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STUDIES ON EXPERIMENTAL COLON TUMORIGENESIS IN RATS 1. STRAIN DIFFERENCES IN INCIDENCE, LOCATION AND TUMOR TYPES OF 1, 2-DIMETHYLHYDRAZINE-INDUCED TUMORS*)

By

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ABSTRACT

Susceptibility to 1, 2-dimethylhydrazine (DMH) was comparatively studied using two rats strains, Wistar-Furth substrain (WF/O) rats derived from the family with a high incidence of spontaneous colon tumor, and Long-Evans (LE) rats. Ten-week-old rats of both sexes were injected with DMH (20 mg/kg body weight) weekly for 20 weeks, and sacrificed at the 10th, 15th, 20th, and 25th week after the last injection. LE rats developed colon tumors more frequently in terms of crude incidence (21/21) ($p<0.01$) and the number of tumors per rat (6.1) ($p<0.05$) than those (17/25, 2.5) in WF/O rats. The predilection site of induced tumors was the descending colon in LE rats (59/128), in which adenomas were prevailing, where only a small number of tumors were found in WF/O rats (8/42). In both strains ascending colon was far more crowded with poorly differenciated adenocarcinoma than other sites of the colon (8/19, 14/34). It is likely that a resistant rat strain (WF/O) for DMH carcinogenesis has emerged from a parent rat strain prone to develop colonic tumors spontaneously.

INTRODUCTION

The incidence of human colon cancer has been increasing in Japan[1] as in western countries[2]. Therefore it has become more important to investigate colon cancer experimentally as well as clinically. Epidemiological data have suggested that major causative factors are associated with diet and, in particular, high fat diet[3]. Cycasin[4] and its relative compounds, such as dimethylazoxymethanol (MAM), azoxymethan (AOM) and 1, 2-dimethylhydrazine (DMH)[5], and some other direct acting carcinogens[6] were found to induce a high incidence of colon tumors in rats[4-6], mice[7] and hamsters[8]. Many experimental studies using these chemical compounds have shown the important role of dietary fat in colonic tumorigenesis[9-11].

On the other hand, as is the case in human familial polyposis genetic factor is presumably an important causative factor[12]. Miyamoto and Takizawa reported a prevalence of colon carcinoma in Wistar Furth substrain rats, which seems to be comparable to human familial polyposis in some respects[13].

A litter from a cancer-bearing parent was

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made available to our laboratory by the courtesy of Dr. Miyamoto, Osaka University, in October, 1975. Thereafter, they have been maintained by brother sister mating. However, not a single case of colon cancer developed among 120 rats during 6 successive generations. The present study is undertaken to know the susceptibility of these offsprings to DMH, and to compare the results with those obtained in other rat strain. Independent studies to check the response of this strain rat to the same carcinogen with different schedules are made by Kawasaki et al.[14] and by Maeura et al.[15]

MATERIALS AND METHODS

Animals Ten litters comprising 25 males and 27 females of 10-week-old WF/O rats, which were third, forth, and fifth generations from the parents given by Dr. Miyamoto, were devided into two groups; a carcinogen treated group and a non-treated control group. Five litters, composed of 17 males and 22 females, of 10-week-old Long-Evans (LE) strain rats, which were made available from National Institute of Genetics in Mishima, were also divided into two groups as WF/O rats. The animals were housed in plastic cage and given commercial diet (Oriental MF) and water freely.

Carcinogen 1, 2-Dimethylhydrazine dihydrochloride (DMH, Nakarai Chemicals, Ltd., Kyoto) adjusted to pH 6.5 with 7% w/v $NaHCO_3$ solution was injected subcutaneously on the back weekly for 20 weeks at a dose of 20 mg/kg body weight[16]. Three to 8 rats of both sexes were sacrificed at the 10th, 15th, 20th, and 25th week after the last injection of DMH.

Pathology Tissue specimens were obtained from the representative segments of the gastrointestinal tract including both tumorous and non-tumorous areas, fixed in 10% neutral formalin, and then embedded in paraffin. Deparaffinized sections were stained with Hematoxylin and Eosin. The data were analyzed by χ^2 test or "t" test.

