Hiroshima Journal of Medical Sciences
Vol.34, No.4, 387~393, December, 1985
HIJM 34-56

Effects of Feeding Regimen on 1,2-Dimethylhydrazine-Induced Intestinal Carcinogenesis in Rats

Hiromichi SUMIYOSHI

The First Department of Pathology, Hiroshima University School of Medicine, 1-2-3, Kasumi, Minami-ku, Hiroshima 734, Japan

(Director: Prof. Eiichi TAHARA)

(Received August 23, 1985)

Key words: 1,2-dimethylhydrazine, Feeding regimen, Intestinal carcinogenesis

ABSTRACT

The effects of feeding regimens on 1,2-dimethylhydrazine (DMH)-induced intestinal carcinogenesis were investigated in male Wistar strain rats. The development of intestinal tumors in rats fed the semipurified powdered diet (SPD) required a longer latent time than that of rats fed the basal pelleted diet (BPD) and the incidence of the tumors, especially adenomas and well differentiated adenocarcinomas, also significantly decreased in the SPD group. However, the incidence of liver cell carcinomas was higher in the SPD group than in the BPD group.

Total bile acid levels in bile were not affected by the diet, but bile acid secretion per hour in the SPD group significantly decreased due to the decrease of bile secretion. Moreover, in the SPD group the colonic epithelial cell proliferation was depressed and the cell cycle time was prolonged when compared to that in the BPD group. These changes might result in a significant decreased incidence of adenomas and well differentiated adnocarcinomas in the SPD group.

These results indicate that feeding regimens play an important role in the proliferation of intestinal epithelial cells and the bile secretion and that they influence not only the development of intestinal tumors but also the organospecific carcinogenesis induced by DMH.

Epidemiologic studies have shown that dietary factors play an important role in the etiology of human colorectal cancer[15,27,29]. The results agree with animal model studies which showed that the incidence of chemical carcinogen-induced colonic carcinomas was increased by high concentrations of dietary fat and protein[1,16,18,20,24], but was decreased by dietary fiber[17,26,28]. Futhermore, bile acid has been well known to promote colorectal carcinogenesis[9,13]. However, the relationship between different feeding regimens and colonic carcinogenesis has been poorly defined, although the influence of each nutritional factor on colonic carcinogenesis has been well investigated.

Subcutanous injections of DMH has been shown to induce colonic adenocarcinomas in rats and mice with marked organ specificity[4,5]. The organospecific carcinogenesis of DMH and its metabolites is altered by dosage, route, speceis of rodent and immunosupressive treatment[5,10,14,25]. However, the effect of feeding regimen on organospecific carcinogenesis induced by DMH has not been elucidated.

The present study was designed to investigate the effect of SPD on organospecific carcinogesis, proliferation and renewal of intestinal epithelial cells, and bile secretion in rats treated with DMH.

MATERIALS AND METHODS

Animals, diets and carcinogen; 4-week-old inbred Wistar strain male rats weighing 60-80g were employed. They were descendants of a

colony obtained in 1961 from the Insitute of Experimental Gerontology in Basal, Switzerland. Animals were housed in a room maintained at 24 ± 2°C and 55 ± 5 % humidity with 14 hr of light and 10 hr of darkness throughout the experiments.

The basal pelleted diet (BPD, CE-2) was purchased from Japan Clea (Osaka, Japan). The semipurified diet (SPD), obtained from Oriental Yeast Co. Ltd. (Tokyo, Japan), consisted of the following ingredients: β-starch, 38 %; vitamin free casein, 25 %; α-starch, 10 %; cellulose powder, 8 %; linol-salad-oil, 6 %; sucrose, 5 %; mineral mix (Oriental formula), 5 % and vitamin mix (Oriental formula), 2 %. The analytical value of major components in both diets are shown in Table 1. Both diets contained approximately the same amount of vitamins and minerals.

1,2-Dimethylhydrazine (DMH) dihydrochloride, purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA), was dissolved with distilled water containing 0.01 % EDTA and the pH was adjusted to 6.5 with $NaHCO_3$.

