Chemopreventive Effect of *N*-Homocysteine Thiolactonyl Retinamido Cobalamin on Carcinogenesis by Ethyl Carbamate in Mice

(42931)

KILMER S. MCCULLY*,[1] AND MICHAEL P. VEZERIDIS†

Veterans Administration Medical Center, Providence, Rhode Island 02908 and Departments of Pathology and Surgery,† Brown University, Providence, Rhode Island 02912*

Abstract. **Because of abnormalities of metabolism of homocysteine thiolactone and methionine in malignant cells, and because of the chemopreventive activity of *N*-homocysteine thiolactonyl retinamide against chemical carcinogenesis by ethyl carbamate in mice, the cobalamin derivative of this retinamide was prepared and tested for chemopreventive activity. The substance, *N*-homocysteine thiolactonyl retinamido cobalamin, was found to have a different UV-visible absorption spectrum from that of 5′-deoxyadenosyl cobalamin or *N*-homocysteine thiolactonyl retinamide. Spectral analysis suggests a ratio of 2 mol of retinamide/mol of cobalamin within the molecule. To demonstrate chemopreventive activity, ethyl carbamate was given in a dose of 2 mg/animal to A/J mice (15–18 g) weekly over a period of 10 weeks to induce pulmonary tumors. A total dose of *N*-homocysteine thiolactonyl retinamido cobalamin of 60 mg/kg, given for a total of 16 weeks, decreased by one fourth ($P < 0.05$) the number of pulmonary tumors induced by ethyl carbamate. An equimolar dose of 5′-deoxyadenosyl cobalamin (40 mg/kg) increased the number of tumors by one third ($P < 0.001$), and an equimolar dose of *N*-homocysteine thiolactonyl retinamide (20 mg/kg) had no effect on the number of pulmonary tumors. No mortality was observed in the experiment. When the ethyl carbamate was given in a single dose of 20 mg/animal, all three substances produced significant mortality in doses of 0.75–30 mg/kg. In the survivors of this experiment, doses of 0.75–30 mg/kg of *N*-homocysteine thiolactonyl retinamido cobalamin decreased the number of pulmonary tumors induced by ethyl carbamate to 52–82% of controls ($P < 0.01$). The results show that *N*-homocysteine thiolactonyl retinamido cobalamin has chemopreventive activity against chemical carcinogenesis by ethyl carbamate in mice.** [P.S.E.B.M. 1989, Vol 191]

Homocysteine thiolactone, a metabolite of the essential amino acid, methionine (1), is metabolized abnormally in malignant cells (2). Normal cells metabolize homocysteine thiolactone to phosphoadenosine phosphosulfate and sulfate ester (3). Because malignant cells are unable to convert homocysteine thiolactone to sulfate ester, excess homocysteine thiolactone accumulates within malignant cells and reacts with free amino groups of proteins (2), nucleic acids, and glycosaminoglycans to form peptide-bound homocysteinyl groups, a reaction known as thiolation (4–6). Free homocysteine thiolactone is present in human malignant tumors, as shown by direct extraction and chromatography (7).

The inability of malignant cells to convert homocysteine thiolactone to sulfate ester is attributed to cellular deficiency or inability to form an *N*-substituted derivative of homocysteine thiolactone (2). The nature of this derivative was investigated by chemical synthesis of *N*-substituted derivatives of homocysteine thiolactone formed from pyridoxal, arachidonic acid, maleimide, and oxalyl chloride (8–10). These studies showed that such *N*-substituted derivatives are antineoplastic if they are soluble in lipid and contain a conjugated double bond system and a carbonyl group adjacent to the amide nitrogen of homocysteine thiolactone. Furthermore, a transitional metal atom, rhodium, is re-

[1] To whom correspondence should be addressed at Laboratory Service, VA Medical Center, Providence, RI 02908.

Received September 6, 1988. [P.S.E.B.M. 1989, Vol 191]
Accepted March 3, 1989.

0037-9727/89/1914–0346$2.00/0

quired for the antineoplastic activity of the oxalyl derivative of homocysteine thiolactone (10).