RESULTS

Changes in Body Weight, Signs and Symptoms of Intestinal Tumor-bearing Rats Weight gain in WF/O rats and LE rats receiving multiple injections of DMH were similar to that of the untreated control animals except for a short period from 27-week-old to 28-week-old, during which male WF/O rats weighed about 50 gr. less than controls ($p<0.05$). Animals bearing intestinal tumors tended to show a marked weight loss, persistent diarrhea, or abdominal distension with ascites and tumor masses, re-

Table 1. The Incidence of Intestinal Tumors in WF/O and LE Rats Administered with Dimethylhydrazine

Strain	Sex	Treatment	No. of animals with tumor	No. of animals with colon tumor	Total no. of colon tumor	No. of animals with small-intestine tumor	Total no. of small-intestine tumor
WF/O	M	DMH	11(13)[a]	10(77)%	25(2.5)[b]	4	5(1.3)[b]
WF/O	F	DMH	7(12)	7(58)	17(2.4)	2	3(1.5)
Total			18(25)	17(68)	42(2.5)	6	8(1.3)
LE	M	DMH	9(9)	9(100)	83(9.2)	1	2(2.0)
LE	F	DMH	12(12)	12(100)	45(3.8)	2	3(1.5)
Total			21(21)	21(100)	128(6.1)	3	5(1.7)
WF/O	M	control	0(12)	0	0	0	0
WF/O	F	control	1(15)	0	0	0	0
Total			1(27)	0	0	0	0
LE	M	control	0(8)	0	0	0	0
LE	F	control	0(10)	0	0	0	0
Total			0(18)	0	0	0	0

a) Figures in parentheses indicate effective number of animals.
b) Figures in parentheses indicate average number of tumor per animal.

gardless of strains or sexes.

Incidence of Tumors (Table 1) All the intestinal tumors developed in DMH-treated rats of both strains were shown in Table 1. The crude incidence of colon tumor was 68% (17/25) in WF/O rats and 100% (21/21) in LE rats. The average number of colon tumors per rat was 2.5 in WF/O rats, the maximum being 7. In LE rats they were 6.1, and 15, respectively. The number of tumors in the small intestine was 8 in 6 WF/O rats, and 5 in 3 LE rats. As for sex difference both the crude incidence and the number of tumors per rat were higher in male than in female.

Other than alimentary tract tumors, one case of nephroblastoma in a female WF/O rat and one case of squamous cell carcinoma of auditory meatus in female LE rat were found in DMH treated group. In the control group, one mammary adenocarcinoma was found in a 50-week-old female WF/O rat.

Histopathology In the present study we attempted to classify colonic and small intestinal tumors of the rat into three groups. They were (1) adenomas, (2) well and moderately differentiated adenocarcinomas, and (3) poorly differentiated adenocarcinomas.

In the present experiment most of adenomas (Fig. 1) and well differentiated adenocarcinomas (Fig. 2, 3) were sessile or polypoid whereas moderately differentiated adenocarcinomas (Fig. 4) and poorly differentiated adenocarcinomas (Fig. 5, 6) tended to be rather flat. Most of well or moderately differentiated adenocinomas were invasive under lamina muscularis mucosae, and only a small number of tumors are diagnosed as carcinoma *in situ*.

Histological types of colonic and small intestinal tumors occured in WF/O and LE rats were shown in Table 2.

As shown in the Table 2, the relative occurrence of adenoma among all types of colonic tumors was higher in LE strain (40%) than that in WF/O strain (15%), while the relative occurrence of carcinoma was higher in WF/O strain than in LE strain.