Table 1. Analytical Value of the Dietary Composition

Component	Content (%)	
	BPD[a]	SPD[b]
Carbohydrate	52.7	53.8
Protein	23.6	22.5
Fat	4.4	6.1
Fiber	4.9	4.4
Ash	6.6	4.2
Water	7.8	9.0

a BPD, basal pelleted diet
b SPD, semipurified powdered diet

Treatment 1; A total of 150 rats were randomly distributed by weight into 4 groups. 25 rats in Group 1 and 40 rats in Group 2 were fed with BPD throughout the experiment, whereas 25 rats in Group 3 and 60 rats in Group 4 were fed with SPD. 2 weeks after acclimation with each diet, all rats in Groups 2 and 4 were given a maximum of 20 weekly s.c. injections of DMH at a dose of 20 mg/kg body weight and rats in Groups 1 and 3 were injected with the vehicle as control. 5 rats each in Groups 1 and 3, 8 rats each in Group 2 and 12 rats each in Group 4 were sacrificed at 10, 15, 20, 25 and 30 weeks after starting DMH injection. The main organs including the intestinal tract were fixed in 10% buffered formalin. The paraffin sections (4.5 μm in thickness) were stained with hematoxylin and eosin, and PAS, and examined histologically.

Treatment 2; 40 rats were randomly divided into 2 groups of 20 rats each, and fed with BPD or SPD throughout the experiment. After 10 weeks of acclimation with each diet, all rats were given 10 weekly s.c. injections of DMH at a dose of 20 mg/kg body weight.

1 week after the last DMH injection, 6 rats in each group were anesthetized with urethane and the polyethylene tube was canulated into the bile duct. The volume of bile secreted for 2 hr was measured. Total bile acid was determined by enzyme-fluorometoric assay using Neo Sterogrnost-3 kit (Daiichi Chemical Co. Ltd., Tokyo, Japan).

The remaining rats in both groups were injected i.p. with 1 mCi of [^{3}H]thymidine (Amersham, England) per kg body weight and were sacrificed at 1 hr, 2 day and 5 day intervals, respectively. The intestinal tract was removed and fixed with 10 % buffered formalin. After fixation representative specimens of duodenum, jejunum, ileum, cecum, proximal colon and distal colon were taken and half of them were employed to determine the incorporation of [^{3}H]thymidine into DNA in the mucosa. DNA was extracted from each scraped mucosa by the method of E.R. Burns et al[2]. The remains were embedded in paraffin and sections were prepared for microautoradiography conducted by the method using NR-M2 emulsion (Konishiroku Co., Japan). The remaining intestinal tract and other organs were examined histologically.

The data obtained were evaluated by Student's t test or X^2 test.

RESULTS

Time course of DMH-induced tumor incidences

The body weights of animals fed the two diets and treated with DMH are shown in Fig. 1. The body weight of animals in the SPD group was no different from that of the BPD group. However, DMH-treated animals gained less weight than did the vehicle treated animals in both diet groups.

Fig. 2 shows the DMH-induced intestinal tumor incidences in animals fed with each diet.

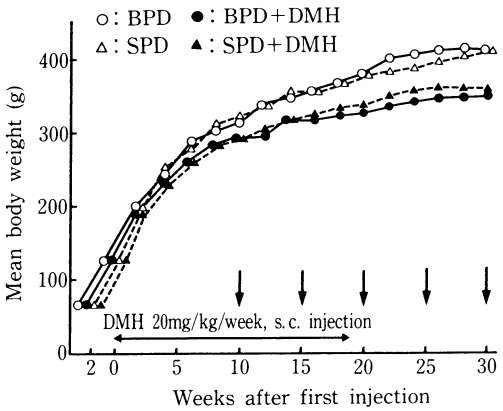

Fig. 1. Average body weights of rats fed BPD or SPD and treated with DMH or vehicle. The arrows show the time of sacrifice

