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Chemopreventive Effects of Taurine on Diethylnitrosamine and Phenobarbital-induced Hepatocarcinogenesis in Male F344 Rats

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Modifying effects of taurine, a naturally occurring organosulfur compound, on diethylnitrosamine (DEN) and phenobarbital (PB)-induced hepatocarcinogenesis were examined in rats. Male F344 rats, 5 weeks old, were divided into 8 groups. Rats of groups 1 through 5 were given i.p. injections of DEN (100 mg/kg body weight) once a week for 3 weeks from one week after the start of the experiment. Of them, animals of group 2 received taurine mixed in a basal diet at a concentration of 2000 ppm for the initial 4 weeks, and those of groups 3 and 5 were given the agent starting 4 weeks after the beginning of experiment until the end (24 weeks). Rats in groups 1, 4, 7 and 8 were kept on the basal diet throughout the experiment (24 weeks). Group 6 was given taurine throughout the experiment and group 8 was treated as a vehicle control. Animals of groups 1, 2, 3 and 7 received PB in drinking water at a dose of 500 ppm from one week after the end of carcinogen or vehicle treatment. Liver neoplasms were recognized only in DEN-treated groups. The incidence and average number of liver neoplasms of group 3 were significantly lower than those of group 1. The number of glutathione S-transferase placental form (GST-P)-positive foci of group 2 or 3 was significantly smaller than that of group 1 ($P<0.01$ or $P<0.005$). The average and unit areas of GST-P-positive foci in groups 2 and 3 were also significantly smaller than those in group 1 ($P<0.005$ and $P<0.0001$, $P<0.0001$ and $P<0.001$, respectively). In this study, the level of ornithine decarboxylase activity in non-neoplastic liver tissue was reduced by taurine treatment in both the initiation and postinitiation phases. These results suggest that taurine could be a chemopreventive agent for liver neoplasia.

Key words: Taurine — Chemoprevention — Hepatocarcinogenesis — GST-P — ODC

Chemoprevention embraces the concept that non-toxic, non-carcinogenic synthetic chemicals or naturally occurring products can inhibit the process of carcinogenesis. Several naturally occurring organosulfur chemicals have been reported to exert chemopreventive effects in animal models of carcinogenesis.[1-9] Taurine (2-aminoethanesulfonic acid, $C_2H_7NO_3S$) is an organosulfur compound and is contained in a variety of animals and plants.[10-12] Neural fibers of squid contain a large amount of taurine, and the taurine concentration in urine of Japanese is higher than that of people in Western countries.[13-16] Several biological functions of taurine have been established, including anti-oxidative, anti-atherosclerotic, and anti-hypertensive effects.[17-19] Since taurine has a cholagogic effect and activates enzymes in the hepatocyte, this agent is clinically used as a protecting agent of the liver.[20] Recently, a chemopreventive effect of taurine on rat large bowel carcinogenesis was reported.[21] In that study, the glutathione S-transferase (GST) level was increased in the liver of rats exposed to taurine. GST is one of the phase II enzymes and has been considered to increase metabolism, detoxification and elimination of carcinogens.[20, 22] Several other organosulfur compounds possessing anti-carcinogenic activity were also reported to increase phase II enzymes.[20-22]

In the present study, possible modifying effects of taurine on diethylnitrosamine (DEN)-induced hepatocarcinogenesis were examined in rats. The frequency of glutathione S-transferase placental form (GST-P)-positive foci as well as that of neoplasms of the liver, was analyzed.[23] Activity of ornithine decarboxylase (ODC), which is considered to correlate to the regulation of cell proliferation,[23, 24] was also assayed in the liver tissues.

MATERIALS AND METHODS

Animals, diet, and chemicals Weanling male F344 rats were purchased from Shizuoka SLC Co., Shizuoka. DEN and taurine were provided by Nacalai Tesque Inc., Kyoto. Phenobarbital (PB) was purchased from Maruishi Pharm. Co., Osaka. The basal diet CE-2 was obtained from CLEA Japan Inc., Tokyo.

