Hiroshima Journal of Medical Sciences
Vol.34, No.3, 363~369, September, 1985
HIJM 34-52

Effects of Vitamin E Deficiency on 1,2-Dimethylhydrazine-Induced Intestinal Carcinogenesis in Rats

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(Received June 25, 1985)

Key words: 1,2-dimethylhydrazine, Vitamin E deficiency, Intestinal carcinogenesis

ABSTRACT

The effects of vitamin E deficiency on intestinal carcinogenesis and intestinal epithelial cell kinetics were investigated in male Wistar strain rats treated with 1,2-dimethylhydrazine (DMH) dihydrochloride. In the time course study of intestinal tumors induced by DMH (20 mg/kg, 20 weekly s.c. injections), at the early stage when no intestinal tumor was observed in control rats, tumor-bearing rats were detected in the vitamin E deficient group. Moreover, in rats treated with DMH (20 mg/kg, 3 weekly s.c. injections) after vitamin E deficient status, the incidence of rats with intestinal tumors was significantly higher in the vitamin E deficient group (58.3 %) than in the sufficient group (8.3 %) at 15 weeks after DMH treatment ($p<0.05$). However, after 45 weeks the incidence of tumors was the same in the both groups. In the cell kinetics study at 15 weeks after DMH treatment, DNA synthsis in the intestinal mucosa increased significantly in the vitamin E deficient group compared to that of the sufficient group. Nevertheless, the life span of intestinal epithelium was shorter in vitamin E deficient rats than in sufficient rats.

These results suggest that vitamin E deficiency promotes the initiation in colon carcinogenesis induced by DMH but inhibits the growth of the tumor.

Epidemiologic studies have shown that dietary factors play an important role in human colon carcinogenesis[15,33,35]. Animal model studies also indicate that high concentrations of dietary fat and protein increase the incidence of colon tumors induced by chemical carcinogens[2,16,18,21,28], while dietary fiber decreases its incidence[17,31,32]. In addition to these major nutritional elements, micronutrients such as vitamin A and selenium play a modifying role in colon carcinogenesis[11,13,14]. Also, vitamin E, which has antioxidant properties and stabilizes the cell membrane[12,25,26], has been shown to decrease the incidence of colon tumor induced by 1,2-dimethylhydrazine (DMH)[5]. However, Toth et al[29] suggested that a supplement of vitamin E enhanced the intestinal tumorigenecity of DMH in mice. The contradictory effects of vitamin E on experimental carcinogenesis in the other organ have been also reported[8-10,22,23,24].

In this study, we investigated the effect of vitamin E deficiency on intestinal carcinogenesis and on intestinal epithelial cell kinetics in male rats treated with DMH in an attempt to clarify the relation between vitamin E deficiency and carcinogenesis.

MATERIALS AND METHODS

Animals, diets and carcinogen; 4-week-old inbred Wistar strain male rats weighing 60-80 g were employed. They were descendants of a colony obtained in 1961 from the Institute of

Experimental Gerontology in Basel, 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.

Semipurified powdered diet (SPD) containing 10 mg vitamin E per 100 g as control diet and SPD without vitamin E (below 0.5 mg/100 g) as vitamin E deficient diet (VED) were obtained from Oriental Yeast Co. Ltd. (Tokyo, Japan). The composition of the diets is shown in Table 1.

DMH dihydrochloride perchased 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 $NaHCO_3$ before use.

Treatment 1; A total of 175 rats were randomly divided into 4 groups. 25 rats in Group 1 and 60 rats in Group 2 were fed on SPD throughout the experiment, whereas 25 rats in Group 3 and 65 rats in Group 4 were fed on VED. 2 weeks after acclimation with each diet, rats in Group 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, 12 rats each in Group 2 and 13 rats each in Group 4 were sacrificed at 10, 15, 20, 25 and 30 weeks after the first DMH injection, respectively and main organs including 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; 79 rats were randomly divided into 2 groups. 38 rats in Group 1 were fed on the SPD and 41 rats in Group 2 were fed on VED throughout the experiment. In order to determine the vitamin E deficient status before DMH treatment, 5 weeks after the acclimation with each diet, 5 rats each in both groups were sacrificed and serum vitamin E level was measured by the fluorometric assay[27]. The remaining rats were given 3 weekly s.c. injections of DMH at a dose of 20 mg/kg body weight.