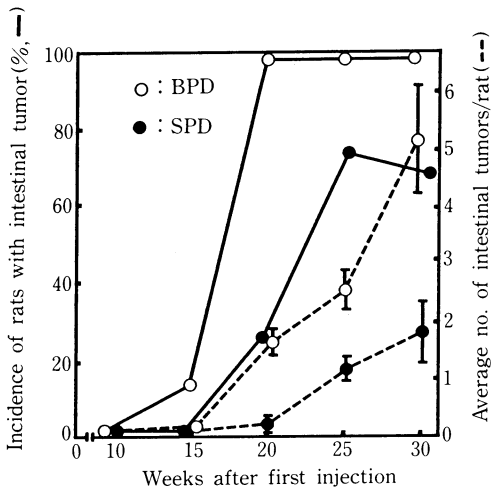

Fig. 2. Time course of DMH-induced intestinal tumor incidences. Animals were fed with BPD (○) or SPD (●) throughout the experiment and given a maximum of 20 weekly s.c. injections of DMH at a dose of 20 mg/kg. Bars, S.E.

No tumor were detected in vehicle-treated groups. Incidences of intestinal tumors in both groups of rats which were treated with DMH increased as the experiment progressed. In the BPD group, an intestinal tumor was detected in 1 of the 8 rats on the 15th week after starting the DMH-treatment, whereas rats without any intestinal tumors were observed in the SPD group. On the 20th week, intestinal tumors developed in all rats fed BPD, but 3 of the 12 rats in the SPD group had intestinal tumors. On the 25th and 30th week, intestinal tumors were observed in all rats in the BPD group and in most of the rats in the SPD group. On the 20th, 25th and 30th week, the number of tumors per rat was significantly higher in the BPD group than in the SPD group ($P<0.05$).

The histologic type and localization of tumors in rats sacrificed on the 25th and 30th week are summarized in Table 2. The incidence of adenomas and differentiated adenocarcinomas was significantly lower in the SPD group than in the BPD group ($P<0.05$). The incidence of poorly differentiated adenocarcinoma including signet ring cell carcinoma, however, was not significantly different between the two diet groups. The localization of tumors in both groups was the same.

The other DMH-induced tumor incidences are shown in Table 3. Both vehicle-treated groups were observed to be tumor free. All liver tumors were liver cell carcinomas. In comparison with the BPD group, liver tumors were observed more frequently in the SPD group. Tumors of the ear cannal were squamous cell carcinomas and its incidence did not differ between the two diet groups.

The effect of interactions between DMH-treatment and feeding regimens on intestinal mucosa and bile secretion.

Body weights of animals fed the two diets did not differ. However, the length of the colon in the SPD group was shorter than that of the BPD group, and the height of the crypts also markedly decreased in the SPD group (Table 4).

The effect of SPD on bile secretion is shown in Table 5. Bile secretion of rats fed SPD significantly decreased in comparison with that of the BPD group ($P<0.01$). Bile acid levels were not affected by the diet, but in the SPD group its secretion per hr decreased due to the depression of bile secretion.

The incorporation of [^{3}H]thymidine into DNA in intestinal mucosa at the first hr after its injection was as follows; the highest was in cecum, the second in ileum, the third in proximal and distal colon, respectively (Table 6). The [^{3}H]thymidine incorporation in cecum and colon was lower in the SPD group than those in the BPD group. Also, the number of labeled epithelial cells per crypt in cecum and colon

Table 2. Histologic Type and Localization of DMH-induced Intestinal Tumors in Rats Sacrificed at 25 and 30 weeks

Group	Histologic type	Localization of tumors[a]			No. of tumors/rat	
		Small intestine	Colon	Total	25 weeks	30 weeks
BPD	Adenoma	10	9	19	0.75±0.31[b](8)[c]	1.63±0.32(8)
	Adenocarcinoma					
	well[d]	9	14	23	0.75±0.25	2.13±0.40
	por[e]	7	12	19	1.00±0.32	1.38±0.32
	total	16	26	42	1.75±0.37	3.50±0.75
SPD	Adenoma	3	4	7	0.17±0.11(12)[f]	0.42±0.19(12)[f]
	Adenocarcinoma					
	well	3	6	9	0.33±0.14[f]	0.42±0.19[f]
	por	9	10	19	0.67±0.19	0.91±0.31
	total	12	16	28	1.00±0.21[f]	1.33±0.47[f]

a Data are presented the amount of tumors in rats sacrificed at 25 and 30 weeks.
b Mean±S.E.
c Value in parentheses, the number of rats sacrificed.
d well, well differentiated adenocarcinoma including moderately differentiated adenocarcinoma.
e por, poorly differentiated adenocarcinoma including signet ring cell carcinoma.
f Significantly different from the BPD group ($P<0.05$).