Experimental procedure The experimental design is shown in Fig. 1. A total of 135 rats, 5 weeks of age, were divided into 8 groups. Groups 1 (20 rats), 2 (20 rats), 3 (21 rats), 4 (24 rats) and 5 (20 rats) were given i.p. injection of DEN (100 mg/kg body weight) once a week for 3 weeks starting one week after the beginning of the experiment, and groups 6 (8 rats), 7 (8 rats) and 8 (14 rats) were given i.p. injection of saline, instead of the carcinogen. Rats of group 1 were given PB in drinking water (500 ppm) starting 4 weeks after the beginning of

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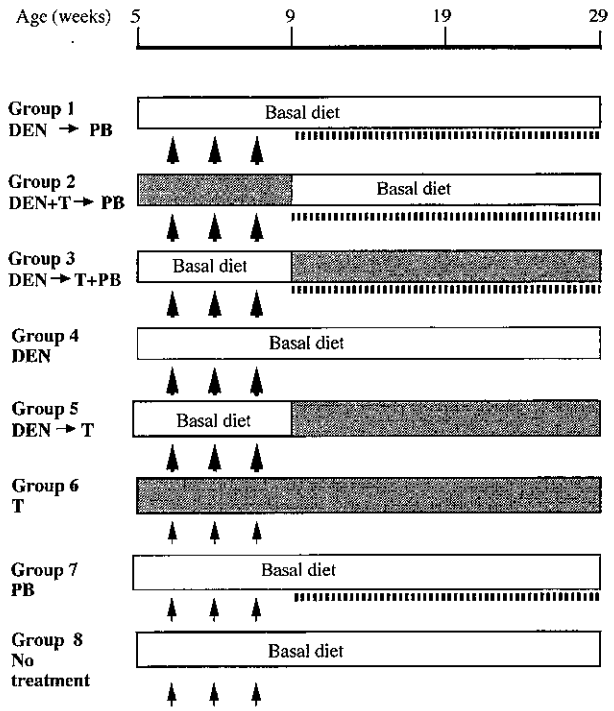

Fig. 1. Experimental design. ▒, T: taurine, 2000 ppm in diet; ||||||||||, PB: phenobarbital, 500 ppm in drinking water; ▲, DEN: diethylnitrosamine, i.p., 100 mg/kg body weight, once a week for 4 weeks; ▲, saline.

the experiment. Animals of group 2 were exposed to taurine mixed in the basal diet CE-2 at a concentration of 2000 ppm in the initiation phase and received PB during the promotion phase. Those of group 3 received PB and taurine in the promotion phase. Group 4 was kept on the basal diet. Group 5 was given taurine in the promotion phase. Group 6 was treated with taurine alone throughout the experiment. Group 7 received PB alone during the promotion phase. Group 8 was treated as a vehicle control. All animals were housed three to four to a wire cage and had free access to water and diet under controlled environmental conditions of humidity (50±10%), lighting (12 h light/dark cycle) and temperature (23±2°C). The experimental diet mixed with taurine was prepared weekly and stored in a cold room. At termination of the experiment, complete autopsies were performed after the animals were killed by ether inhalation. At autopsy, the location, number and size of liver tumors were recorded. Two sets of liver sections were made from each lobe. One set of the slices was fixed in cold acetone and the other set was fixed in 10% buffered formalin, embedded in paraffin blocks, and processed for routine histological observation with the use of hematoxylin and eosin stain.

GST-P staining and counting The liver sections from acetone-fixed tissues were stained for GST-P. An immunohistochemical staining for GST-P was carried out using the avidin-biotin-peroxidase complex method (Vectastain ABC kit, Vector Lab. Inc., Burlingame, CA). Anti-GST-P antibody was kindly provided by Dr. K. Satoh, Hirosaki University School of Medicine, Hirosaki. The areas of GST-P-positive foci and number of foci/cm^2 were measured by means of an image analyzer with a microscope (IPAP, Sumitomo Chemical Co., Ltd., Osaka). GST-P-positive foci were defined as foci of positive cells of more than 0.01 mm^2 in area.