The retinamide formed from *trans*-retinoic acid and homocysteine thiolactone, *N*-homocysteine thiolactonyl retinamide, is soluble in lipid and contains a conjugated double bond system and a carbonyl group adjacent to the amide nitrogen atom (11). In high doses, *N*-homocysteine thiolactonyl retinamide decreases the growth of transplanted rhabdomyosarcoma and counteracts the carcinogenicity of ethyl carbamate in mice (11). Methyl cobalamin, the coenzyme required for methionine biosynthesis from methyltetrahydrofolate and homocysteine, accumulates in malignant tumors and in the livers of rats treated with the carcinogen, diethyl nitrosamine (12). Although methyl cobalamin was increased, the total cobalamin of these liver tumors was found to be deficient (12). These findings and the chemopreventive and antineoplastic effects of *N*-homocysteine thiolactonyl retinamide (11) suggest that a molecular form of cobalamin containing *N*-homocysteine thiolactonyl retinamide may be deficient in malignant tumors. To investigate this possibility, the substance, *N*-homocysteine thiolactonyl retinamido cobalamin, was synthesized and assayed for chemopreventive activity against chemical carcinogenesis by ethyl carbamate in mice.

Materials and Methods

N-homocysteine thiolactonyl retinamide (NHTR) was synthesized as previously described (11). 5′-Deoxyadenosyl cobalamin (Ad-Cob; coenzyme B_{12}) was obtained from Sigma Chemical Co., St. Louis, MO. To form *N*-homocysteine thiolactonyl retinamido cobalamin, $(NHTR)_2Cob$, 10 mg (0.025 mmol) of NHTR were dissolved in 100 ml of ethanol at 37°C and 20 mg (0.0125 mmol) of Ad-Cob were added, protecting the mixture from direct light. Mixing and warming to 37°C produced a clear, deep salmon tan solution. In some experiments 0.005 ml of concentrated HCl was added to prevent precipitation of Ad-Cob. To transfer $(NHTR)_2Cob$ to a nonvolatile, nontoxic solvent, 20 ml of propylene glycol were added, and the ethanol was evaporated under reduced pressure at 37°C. The UV-visible absorption spectra of $(NHTR)_2Cob$, Ad-Cob, and NHTR in propylene glycol at pH 2 (Figs. 1–4) was obtained on a Beckman model DU-7 spectophotometer, equipped with computerized data analysis.

The mouse pulmonary tumor assay (13) was used to study the effect of $(NHTR)_2Cob$ on chemical carcinogenesis by ethyl carbamate. Female mice of the A/J strain, weighing 15–18 g, were obtained from the National Cancer Institute, Frederick, MD. The animals were kept in polycarbonate cages with Agway Prolab Chow 3500 and water supplied *ad libitum*. Temperature, humidity, and a 12-hr light/dark cycle were controlled daily, and body weight was recorded weekly. In the first experiment, 2 mg of ethyl carbamate in 0.2 ml of water were injected ip each week for 10 weeks. The day after each injection of carcinogen, 0.05 ml of propylene glycol alone (Group 1) or vehicle containing equimolar doses of $(NHTR)_2Cob$ (0.075 mg, Group 2), Ad-Cob (0.05 mg, Group 3), or NHTR (0.025 mg, Group 4) were injected ip. The weekly injection of the test compounds was continued for 6 additional weeks after the last dose of carcinogen. After sacrifice, the lungs were fixed in 10% buffered formalin, and the lung tumors were counted and the diameters were measured with an ocular micrometer mounted in a dissecting microscope. In the second experiment, 20 mg of ethyl carbamate in 0.5 ml of water was injected ip on Day 1. Group 5 received no further injections and Group 6 received vehicle only. In Group 10, 0.1 ml of propylene glycol containing 0.150 mg of $(NHTR)_2Cob$ was injected ip 4 hours later, and on Days 2, 4, and 6, for a total of four doses. In Group 9, the same dose of $(NHTR)_2Cob$ was given on Days 2, 4, and 6; in Group 8 the same dose of $(NHTR)_2Cob$ was given on Day 2; and in Group 7, 0.1 ml of propylene glycol containing 0.015 mg of $(NHTR)_2Cob$ was injected on Day 2. In Group 11, 0.1 ml of propylene glycol containing 0.1 mg of Ad-Cob was given on Day 2. In Group 12, 0.1 ml of propylene glycol containing 0.05 mg of NHTR was given on Day 2. In Group 13, 0.1 ml of propylene glycol containing 0.05 mg of NHTR was given on Day 2 and 0.1 ml of propylene glycol containing 0.1 mg of Ad-Cob was given on Day 4. The animals were observed for a total of 16 weeks, and the pulmonary tumors were counted and measured, as in experiment 1.

