Retarding Effect of Vitamin Deficient and Cholesterol Free Diets on Growth of Sarcoma 180.* (29170)

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The growth of solid Sarcoma 180 in CF_1 white Swiss mice was shown to be significantly inhibited by feeding a diet deficient in vitamin B_6(1). Tumor retardation was reversed either by admixing Vit. B_6 with the deficient diet or by administering Vit. B_6 parenterally(2). Pyridoxamine dihydrochloride was stimulatory to the growth of ascitic and solid Sarcoma 180 in CF_1 mice, revealing the B_6-avidity of this tumor system and pointing to the existence of an exploitable biochemical difference of this tumor from normal host tissue(2). Pyridoxine antagonists such as 4-deoxypyridoxine and isonicotinic acid hydrazide, were inhibitory for transplantable tumors in mice fed with pyridoxine deficient diets(3-7). However, these antagonists were relatively ineffective as tumor retarding agents when the tumor bearing animals were maintained on a complete diet(7) indicating that their action was readily nullified by the corresponding vitamin in the diet. Recently, we reported that a relatively nontoxic chemical antagonist of Vit. B_6, L-penicillamine (β-mercaptovaline), retarded the growth of solid form Sarcoma 180 in mice whether they were maintained on a complete diet or on pyridoxine deficient diet(2). D-Penicillamine has been in clinical use as a chelating agent for treatment of hepatolenticular degeneration. The L-, D- and DL- forms of penicillamine were all found to be active as growth retarding agents for Sarcoma 180, the L-form being the most active. Tumor retardation was produced by L-penicillamine whether therapy was initiated 24 hours after sarcoma implantation or was delayed for 96 hours. L-penicillamine was equally as active as 5-fluorouracil in retarding the growth of solid form Sarcoma 180 in mice maintained on pyridoxine deficient diet and was less toxic. The tumor retarding effect of L-penicillamine was almost completely nullified *in vivo* by pyridoxamine(2). L-penicillamine retained its tumor growth-retarding action when it was administered orally *via* the drinking water or by stomach intubation to solid Sarcoma 180 bearing mice maintained on a complete diet(8). *Complete regression of solid tumors occurred in over half of the treated mice on complete diet given prolonged oral treatment with L-penicillamine* (8). L- penicillamine was also active by subcutaneous and intraperitoneal routes.

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Because of the retarding effect of L-penicillamine and pyridoxine deficient diet on the growth of Sarcoma 180, it became important to determine whether this rodent tumor system possessed other vitamin avidities that could be similarly exploited in controlling tumor growth.

Methods. Fifteen individual diets were employed, each specifically deficient in one vitamin (Table I)(9).

The basic vitamin-free diet (Nutritional Biochemicals Corp.) contained the following ingredients:

Vitamin-free casein	18%
Sucrose	68%
Vegetable oil	10%
Salt Mixture No. 2, USP (XIII)	4%

A vitamin fortification mixture (NBC) minus one vitamin, in each instance, was used in supplementing the basic diet:

Vit. A conc. (200,000 units/g)	4.5 g/100 lb diet
Vit. D conc. (400,000 units/g)	.25
Alpha tocopherol	5.0
Ascorbic acid	45.0
Inositol	5.0
Choline chloride	75.0
Menadione	2.25
P-aminobenzoic acid	5.0
Niacin	4.5
Riboflavin	1.0
Pyridoxine hydrochloride	1.0
Thiamine hydrochloride	1.0
Calcium pantothenate	3.0 g
Biotin	20 mg
Folic acid	90 mg
Vit. B_{12}	1.35 mg

Cholesterol-free diet (NBC) contained the following ingredients: vitamin-free casein 25%; Sucrose 65%; Alphacel (non-nutritive cellulose) 6%; Salt mixture USP XIV 4%, plus the following vitamins/100 lb of diet:

Thiamine HCl	1 g
Riboflavin	1 g
Calcium pantothenate	3.2 g
Niacin	4 g
Pyridoxine HCl	1 g
Menadione	1.8 g
Alpha tocopherol	5 g
Folic acid	100 mg
Biotin	20 mg
Choline chloride	76 g
P-aminobenzoic acid	5 g
Inositol	5 g
Vit. A acetate (powder)	1.4 g
Granular Calciferol	125 mg

An all *vitamin-free, cholesterol-free diet* (NBC) consisted of the above cholesterol-free diet without the added vitamins.