Measurement of ODC activity At necropsy, non-neoplastic tissues were trimmed from the liver. The samples were immediately frozen in liquid nitrogen and stored at −70°C. The specimens were pooled and homogenized in 0.25 ml of a buffer containing 0.25 *M* sucrose, 50 m*M* Tris-HCl (pH 7.5), 1 m*M* EDTA, 0.4 m*M* pyridoxal 5′-phosphate, and 1 m*M* dithiothreitol in an Ultra-Turrax tissue homogenizer. They were then centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant was assayed for ODC activity by a modification of the micro method of Lans *et al.*[25] in an Eppendorf microfuge in a final volume of 40 μl. The reaction mixture contained 50 m*M* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4), 1 m*M* EDTA, 0.25 m*M* pyridoxal 5′-phosphate, 1 m*M* dithiothreitol, and 130 μ*M* [1-^{14}C]ornithine (40.6 mCi/mmol; Amersham International plc, Amersham, UK). The reaction was initiated with 20 μl of the supernatant at 37°C for 60 min, and the liberated $^{14}CO_2$ was collected on a paper filter bearing 10 μl of 10% potassium hydroxide, on the top of the microtubes. The reaction was terminated by adding 10 μl of 6 *N* hydrochloric acid. Then the sample was incubated for 15 min to collect $^{14}CO_2$ completely. At the end of this procedure, the filter paper with the captured $^{14}CO_2$ was removed, immersed in a scintillation vial, and counted for radioactivity in 10 ml of scintillation cocktail. One enzyme unit was defined as 1 pmol of $^{14}CO_2$ released/mg protein/h. Protein content was measured with a Bio-Rad protein assay kit (Bio-Rad, Richmond, CA), utilizing bovine serum albumin as a standard.

Statistical analysis Differences of incidence or multiplicity of pathological lesions in the liver among the groups were analyzed by the χ^2 test, Fisher's exact probability test, Welch's *t* test or Student's *t* test.

RESULTS

Body weight and liver weight Exposure to taurine significantly reduced the body weights, liver weights and relative liver weights compared to the other groups (Table I). The three values of group 3 were respectively smaller than those of group 1 ($P<0.01$, $P<0.0001$, $P<0.0001$),

Table I. Body and Liver Weights of Rats in Each Group

Group	Treatment	No. of rats	Body weight (g)	Liver weight (g)	Relative liver weight (%)
1	DEN→PB	20	322.7±29.5[a]	15.9±1.9	4.96±0.42
2	DEN+T→PB	19	315.0±35.3	15.1±1.7	4.80±0.24
3	DEN→T+PB	21	293.2±35.1	12.4±3.1	4.19±0.67
4	DEN	24	325.8±18.8	11.7±1.2	3.59±0.47
5	DEN→T	18	310.2±18.0	10.6±1.0	3.40±0.25
6	T	8	326.8±18.3	10.5±0.8	3.16±0.21
7	P	8	357.0±23.9	16.1±2.4	4.51±0.59
8	No treatment	14	351.6±13.8	11.9±0.9	3.39±0.24

a) Mean±SD.

Table II. Tumor Incidence in the Liver

Group	Treatment	Hepatocellular adenoma (%)	Hepatocellular carcinoma (%)	Total (%)
1	DEN→PB	19/20 (95)	14/20 (70)	20/20 (100)
2	DEN+T→PB	12/19 (63)[a]	13/19 (68)	17/19 (90)
3	DEN→T+PB	7/21 (33)[b]	13/21 (62)	13/21 (62)[c]
4	DEN	3/24 (13)[b]	7/24 (29)[d]	10/24 (42)[b]
5	DEN→T	4/18 (22)	4/18 (22)	6/18 (33)

a) Significantly different from group 1 by χ^2 test $P<0.02$.
b) Significantly different from group 1 by χ^2 test $P<0.0001$.
c) Significantly different from group 1 by χ^2 test $P<0.005$.
d) Significantly different from group 1 by χ^2 test $P<0.01$.