15 weeks after the last DMH injection, 12 rats each in both groups were randomly selected out and were injected i.p. with 1 mCi of [^{3}H]thymidine (Amersham, England) per kg body weight. 6 rats each in both groups were sacificed at 1hr and 5 day intervals. 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 they 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 Burns et al[3]. The remaining intestinal tracts were examined histologically.

The other rats were sacrificed at 45 weeks after the last DMH injection. Histological examinations were performed in the same manner as in Treatment 1.

Table 1. Composition of the Experimental Diets

Ingredient	Content (g/100g)	
	SPD	VED
α-starch	10	
ß-starch	38	
Vitamin free casein	25	
Sucrose	5	
Linol salad oil	6	
Cellulose	8	
Mineral mix[a]	6	
Vitamin mix[b]	2	
Vitamin E	10 mg	ND[c]

a Mineral mix contains 14.56 g $CaHPO_4 \cdot 2H_2O$; 25.72 g of KH_2PO_4; 9.35 g of NaH_2PO_4; 4.66 g of NaCl; 35.09 g of Ca-lactate; 3.18 g of Fe-citrate; 7.17 g of $MgSO_4$; 0.11 g of $ZnCO_3$; 0.12 g of $MnSO_4 \cdot 4\text{-}6H_2O$; 0.03 g of $CuSO_4 \cdot 5H_2O$ and 0.01 g of KI per 100 g.

b Vitamin mix contains 100 mg of vitamin A; 0.25 mg of vitamin D; 520 mg of vitamin K; 120 mg of vitamin $B_1 \cdot HCl$; 400 mg of vitamin B_2; 80 mg of vitamin $B_6 \cdot HCl$; 0.05 mg of vitamin B_{12}; 3,000 mg of vitamin C; 2.0 mg of biotin; 20 mg of folic acid; 500 mg of Ca-panthotate; 500 mg of p-aminobenzoic acid; 600 mg of nicotinic acid; 600 mg of inositol; 20,000 mg of choline chloride and cellulose powder per 100 g.

c ND, not detectable (below 0.5 mg/100 g).

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

RESULTS

Time course of DMH-induced tumor incidences

Body weights of animals fed the two diets and treated with DMH are shown in Fig. 1. Body weights of animals were not different between the SPD group and the VED group. However, DMH-treated animals gained less weight than did the vehicle treated animals in both diet groups.

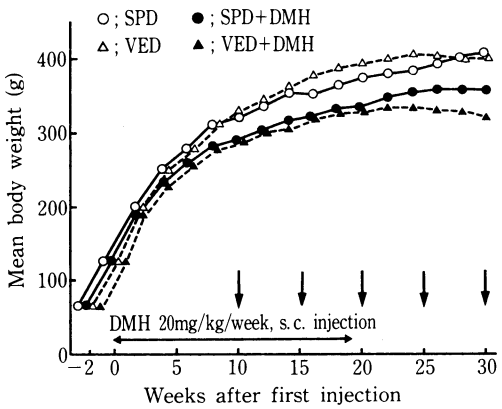

Fig. 1. Average body weight of rats fed SPD or VED and treated with DMH or the vehicle. The arrows show the time of sacrifice.

Table 2 shows the time course of DMH-induced tumor incidences in rats fed SPD and VED. The incidence of rats with intestinal tumors in both groups treated with DMH increased as the experiment progressed. In the VED group, intestinal tumors were detected in 2 of the 13 rats on the 10th week and 3 of the 13 rats on the 15th week after the first DMH injection, whereas no rat with intestinal tumor was observed in the SPD group. On the 20th week, the incidence of rats with intestinal tumors in the VED group was higher tendency than that in the SPD group ($0.05<p<0.1$) and the number of intestinal tumors per rats were significantly higher in the VED group than in the SPD group ($p<0.05$). However, after 25 and 30 weeks, the incidence of intestinal tumors was not significantly different between the SPD group and the VED group. There was also no difference in the histologic type and the localization of intestinal tumors between the SPD group and the VED group.

Liver tumors, all of which were liver cell carcinomas, developed in the SPD group after the 25th week, while they developed in the VED group after the 20th week (Table 2). Ear canal tumors, which were squamous cell carcinomas, developed in the SPD group on the 30th week and in the VED group after the 25th week. However, the incidences of liver tumors and ear canel tumors were not significantly different between both groups.

