

# Inhibitory Effects of Selenium on the Growth of L1210 Leukemic Cells<sup>1</sup>

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## ABSTRACT

Selenium has been shown to inhibit L1210 cells both *in vitro* and *in vivo*. The death of L1210 cells *in vitro* as indicated by trypan blue exclusion was dependent upon the form and concentration of selenium tested. Incubation of L1210 cells in buffer containing selenium at 1  $\mu\text{g}/\text{ml}$  for 1 hr prior to inoculation into mice significantly retarded the ability of the cells to propagate *in vivo*. Sodium selenite injected i.p. increased the longevity of mice inoculated with L1210 cells. Administration of 40  $\mu\text{g}$  selenium as sodium selenite daily for 7 days resulted in a 65% increase in longevity of mice inoculated with  $10^5$  L1210 cells. Injections of sodium selenite at doses of 40  $\mu\text{g}/\text{day}$  or less for 7 days did not significantly alter growth, liver weight, or red and white blood cell counts. The efficacy of selenium therapy was dependent upon the total number of tumor cells given in the initial inoculum. Selenium administration as sodium selenite was shown to be more effective in increasing the longevity of L1210-inoculated mice than was treatment with sodium selenate, selenocystine, or selenomethionine. Sodium selenite treatment at 20, 30, or 40  $\mu\text{g}/\text{day}$  in mice inoculated with  $10^2$  cells resulted in 50, 80, and 90% cures, respectively. Supplementation of the drinking water with 3 ppm selenium as sodium selenite increased the longevity of L1210-inoculated mice by approximately 30%. Combined therapy with selenium (30  $\mu\text{g}/\text{day}$ ) and methotrexate resulted in a significantly longer life span of L1210-treated mice than resulted from either compound administered separately.

## INTRODUCTION

Considerable epidemiological data suggest that cancer mortality is inversely correlated with selenium consumption (16, 19, 20). Furthermore, recent reports clearly show that selenium can retard the growth of chemically induced as well as spontaneous tumors (2, 5-7, 17, 18, 22). Consequently, appreciable experimental evidence does suggest that selenium may have a preventive role in the etiology of cancer. Weisberger and Suhrlund (25) also demonstrated that selenium could retard the growth of Murphy lymphosarcoma tumor cells. Poirier and Milner (15) have demonstrated that selenium can retard or completely prevent the growth of Ehrlich ascites tumor cells *in vivo*.

Greeder and Milner (4) recently showed that the ability of selenium to inhibit or prevent the growth of Ehrlich ascites tumor cells was highly dependent upon the form and quantity of selenium administered. The only significant alteration that has been observed in mice treated with quantities of selenium at dosages of 40  $\mu\text{g}$  3 times weekly was a slight reduction in intestinal weights. The reduction in intestinal weights was not

attributable to a reduction in the intestinal macroconstituents, suggesting that selenium may have altered rapidly dividing cells (4).

The L1210 cell line is known to be susceptible to antifolics and to purine and pyrimidine analogs (1, 3, 9, 10). Selenopurines have also been shown to retard the growth of L1210 leukemic cells (11). However, other forms of selenium have not been examined adequately.

Therefore, the present studies were conducted to examine the influence of: (a) various dosages and forms of selenium on the growth of L1210 cells; (b) the route of administration of selenium on the mean survival time of the mice inoculated with L1210 cells; and (c) the quantity of tumor cells inoculated on the efficacy of selenium.

## MATERIALS AND METHODS

Male CDF mice (20 to 25 g; Harlan Industries, Indianapolis, Ind.) were used in all studies. All mice were given water and Purina mouse chow *ad libitum* and were housed 5/cage in a room with controlled temperature and lighting. L1210 cells have been continuously maintained in our laboratory for the past year. The original inoculum of cells was a gift of Dr. J. Mayo, National Cancer Institute.