Table III. Tumor Multiplicity of the Liver

Group	Treatment	No. of rats	Average No. of adenoma	Average No. of carcinoma	Average No. of tumor
1	DEN→PB	20	3.15±2.21[a]	2.00±1.92	5.15±3.92
2	DEN+T→PB	19	0.95±0.83[b]	1.00±0.86[d]	1.95±1.00[b]
3	DEN→T+PB	21	0.48±0.79[c]	1.33±1.43	1.81±1.89[c]
4	DEN	24	0.13±0.33[c]	0.38±0.63[b]	0.50±0.65[c]
5	DEN→T	18	0.22±0.42	0.22±0.42	0.44±0.68

a) Mean±SD.
b) Significantly different from group 1 by Welch's t test $P<0.001$.
c) Significantly different from group 1 by Welch's t test $P<0.0001$.
d) Significantly different from group 1 by Welch's t test $P<0.05$.

and those of group 5 were smaller than those of group 4 ($P<0.05$, $P<0.01$, $P<0.05$). Furthermore, the values of group 6 were also smaller than those of group 8 ($P<0.001$, $P<0.001$, $P<0.05$).

Incidence and multiplicity of neoplasms One rat in group 2 and two rats in group 5 died of pneumonia before termination of the experiment, but no neoplasms were found among them. Incidence and multiplicity of liver neoplasms are shown in Tables II and III respectively. Liver tumors were only recognized in DEN-treated groups. The neoplasms induced by DEN were hepatocellular in origin. No hepatocellular neoplasm was found in the vehicle-treated group (group 8). The incidences of adenoma of groups 2, 3 and 4 were significantly lower than those of group 1 ($P<0.02$, $P<0.0001$ and $P<0.0001$, respectively). The incidences of total liver neoplasms of groups 3 and 4 were significantly lower than that of group 1 ($P<0.005$ and $P<0.0001$, respectively). Fur-

Table IV. Quantitative Analysis of GST-P-positive Foci

Group	Treatment	Density (/cm^2)	Unit area of GST-P-positive foci (%)	Average area of GST-P-positive (cm^2)
1	DEN→PB	46.6±7.6[a]	6.5±1.8	0.14±0.04
2	DEN+T→PB	37.9±10.3[b]	4.9±1.5[c]	0.13±0.03[d]
3	DEN→T+PB	31.8±9.0[c]	3.7±1.3[c]	0.12±0.03[c]
4	DEN	21.6±6.3[c]	2.1±1.0	0.09±0.03
5	DEN→T	18.0±6.2[e]	1.8±0.9	0.10±0.02

a) Mean±SD.
b) Significantly different from group 1 by Student's *t* test $P<0.01$.
c) Significantly different from group 1 by Student's *t* test $P<0.0001$.
d) Significantly different from group 1 by Student's *t* test $P<0.05$.
e) Significantly different from group 4 by Student's *t* test $P<0.05$.

thermore, that of hepatocellular carcinoma of group 4 was significantly lower than that of group 1 ($P<0.01$). No significant difference of the incidence of these neoplasms was found between groups 4 and 5. The incidence of total hepatocellular neoplasms of group 2 was rather lower than that of group 1, although the difference was not statistically significant. The multiplicities of adenoma, carcinoma and total tumors of groups 2 and 4 were significantly smaller than the corresponding values of group 1 ($P<0.001$, $P<0.05$ and $P<0.001$, and $P<0.0001$, $P<0.001$, and $P<0.0001$, respectively). The multiplicities of adenoma and total tumor of group 3 were significantly smaller than those of group 1 ($P<0.0001$ and $P<0.0001$). No significant differences in the incidence and multiplicity of tumors were present between groups 4 and 5 (Table III).

Frequency of hepatocellular foci Three types of preneoplastic hepatocellular foci (clear, eosinophilic and basophilic) positive for GST-P were found in all groups exposed to DEN. A few liver cell foci were also recognized in some animals given vehicle treatment. The results of quantitative analysis of the frequency of GST-P-positive foci are summarized in Table IV. The density and unit area of GST-P-positive foci of groups 2, 3 and 4 were significantly smaller than those of group 1 ($P<0.01$ and $P<0.01$, $P<0.0001$ and $P<0.0001$, $P<0.0001$ and $P<0.0001$, respectively). The average area of GST-P-positive foci of group 1 was significantly larger than the corresponding values of groups 3 and 4 ($P<0.05$ and $P<0.0001$, respectively). The density of group 5 was significantly lower than that of group 4 ($P<0.05$) (Table IV).