Complete diet consisted of Rockland mouse pellet diet (Teklad, Monmouth, Ill.) containing protein 24.2%, fat 4.1%, fiber 4.8%, carbohydrate 56.2%, nitrogen free extract 52.0%, ash 7.7%; and containing soybean oil meal, fish meal, dried whole milk, irradiated Brewer's type yeast, ground oats, wheat standard, middlings oat meal, ground yellow corn, ground barley, feeding oat meal, ground whole wheat, dehydrated alfalfa meal, 1% animal fat (preserved with propylene glycol, butylated hydroxyanisole, propyl gallate, citric acid), steamed bone meal ½%, calcium carbonate 1%, salt 1%, plus the following vitamins/100 lb of diet:

Vit. A	610 USP units
Thiamine	.22– .46 mg
Riboflavin	.44– .65 mg
Pyridoxine	.21– .27 mg
Pantothenic acid	1.0 – 1.5 mg
Choline	480 –492 mg
Inositol	61 mg
Vit. D	148 USP units
Vit. E	1.32 intern. units
P-aminobenzoic acid	.078 mg
Niacin	6.6 mg
Vit. C	3.6 mg
Carotene	.42 mg

Complete diet also contained the following amino acids:

Lysine	1.15%
Methionine	.40%
Cystine	.29%
Tryptophan	.27%
Arginine	1.39%
Histidine	.53
Isoleucine	1.20
Leucine	1.74
Phenylalanine	1.03
Threonine	.87
Valine	1.14
Tyrosine	.72

The technique of tumor transplantation and methods of evaluation of tumor growth were as previously described for solid Sarcoma 180(2).

Four hundred and forty 20 g white Swiss CF_1 male mice previously maintained on complete diet, each received right flank subcutaneous implantation of 1×10^6 ascitic Sarcoma 180 cells/mouse. The mice were divided into 15 groups and were fed with deficient and control diets (with water) *ad libitum* for 14 days. The animals were sacrificed on the 15th day and their solid tumors were excised and weighed.

Results. Significant retardation of growth of solid Sarcoma 180 was produced by 5 deficient diets. These were, in order of their effectiveness, (a) all vitamin-free, cholesterol-free diet, 76% tumor inhibition (b) pyridoxine deficient diet, 53% inhibition (c) cholesterol-free diet, 49% inhibition (d) thiamine deficient diet, 48% inhibition and (e) riboflavin deficient diet, 45% inhibition (Table I, Fig. 1). In 3 diet groups, weight loss was not a large enough factor to affect the size of the tumor masses(10), nor were the defi-

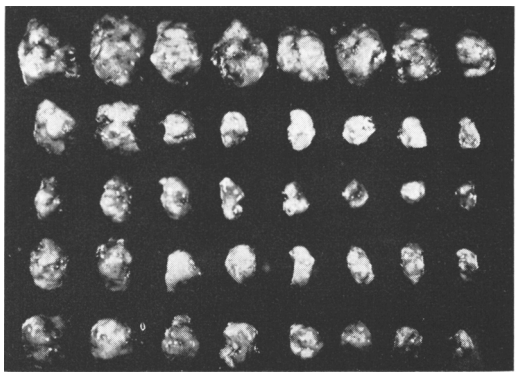

FIG. 1. Growth retarding effect of vitamin deficient and cholesterol free diets on solid Sarcoma 180. Sarcomas excised 2 wk after subcutaneous implantation of 1×10^6 ascitic cells/mouse. Top row: Complete diet. 2nd row: Pyridoxine deficient diet. 3rd row: All vitamin-free, cholesterol-free diet. 4th row: Low magnesium, pyridoxine deficient diet. Bottom row: Riboflavin deficient diet.

cient diets attended by a significant mortality. In the group of mice maintained on low protein diet, however, there was 82% tumor inhibition and 33% weight loss. This weight loss could account for a large part of the tumor inhibition (62%)(10), but not all.