Experiment 1 examined the influence of concentration and form of selenium on the viabilities of the L1210 cells *in vitro*. Leukemic cells harvested from the peritoneal cavity of inoculated mice were washed twice in KRP<sup>2</sup> buffer (15) before use. The cells were diluted to approximately  $10^7$  cells/ml with KRP containing glucose (90 mg/100 ml) and supplemental selenium (0, 1, 5, or 10  $\mu\text{g}/\text{ml}$ ) as sodium selenite. Incubations were performed in a metabolic shaker bath maintained at 37°. Viabilities were determined hourly for 3 hr by trypan blue exclusion (23). Duplicate counts were made of cells exposed to each of the test solutions per time period using a hemocytometer. In the second phase of this study, L1210 cells ( $10^7/\text{ml}$ ) were incubated in KRP containing glucose alone or glucose plus supplemental selenium (1  $\mu\text{g}/\text{ml}$ ) as sodium selenite. After 1 hr of incubation, the cells were removed from their respective buffers and washed twice with KRP containing no supplemental selenium. Cell numbers and viabilities were determined using a hemocytometer after addition of trypan blue. After appropriate dilution,  $10^3$  viable cells were inoculated into the peritoneal cavity of CDF mice. Ten mice received inoculations of cells from each of the 2 incubation treatments. Mean survival time of the mice after inoculation of the L1210 cells was recorded. All mice were fed a commercial laboratory chow and received no additional treatment. The concentration of selenium in the commercial chow was 0.35  $\mu\text{g}/\text{g}$ . Additionally, the third part of the study examined sodium selenite, sodium selenate, selenium dioxide, and selenocystine at a final selenium concentration of 0.5  $\mu\text{g}/\text{ml}$  for their influence on the viability of L1210 cells

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<sup>2</sup> The abbreviation used is: KRP, Krebs-Ringer phosphate buffer.

( $10^7$ /ml) as a function of time. Viabilities were determined over a 3-hr incubation.

Experiment 2 examined the influence of i.p. injections of sodium selenite on the longevity of mice inoculated with  $10^5$  cells. The quantities of selenium injected i.p. were 0, 20, 30, 40, or 50  $\mu\text{g}$  selenium per day for 6 days. Ten mice received each quantity of selenium examined. Selenium administration began 30 min after inoculation of the mice with the L1210 cells. KRP was administered to all controls and used as the diluent for the selenium solutions. All injections were 0.1 ml and were administered at a site distant from that of tumor inoculation.

The influence of the quantity of tumor cells inoculated on the action of selenium was examined in Experiment 3. Mice were inoculated with  $10^4$ ,  $10^3$ , or  $10^2$  L1210 cells. The influence of 30  $\mu\text{g}$  selenium as sodium selenite on the longevity of the inoculated mice was examined. Selenium administration began 15 hr after inoculation with the tumor cells.

Experiment 4 examined the minimum quantity of sodium selenate that would increase the longevity of L1210 tumor-bearing mice. Mice were inoculated with  $1 \times 10^2$  L1210 cells on Day 0. Mice were given injections of KRP or sodium selenite daily for 10 days at a dose of 20, 30, or 40  $\mu\text{g}$ /mouse. Furthermore, the influence of treatment with 40  $\mu\text{g}$  of selenium as sodium selenate, selenomethionine, and selenocystine was examined.

In 2 studies, the influence of methotrexate (25 mg/kg; a gift from Dr. R. Donaldson, Veterans Administration Hospital, St. Louis, Mo.) with or without supplemental sodium selenite on the longevity of mice treated with varying quantities of L1210 cells was examined. Experiment 5A examined the influence of selenium, methotrexate, or combinations of each on mice inoculated with  $10^5$  L1210 tumor cells. Methotrexate (25 mg/kg) was administered i.p. 2 and 6 days after tumor inoculation. Selenium and methotrexate were administered at sites distant from each other to avoid direct interaction. Selenium as sodium selenite at various dosages was administered on Days 0, 1, 2, 4, 6, 8, and 10. In Experiment 5B, 20 mice were assigned to each treatment and received  $10^3$  viable tumor cells on Day 0. In this experiment, methotrexate (25 mg/kg) was administered i.p. at 3 and 8 days after inoculation with tumor cells. Selenium (30  $\mu\text{g}$ /day) was administered i.p. on the day of tumor inoculation and was continued daily for 10 days. Control mice received daily injections of KRP.