Significant difference between means was accepted at the $P = 0.05$ level, calculated from Student's t test. Linear regression analysis was used to study the correlations between log dose, weight gain, and numbers of pulmonary tumors compared with vehicle controls (Groups 6–9).

Results

The formation of *N*-homocysteine thiolactonyl retinamido cobalamin is shown by increased absorption between 600 and 900 nm, where neither *N*-homocysteine thiolactonyl retinamide nor 5′-deoxyadenosyl cobalamin have any appreciable absorption (Figs. 1 and 2). The difference spectrum between *N*-homocysteine thiolactonyl retinamido cobalamin and 5′-deoxyadenosyl cobalamin reveals a complex absorption band between 200 and 400 nm, a peak at 410 nm, and a broad flat absorption band from 490 to 560 nm and a more intense flat band from 560 to 900 nm (Fig. 3). The absorption of *N*-homocysteine thiolactonyl retinamido cobalamin at 640 nm reaches a maximum at a mole ratio of 2:1, when the absorption is plotted as a function of mole ratio of *N*-homocysteine thiolactonyl retinamide to 5′-deoxyadenosyl cobalamin (Fig. 4).

Weekly doses of *N*-homocysteine thiolactonyl

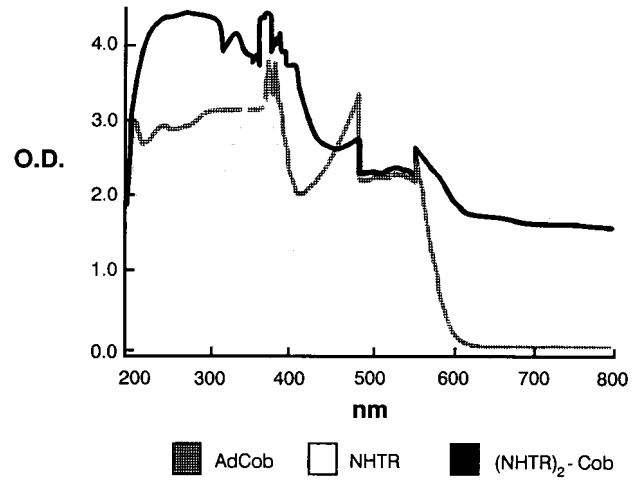

Figure 1. The UV-visible absorption spectra of $(NHTR)_2Cob$, NHTR, and Ad-Cob are presented in the range from 200 to 800 nm.

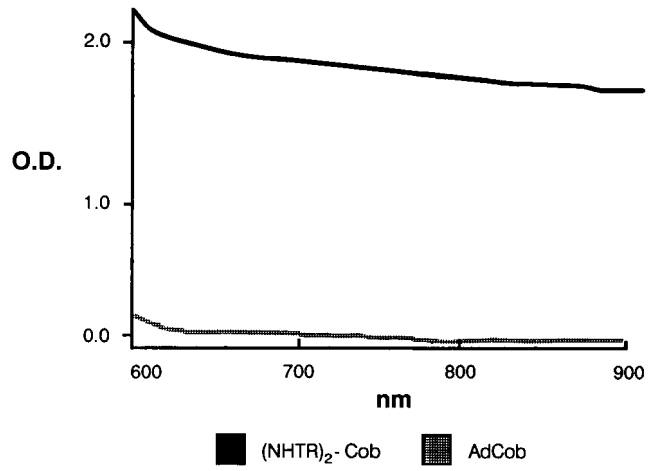

Figure 2. The visible absorption spectra of $(NHTR)_2Cob$ and Ad-Cob are presented in the range from 600 to 900 nm.