Distribution and the Histological Types of the Intestinal Tumors in Relation to the Sites

Table 2. The Histological Types of Intestinal Tumors in Relation to the Sites

Strain	Site	Total no. of intestinal tumors	Histological type			Not examined
			Adenoma	Adenocarcinoma		
				well & moderately differentiated	poorly differentiated	
WF/O (25)[a]	Cecum & ascending colon	19(3)[b]	3	7	8(3)[b]	1
	Pars flexura lienalis	15(1)[b]	1	11	3(1)[b]	0
	Descending colon	8(1)[b]	2	5	1(1)[b]	0
	Total colon	42	6(15)[c]	23(56)[c]	12(29)[c]	1
	Small intestine	8(2)[b]	4	2	2(2)[b]	0
LE (21)[a]	Cecum & ascending colon	34(5)[b]	7	12(2)[b]	14(3)[b]	1
	Pars flexura lienalis	35	9	15	2	9
	Descending colon	59(1)[b]	25	17	2(1)[b]	15
	Total colon	128	41(40)[c]	44(43)[c]	18(17)[c]	25
	Small intestine	5(1)[b]	1	1	3(1)[b]	0

a) Figures in parentheses indicate number of rats examined.
b) Figures in parentheses indicate number of metastatic tumors.
c) Figures in parentheses indicate the percentage of the tumors per total number of tumors examined.

(Table 2) The colon is divided arbitrarily into three parts, i. e. ascending colon (including the cecum), pars flexura lienalis, and descending colon.

In 25 WF/O rats, there were 19 tumors in the ascending colon, 15 in the pars flexura lienalis, and 8 in the descending colon. Poorly differentiated adenocarcinomas were more prevalent in the ascending colon (8/19), whereas well and moderately differentiated adenocarcinomas were frequently found in the pars flexura lienalis and descending colon.

In 21 LE rats, there were 34 tumors in the ascending colon, 35 in the pars flexura lienalis, and 59 in the descending colon. Poorly differentiated adenocarcinomas were more prevailing in the ascending colon, and well and moderately differentiated adenocarcinomas were evenly distributed, and benign form of tumors (adenomas) were found mostly in the descending colon.

The major difference in tumor distribution between the two rat strains was as follows: The large portion of tumors were found in the descending colon in LE rats, as compared to those in WF/O rats in which tumors developed more frequently in the ascending colon. In the ascending colon, poorly differentiated adenocarcinoma was the major histological type in both strains.

Incidence of Metastatic Tumors in WF/O and LE Rats with Colon Tumors (Table 2). Seven cases of metastatic tumors were found in 18 WF/O rats, and they were all from poorly differentiated adenocarcinomas (especially mucocellular type). In 21 LE rats seven cases of metastatic tumors were found, and 5 cases of which were from poorly differentiated adenocarcinomas, and the remaining 2 were from moderately differentiated adenocarcinomas.

DISCUSSION

The present study showed that in LE strain rats both the incidence of colon tumor ($p<0.01$) and the average number of tumors per rat ($p<0.05$) were significantly higher than those in WF/O rats. WF/O rats, in which spontaneous colon tumors had been reported to occur, were found to be less susceptible to DMH than LE rats in this experiment contrary to our expectation.

However, the degree of resistance in WF/O rats was not so great as shown in C57BL/Ha mice, in which colon cancer induction by DMH was virtually nil[17]. Genetic study using F_1, F_2 and backcross of highly susceptible ICR/Ha and resistant C57BL/Ha mice suggested that colon carcinogenesis by DMH was controlled by a dominant autosomal gene[18]. In a study using WF/O strain, Miyamoto showed that incidence of spontaneous colon cancer was about 20% and sug gested that multiple unknown genes were operative to develop cancer (personal communication). It might be still premature to relate these genetic traits in this strain rat to their low susceptibility to a chemical carcinogen, since no case of spontaneous colon cancer was obtained in this study as yet. Nevertheless, it is likely that this substrain is resistant to DMH.

In the study of colon tumorigenesis by methylazoxymethanol using DMH-resistant rats (Lobund Wistar rats) and DMH-sensitive rats (Sprague-Dawley rats), Pollard et al. concluded that difference in susceptibility was partly related to metabolic activation of the DMH[19]. There may be some differences in metabolic activation of DMH or in alkylation of DNA by DMH metabolites[20] and succeeding DNA repairment between WF/O and LE rats.