The influence of selenium supplement in drinking water on the longevity of mice inoculated with L1210 cells was examined in Experiment 6. Water containing less than 0.01  $\mu\text{g}$  selenium per ml was used throughout this study. All mice were acclimated to the respective water treatments 2 weeks prior to inoculation with tumor cells. Selenium supplements were 0, 1, 3, 5, or 10 ppm. Ten mice were acclimated to each water treatment. Selenium was added to the drinking water as sodium selenite.

## RESULTS

Incubation of L1210 cells with selenium was found to significantly reduce viabilities as indicated by trypan blue exclusion (Table 1). Increasing quantities of selenium in the incubation media resulted in a progressive decrease in cell viabilities within 1 hr. Longer-term incubation resulted in a complete loss

of viability in these cells. The L1210 cells incubated with selenium (0.5  $\mu\text{g}$ /ml) in various forms were also found to have decreased viability. The order of relative toxicity of the selenium compounds after 3 hr of incubation was selenium dioxide > sodium selenite > selenocystine > sodium selenate, based on trypan blue exclusion (Table 1).

In the recovery phase of this study, mice were inoculated with  $10^3$  viable L1210 cells previously incubated in media with (1  $\mu\text{g}$ /ml) or without selenium. Viabilities as determined by trypan blue exclusion were decreased approximately 25% during the hr of incubation. The mean survival time for mice inoculated with L1210 cells incubated without supplemental selenium was  $333 \pm 16$  (S.D.) hr. Only 2 of the 10 mice that received cells incubated in media containing selenium (1  $\mu\text{g}$ /ml) died. Death occurred in these mice  $375 \pm 24$  hr after inoculation with the tumor cells. The remaining 8 animals are considered cured and non-tumor-bearing, since 60 days have elapsed since their inoculation with the cells.

The influence of varying quantities of selenium on the longevity of mice inoculated with  $10^5$  L1210 cells was also examined. Selenium (up to 40  $\mu\text{g}$ /day) did not significantly alter the growth of the non-tumor-bearing mice during the 7-day experimental period (Table 2). However, at 50  $\mu\text{g}$ /day, there

Table 1

Effect of selenium supplement in vitro on viabilities of L1210 cells

Results are means for 2 flasks per treatment per time period. Vertical means not sharing a common superscript letter differ by  $p < 0.05$ .

Treatment	Selenium concentration ( $\mu\text{g}/\text{ml}$ )	% of viability		
		1 hr	2 hr	3 hr
Experiment 1A				
Control		95.2 <sup>a</sup>	73.5 <sup>a</sup>	75 <sup>a</sup>
Sodium selenite	1	27.5 <sup>b</sup>	27.3 <sup>b</sup>	0 <sup>b</sup>
Sodium selenite	5	40.8 <sup>c</sup>	14.3 <sup>c</sup>	0 <sup>b</sup>
Sodium selenite	10	28.3 <sup>c</sup>	16.7 <sup>c</sup>	0 <sup>b</sup>
Pooled S.E.		5.5	3.5	6.5
Experiment 1B				
Control		95.2 <sup>a</sup>	95.8 <sup>a</sup>	94.6 <sup>a</sup>
Sodium selenite	0.5	95.4 <sup>a</sup>	64.6 <sup>c</sup>	37.7 <sup>d</sup>
Sodium selenate	0.5	95.4 <sup>a</sup>	93.9 <sup>a</sup>	82.4 <sup>b</sup>
Selenodioxide	0.5	94.8 <sup>a</sup>	94.1 <sup>a</sup>	10.0 <sup>e</sup>
Selenocystine	0.5	89.7 <sup>b</sup>	81.8 <sup>b</sup>	70.8 <sup>c</sup>
Pooled S.E.		1.3	2.8	4.2