Results of ODC assay Table V indicates the ODC activities in the liver tissues without macroscopic tumors. Treatment with DEN alone (group 4) and PB alone (group 7) significantly increased liver ODC activity compared to untreated rat liver ($P<0.0001$ and $P<0.01$, respectively). In rats given DEN followed by PB, a slight increase in ODC activity was seen compared to group 4 (DEN alone). When taurine was fed during or after DEN exposure, ODC activity was significantly decreased ($P<0.05$, $P<0.0001$) and the decrease was prominent in rats given taurine after DEN treatment. However, in this experiment, feeding of taurine alone significantly increased liver ODC activity ($P<0.0005$), as seen in rats treated with PB alone.

Table V. ODC Activity of Rat Liver

Group	Treatment	No. of rats	ODC activity (pmol $^{14}CO_2$/mg protein/h)
1	DEN→PB	20	39.03±4.83[a]
2	DEN+T→PB	19	24.47±11.15[b]
3	DEN→T+PB	21	13.68±7.99[c]
4	DEN	24	30.93±3.66
5	DEN→T	18	19.13±5.00[d]
6	T	8	25.58±5.25
7	PB	8	10.16±2.85[e]
8	No treatment	14	4.74±0.57[f, g]

a) Mean±SE.
b) Significantly different from group 1 by Student's *t* test $P<0.05$.
c) Significantly different from group 1 by Student's *t* test $P<0.0001$.
d) Significantly different from group 4 by Student's *t* test $P<0.01$.
e) Significantly different from group 6 by Student's *t* test $P<0.01$.
f) Significantly different from group 6 by Student's *t* test $P<0.001$.
g) Significantly different from group 7 by Student's *t* test $P<0.05$.

DISCUSSION

The results of the present study clearly indicate that taurine inhibits DEN-induced hepatocarcinogenesis in rats when given during the initiation or promotion phase.

The inhibitory effect of taurine was particularly apparent in the promotion phase. Results of quantitative analysis of altered liver cell foci were in agreement with the data on frequency of liver neoplasms. These liver cell foci are generally recognized as preneoplastic lesions in the lineage of hepatocellular carcinoma development and are considered to reflect the carcinogenic potential due to the consistent manner in which liver cell foci appear during the post-initiation stage of hepatocarcinogenesis.[26, 27]

The experimental diets used slightly retarded body weight gain. Although no clear evidence of toxicity was found histologically in taurine-treated animals, such weight loss suggests a mild toxic effect of the experimental diet. The dose of taurine used here may be close to the maximum tolerated dose. Calorie intake and body weight have been considered to be related to tumor incidence. Albanes[28] has reported that about 15.3% calorie restriction causes 20.2% tumor reduction and 11% weight loss reduces tumor incidence by 3.7%. In this study, 9.1% body weight reduction was present in group 3 compared to group 1, while the tumor incidence was reduced to 62% in group 3. Thus, the reduction of tumor incidence was not primarily due to body weight loss. Taurine also decreased expression of enzyme-altered foci. We have reported that restricted caloric intake reduces the development of GST-P-positive foci.[29] In that experiment, the body weight was decreased to 76.7% by a 70% calorie-restricted diet, and the density of GST-P-positive foci was reduced to 37.6%, while the average area of foci was decreased to 53% at the termination. In the present experiment, the density of GST-P-positive foci was reduced to 68.1%, and the average area of foci was decreased to 83.4%, although the body weight was reduced only to 90.9%. Accordingly, the reduction of GST-P-positive foci was more marked than the decrease of the body weight, and the changes of the body weight and the liver weight were not clearly related with the number and volume of GST-P-positive foci.