To help verify the specific avidities of Sarcoma 180 for the vitamins, pyridoxine, thiamine, riboflavin and for cholesterol, reversal experiments were conducted with specifically deficient diets to which varying concentrations of the corresponding vitamins and cholesterol had been separately added. It was found that thiamine reversed the tumor retarding action of thiamine-deficient diet (Table II). Thiamine appeared to have a stimulatory effect on the development of tumor mass in mice maintained on thiamine deficient diet, and also appeared to increase the mortality in the tumor bearing animals. Ad-

TABLE I. Effect of Specific Dietary Deficiencies on Growth of Solid Sarcoma 180 in CF_1 Mice.*

Deficient diet†	Avg change in body wt§ (g)	(%)	Avg tumor wt (g ± S.D.)	Tumor inhib (%)	Mortality
Control (complete diet) ‡	+6.5	+31.2	2.70 ± .73	0	4/60
Vit A deficc	+5.3	+21.8	2.30 ± .63	15	0/20
Thiamine "	+ .8	+ 3.8	1.40 ± .70	48	1/30
Riboflavin "	+3.0	+14.6	1.50 ± .85	45	1/40
Niacin "	+5.1	+24.8	2.17 ± .88	20	1/20
Pyridoxine "	+2.1	+10.2	1.27 ± .42	53	2/40
Pantothenic acid "	+2.1	+10.2	2.56 ± .85	5	0/20
Vit B_{12} "	+5.7	+27.7	1.85 ± .61	32	0/20
Biotin "	+5.3	+25.8	2.38 ± .82	12	1/20
Folic acid "	− .3	− 1.2	2.29 ± .76	15	0/20
Choline "	+1.1	+ 4.4	2.17 ± .60	20	1/20
All vit-free diet‖	−1.5	− 7.3	.64 ± .33	76	3/30
Low protein diet (casein 8.0%)	−6.7	−32.7¶	.49 ± .05	82	6/20
Cholesterol-free diet	−3.6	−14.4**	1.36 ± .27	49	2/40
Low iron	+3.7	+14.5	2.64 ± .63	2	0/20
" magnesium	+5.5	+25.5	2.60 ± .70	2	0/20

* All mice maintained on their respective diets for 14 days following subcutaneous implantation of 1×10^6 ascitic Sarcoma 180 cells/mouse, tumors excised on 15th day.
† Nutritional Biochemicals Corp., Cleveland, Ohio; fed *ad libitum*.
‡ Rockland Farms Mouse Pellet.
§ Initial wt 20 g.
‖ Cholesterol-free diet without added vitamins.
¶ This weight loss would account for 62% tumor inhibition[10].
** This weight loss would account for 18% tumor inhibition[10].

TABLE II. Effect of Thiamine Deficient Diet on Growth of Solid Sarcoma 180 in CF_1 Mice and Reversing Effect of Thiamine.*

Diet	Avg change in body wt† (g)	(%)	Avg tumor wt (g ± S.D.)	Tumor inhib (%)	Tumor stim (%)	Mortality
Complete diet‡	+8.0	+33	2.86 ± .76			0/10
Complete diet plus 5 mg thiamine/mouse/day × 14 d subcut	+5.5	+21	2.96 ± 1.17			3/10
Thiamine defic diet§	+1.4	+ 6	1.67 ± .74	41		1/10
Idem plus 0.1 mg B_1/100 g diet	+6.0	+27	2.75 ± 1.42	3		5/10
Idem plus 1.0 mg B_1/100 g diet	+5.0	+23	2.96 ± .74	0	1	3/10
Idem plus 10 mg B_1/100 g diet	+0.3	+ 1	3.86 ± 1.85	0	35	3/10

* All mice maintained on their respective diets for 14 days following subcut implantation of 1×10^6 ascitic S-180 cells/mouse; tumors excised on 15th day.
† Initial avg wt 23 g.
‡ Rockland Farms mouse pellet diet.
§ Nutritional Biochemicals Corp.

mixture of riboflavin with riboflavin-deficient diet produced a similar reversing effect on tumor retardation (Table III). Addition of congeners of Vit. B_6, *i.e.*, pyridoxine, pyridoxal and pyridoxamine at dose levels of 1, 10 and 100 mg/kg reversed the tumor retarding action of pyridoxine-deficient diet on solid form Sarcoma 180 (Table IV). All three B_6 congeners, at a dose level of 100 mg/kg, produced larger tumors than appeared with complete diet. The foregoing data indicate that solid Sarcoma 180 growing in CF_1 mice, possesses avidities for Vit. B_1, B_2, B_6, and possibly B_{12}, while it is relatively unaffected by deficiencies of Vit. A, niacin, pantothenic acid, biotin, folic acid and choline. The feeding of vitamin-free, cholesterol-free defined diet caused 71% tumor inhibition of solid Sarcoma 180 in mice, which we attribute mainly to deficiencies of Vit. B_1, B_2 and B_6 and cholesterol (Table V). Daily subcutaneous injections of Vit. B complex partially re-