Table 2

Effect of daily injections of graded quantities of  $\text{Na}_2\text{SeO}_3$  upon mice inoculated with  $10^5$  L1210 cells

KRP or  $\text{Na}_2\text{SeO}_3$  were administered daily for 6 days after tumor cell inoculation. Vertical mean values not sharing a common superscript letter differ by  $p < 0.05$ . Means are for 5 non-tumor-bearing and 10 tumor-bearing mice per treatment, except for the 50- $\mu\text{g}/\text{day}$   $\text{Na}_2\text{SeO}_3$  therapy, where means are for 4 non-tumor-bearing and 7 tumor-bearing mice per treatments.

Treatment	Non-tumor bearing		Tumor bearing	
	Growth (g/7 days)	Liver wt (mg)	Mean survival time (hr)	Increase in mean survival time above controls (%)
Controls				
KRP	$0.6 \pm 0.2^a$	$881 \pm 35^a$	$174 \pm 15^a$	
$\text{Na}_2\text{SeO}_3$				
20 $\mu\text{g}/\text{day}$	$0.4 \pm 0.3^a$	$910 \pm 40^a$	$250 \pm 12^b$	43
30 $\mu\text{g}/\text{day}$	$0.2 \pm 0.2^a$	$890 \pm 43^a$	$268 \pm 8^{b,c}$	54
40 $\mu\text{g}/\text{day}$	$0.3 \pm 0.3^a$	$1080 \pm 75^a$	$286 \pm 15^c$	65
50 $\mu\text{g}/\text{day}$	$-1.9 \pm 0.5^b$	$900 \pm 25^a$	$290 \pm 35^c$	67

<sup>a</sup> Mean  $\pm$  S.E.

was a significant reduction in growth of the mice. Selenium at this concentration was apparently toxic to the mice, since 4 of the 15 animals died within 3 days after initiation of the injections of selenium. No significant alteration in liver weight was detected in animals treated with selenium as compared to that of controls at any of the concentrations of selenium examined. Longevity of the tumor-bearing control animals treated with KRP was approximately 8 days (Table 2). Treatment with 20 µg of selenium resulted in a 43% increase in longevity of the mice. Increasing the quantity of injected selenium from 20 to 40 µg resulted in a further significant increase in longevity. The increase in longevity resulting from a 40-µg/day dose was 65% greater than that observed with KRP-treated controls. Examination of non-tumor-bearing control mice for blood histology revealed that there were no significant alterations in total RBC and WBC between control and selenium-treated mice. The mean counts for RBC from mice treated with buffer or selenium (40 µg/day) for 7 days were 6.5 ± 0.3 (S.E.) and 6.9 ± 0.2 × 10<sup>6</sup> cells/cu mm. WBC were 11.3 ± 1.4 and 10.7 ± 0.6 × 10<sup>3</sup> cells/cu mm for control and sodium selenite-treated animals, respectively.

Inoculation of mice with varying quantities of tumor cells was also examined as a factor influencing selenium efficacy (Table 3). Inoculation of decreasing quantities of tumor cells from 10<sup>4</sup> to 10<sup>2</sup> resulted in a significant increase in mean survival time of the mice. Selenium administration significantly increased the longevity of the mice, regardless of the initial cell number. Of interest were the mice receiving 10<sup>2</sup> cells. One of the 10 animals treated with selenium (30 µg/day) as sodium selenite died 410 hr after the initial tumor inoculum. The remaining 9 mice are still alive approximately 7 months after selenium treatment was discontinued.