Factors other than genetics capable of modifying the incidence of carcinogen-induced colon cancer might be age, sex[21], diet,[10,11,22] and intestinal flora[23,24].

According to Balish et al.[25] male rats are more susceptible to colon cancer induction by direct-acting carcinogen (intrarectal injection of N-methyl-N'-nitro-N-nitrosoguanidine). In the present study male rats tended to be more susceptible than female rats. In female rats of BD-IX strain, the incidence of colon cancer dropped with increasing age when DMH treatment was started; 39% in 35-day-old group, 30% in 120-day-old group, and nil in 210-day-old group[21]. Because age, and diet were matched in the present study, we could exclude the possibility of fluctuation in tumor incidence due to these factors.

In order to clarify the malignancy of each tumor in a simple manner, tumors are classified to adenoma, well or moderately differentiated adenocarcinoma and poorly differentiated adenocarcinoma without using the well known classification by Pozharrisski[26] or by Lingeman &

Garner[27]. (Previously we used the diagnosis of adenoma, tubular carcinoma, mucinous carcinoma respectively[28].) In this experiment 45% (19/42) of all colonic tumors occured in the ascending colon in WF/O rats, 44% (8/18) of them examined histologically were poorly differentiated adenocarcinomas. In LE rats 46% (59/128) of all colonic tumors occured in the descending colon and 57% (25/44) of them examined were adenomas. As for poorly differentiated adenocarcinomas in LE rats, a higher incidence (14/33) (42%) was noted in the ascending colon as compared to those in the pars flexura lienalis (2/26) or descending colon (2/44). Thus poorly differentiated adenocarcinomas were found more frequently in the ascending colon than any other site of the colon in both strains. Others also observed the higher frequency of poorly differentiated adenocarcinomas in the ascending colon in Fischer strain[29] or non inbred albino rats[30]. The difference in cell kinetics between ascending and descending colon tumors and some other features in DMH tumorigenesis will be discussed in a following paper.

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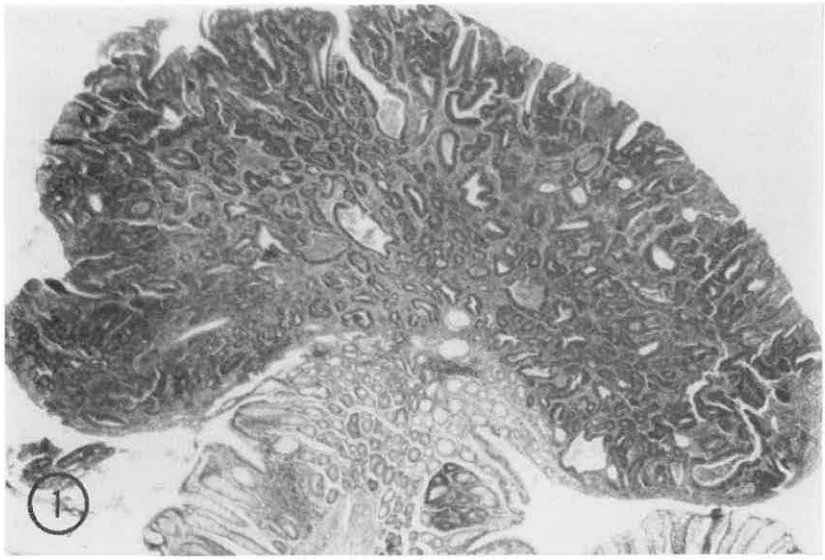
Figure Legends