Table 2. Time Couse of DMH-Induced Tumor Incidences[a]

Group	Weeks	No. of rats	Intestinal tumor				No. of rats with liver tumor	No. of rats with ear canal tumor
			No. of rats with tumors	No. of tumors/rat				
				Total	Adenoma	Adeno-carcinoma		
SPD	10	12	0 (—)	—	—	—	0	0
	15	12	0 (—)	—	—	—	0	0
	20	12	2 (16.7 %)	0.17±0.11[b]	—	0.17±0.11	0	0
	25	12	9 (75.0 %)	1.17±0.24	0.17±0.11	1.00±0.21	6 (50 %)	0
	30	12	8 (66.7 %)	1.75±0.45	0.42±0.19	1.33±0.36	6 (50 %)	3 (25 %)
VED	10	13	2 (15.4 %)	0.15±0.10	—	0.15±0.10	0	0
	15	13	3 (23.1 %)	0.23±0.12	—	0.23±0.12	0	0
	20	13	8 (61.5 %)	1.00±0.32[c]	0.15±0.10	0.85±0.33	3 (23 %)	0
	25	13	10 (76.9 %)	1.38±0.33	0.23±0.17	1.15±0.32	8 (62 %)	1 (8 %)
	30	13	11 (84.6 %)	1.92±0.37	0.08±0.08	1.85±0.38	8 (62 %)	4 (31 %)

a Animals were given weekly s.c. injections of DMH (a maximum of 20 injections) at a dose of 20 mg/kg and sacrificed at 10, 15, 20, 25 and 30 weeks after the first DMH injection.

b Mean±S.E.

c Significantly different from the SPD group ($p<0.05$).

In rats treated with the vehicle, no tumor occurred in both the SPD and VED group throughout the experiment.

Incidence of tumors in rats treated with DMH after vitamin E deficient status and intestinal epithelial cell kinetics

The serum vitamin E level was significantly lower in the VED group than in the SPD group before and after DMH treatment (Table 3).

Table 4 shows the incidence of intestinal tumors in rats fed SPD and VED on the 15th and 45th week after DMH treatment. In the VED group, intestinal tumors were detected in 7 (58.3 %) of the 12 rats on the 15th week after DMH treatment, whereas in the SPD group they were observed in one (8.3 %) of the 12 rats. There was a significant difference in the incidence of rats with intestinal tumors between both groups ($P<0.05$). However, after 45 weeks the incidence was not significantly different between the SPD group (47.6 %) and the VED group (45.8 %). Moreover, there was no difference in the invasive stage of adenocarcinomas between the SPD group and the VED group (Table 5). The number, the histologic type and the localization of intestinal tumors also were not different between both groups.

The incorporation of [^{3}H]thymidine into DNA in the intestinal mucosa is summarized in Table 6. At the first hour, the [^{3}H]thymidine incorporation was higher in the VED group than in the SPD group in all segments of intestine examined. However, on the 5th day it was eliminated more rapidly in the VED group and the ratio of [^{3}H]thymidine incorporation on the 5th day/first hour was significantly lower in the VED group than in the SPD group. These results showed that in vitamin E deficient rats DNA synthesis of the intestinal epithelium more increased but the life span of them was shorter when compared to those of vitamin E sufficient rats.

Extra-intestinal tumor was not found on the 15th week. On the 45th week an ear canal tumor was observed in one of the 21 rats in the SPD group, and a renal tumor was observed in one of the 24 rats in the VED group.

Table 3. Serum Vitamin E Level before DMH Treatment and at Sacrificed

Group	Serum vitamin E level (mg/dl)		
	before DMH treatment	after DMH treatment	
		15 weeks	45 weeks
SPD	0.90 ± 0.05[a] (n=5)	0.90 ± 0.04 (n=12)	0.85 ± 0.03 (n=21)
VED	0.18 ± 0.06 (n=5)[b] (2)[c]	0.11 ± 0.02 (n=12)[b] (9)[c]	ND (n=24)[d]

a Mean ± S.D.
b Significantly different from the SPD group ($p<0.01$).
c Number of rats whose serum vitamin E were not detectable (below 0.10 mg/dl).
d ND, not detectable in all rats.