Similar results were obtained in Experiment 4, in which selenium therapy was given at 30 or 40 µg/day for 10 days. Interestingly, 20 µg selenium per day for 10 days resulted in apparent tumor cell cures in 50% of the mice (Table 4). The order of efficacy for the forms of selenium tested was sodium selenite >> selenocystine > selenomethionine = sodium selenate. These data agree relatively well with data obtained in Experiment 1B.

Data for Experiment 5 are presented in Table 5. In both sections of this study, selenium and methotrexate were capable of increasing the longevity of tumor-bearing mice. The combined administration of selenium and methotrexate resulted in

a further increase in longevity. Although the data do not convincingly reveal a synergistic effect, clearly, the response to both compounds is greater than is the response to each compound administered separately (Table 5). This response was evident in mice inoculated with either 10<sup>5</sup> or 10<sup>3</sup> tumor cells. Mean survival times of greater than 60 days in these studies were detected only in mice receiving both compounds.

Supplementation of the drinking water with selenium at 3 or more ppm reduced the growth of the CDF mouse (Table 6). At 3 and 5 ppm selenium in drinking water, an approximately 40% reduction in weight gain was observed. At 10 ppm, a significant further depression in growth rate occurred. A significant increase in longevity occurred in tumor-bearing mice receiving 3 ppm selenium added to the drinking water. A 30.3% increase in longevity was observed at this concentration of selenium compared to that of control. Further supplement with selenium at quantities higher than 3 ppm resulted in a decline in the longevity of the animals. However, mice given 5 and 10 ppm selenium in water had a significantly increased mean survival

Table 4

Effect of form of selenium on the longevity of mice inoculated with 10<sup>2</sup> L1210 cells

All mice received 1.06 × 10<sup>2</sup> tumor cells on Day 0. All solutions were given daily for 10 days in a total daily volume of 0.1 ml. Means ± S. E. not sharing a common superscript letter differ by p < 0.05 and represent 10 mice/treatment.

	Dosage of selenium (µg/day)	Mean survival time (hr)	60-day survival (%)	Wt gain <sup>a,b</sup> (g/10 days)
Control	0	296 ± 4 <sup>a,b</sup>	0	0.7 ± 0.4 <sup>a</sup>
Na <sub>2</sub> SeO <sub>3</sub>	20	392 ± 11 <sup>b,c</sup>	50	0.4 ± 0.3 <sup>a</sup>
Na <sub>2</sub> SeO <sub>3</sub>	30	410 ± 10 <sup>c</sup>	80	0.3 ± 0.5 <sup>a</sup>
Na <sub>2</sub> SeO <sub>3</sub>	40	437 <sup>c</sup>	90	-0.5 ± 0.9 <sup>a</sup>
Na <sub>2</sub> SeO <sub>4</sub>	40	319 ± 8 <sup>a</sup>	0	0.9 ± 0.4 <sup>a</sup>
Selenocystine	40	361 ± 14 <sup>b</sup>	0	-0.6 ± 0.4 <sup>a</sup>
Selenomethionine	40	321 ± 7 <sup>a</sup>	0	0.3 ± 0.4 <sup>a</sup>

<sup>a</sup> Initial body weights were 20 to 25 g.  
<sup>b</sup> Mean ± S.E.

Table 5

Effect of methotrexate with or without selenium therapy on the longevity of L1210 tumor-bearing mice

See "Materials and Methods" for experimental design. Methotrexate was administered at 25 mg/kg on Days 2 and 6 after inoculation in Experiment 5A and on Days 3 and 8 in Experiment 5B. Selenium was administered as sodium selenite on Days 0, 1, 2, 4, 6, 8, and 10 in Experiment 5A and daily for 10 days in Experiment 5B. Means not sharing a common superscript letter differ by p < 0.05.