retinamido cobalamin significantly decreased the number of pulmonary tumors induced in strain A/J mice by ethyl carbamate, when the carcinogen was given in 10 weekly doses (Table I). The number of tumors was reduced by about one fourth by a total dose of 60 mg/kg of *N*-homocysteine thiolactonyl retinamido cobalamin, compared with animals treated with vehicle only ($P < 0.05$). An equimolar dose of *N*-homocysteine thiolactonyl retinamide (20 mg/kg) had no effect on pulmonary carcinogenesis, but an equimolar dose of 5′-deoxyadenosyl cobalamin (40 mg/kg) increased the number of pulmonary tumors by one third, compared with animals treated with vehicle only ($P < 0.001$). No mortality was observed with any of these substances in this experiment, but both *N*-homocysteine thiolactonyl retinamido cobalamin and 5′-deoxyadenosyl cobalamin significantly inhibited weight gain compared with animals treated with vehicle only.

When an equivalent quantity of ethyl carbamate (20 mg/animal) was given to strain A/J mice in a single dose, almost four times as many pulmonary tumors were induced compared with the results when the carcinogen was given in 10 weekly doses (Table I). Single doses of *N*-homocysteine thiolactonyl retinamido cobalamin significantly inhibited the formation of pulmonary tumors to 82% and 64% of controls at 0.75 and 7.5 mg/kg doses, respectively. Multiple doses of the compound decreased the number of pulmonary tumors to 52 and 65% of controls at total doses of 22.5 and 30 mg/kg, respectively ($P < 0.01$). In the animals treated with varying doses of the compound, compared with vehicle controls (Groups 6–9), both weight gain and number of pulmonary tumors are correlated inversely with log dose ($r = 1.00$, $P < 0.005$) and weight gain is correlated with the number of pulmonary tumors ($r = 0.98$, $P < 0.005$). There was no effect on the number of pulmonary tumors or weight gain when single doses of 5′-deoxyadenosyl cobalamin or *N*-homocysteine thiolactonyl retinamide were given alone (Groups 11 and 12) or on separate days (Group 13) at 5.0 and 2.5 mg/kg, respectively.

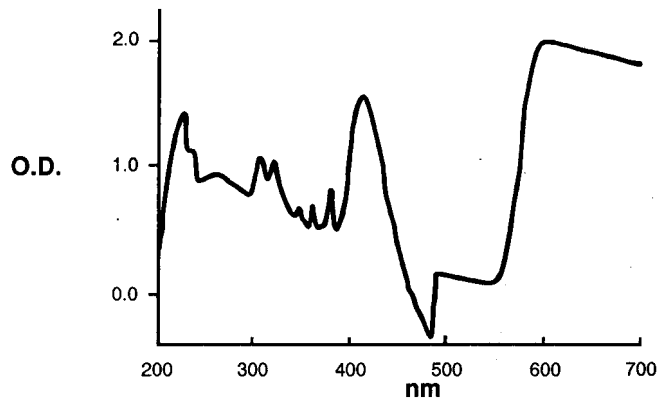

Figure 3. The UV-visible differential absorption spectrum is determined by the difference between the spectra of $(NHTR)_2Cob$ and Ad-Cob in the range from 200 to 700 nm.

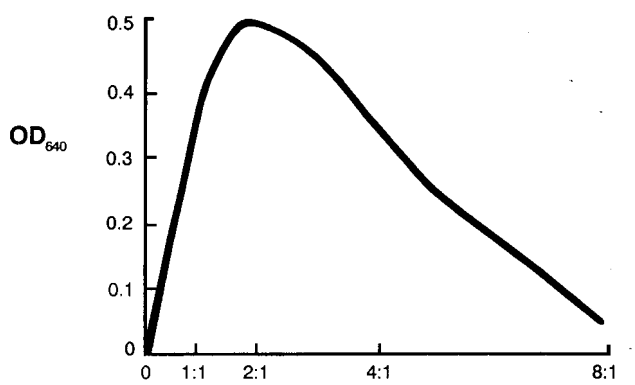

Figure 4. The relative absorption of *N*-homocysteine thiolactonyl retinamido cobalamin at 640 nm is plotted against the mole ratio of *N*-homocysteine thiolactonyl retinamide to 5′-deoxyadenosyl cobalamin.