Table 4. Incidence of Intestinal Tumors in Rats Treated with DMH after Vitamin E Deficient Status[a]

Sacrificed at	Group	No. of rats	No. of rats with tumor	No. of tumors/rat		
				Total	Adenoma	Adenocarcinoma
15 weeks	SPD	12	1 (8.3 %)	0.08 ± 0.08[b]	—	0.08 ± 0.08
	VED	12	7 (58.3 %)[c]	0.75 ± 0.22[c]	0.08 ± 0.08	0.67 ± 0.18[c]
45 weeks	SPD	21	10 (47.6 %)	0.76 ± 0.19	0.05 ± 0.05	0.71 ± 0.18
	VED	24	11 (45.8 %)	0.67 ± 0.18	0.08 ± 0.06	0.59 ± 0.16

a Animals were given 3 weekly s.c. injections of DMH at a dose of 20 mg/kg and sacrificed at 15 and 45 weeks after the DMH treatment.
b Mean ± S.E.
c Significantly different form the SPD group ($p<0.05$).

Table 5. Invasive Stage of Adenocarcinomas in Rats Treated with DMH after Vitamin E Deficient Status[a]

Sacrificed at	Group	No. of adeno-carcinomas	Depth of invasion[b]		
			m+sm	pm	ss
15 weeks	SPD	1	1	0	0
	VED	9	8	0	1
45 weeks	SPD	13	10	0	3[c]
	VED	14	12	1	1

a Animals were given 3 weekly s.c. injections of DMH at a dose of 20 mg/kg and sacrificed at 15 and 45 weeks after the DMH treatment.
b m+sm, mucosa and submucosa; pm, muscularis propria and ss, subserosa.
c One of the three adenocarcinomas metastasized to the mesenteric lymph node.

Table 6. Effect of Vitamin E Deficiency on [^{3}H]thymidine Incorporation in the Intestinal Mucosa of DMH-Treated Rats[a]

Group	Sacrificed at	No. of rats	[^{3}H]thymidine incorporation (cpm/mg wet weight)					
			Duodenum	Jejunum	Ileum	Cecum	Proximal colon	Distal colon
SPD	1st hr	6	976±164[b]	1000±194	2049±205	2588±359	1215±160	1122±158
	5th day	6	234± 65	203± 66	550± 75	1092±210	815±168	786±181
			(23.9 %)[c]	(20.3 %)	(26.8 %)	(42.2 %)	(67.1 %)	(70.1 %)
VED	1st hr	6	1498±216[d]	1296±211[d]	2482±340[d]	3418±532[d]	1836±377[d]	1426±396
	5th day	6	158± 21[d]	147± 32	359± 57[d]	766±166[d]	680±123	559±196
			(10.5 %)	(9.8 %)	(14.5 %)	(22.4 %)	(37.0 %)	(39.2 %)

a 15 weeks after the DMH treatment, animals were sacrificed at 1 hr or 5 days after the [^{3}H]thymidine (1 mCi/kg) i.p. injection.
b Mean±S.D.
c Values in parentheses, the percent-ratio of [^{3}H]thymidine incorporation on the fifth day/first hr.
d Significantly different from the SPD group ($p<0.05$).

DISCUSSION

The relationship between vitamin E and tumorigenesis induced by chemical carcinogens has been reported with contradictory results[5,8-10,22,23,29,34]. Cook et al[5] showed that the incidence of intestinal tumors in mice treated with DMH was decreased by a supplement of vitamin E. Moreover, in the other experimental tumor systems, the inhibitory effect of vitamin E on carcinogenesis has been demonstrated[3,22-24]. In the present study, at the early stage when no intestinal tumors developed in vitamin E sufficient rats treated with DMH, the tumors had already occurred in vitamin E deficient rats. Moreover, in rats treated with DMH after vitamin E deficient status, the incidence of intestinal tumors on the 15th week was significantly higher than that in vitamin E sufficient rats. These results suggest that vitamin E deficiency causes the shorter latent period of intestinal tumors induced by DMH. Therefore, vitamin E might inhibit the development of tumor. Vitamin E, which has antioxidant properties and scavenges free radicals[12,25,26], might neutralize or reduce ultimate carcinogen (free radical) which is the metabolite of DMH. However, the inhibitory mechanism of vitamin E on DMH-induced intestinal carcinogenesis has not been clarified in the present study.

