells in the lamina propria and tunica submucosa. (Hematoxylin-eosin stain, ×260)

WF/O rats older than 13 months of age dysplastic epithelia passed through the muscularis mucosae but did not invade the muscularis propria (Fig. 3). These submucosal glands stained strongly positive with PAS and HID-AB method, by the latter method these submucosal epithelia were shown to have both sialo-and sulfomucin. Many fibrous tissues were seen around these glands. No metastasis was observed in the mesenteric lymph nodes. However, they were enlarged and packed with many plasma cells. In these nine cases, no microorganisms were found in their submucosal cells by Giemsa staining.

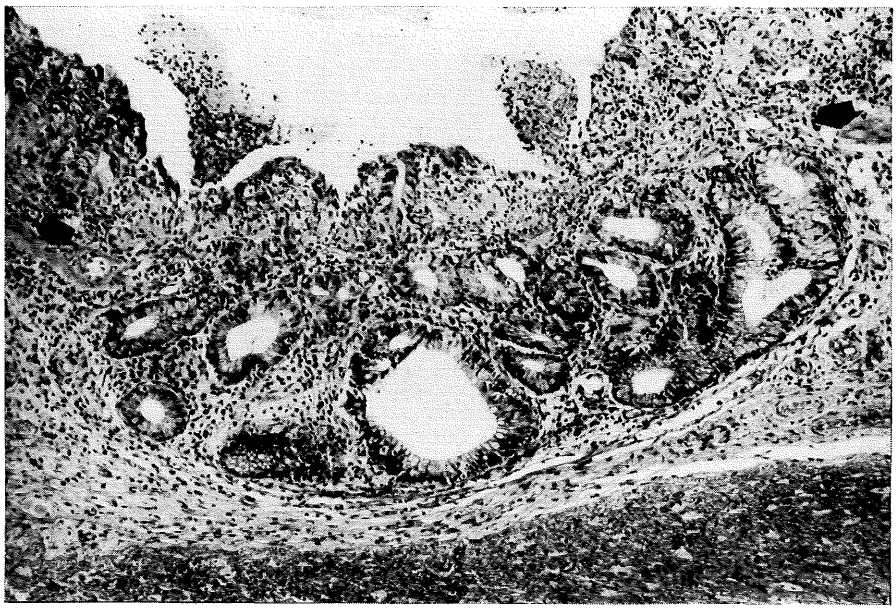

**Fig. 3.** Microscopic appearance of a submucosal gland of the caecum of a WF/O rat sacrificed 21 months after birth. Arrows indicate the lamina muscularis mucosae. (Hematoxylineosin stain, ×130)

*Other tumors*

Five mammary tumors and two hemangiomas of mesenteric lymph nodes were observed in WF/O rats older than 13 months of age.

## DISCUSSION

Miyamoto reported that about 30% of WF/O rats in Osaka University developed a well-differentiated tubular adenocarcinoma in the ascending colon within a year[10]. In this experiment, however, we could not detect spontaneous colon cancer in our WF/O rats bred in our laboratory in spite of the longer observation period. However, there was a high incidence of dysplastic epithelium in the caecum with little change in the ascending colon. In LE rats no caecal dysplasia was observed, but in $F_1$ of WF/O and LE rats the incidence of caecal dysplasia was 10%, an incident being about one fourth of the incidence in WF/O rats of the same age. These results suggest that genetic factor(s) play an important role in the occurrence of caecal dysplasia. In nine animals older than 13 months of age, dysplastic glands passed through the muscularis mucosae. Because none of herniated glands invaded the muscularis propria and none of them showed any morphological changes suggestive of cancer, we considered these glands to be simple benign herniation. In case of porcine intestinal adenomatosis, epithelial dysplasia was seen with infiltration of the epithelium into underlying tissues and spread *via* the lymphatics, to drainage lymph nodes[5,13]. This condition was thought to be a transient disorder of growth and maturation associated with the presence of intracellular bacterium. In the present experiment no such organisms were found in the submucosal glands by Giemsa staining. In apes and monkeys suffering from inflammatory disease of the colon, herniation of the mucosae has been reported[14].

WF/O rats in Osaka University showed atypical glands in the ascending colon, which developed to local hyperplasia and then spread into neighbour crypts. These glands invaded the underlying submucosa, and finally turned into a cancer[11]. Our WF/O rats may have a genetic trait that caecal epithelium is sensitive to unknown factors and becomes dysplastic, but does not develop cancer. As to the etiology of spontaneous occurrence of colon cancer in rats, some authors have suggested infective etiology[4,7], and Miyamoto[8-10] has considered genetic factors to be important. The reason for the lower incidence of spontaneous tumor in our laboratory could not be elucidated, but

these dysplastic lesions in the caecum may be a precondition rising to the burst of spontaneous tumors of the proximal part of the colon in WF/O rats with the aid of some promoting factors.

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