	Total mice	Mean survival time (hr)	% of increase above controls	Final wt (g)
Experiment 5A (10 <sup>5</sup> cells)				
Control	10	180 ± 7 <sup>a</sup>		23.8 ± 0.7 <sup>a</sup>
Selenium (20 µg)	10	206 ± 9 <sup>b</sup>	14	23.3 ± 0.7 <sup>a</sup>
Selenium (40 µg)	10	220 ± 3 <sup>b</sup>	22	24.1 ± 0.4 <sup>a</sup>
Methotrexate	10	270 ± 4 <sup>c</sup>	50	24.9 ± 0.7 <sup>a</sup>
Methotrexate + selenium (20 µg)	10	288 ± 3 <sup>c</sup>	60	23.7 ± 0.5 <sup>a</sup>
Methotrexate + selenium (30 µg)	10	313 ± 9 <sup>d</sup>	74	23.0 ± 0.6 <sup>a</sup>
Methotrexate + selenium (40 µg)	10	309 ± 3 <sup>d</sup>	72	24.4 ± 0.5 <sup>a</sup>
Experiment 5B (10 <sup>3</sup> cells)				
Control	20	240 ± 7 <sup>a</sup>		26.6 ± 0.5 <sup>a</sup>
30 µg selenium	20	350 ± 9 <sup>b</sup>	46	26.3 ± 0.4 <sup>a</sup>
Methotrexate	20	401 ± 12 <sup>c</sup>	67	25.3 ± 0.6 <sup>a</sup>
30 µg selenium + methotrexate	20	447 ± 8 <sup>d</sup>	86	23.9 ± 0.8 <sup>a</sup>

<sup>a</sup> Mean ± S.E.  
<sup>b</sup> Two mice were alive 60 days after the termination of the study.

Table 3

Effect of tumor inoculum of L1210 cells on the inhibitory effect of Na<sub>2</sub>SeO<sub>3</sub>

Mice received either KRP or Na<sub>2</sub>SeO<sub>3</sub> (30 µg/day) for 6 days starting 15 hr after the time of tumor inoculation. Mean survival time ± S. E. of controls increased (p < 0.05, as indicated by superscript letters) for each decrease in initial inoculum.

Inoculum (cells/mouse)	Treatment	Mean survival time (hr)	% of increase above controls	210-day survivors
10 <sup>4</sup>	Control	218 ± 8 <sup>a</sup>		0/10
	Na <sub>2</sub> SeO <sub>3</sub>	248 ± 8 <sup>b</sup>	15	0/10
10 <sup>3</sup>	Control	241 ± 5 <sup>a</sup>		0/10
	Na <sub>2</sub> SeO <sub>3</sub>	298 ± 9 <sup>b</sup>	23	1/10
10 <sup>2</sup>	Control	293 ± 8 <sup>a</sup>		0/10
	Na <sub>2</sub> SeO <sub>3</sub> <sup>b</sup>	>4320 <sup>b</sup>	>40	9/10

<sup>a</sup> Mean ± S.E.  
<sup>b</sup> One mouse died 410 hr after inoculation. The remaining mice were alive 7 months after receiving their last Na<sub>2</sub>SeO<sub>3</sub> injection.



Table 6

Effect of selenium supplement to drinking water on mice inoculated with L1210 cells

All mice were acclimated to their respective water treatments for 2 weeks prior to tumor inoculation. Selenium was administered as sodium selenite. All mice were treated with  $10^4$  cells. Means not sharing a common superscript letter differ by  $p < 0.05$ .

Water treatment	Non-tumor bearing		Tumor bearing	
	Growth (g/10 day)	Mean survival time (hr)	Mean survival time (hr)	% of increase above controls
Controls	$4.0 \pm 0.4^a$	$248 \pm 8^a$		
Selenium				
1 ppm	$4.1 \pm 0.5^a$	$264 \pm 7^b$		6.5
3 ppm	$2.5 \pm 0.3^b$	$323 \pm 10^c$		30.3
5 ppm	$2.5 \pm 0.5^b$	$294 \pm 10^{b,c}$		18.7
10 ppm	$0.1 \pm 0.3^c$	$278 \pm 8^b$		10.0

<sup>a</sup> Mean  $\pm$  S.E. for 5 non-tumor-bearing and 10 tumor-bearing mice per treatment.

time compared to that of unsupplemented control tumor-bearing mice (Table 6).