In hepatocarcinogenesis in rodents, enhanced cell proliferation is known to play an important role.[12, 13] In this study, exposure of taurine during either the initiation or the promotion phase of the hepatocarcinogenesis decreased ODC activity in hepatocytes from the non-neoplastic area. Such a decrease was prominent in rats treated with DEN and PB, and paralleled the reduction of tumor incidence. ODC induction by exposure to xenobiotics is reported to precede cell proliferation in cells from various organs[30]; the activity is regarded as a reliable marker for cell proliferation[31, 32] and increase of this enzyme activity is considered to be related to tumor promotion.[33, 34] In the present experiment, enhancement of ODC activities by PB treatment suggests a clear association between ODC activity and promoting action of PB in the liver. There is evidence that suggests cell proliferation is also concerned with increase of tumor initiation.[24, 35–37] In hepatocarcinogenesis, liver cell proliferation is apparent in several stages, including initiation.[33, 35, 38] In this study, DEN treatment increased ODC activities. Conceivably, ODC activity has an important role in the initiation as well as promotion phase, and decrease of the enzyme activity by exogenous factors may be related to suppression of carcinogenesis. Although taurine suppressed the increased ODC activity induced by DEN and/or PB, it caused a significant increase of ODC activity in rats treated without DEN and PB. This increase was unexpected and the reasons for it are not known. Further studies are necessary to explain this phenomenon.

Oral administration of taurine has been reported to provide protection against lipid peroxidation induced by toxic doses of taumustin and isoprenaline.[34] Recently, Reddy *et al.* reported that taurine has a chemopreventive effect on azoxymethane-induced colon carcinogenesis.[21] They emphasized the significant increase of phase II enzymes, GST and NAD(P)H-dependent quinone reductase (NAD(P)H:QR) induced by taurine. Several other organosulfur compounds have also been speculated to reduce carcinogenesis by detoxification of carcinogens via increased activity of phase II enzymes.[20, 22]

Taurine is also known to increase prostaglandin (PG) I_2.[39, 40] An opposite effect of taurine on PG E_2 has also been reported.[41] These findings suggest that taurine participates in the metabolism of PGs synthesized in both normal parenchymal and non-parenchymal regions of the liver.[33, 42] A suppressive effect of PG synthesis inhibitor on rat hepatocarcinogenesis has been reported.[26] The possibility that taurine reduces synthesis of PG could be relevant to the chemopreventive effects of taurine.

In conclusion, the results of the present investigation indicate that taurine could be a promising chemopreventive agent against liver neoplasms.

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REFERENCES

1) Wattenberg, L. W. Guest editorial: Inhibition of chemical carcinogenesis. *J. Natl. Cancer Inst.*, **48**, 1425–1430 (1978).
2) Pitot, H. C., Barsness, L. and Kitagawa, T. Stages in the process of hepatocarcinogenesis in the rat liver. *In* "Carcinogenesis, Vol. 2: Mechanisms of Tumor Promotion and Carcinogenesis," ed. T. J. Slaga, A. Sivak and R. K. Boutwell, pp. 433–442 (1978). Raven Press, New York.