Fig. 1-6 All rats were treated with DMH. Histologic sections were stained with Hematoxylin and Eosin.
Fig. 1. Adenoma in the descending colon of a LE strain rat. Polypoid protrusion with the stalk and the abundance of tubular adenomatous architecture were seen. No invasion of neoplastic glands were seen at the stalk and adjacent areas. ×20.
Fig. 2. Highly differenciated adenocarcinoma in the pars flexura lienalis of a LE strain rat. Invasion of neoplastic glands or cells could be seen in the submucosal layer by high power of view. ×20.
Fig. 3. High power view of Fig. 2. m: lamina muscularis mucosae. ×100.
Fig. 4. Moderately differentiated adenocarcinoma in the ascending colon of a WF/O strain rat. Increase of neoplastic glands and associated proliferation of fibrous tissues occupied the whole wall from the mucosa to subserosa. ×20.
Fig. 5. Poorly differentiated adenocarcinoma in the ascending colon of a WF/O strain rat. Lymphoid follicles in the colon were partially demolished with invasion of neoplastic tissues. ×20.
Fig. 6. High power view of Fig. 5. ×200.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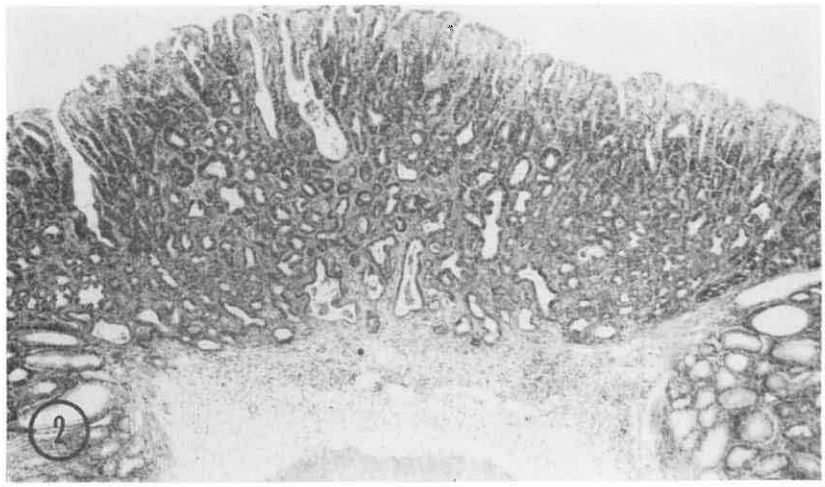
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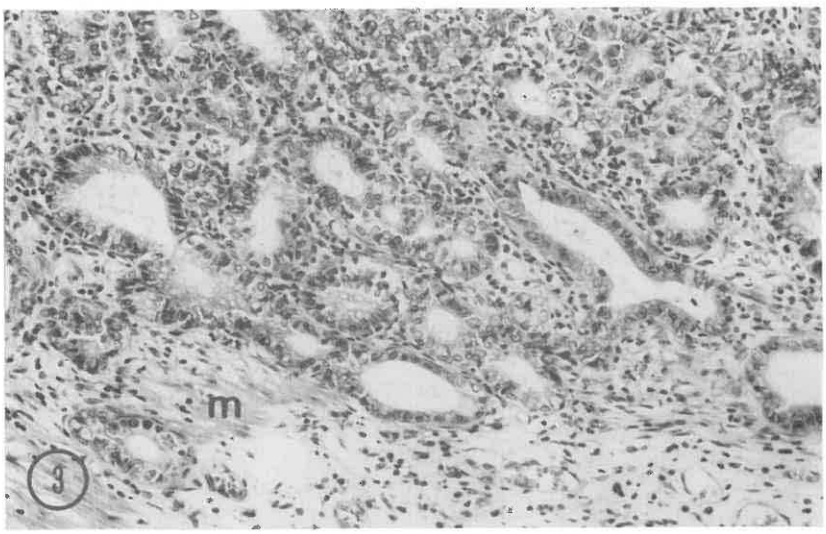
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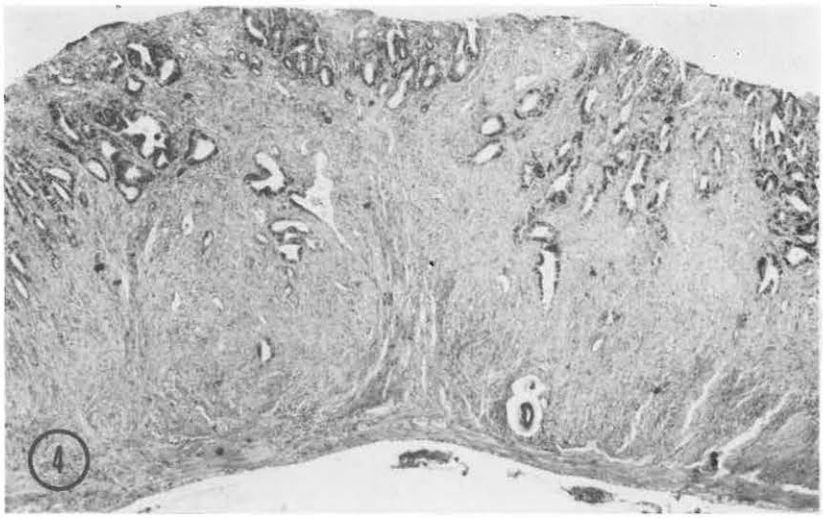
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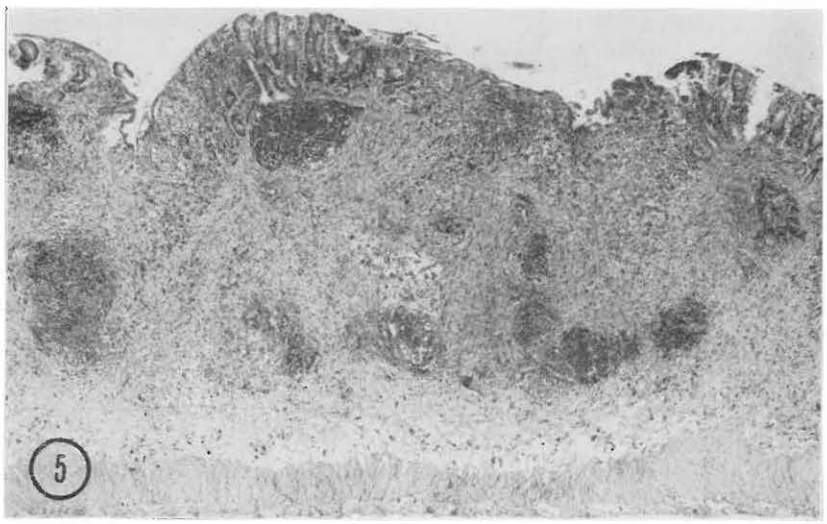
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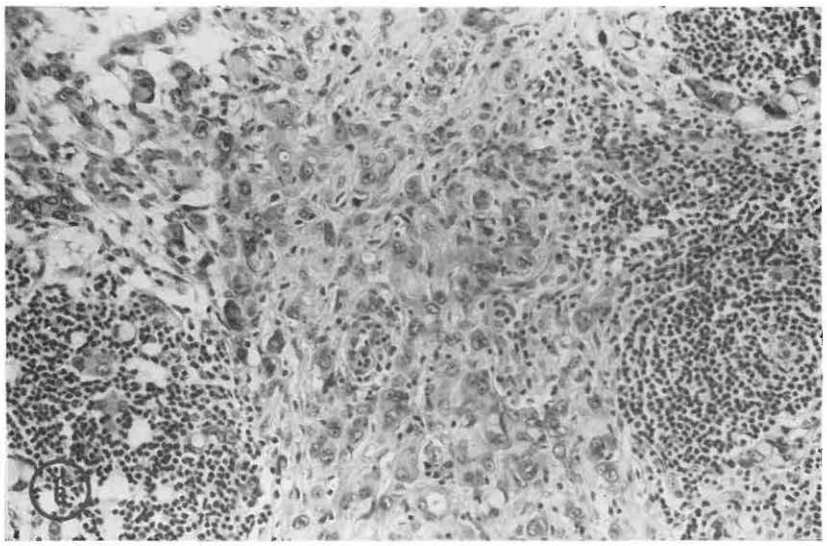
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