TABLE III. Effect of Riboflavin Deficient Diet on Growth of Solid Sarcoma 180 in CF_1 Mice and Reversing Effect of Riboflavin.*

Diet	Avg change in body wt† (g)	(%)	Avg tumor wt (g ± S.D.)	Tumor inhib (%)	Tumor stim (%)	Mortality
Complete diet‡	+8.0	+33	2.86 ± .76			0/10
Idem plus 5 mg B_2/mouse/day × 14 d subcut	+2.0	+ 8	2.82 ± 1.06			2/10
Riboflavin defic diet§	+3.3	+14	1.99 ± .80	30		2/10
Idem plus 0.1 mg B_2/100 g diet	+5.0	+21	3.51 ± 1.29	0	23	5/10
Idem plus 1.0 mg B_2/100 g diet	+1.8	+ 8	3.50 ± 1.18	0	23	3/10
Idem plus 10 mg B_2/100 g diet	+5.5	+24	3.23 ± 2.40	0	11	3/10

* All mice maintained on their respective diets for 14 days following subcut implantation of 1 × 10^6 ascitic S-180 cells/mouse; tumors excised on 15th day.
† Initial avg wt 23 g.
‡ Rockland Farms mouse pellet diet.
§ Nutritional Biochemicals Corp.

TABLE IV. Reversing Effect of Congeners of Vitamin B_6 on the Tumor Retarding Action of Pyridoxine Deficient Diet on Solid Sarcoma 180 in CF_1 Mice.*

Diet		Avg change in body wt† (g)	(%)	Avg tumor wt (g ± S.D.)	Tumor inhib (%)	Mortality
Complete diet‡		+6.7	+32	2.03 ± .56		1/10
Pyridoxine defic diet§		+1.2	+ 5.7	.68 ± .11	62	1/10
Pyridoxine deficient diet plus— Pyridoxine	1 mg/kg diet	+5.1	+24	1.21 ± .34	40	1/10
	10 " "	+6.0	+28	1.64 ± .74	19	0/10
	100 " "	+5.0	+24	2.13 ± .89	0	0/10
Pyridoxal	1 " "	+5.5	+26	1.98 ± .68	2	0/10
	10 " "	+5.0	+24	2.16 ± .74	0	0/10
	100 " "	+7.8	+37	2.83 ± .71	0	2/10
Pyridoxamine	1 " "	+5.0	+24	1.85 ± .82	9	0/10
	10 " "	+6.0	+28	1.90 ± .35	6	0/10
	100 " "	+5.2	+25	2.35 ± .87	0	2/10

* All mice maintained on their respective diets for 14 days, following subcut implantation of 1 × 10^6 ascitic S-180 cells/mouse; tumors exised on 15th day.
† Initial avg wt 21 g.
‡ Rockland Farms mouse pellet diet.
§ Nutritional Biochemicals Corp. B_6 congeners mixed with diet in quantities indicated.

TABLE V. Effect of Vitamin-free, Cholesterol-free Diet on Growth of Solid Sarcoma 180 in CF_1 Mice and Partial Reversing Effect of Vitamin B Complex Injections.*

Diet	Vit B complex dilution†	Avg change in body wt‡ (g)	(%)	Avg tumor wt (g ± S.D.)	Tumor inhib (%)	Mortality
Complete diet§	0	+8.0	+40	2.11 ± .55		2/10
Vit-free‖ cholesterol-free	0	+1.0	+ 5	.61 ± .07	71	1/10
"	1/1000	+2.0	+10	1.01 ± .39	52	3/10
"	1/100	+2.0	+10	1.21 ± .55	43	2/10
"	1/10	+3.0	+15	1.67 ± .19	21	5/10

* All mice maintained on their respective diets for 14 days, following subcut implantation of 1 × 10^6 ascitic S-180 cells/mouse; tumors excised on 15th day.