3) Pitot, H. C., Barsness, L., Goldsworthy, T. and Kitagawa, T. Biochemical characterization of stages of hepatocarcinogenesis after a single dose of diethylnitrosamine. *Nature,* **271,** 456–458 (1978).
4) Wattenberg, L. W. Chemoprevention of cancer. *Cancer Res.,* **45,** 1–8 (1985).
5) Graham, S. Toward a dietary prevention of cancer. *Epidemiol. Rev.,* **5,** 38–50 (1983).
6) Freudenheim, J. L. and Graham, S. Toward a dietary prevention of cancer. *Epidemiol. Rev.,* **11,** 229–235 (1989).
7) Salt, D. B. and Farber, E. New principle for the analysis of chemical carcinogenesis. *Nature,* **263,** 701–703 (1976).
8) Peraino, C., Staffeldt, E. F. and Ludeman, V. A. Early appearance of histochemically altered hepatocyte foci and liver tumors in female rat treated with carcinogens one day after birth. *Carcinogenesis,* **2,** 463–465 (1981).
9) Williams, G. M. The pathogenesis of rat liver cancer caused by chemical carcinogenesis. *Biochim. Biophys. Acta,* **605,** 167–189 (1980).
10) Kochakian, C. D. Taurine and related sulfur compounds in the reproductive tract of marine animals. *In* "Taurine," ed. R. Huxtable and A. Barbeau, pp. 375–378 (1976). Raven Press, New York.
11) Uhe, A. M., Collier, G. R. and O'dea, K. A comparison of the effects of beef, chicken and fish protein on satiety and amino acid profiles in lean male subjects. *J. Nutr.,* **122,** 467–472 (1992).
12) Suyama, M. and Yoshizawa, Y. Free amino acid composition of the skeletal muscle of migratory fish. *Bull. Jpn. Soc. Sci. Fish.,* **39,** 1339–1343 (1973).
13) Mizushima, S. and Yamori, Y. Nutritional improvement, cardiovascular diseases and longevity in Japan. *Nutr. Health,* **8,** 97–105 (1992).
14) Yamori, Y., Nara, Y., Mizushima, S., Mano, M., Sawamura, M., Kihara, M. and Hiroe, R. International cooperative study on the relationship between dietary factors and blood pressure: a preliminary report from the Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study. The CARDIAC Cooperative Study Research Group. *Nutr. Health,* **8,** 77–90 (1992).
15) Yamori, Y., Nara, Y., Mizushima, S., Sawamura, M. and Horie, R. Nutritional factors for stroke and major cardiovascular diseases: international epidemiological comparison of dietary prevention. *Health Rep.,* **6,** 22–27 (1994).
16) Yamori, Y., Nara, Y., Mizushima, S., Murakami, S., Ikeda, K., Sawamura, M., Nabika, K. and Horie, R. Gene-environment interaction in hypertension, stroke and atherosclerosis in experimental models and supportive findings from a world-wide cross-sectional epidemiological survey: a WHO-cardiac study. *Clin. Exp. Pharmacol. Physiol.,* **20,** 43–52 (1992).
17) Petty, M. A., Kintz, J. and DiFrancesco, G. F. The effects of taurine on atherosclerosis development in cholesterol-fed rabbits. *Eur. J. Pharmacol.,* **180,** 119–127 (1990).
18) Trachtman, H., Futterweit, S. and Bienkowski, R. S. Taurine prevents glucose-induced lipid peroxidation and increased collagen production in cultured rat mesangial cells. *Biochem. Biophys. Res. Commun.,* **191,** 759–765 (1993).
19) Aruoma, O. I., Halliwell, B., Hoey, B. M. and Butler, J. The antioxidant action of taurine, hypotaurine and their metabolic precursors. *Biochem. J.,* **256,** 251–255 (1988).
20) Talalay, P. The role of enzyme induction against carcinogenesis. *In* "Cancer Chemoprevention," ed. L. W. Wattenberg, M. Lipkin, C. W. Boone and G. J. Keroff, pp. 469–478 (1992). CRC Press, Boca Raton, FL.
21) Reddy, B. S., Rao, C. V., Rivenson, A. and Kelloff, G. Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res.,* **53,** 3493–3498 (1993).
22) Wattenberg, L. W. Chemoprevention of cancer by naturally occurring and synthetic compounds. *In* "Cancer Chemoprevention," ed. L. W. Wattenberg, M. Lipkin, C. W. Boone and G. J. Keroff, pp. 19–39 (1992). CRC Press, Boca Raton, FL.
23) Kojima, T., Tanaka, T., Kawamori, T., Hara, A. and Mori, H. Chemopreventive effects of dietary D, L-α-difluoromethylornithine, an ornithine decarboxylase inhibitor, on initiation and postinitiation stages of diethylnitrosamine-induced rat hepatocarcinogenesis. *Cancer Res.,* **53,** 3903–3907 (1993).
24) Tanaka, T., Kojima, T., Yoshimi, N. and Mori, H. Chemoprevention of diethylnitrosamine-induced hepatocarcinogenesis by a simple phenolic acid protocatechuic acid in rats. *Cancer Res.,* **53,** 2775–2779 (1993).
25) Lans, J. I., Jaszewski, R., Arlow, F. L., Tureaud, J., Luk, G. D. and Majumder, A. P. N. Supplemental calcium suppresses colonic mucosal ornithine decarboxylase activity in elderly patients with adenomatous polyps. *Cancer Res.,* **51,** 3416–3419 (1991).
26) Tanaka, T., Iwata, H., Niwa, K., Mori, Y. and Mori, H. Inhibitory effect of ellagic acid on N-2-fluorenylacetamide-induced liver carcinogenesis in male ACI/N rats. *Jpn. J. Cancer Res.,* **79,** 1297–1303 (1988).
27) Tanaka, T., Mori, Y., Morishita, Y., Hara, A., Ohno, T., Kojima, T. and Mori, H. Inhibitory effect of sinigrin and indole-3-carbinol on diethylnitrosamine-induced hepatocarcinogenesis in male ACI/N rats. *Carcinogenesis,* **11,** 1403–1406 (1990).
28) Albanes, D. Total calories, body weight, and tumor incidence in mice. *Cancer Res.,* **47,** 1987–1992 (1987).
29) Sugie, S., Tanaka, T., Mori, H. and Reddy, B. S. Effect of restricted caloric intake on the development of the azoxymethane-induced glutathione S-transferase placental form positive hepatocellular foci in male F344 rats. *Cancer Lett.,* **68,** 67–73 (1993).
30) Kitchin, K. T., Brown, J. L. and Kulkarni, A. P. Ornithine decarboxylase induction and DNA damage as predictive assay for potential carcinogenicity. *In* "Chemically Induced Cell Proliferation: Implications for Risk Assessment," ed. B. E. Boutwell, T. J. Slaga, W. Farland and M. McClain, pp. 137–144 (1991). Wiley-Liss Inc., New York.
31) Pegg, A. E. Recent advances in biochemistry of poly-