The increase of colonic cell proliferation has been shown to be related to an increased incidence of chemical carcinogen induced tumors[4]. On the one hand, vitamin E deficiency has been shown to result in the increase of DNA synthesis and activation of DNA polymerase in the liver and muscle[6,7,24]. In the present study also, the increase of DNA synthesis of the intestinal mucosa was observed in vitamin E deficient rats treated with DMH in comparison with that of vitamin E sufficient rats. This change might also

lead to the shorter latent period of DMH-induced intestinal tumor and its higher incidence in the early stage in vitamin E deficient rats.

On the other hand, Baker et al[1] reported the elevated tissue content of vitamins in colon adenocarcinomas as compared with that in normal adjacent mucosa. Furthermore, the deficiency of vitamins such as riboflavin and vitamin B_6 has been shown to inhibit the growth of tumors[19,20,30]. The possibilities would seem to be; 1) the tumor needs extra vitamins for accelerated growth, and 2) vitamin E deficiency depresses the tumor growth. Indeed, although at the early stage the incidence of intestinal tumors in vitamin E deficient group was significantly higher than that of vitamin E sufficient group, at the late stage the incidence and the invasive stage of intestinal tumors was not different between vitamin E deficient group and sufficient group. Tumor growth was considered to be inhibited by the vitamin E deficiency, because tumor cells should have a short life span as well as normal intestinal epithelium in vitamin E deficient rats. Therefore, a supplement of vitamin E might be a beneficial factor for tumor growth. It may agree with the enhanced effect of vitamin E on mice intestinal carcinogenesis induced by DMH[29].

These results suggest that vitamin E deficiency promotes the initiation in carcinogenesis but inhibits the tumor growth. Consequently, the relationship between vitamin E and carcinogenesis seems to vary with the stage of DMH-induced colon carcinogenesis.

ACKNOWLEDGMENT

I am greatly indebted to Professor Eiichi Tahara, 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 First Department of Pathology, Hiroshima University School of Medicine, for thier valuable suggestions.

Some sections of this manuscript were presented at the 73rd Annual Congress of the Japanese Pathological Society, April 3-5, 1984, Tokyo and the third International Congress on Cell Biology, August 26-31, 1984, Tokyo, Japan.

REFERENCE

1. **Baker, H., Frank, O.F., Chen, R., Feingold, S., DeAngelis, B. and Baker, E.R.** 1981. Elevated vitamin levels in colon adenocarcinoma as compared with metastatic liver adenocarcinoma from colon primary and normal adjacent tissue. Cancer **47**: 2883-2886.
2. **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.
3. **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**: 1538-1544.
4. **Cohen, B.I., Raicht, R.F., Deschner, E.E., Takahashi, M., Sarwal, A.N. and Fazzini, E.** 1980. Effect of cholic acid feeding on N-methyl-N-nitrosourea-induced colon tumors and cell kinetics in rats. J. Natl. Cancer Inst. **64**: 573-578.
5. **Cook, M.G. and McNamara, P.** 1980. Effect of dietary vitamin E on dimethylhydrazine-induced colonic tumors in mice. Cancer Res. **40**: 1329-1331.
6. **Devi, A., Raina, P.L. and Singh, A.** 1965. Abnormal protein and nucleic acid metabolism as a cause of cataract formation induced by nutritional deficiency in rabbits. Brit. J. Ophthal. **49**: 271-275.
7. **Dinning, J.S.** 1962. Nucleic acid metabolism in vitamin E deficiency. Vitamins and Hormones **20**: 511-519.
8. **Epstein, S.S., Andrea, J., Forsyth, J. and Mantel, N.** 1667. The null effect of antioxidants on the carcinogenecity of 3,4,9,10-dibenzpyrene to mice. Life Sci. **6**: 225-233.
9. **Haber, S.L. and Wissler, R.W.** 1962. Effect of vitamin E on carcinogenecity of methylcholanthrene. Proc. Soc. Exp. Biol. Med. **111**: 774-775.
10. **Horvath, P.M. and Ip, C.** 1983. Synergistic effect of vitamin E and selenium in the chemoprevention of mammary carcinogenesis in rats. Cancer Res. **43**: 5335-5341.
11. **Jacobs, M.M., Forst, C.F. and Beams, F.A.** 1981. Biochemical and clinical effects of selenium on dimethyl-hydrazine-induced colon cancer in rats. Cancer Res. **41**: 4458-4465.
12. **McCay, P.B., Royer, J.L., Pfeifer, P.M., May, H.E. and Gilliam, H.E.** 1971. A function of α-tocopherol: Stabilization of the microsomal membrane from radical attack during TPNH-dependent oxidations. Lipids **6**: 297-306.
13. **Narisawa, T., Reddy, B.S., Wong, C.Q. and Weisburger, H.** 1976. Effect of vitamin A deficiency on rat colon carcinogenesis by N-methyl-N'-nitro-N-nitrosoguanidine. Cancer Res **36**: 1379-1383.
14. **Nigro, N.D., Bull, A.W., Wilson, P.S., Spullier, B.K. and Alousi, M.A.** 1982. Combined in-