† 0.2 ml/mouse/day for 14 days, Plebex (Wyeth Laboratories, Inc., Phila., Pa.), includes thiamine hydrochloride 10 mg/ml, riboflavin 2 mg/ml, niacin 100 mg/ml, pyridoxine 5 mg/ml, calcium pantothenate 5 mg/ml, adm subcut.

‡ Initial avg wt 20 g.

§ Rockland Farms mouse pellet diet.

‖ Vitamin free casein 25%, sucrose 65%, alphacel (nonnutritive cellulose) 6%, salt mixture U.S.P. XIII 4%.

versed the tumor retarding effect of this deficient diet, presumably that part of the inhibition produced by vitamin deficiencies.

Effect of cholesterol. Two separate feeding experiments with cholesterol-free diet showed 49 and 41% inhibition of development of solid Sarcoma 180, respectively (Tables I and VI). Addition of 1, 2 and 4% U.S.P. cholesterol to cholesterol-free diet, nullified this effect (Table VI). Also, addition of cholesterol to complete diet produced a stimulatory effect on tumor weight without a corresponding increase in body weight.

Discussion. Although the diet is the ultimate source of tumor nutrients, it is presumed that the immediate source of nourishment for the cancer is the body fluids of its host(11). That tumors require known vitamins is also assumed from their ubiquitous presence in cancer tissues and the present knowledge of the crucial role of vitamins in cellular metabolic processes. Our studies of the growth of Sarcoma 180 in white Swiss mice have shown an increased need or avidity of the tumor for Vit. B_1, B_2 and B_6, and for cholesterol. Tumor development *in vivo* in a rodent host, was found to be profoundly affected by an excess or deficiency of a single vitamin given either in the diet or parenterally. The fact that an already rapidly growing animal tumor such as Sarcoma 180 can be further stimulated *in vivo* by Vit. B_6 and can be significantly retarded by a B_6 antagonist, such as L-penicillamine(2), raises the important question whether this is a general phenomenon common to other mammalian tumors, or an isolated laboratory occurrence. In support of the former idea, is the information that pyridoxine-deficient diet has produced inhibition, to a varying degree, of the

TABLE VI. Effect of Cholesterol-free Diet on the Growth of Solid Sarcoma 180 in CF_1 Mice and Reversing Effect of Cholesterol.*

Diet	Avg change in body wt† (g)	(%)	Avg tumor wt (g ± S.D.)	Tumor inhib (%)	Tumor stim (%)	Mortality
Complete diet‡	+7.3	+31	1.61 ± .35			0/12
Idem plus 2% cholesterol	+4.8	+21	2.03 ± .50		26	1/12
Cholesterol-free diet	− 2.1	− .9	.95 ± .19	41		0/12
Idem plus 1% cholesterol	+3.4	+15	1.78 ± .33		10	1/12
Idem plus 2% cholesterol	+3.3	+14	2.15 ± .55		33	5/12
Idem plus 4% cholesterol	+3.9	+17	2.42 ± .70		50	4/12

* All mice maintained on their respective diets for 10 days, following subcut implantation of 1 × 10^6 ascitic S-180 cells/mouse; tumors excised on 11th day.

† Initial avg wt 23 g.

‡ Rockland Farms mouse pellet diet.

growth *in vivo* of other animal tumors, such as Adenocarcinoma 755, Ehrlich carcinoma (solid), Murphy-Sturm lymphosarcoma, leukemias L1210 and L4946, Ehrlich carcinoma clone 2 (ascites) and plasma cell tumor 70429(12). In view of this, we conclude that the data justify a widening of the study of the effect of vitamin deficient diets on animal tumor systems. It also justifies a study of the vitamin avidities of human cancer systems to reveal biochemical differences of tumor and host tissue and to provide exploitable biochemical targets.

Cholesterol deficiency. The retarding effect of a cholesterol-free diet on solid Sarcoma 180 was of the order of that produced separately by pyridoxine deficient diet and L-penicillamine. The effect of added cholesterol in reversing the tumor retarding effect of the cholesterol-free diet helped to verify the finding that restriction of cholesterol alone was responsible for the tumor retardation.