amines in eukaryotes. *Biochem. J.*, **234**, 249–262 (1986).
32) Pegg, A. E. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res.*, **48**, 759–774 (1988).
33) Liu, P., Kawada, N., Mizoguchi, Y. and Morisawa, S. Arachidonate metabolism in D-galactosamine or carbon tetrachloride-induced acute and chronic liver injuries in rats. *Gastroenterol. Jpn.*, **27**, 624–631 (1992).
34) Pierson, H. F., Fisher, J. M. and Rabinovitz, M. Modulation by taurine of the toxicity of taumustine, a compound with antitumor activity. *J. Natl. Cancer Inst.*, **75**, 905–909 (1985).
35) Popp, J. A. and Marsman, D. S. Chemically induced cell proliferation in liver carcinogenesis. *In* "Chemically Induced Cell Proliferation: Implications for Risk Assessment," ed. B. E. Boutwell, T. J. Slaga, W. Farland and M. McClain, pp. 389–395 (1991). Wiley-Liss Inc., New York.
36) Olson, J. W. and Russell, D. H. Prolonged induction of hepatic ornithine decarboxylase and its relation to cyclic adenosine 3′:5′-monophosphate-dependent protein kinase activation after a single administration of diethylnitrosamine. *Cancer Res.*, **39**, 3074–3079 (1979).
37) Olson, J. W. and Russell, D. H. Prolonged ornithine decarboxylase induction in regenerating carcinogen-treated liver. *Cancer Res.*, **40**, 4373–4380 (1980).
38) Pathirana, C. and Grimble, R. F. Taurine and serine supplementation modulates the metabolic response to tumor necrosis factor α in rats fed a low protein diet. *J. Nutr.*, **122**, 1369–1375 (1992).
39) el Tahir, K. E., Ageel, A. M. and Abu-Jayyab, A. R. Effect of taurine on arterial, uterine and cardiac PGI_2 and TXA_2 synthesis in the rat. *Prostaglandins*, **33**, 17–24 (1987).
40) Myers, S., Bartula, L. and Kalley-Taylor, B. The role of prostaglandin I_2 and biliary lipids during evolving cholocystitis in the rabbit. *Gastroenterology*, **104**, 248–255 (1993).
41) Kimura, H., Okamoto, K. and Sakai, Y. Modulatory effects of prostaglandin D_2, E_2 and $F_{2\alpha}$ on the postsynaptic actions of inhibitory and excitatory amino acids in cerebellar Purkinje cell dendrites *in vitro*. *Brain Res.*, **330**, 235–244 (1985).
42) Kuiper, J., Casteleyn, E. and Van Berkel, T. J. Regulation of liver metabolism by intercellular communication. *Adv. Enzyme Regul.*, **27**, 193–208 (1988).