Table 3. The Incidence of Liver and Ear Canal Tumors Induced by DMH[a]

Group	Organ	10, 15 and 20 weeks[b]		25 weeks		30 weeks	
		No. of rats	No. of rats with tumor	No. of rats	No. of rats with tumor	No. of rats	No. of rats with tumor
BPD	Liver	24	0	8	1 (12.5 %)	8	0
	Ear canal		0		1 (12.5 %)		2 (25 %)
SPD	Liver	36	0	12	6 (50 %)	12	6 (50 %)
	Ear canal		0		0		3 (25 %)

a Animals were given a maximum of 20 weekly s.c. injections of DMH at a dose of 20 mg/kg and were sacrificed at 10, 15, 20, 25 and 30 weeks after the start of DMH injection.
b 8 rats each in the BPD group and 12 rats each in the SPD group were sacrificed at 10, 15 and 20 weeks.

Table 4. Effect of Feeding Regimen on Length and Crypt Height of the Colon[a]

Group	No. of rats	Body weight (g)	Length of colon (mm)	Height of crypt (μm)		
				Cecum	Proximal colon	Distal colon
BPD	20	345±24[b]	287±10	304±21	287±15	250±38
SPD	20	394±34	240± 7[c]	233±29[c]	168±15[c]	193±37[c]

a After 10 weeks of acclimation with each diet, animals were given 10 weekly s.c. injections of DMH at a dose of 20 mg/kg.
b Mean±S.D.
c Significantly different from the BPD group ($P<0.01$).

Table 5. Effect of Feeding Regimen on Bile Secretion[a]

Group	No. of rats	Body weight (g)	Bile secretion (μl/hr)	Bile acid	
				nmole/ml	nmole/hr
BPD	6	348±34[b]	1051±218	29.9±2.4	31.3±5.8
SPD	6	351±37	530±112[c]	31.4±5.1	16.6±3.6[c]

a After 10 weeks of acclimation with each diet, animals were given 10 weekly s.c. injections of DMH at a dose of 20 mg/kg.
b Mean±S.D.
c Significantly different from the BPD group ($P<0.01$).

Table 6. Effect of Feeding Regimen on [^{3}H]Thymidine Incorporation in the Intestinal Mucosa[a]

Group	Sacrificed at	No. of rats	[^{3}H]thymidine incorporation (cpm/mg wet weight)					
			Duodenum	Jejunum	Ileum	Cecum	Proximal colon	Distal colon
BPD	1st hr	5	1142±140[b]	1812±198	1820±190	2710±238	1530±184	1716±245
	2nd day	4	1066±155	2092±334	1885±136	2803±255	1582±231	1804±184
	5th day	5	165± 21	218± 52	370± 35	445± 40	670± 79	637± 89
			(14.4 %)[c]	(12.0 %)	(20.3 %)	(16.4 %)	(43.8 %)	(37.1 %)
SPD	1st hr	5	1090±245	1208±158	1862±296	2405±308	1268±183	1484±252
	2nd day	4	1002±187	1109±266	1836±337	2354±214	1208±378	1491±173
	5th day	5	262± 75	253± 33	594±154	991±201	851±186	905±193
			(23.9 %)	(20.9 %)	(31.9 %)	(41.2 %)	(67.1 %)	(61 %)

a After 10 weeks of acclimation with each diet, animals were given 10 weekly s.c. injections of DMH at a dose of 20 mg/kg. 1 week after the last DMH injection, animals were i.p. injected 1 mCi of [^{3}H] thymidine per kg body weight and were sacrified at 1 hr, 2 day and 5 day intervals.
b Mean±S.E.
c Values in parentheses, the percent-ratio of [^{3}H]thymidine incorporation on the fifth day/first hour.