Table I. Chemopreventive Effect of *N*-Homocysteine Thiolactonyl Retinamido Cobalamin on Carcinogenesis by Ethyl Carbamate in Mice[a]

Group	Treatment by ethyl carbamate	Test compound	Treatment by test compound	Total dose (mg/kg)	Survivors/ total	Weight gain (g ± SE)	*P*	Tumors/ animal (*n* = SE)	*P*
1	2 mg/week × 10	Vehicle	Weekly × 16	—	20/20	5.9 ± 0.15	—	7.5 ± 0.75	—
2	2 mg/week × 10	$(NHTR)_2Cob$	Weekly × 16	60	21/21	3.8 ± 0.53	0.01	5.6 ± 0.53	0.045
3	2 mg/week × 10	Ad-Cob	Weekly × 16	40	20/20	4.8 ± 0.25	0.01	9.8 ± 0.23	0.0008
4	2 mg/week × 10	NHTR	Weekly × 16	20	20/20	5.3 ± 0.53	NS	6.9 ± 1.10	NS
5	20 mg, Day 1	—	—	—	20/20	8.5 ± 0.75	—	26.7 ± 1.29	—
6	20 mg, Day 1	Vehicle	—	—	19/20	7.5 ± 0.70	NS	27.0 ± 1.03	—
7	20 mg, Day 1	$(NHTR)_2Cob$	Day 2	0.75	17/20	6.5 ± 0.61	NS	22.2 ± 1.61	0.01
8	20 mg, Day 1	$(NHTR)_2Cob$	Day 2	7.5	16/20	6.0 ± 0.88	NS	17.4 ± 1.71	0.001
9	20 mg, Day 1	$(NHTR)_2Cob$	Days 2, 4, and 6	22.5	6/20	5.7 ± 2.76	NS	14.0 ± 1.76	0.0000001
10	20 mg, Day 1	$(NHTR)_2Cob$	Days 1, 2, 4, and 6	30	3/20	5.4	—	17.7 ± 1.20	0.006
11	20 mg, Day 1	Ad-Cob	Day 2	5.0	12/20	7.1 ± 0.50	NS	24.0 ± 1.44	NS
12	20 mg, Day 1	NHTR	Day 2	2.5	12/20	7.1 ± 0.90	NS	25.9 ± 1.70	NS
13	20 mg, Day 1	NHTR, Ad-Cob	Day 2, 4	2.5; 5.0	14/20	8.1 ± 1.70	NS	26.2 ± 1.70	NS

[a] An aqueous solution containing 20 mg of ethyl carbamate (0.5 ml) or 2 mg of ethyl carbamate (0.2 ml) was injected ip into A/J female mice (15–18 g) in a single dose or weekly for 10 weeks, for a total dose of 20 mg/animal. The next day after each carcinogen injection and weekly for a total of 16 weeks, 0.05 ml of propylene glycol containing equimolar doses of $(NHTR)_2Cob$, Ad-Cob, or NHTR was injected ip (Groups 1–4). Four hours after ethyl carbamate was injected, 0.1 ml of propylene glycol containing $(NHTR)_2Cob$ was injected ip into Group 10. On Day 2, 0.1 ml of propylene glycol containing $(NHTR)_2Cob$, Ad-Cob, or NHTR was injected into Groups 7–13. On Day 4 $(NHTR)_2Cob$ and Ad-Cob were injected into Groups 9, 10, and 13. Group 13 received NHTR on Day 2 and Ad-Cob on day 4. $(NHTR)_2Cob$ was injected into Groups 9 and 10 on Day 6. Fatalities occurred as follows: Group 6, Week 12; Group 7, Day 6 (2) and Week 9; Group 8, Day 6, Day 8, Week 8, and Week 10; Group 9, Day 6 (4), Day 7 (2), Day 8 (4), Day 9, and Day 10 (3); Group 10, Day 3, Day 6 (2), Day 7 (4), Day 9 (4), Day 10, Day 11 (2), and Day 13 (3); Group 11, Day 6 (2), Day 7, Day 8, Day 9 (2), Week 9, and Week 10; Group 12, Day 6 (4), Day 8 (2), Day 13, and Week 5; Group 13, Day 6 (3), Day 7 (2), and Day 8. Body weight was recorded weekly, and average weight gain was calculated from initial and final weights. After 16 weeks the lungs were dissected, fixed in formalin, and the tumors were enumerated; the diameters were measured, using an ocular micrometer mounted in a dissecting microscope. The probablility values were calculated from Student's *t* test. No differences were found in tumor diameters among the groups (data not shown).