The stimulatory effect of cholesterol on size and weight of solid Sarcoma 180 in mice fed with a complete diet raises many questions. Does an increased intake of cholesterol stimulate the growth *in vivo* of other animal tumors or of human cancers? Is the effect of cholesterol on tumor growth direct or indirect? Is the incidence of cancer any different in persons with hypocholesterolemia than in those with hypercholesterolemia, or in the lean *vs* the obese? Also, might it be possible to retard the development of animal and human tumors by the simple expedient of feeding cholesterol-free diet or administering anti-cholesterol agents? What other factors of diet affect cholesterol content, and what is their effect on animal and human tumor growth? We are now studying the effect of cholesterol-free diet on the growth of several other animal tumors.

Review of the literature on the role of cholesterol and fats in tumors reveals that neoplastic, as well as normal cells, require lipids of various types for growth and survival. Malignant neoplasms contain a much higher percentage of fatty substances—neutral fat, phospholipid and cholesterol than benign tumors or the tissues in which they grow(13, 14). A high phospholipid content appears characteristic of malignancy(13). These phospholipids are cephalin, lecithin, sphingomyelin and acetalphospholipids, which are common to noncancerous host tissue(13). Unsaturated fatty acids appear to predominate in the phospholipids of rat carcinosarcoma 256(15).

The exact role of lipids in tumor metabolism is unclear. They are believed to be involved in cell structure and to be oxidized as sources of energy. A number of workers have shown that tumor cells will oxidize fatty acids *in vitro*(16,17,18) and there seems to be good evidence for assuming that fatty acids are physiological substrates for tumor respiration. Endogenous respiration in Ehrlich ascites tumor cells was attributed to oxidation of fatty acids, amounting to as much as 30% of the aerobic respiration, even in the presence of glucose(17). Injection of P^{32} into tumor bearing mice and rats showed rapid rise of labelled P in tumor phospholipid to the same magnitude as the more active tissue such as liver and kidney than to that of the less active tissue such as muscle(19, 20, 21). Also, labelled phosphorus was retained in the tumor for longer periods, unlike normal tissues(22). Further studies by Haven showed rapid turnover of lecithin, cephalin(23) and sphingomyelin(24) in tumors.

There is considerable evidence that dietary lipids are utilized by tumors, since changes in type and amount of dietary fats cause alterations in the kind and sometimes in the amount of tumor lipid(11,25,26). It is known that lipid content of the host decreases as tumor grows(27,28), the loss being mainly in neutral fat(29,30). Studies of cholesterol-C^{14} metabolism in rats bearing Yoshida ascites tumors revealed that tumor cholesterol synthesized in the host is deposited to a greater degree in the tumor, which acts to trap cholesterol from the circulation(31). The feeding of cholesterol with lard or raw egg yolk was shown to cause increased incidence of lymphosarcoma and lung adenocarcinoma in mice(32). However, subcutaneous injection of cholesterol in an oily vehicle was not carcinogenic *in situ* in mice or rats(33).

The retarding effect of vitamin deficient and cholesterol-free diets on Sarcoma 180

shown by our study and the effect of pyridoxine deficient diet reported for Sarcoma 180 and many other tumors(1-7), illustrate the need for a broad experimental approach to cancer etiology and chemotherapy. This would encompass factors in the diet such as total calories, proteins, fats, carbohydrates, vitamins and antivitamins, carcinogenic and anticarcinogenic compounds, sterols, hormones and antimetabolites. When these are controlled and known, as in the present study, the effect of individual factors becomes much clearer.

Summary. By using 15 individual diets, each deficient in a single vitamin, it was possible to ascertain the vitamin avidities of growing solid Sarcoma 180 in CF_1 white Swiss mice. Significant retardation of growth was produced separately by diets deficient in thiamine, riboflavin and pyridoxine. In each instance, the growth retarding effect of the diet could be reversed by admixing the corresponding vitamin with the diet. A diet deficient in cholesterol caused 49% inhibition of sarcoma growth without significant mortality. This was equivalent to the growth inhibition produced separately by pyridoxine deficient diet and L-penicillamine. An all vitamin-free, cholesterol-free diet caused 76% tumor inhibition with 10% mortality. Admixture of cholesterol to both cholesterol-free diets, reversed their tumor retarding effects. The need for a broad experimental approach to cancer etiology and chemotherapy was discussed.

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