Table 7. Effect of Feeding Regimen on DNA Synthesis in the Colon[a]

Group	No. of rats	No. of labeled cells/crypt		
		Cecum	Proximal colon	Distal colon
BPD	5	36.6±4.1[b]	19.7±3.1	19.9±5.2
SPD	5	25.8±2.4[c]	8.8±2.7[c]	12.3±2.2[c]

a After 10 weeks of acclimation with each diet, animals were given 10 weekly s.c. injections of DMH at a dose of 20 mg/kg. 1 week after the last DMH injection, animals were i.p. injected with 1 mCi of [^{3}H]thymidine per kg body weight and were sacrified 1 hr after its injection.
b Mean±S.D.
c Significantly different from the BPD group ($P<0.05$).

clearly decreased in the SPD group (Table 7). On day 2 the incorporation of [^{3}H]thymidine in all segments of intestine was approximately equivalent to that at the first hr in both groups. Most of the incorporated [^{3}H]thymidine in the BPD group was eliminated from the intestinal mucosa on day 5, but in the SPD group it was eliminated more slowly and the ratio of [^{3}H]thymidine incorporation on fifth day/first hr was two fold of the ratio of the BPD group (Table 6).

Furthermore, in this treatment no tumor was observed in the SPD group, but signet ring cell carcinoma in proximal colon was detected in one of the 20 rats in the BPD group.

DISCUSSION

The colonic mucosal atrophy and the decrease

in colonic epithelial cell proliferation are known to occur in rats fed a liquid diet or a synthetic diet[8,11,21,22]. On the one hand, administration of DMH to rats has been shown to produce colonic mucosal hyperplasia, an increase in the size of the proliferation zone, an increase in colonic crypt cell production and a shortness of cell cycle time[19,23]. However, D. W. Heitman et al[7] reported that an increase in colonic epitherial cell proliferation induced by DMH was not observed in rats which were maintained on a parenteral nutrition. In the present study, the incidence of intestinal tumors induced by DMH was significantly lower in the SPD group than in the BPD group. Moreover, in rats fed SPD and treated with DMH the proliferation of colonic epithelial cells was supressed and the colonic epithelial cell cycle time was prolonged. These mucosal changes which occurred in the SPD group might result in a decreased incidence of DMH-induced intestinal tumors, especially adenomas and well differentiated adenocarcinomas. W. M. Castleden[3] also demonstrated the prolonged survival and decrease in intestinal tumors in DMH-treated rats fed a liquid diet.

Bile acids are well known to promote the development of intestinal tumors induced by N-methyl-N'-nitro-N-nitrosoguanidine or DMH in rats[9,13]. In the present study, the bile acid secretion of rats in the SPD group decreased due to the decrease in bile secretion, while the bile acid level was not affected by the diet. The decrease in bile acid secretion could possibly suppress the development of intestinal tumors in the SPD group.

E. S. Fiala[6] reported that DMH was metabolized in the liver through the hydroxylation of azoxymethane to methylazoxymethanol (MAM). The exclusive production of colorectal tumors by DMH or azoxymethane is suggested to be attributable to tissue-specific enzymes which release methylating radical from MAM, to receptors to MAM, or to an absence of detoxifying enzymes[6,30]. Furthermore, the organospecific carcinogenesis induced by DMH and azoxymethane have been shown to be altered by dosage, route and speceis of rodent[5,14,25]. In the present study, 20 weekly s.c. injections of DMH (20 mg/kg) specifically induced the intestinal tumors in rats fed BPD, but in rats fed SPD intestinal tumors decreased and liver cell carcinomas increased. Why liver cell carcinomas occurred in the SPD group is unknown. The following possibilites, however, are considered; 1) the excretion of MAM from liver cells might be inhibited, 2) detoxifying enzymes of MAM might be reduced in rats fed SPD, because of the liver hypofunctions, and 3) MAM might break down into the ultimate carcinogen in liver cells as well as *in vitro*[12].