In the experiment in which the carcinogen was given in 10 weekly doses, no mortality was observed (Table I). When the carcinogen was given in a single large dose, no mortality was observed in the untreated Group 1 and a single animal died in Group 2 given vehicle only. In the other groups given a single large dose of carcinogen, 5′-deoxyadenosyl cobalamin or *N*-homocysteine thiolactonyl retinamide, given separately or on different days to the same animals, produced significant mortality. *N*-Homocysteine thiolactonyl retinamido cobalamin also produced significant mortality which increased with increasing dose.

In animals given no carcinogen, 0.1 ml of propylene glycol containing 0.1 mg of *N*-homocysteine thiolactonyl retinamido cobalamin was injected intraperitoneally in five female A/J mice every week for 9 weeks for a total dose of 45 mg/kg. The animals were observed for an additional 10 weeks and sacrificed. During the treatment period the animals became very active and restless, and during the observation period they became less active. Weight gain was inhibited during the treatment period (−1.6 g/animal), but the animals gained weight normally during the observation period (+5.0 g/animal). All five animals survived both treatment and observation periods, and autopsy revealed no abnormalities except for scattered peritoneal adhesions.

Discussion

The increased absorption of *N*-homocysteine thiolactonyl retinamido cobalamin between 600 and 900 nm and the difference spectrum between this substance and 5′-deoxyadenosyl cobalamin indicate formation of a complex in which *N*-homocysteine thiolactonyl retinamide is bonded to the cobalt atom of cobalamin. Evidently the 5′-deoxyadenosyl ligand is displaced by formation of this complex. The optimization of absorption at 640 nm by a 2:1 mole ratio of *N*-homocysteine thiolactonyl retinamide to cobalamin suggests that this ligand occupies both axial positions of the octahedral complex of cobalamin. These conclusions need confirmation by detailed analysis of molecular structure by x-ray crystallography and other methods.

The results of the first experiment (Table I) show that the carcinogenic effect of ethyl carbamate was significantly counteracted by *N*-homocysteine thiolactonyl retinamido cobalamin. 5′-Deoxyadenosyl cobalamin was found to be cocarcinogenic, significantly increasing the number of pulmonary tumors induced by ethyl carbamate. There was no effect of *N*-homocysteine thiolactonyl retinamide on carcinogenesis in the dose given. Thus, *N*-homocysteine thiolactonyl retinamide interacts with 5′-deoxyadenosyl cobalamin to produce an anticarcinogenic derivative. These findings suggest the possibility that the previously observed chemopreventive effect of large doses of *N*-homocysteine thiolactonyl retinamide occurs because of increased formation of *N*-homocysteine thiolactonyl retinamido cobalamin (11). The findings also support the suggestion that malignant cells are deficient in an *N*-substituted derivative of homocysteine thiolactone

(2) which contains a carbonyl group and a conjugated double bond system (9–11) adjacent to the amide nitrogen atom which is bound to a transitional metal atom (10). It is also possible that *N*-homocysteine thiolactonyl retinamido cobalamin may prevent carcinogenesis by suppression of metabolic activation of ethyl carbamate.

Homocysteine thiolactone, given either as the free base or as the perchlorate salt (11), and 5′-deoxyadenosyl cobalamin (Table I) are both cocarcinogenic in strain A/J mice given ethyl carbamate. Methyl cobalamin accumulates in malignant tumors and in the liver of animals given a carcinogen (12), and methyl cobalamin increases the growth of transplanted malignant neoplasms (14). By interfering with methyl transfer reactions, a cellular deficiency of *N*-homocysteine thiolactonyl retinamido cobalamin would be expected to cause the accumulation of homocysteine thiolactone (2, 7) and methyl cobalamin (12) and the decreased concentration of methionine observed in malignant cells (15).