## DISCUSSION

These studies clearly show that selenium can alter the growth of L1210 leukemic cells. This retardation in tumor growth as induced by selenium supplementation and indicated by increased longevity of L1210 tumor-bearing mice is without apparent ill consequences to the host. The efficacy of selenium therapy against L1210 cells was dependent upon the dose and form administered. Schroeder and Mitchner (21) have suggested that the valence states may account for differences in relative toxicities of various forms of selenium. The data presented above reveal that sodium selenite is one of the most efficient compounds both *in vitro* and *in vivo* against L1210 cells. Sodium selenate and selenocystine are less effective in altering the growth of this tumor cell line. Greeder and Milner (4) have shown that the selenoamino acids are approximately 10 to 20 times less efficient than is sodium selenite in reducing the growth of Ehrlich ascites tumor cells. Types of tumors as well as form of selenium administered may therefore be important factors in the efficacy of selenium therapy.

Previous studies in this (15) and other laboratories have shown that cell viabilities as determined by dye exclusion are not a sensitive indicator of cell damage. Although L1210 cells appeared viable by dye exclusion test after incubation with selenium, these cells were unable to propagate *in vivo*. However, *in vitro* viabilities determined at low selenium concentrations as a function of time were indicative of the relative potential efficacy of the various forms of selenium.

The mechanism by which selenium retards the growth of transplantable tumors is unknown. Studies are presently being conducted in our laboratory to examine the metabolic effects of selenium on tumor tissue. Clearly, selenium can alter the growth of tumor cells without apparent ill consequences to the host. The action of selenium is not associated with severely depressed growth of the host mouse. Therefore, the response to selenium may represent some specific effect on neoplastic tissue. Selenium is known to localize in neoplastic tissue and has been used diagnostically as a scintigraphic tumor-localizing agent. The mechanism responsible for the increased uptake of selenium by neoplastic tissue is unknown.

Selenium has now been shown to alter the rate of growth of various types of tumors (4, 15, 25). The response to selenium was at least additive when given in conjunction with the chemotherapeutic agent, methotrexate. Mean survival times greater than 60 days were observed only in mice receiving both methotrexate and selenium therapies. The usefulness of selenium as a therapeutic agent in humans clearly deserves further consideration.

These data reveal that p.o. administration of selenium is not as efficient in retarding tumor growth as are i.p. injections. However, a significant increase in longevity occurred in tumor-bearing mice consuming water supplemented with selenium. Selenium supplementation in water at greater than  $3 \mu\text{g}/\text{ml}$  has generally been observed to retard water consumption. A decrease in water consumption may explain the reduction in longevity of tumor-bearing mice consuming water containing more than  $3 \mu\text{g}$  selenium per ml.

Weisberger and Suhland (26) have shown that the administration of selenocystine p.o. at dosages of 50 to 200 mg/day decreased leukocyte counts and spleen size in leukemic patients. Selenium treatment also resulted in severe nausea, vomiting, anorexia, moderate drowsiness, and occasional diarrhea. However, hepatic and renal functions appeared normal despite prolonged administration of selenocystine. Our data suggest that sodium selenite may have been more effective at a lower dosage. Clearly, additional clinical trials are needed.

Although selenium is essential in the diet of humans (24), it has often unfortunately been considered extremely toxic and carcinogenic (14). However, the overwhelming data currently available clearly indicate that selenium may have preventive as well as therapeutic benefits in the etiology of cancer (2, 4-7, 15, 16, 22). Selenium has been shown to reduce the frequency and growth of tumors that are chemically induced, spontaneous, or transplanted. Clearly, selenium deserves attention as a factor in the etiology of cancer in humans.

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