These results suggest that the development of intestinal tumors is influenced by not only each nutritional element such as fat, protein and fiber but also feeding regimen. Furthermore, the organospecific carcinogenesis induced by DMH could be altered by feeding regimen.

ACKNOWLEDGMENT

I am greatly indebted to Professor Eiichi Tahara, the First Department of Pathology, Hiroshima University School of Medicine, for his kind guidance and critical review of this manuscript. I wish to appreciate all the stuff members of the First Department of Pathology, Hiroshima University School of Medicine, for their valuable suggestions.

Some sections of this manuscript were presented at the 43rd Annual Meeting of the Japanese Cancer Association, October 3-5, 1984, Fukuoka, Japan.

REFERENCE

1. **Bull, A. W., Soullier, B. K., Wilson, P. S., Hayden, M. T. and Nigro, N. D.** 1979. Promotion of azoxymethane-induced intestinal cancer by high-fat in rats. Cancer Res. **39**: 4956-4959.
2. **Burns, E. R., Scheving, L. E., Pauly, J. E. and Tsai, T. H.** 1976. Effect of altered lighting regimens, time-limited feeding, and presence of Ehelich ascites carcinoma on the circadian rhythm in DNA synthesis of mouse spleen. Cancer Res. **36**: 1358-1544.
3. **Castleden, W. M.** 1977. Prolonged survival and decrease in intestinal tumours in dimethylhydrazine-treated rats fed a chemically defined diet. Br. J. Cancer **35**: 491-495.
4. **Druckrey, H.** 1970. Production of colonic carcinomas by 1,2-dimethylhydrazine and azoxymethane. In: Carcinoma of the Colon and Antecedent Epithelium (W. J. Burdette ed.), Springfield, II. Charles C Thomas, Publisher, Chap. 20, pp. 267-279.
5. **Druckrey, H.** 1972. Organospecific carcinogenesis in digestive tract. In: Topics in Chemical Carcinogenesis (Nakahara, W., Takayama, S.,

Sugimura, T., et al. eds.), Tokyo, Japan Scientific Societies Press, pp. 73-101.