The results of the second experiment (Table I) show that a single large dose of ethyl carbamate induces toxicity by subsequently administered 5′-deoxyadenosyl cobalamin, *N*-homocysteine thiolactonyl retinamide, and *N*-homocysteine thiolactonyl retinamido cobalamin. No such toxicity was observed when the same quantity of carcinogen was given in 10 weekly doses (Table I). Previous studies showed that the free base of homocysteine thiolactone caused significant mortality and weight loss in mice given ethyl carbamate (11). Some degree of toxicity was also observed when large doses of *N*-homocysteine thiolactonyl retinamide were given in mixed lipid vehicle to mice given ethyl carbamate (11). Homocysteine toxicity in cultured endothelial cells was attributed to accumulation of hydrogen peroxide (16, 17). Malignant tumors have also been found to contain increased concentrations of hydrogen peroxide (18). A possible explanation for hydrogen peroxide accumulation may be the inhibition of cellular respiration by depletion of mitochondrial *N*-homocysteine thiolactonyl retinamido cobalamin. The cobalamin content of liver is principally contained within mitochondria (19). This depletion may be exacerbated by interaction of mitochondrial cobalamin with exogenous homocysteine thiolactone or *N*-homocysteine thiolactonyl retinamide, further depleting the mitochondrial content of *N*-homocysteine thiolactonyl retinamido cobalamin. Furthermore, the carcinogen, ethyl carbamate, may also deplete mitochondrial *N*-homocysteine thiolactonyl retinamido cobalamin, causing toxicity by inhibition of cellular respiration. Further investigation is needed to establish a role for *N*-homocysteine thiolactonyl retinamido cobalamin in cellular respiration.

N-homocysteine thiolactonyl retinamido cobalamin significantly inhibited weight gain both in animals given 10 weekly doses of ethyl carbamate and in animals given no ethyl carbamate. In the experiment with a single large dose of ethyl carbamate, a correlation between weight gain and number of pulmonary tumors and an inverse correlation of these effects with log dose of the compound were observed. These results suggest that metabolic effects of the compound are responsible both for inhibition of weight gain and for its anticarcinogenic effect. Caloric restriction is known to inhibit chemical carcinogenesis (20, 21), but the animals in the present study were fed *ad libitum*. A possible explanation for the present results is that increased cellular respiration induced by exogenous *N*-homocysteine thiolactonyl retinamido cobalamin may promote both weight loss and chemoprevention of carcinogenesis, simulating the effects of caloric restriction. An alternative explanation for the results is that the substance may have caused caloric restriction by decreasing appetite and feeding, producing the anticarcinogenic effect. Although adenosyl cobalamin inhibited weight gain in animals given 10 weekly doses of ethyl carbamate, carcinogenesis was promoted, suggesting that exogenous adenosyl cobalamin may augment the depletion of cellular *N*-homocysteine thiolactonyl retinamido cobalamin caused by ethyl carbamate.

The abnormal metabolism of homocysteine thiolactone in malignant cells (2) and the results of the present study suggest the possibility that carcinogens induce a state of malignant growth by depletion of cellular *N*-homocysteine thiolactonyl retinamido cobalamin. Chemical carcinogens are believed to act by formation of highly electrophilic molecular species (22) which may interact with or decompose *N*-homocysteine thiolactonyl retinamido cobalamin, decreasing its concentration in the physiologic site of action. Some evidence for this formulation is the isolation of homocysteinyl derivatives of *N*-methyl-4-aminoazobenzene from metabolism of *N*-benzoyloxy-*N*-methyl-4-aminoazobenzene and methionine (22). Carcinogenic radiation may also cause depletion of cellular *N*-homocysteine thiolactonyl retinamido cobalamin by reaction with highly electrophilic free radicals which are formed within cells. Electrophilic metal salts, such as nickel subsulfide and nickel oxide, may cause transformation, carcinogenesis, and erythrocytosis (23, 24) by competitive destruction of *N*-homocysteine thiolactonyl retinamido cobalamin. A critically low concentration of cellular *N*-homocysteine thiolactonyl cobalamin may be transmitted to successive neoplastic daughter cells because of competition for cobalamin and *N*-homocysteine thiolactonyl retinamide by accumulated methyl cobalamin (12) or homocysteine thiolactonyl cobalamin, formed from methyltetrahydrofolate or homocysteine thiolactone and 5′-deoxyadenosyl cobalamin. The possible action of carcinogens on cellular *N*-homocysteine thiolactonyl retinamido cobalamin needs further study by methods which permit isolation and

quantitation of the substance from normal and neoplastic tissues.