hibitors of carcinogenesis: Effect on azoxymethane-induced intestinal cancer in rats. J. Natl. Cancer Inst. **69**: 103-107.

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. **Rivlin, R.S., Hornibrook, R. and Osnos, M.** 1973. Effects of riboflavin deficiency upon cocentration of riboflavin, flavin mononucleotide, and flavin dinucleotide in Nobikoff hepatoma in rats. Cancer Res. **33**: 3019-3023.
20. **Rosen, F., Mihich, E. and Nichol, C.A.** 1964. Selective metabolic and chemotherapeutic effects on vitamin B_6 antimetabolites. Vitamins and hormones **22**: 609-640.
21. **Sakaguchi, M., Hiramatsu, Y., Takeda, H., Yamamura, M., Hioki, K., Saito, K. and Yamamoto, M.** 1984. Effect of dietary unsaturated and saturated fasts on azoxymethane-induced colon carcinogenesis in rats. Cancer Res. **44**: 1472-1477.
22. **Shklar, G.** 1982. Oral mucosal carcinogenesis in hamsters: Inhibition by vitamin E.J. Natl. Cancer Inst. **68**: 791-797.
23. **Slaga, T.J. and Bracken, W.M.** 1977. The effects of antioxidants an skin tumor initiation and aryl hydrocarbon hydroxylase. Cancer Res. **37**: 1631-1635.
24. **Srivastava, U. and Mukundan, M.A.** 1966. Biochemical changes in progressive muscular dystrophy. IV. Nucleic acid and protein metabolism int he lenses of animal exhibition nutritional and hereditary muscular dystrophy. Exp. Eye Res. **5**: 98-105.
25. **Tappel, A.L.** 1972. Vitamin E and free radical peroxidation of lipids. Ann. NY Acad. Sci. **230**: 12-28.
26. **Tappel A.L.** 1980. Vitamin E and selenium protection from in vivo lipid peroxidation. Ann. NY Acad. Sci. **355**: 18-29.
27. **Thompson, J.N., Erdody, P. and Maxwell, W.B.** 1973. Simultaneous fluorometric determinations of vitamin A and E in human serum and plasma. Biochem. Med. **8**: 403-414.
28. **Topping, D.C. and Viseck, W.J.** 1976. Nitrogen intake and tumorigenesis in rats injected with 1,2-dimethylhydrazine. J. Nutr. **106**: 1583-1587.
29. **Toth, B. and Patil, K.** 1983. Enhancing effect of vitamin E on murine intestinal tumorigenesis by 1,2-dimethylhydrazine. J. Natl. Cancer Inst. 70: 1107-1111.
30. **Tryfiates, G.P., Larson, L. and Morris, P.H.** 1978. Effect of pyridoxine dose on the growth of transplantable Morris hepatoma 7288 Ctc. J. Nutr. **108**: 417-420.
31. **Watanabe, K., Reddy, B.S., Wiesburger, J.H. and Kritchevsky, D.** 1978. 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.
32. **Wilson, R.B., Hutcheson, D.P. and Wideman, L.** 1977. Dimethylhydrazine-induced colon tumor in rats fed diet containing beef fat or corn oil with and without wheat bran. Am. J. Clin. Nutr. **30**: 176-181.
33. **Weisburger, J.H., Reddy, B.S. and Wynder, E.L.** 1977. Colon cancer: Its epidemiology and experimental production. Cancer **40**: 2414-2420.
34. **Wettenberg, L.W.** 1972. Inhibition of carcinogenic and toxic effect of polycyclic hydrocarbons by phebnolic antioxidant and ethoxyquin. J. Natl. Cancer Inst. **48**: 1425-1430.
35. **Zaridze, D.G.** 1983. Environmental etiology of large-bowel cancer. J. Natl. Cancer Inst. **70**: 389-400.