6. **Fiala, E. S.** 1977. Investigations into the metabolism and mode of action of the colon carcinogens 1,2-dimethylhydrazine and azoxymethane. Cancer **40**: 2346-2445.
7. **Heitman, D. W., Grubbs, B. G., Heitman, T. O. and Cameron, I. L.** 1983. Effect of 1,2-dimethylhydrazine treatment and feeding regimen on rat colonic epithelial cell proliferation. Cancer Res. **43**: 1153-1162.
8. **Janne, P., Carpentier, Y. and Willems, G.** 1977. Colonic mucosal atrophy induced by a liquid elemental diet in rats. Am. J. Dig. Dis. **22**: 808-812.
9. **Koga, S., Kaibara, N. and Takeda, R.** 1982. Effect of bile acids on 1,2-dimethylhydrazine-induced colon cancer in rats. Cancer **50**: 543-547.
10. **Kres, R., Berkvens, J. M. and Weisburger, J. H.** 1975. Immunosupression in primary liver and colon tumor induction with N-hydro-N-2-fluorenylacetamide and azoxymethane. Cancer Res. **35**: 2651-2656.
11. **Morin, C. L., Jing, V. and Bourassa, D.** 1980. Small intestine and colonic changes induced by a chemically defined diet. Dig. Dis. Sci. **25**: 123-128.
12. **Nagasawa, H. T., Shirota, F. N. and Matsumoto, H.** 1972. Decomposition of methylazoxymethane, the aglycone of cycasin, in D_2O. Nature (Lond.) **236**: 234-235.
13. **Narisawa, T., Magadia, N. E., Weisburger, J. H. and Wynder, E. L.** 1974. Promoting effect of bile acids on colon carcinogenesis after instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. J. Natl. Cancer Inst. **55**: 1093-1097.
14. **Narisawa, T., Wong, C. Q. and Weisburger, J. H.** 1976. Azoxymethane-induced hemangiosarcomas in inbred strain-2 guniea pigs. J. Natl. Cancer Inst. **56**: 653-654.
15. **Reddy, B. S., Cohen, L., McCoy, G. D., Hill, P., Weisburger, J. H. and Wynder, E. L.** 1980. Nutrition and its relationship to cancer. Adv. Cancer Res. **32**: 237-345.
16. **Reddy, B. S. and Maeura, Y.** 1984. Tumor promotion by dietary fat in azoxymethane-induced colon carcinogenesis in female F344 rats: Influence of amount and source of dietary fat. J. Natl. Cancer Inst. **3**: 745-750.
17. **Reddy, B. S., Mori, H. and Nicolais, M.** 1981. Effect of dietary wheat bran and dehydrated citrus fiber on azoxymethane-induced intestinal carcinogenesis in Fischer 344 rats. J. Natl. Cancer Inst. **66**: 553-557.
18. **Reddy, B. S., Narisawa, T. and Weisburger, J. H.** 1976. Effect of a diet with high levels of protein and fat on colon carcinogenesis in F344 rats treated with 1,2-dimethylhydrazine. J. Natl. Cancer Inst. **57**: 567-569.
19. **Richards, T. C.** 1981. The effects of the carcinogen, 1-2dimethylhydrazine, on turnover of ^{3}H-thymidine labeled cells from mucosal glands of mouse colon. Anat. Rec. **200**: 299-308.
20 **Sakaguchi, M., Hiramatsu, Y., Takeda, H., Yamamura, M., Hioki, K., Saito, K. and Yamamoto, M.** 1984. Effect of dietary unsaturated and saturated fats on azoxymethane-induced colon carcinogenesis is rats. Cancer Res. **44**: 1472-1477.
21. **Sicar, B., Johnson, L. R. and Lichtenberger, L. M.** 1983. Effect of Synthetic diets on gastrointestinal mucosal DNA synthsis in rats. Am. J. Physiol. **244**: G327-G335.
22. **Storme, G. and Willems, G.** 1981. The effect of liquid elemental diet on cell proliferation in the colon of rats. Cell Tissue Res. **216**: 221-225.
23. **Sunter, J. P., Watson, A. J. and Appleton, D. R.** 1981. Kinetics of the non-neoplastic mucosa of the large bowel of dimethylhydrazine-treated rats. Br. J. Cancer **44**: 35-44.
24. **Topping, D. C., and Viseck, W. J.** 1976. Nitrogen intake and tumorigenesis in rats injected with 1,2-dimethylhydrazine. J. Nutr. **106**: 1583-1587.
25. **Toth, B.** 1972. Morphological studies of angiosarcoma induced by 1,2-dimethylhydrazine in Syrian golden hamster. Cancer Res. **32**: 2818-2827.
26. **Watanabe, K., Reddy, B. S., Wiesburger, J. H. and Kritchevsky, D.** 1979. Effect of dietary alphalfa, pectin, and wheat bran on azoxymethane or methylnitrosourea-induced colon carcinogenesis in F344 rats. J. Natl. Cancer Inst. **63**: 141-145.
27. **Weisburger, J. H., Reddy, B. S. and Wynder, E. L.** 1977. Colon cancer: Its epidemiology and experimental production. Cancer **40**: 2414-2420.
28. **Wilson, R. B., Hutcheson, D. P. and Wideman, L.** 1977. Dimethylhydrazine-induced colon tumor in rats fed diet contaning beef fat or corn oil with and without wheat bran. Am. J. Clin. Nutr. **30**: 176-181.
29. **Zaridze, D. G.** 1983. Enviromental etiology of large-bowel cancer. J. Natl. Cancer Inst. **70**: 389-400.
30. **Zedeck, M. S., Grab, D. J. and Sternberg, S. S.** 1977. Differences in the acute response of the various segments of rat intestine to treatment with the intestinal carcinogen methylazoxymethanol acetate. Cancer Res. **37**: 32-36.