This investigation was supported by a grant from the Veterans Administration, Washington, DC. We thank Paula Laliberte for technical assistance.

1. Spindel E, McCully KS. Conversion of methionine to homocysteine thiolactone in liver. Biochim Biophys Acta **343**:687–691, 1974.
2. McCully KS. Homocysteine thiolactone metabolism in malignant cells. Cancer Res **36**:3198–3202, 1976.
3. McCully KS. Homocysteine metabolism in scurvy, growth and arteriosclerosis. Nature **231**:391–392, 1971.
4. DuVigneaud V, Patterson WI, Hunt M. Opening of the ring of the thiolactone of homocysteine. J Biol Chem **126**:217–231, 1938.
5. Benesch R, Benesch RE. Formation of peptide bonds by ammoniolysis of homocysteine thiolactone. J Am Chem Soc **78**:1597–1599, 1956.
6. Benesch R, Benesch RE. Thiolation of proteins. Proc Natl Acad Sci USA **44**:848–853, 1958.
7. McCully KS, Vezeridis MP. Homocysteine thiolactone in arteriosclerosis and cancer. Res Commun Chem Pathol Pharmacol **59**:107–119, 1988.
8. McCully KS, Clopath P. Homocysteine compounds which influence the growth of a malignant neoplasm. Chemotherapy **23**:44–49, 1977.
9. McCully KS, Vezeridis MP. Antineoplastic activity of N-maleamide homocysteine thiolactone amide encapsulated within liposomes. Proc Soc Exp Biol Med **180**:57–61, 1985.
10. McCully KS, Vezeridis MP. Antineoplastic activity of a rhodium trichloride complex of oxalyl homocysteine thiolactone. Cancer Invest **5**:25–30, 1987.
11. McCully KS, Vezeridis MP. Chemopreventive and antineoplastic activity of N-homocysteine thiolactonyl retinamide. Carcinogenesis **8**:1559–1562, 1987.
12. Linnell JC, Quadros EV, Matthews DM, Morris HP, Poirier LA. Altered cobalamin distribution in rat hepatomas and in the livers of rats treated with diethyl nitrosamine. Cancer Res **37**:2975–2978, 1977.
13. Shimkin MB, Stoner GD. Lung tumors in mice: Application to carcinogenesis bioassay. Adv Cancer Res **21**:1–58, 1975.
14. Arsenyan FG, Myasishcheva NV, Sofina ZP, Raushenbakh MO, Rudakova IP, Chauser EG, Yurkevich AM. Influence of methylcobalamin on the antineoplastic activity of methotrexate. Pharm Chem J **12**:1299–1304, 1978.
15. Hoffman RM. Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. Biochim Biophys Acta **738**:49–87, 1984.
16. Wall RT, Harlan JM, Harker LA, Striker GE. Homocysteine induced endothelial injury *in vitro*: A model for the study of vascular injury. Thromb Res **18**:113–121, 1980.
17. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest **77**:1370–1376, 1986.
18. Frigerio NA, Sherwin LC, Seiler JA. Quantitative determination of peroxide in tumor tissue. US AEC Argonne National Laboratory Reports ANL-7635:148–150, 1969.
19. Beck WS. Biological and medical aspects of vitamin B_{12}. In: Dolphin D, Ed. B_{12}. New York: John Wiley & Sons, Vol **2**:pp1–30, 1982.
20. Tannenbaum A, Silverstone H. Nutrition in relation to cancer. Adv Cancer Res **1**:451–501, 1953.
21. Shimkin MB. Pulmonary tumors in experimental animals. Adv Cancer Res **3**:223–267, 1955.
22. Miller JA. Carcinogenesis by chemicals: An overview—G. H. A. Clowes Memorial Lecture. Cancer Res **30**:559–576, 1970.
23. Sunderman FW Jr. Carcinogenicity of nickel compounds in animals. In: Sunderman FW Jr, Ed. Nickel in the Human Environment. Oxford: Oxford University Press, pp127–142, 1984.
24. Sunderman FW Jr, Hopfer SM, Knight JA, McCully KS, Cecutti AG, Thornhill PG, Conway K, Miller C, Patierno SR, Costa M. Physicochemical characteristics and biological effects of nickel oxides. Carcinogenesis **8**:305–313, 1987.