Selenium and the Transplantable Tumor

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Selenium has been shown to be effective in inhibiting chemically induced or transplantable tumors. Present information suggests this efficacy depends on the quantity and form administered. Organic forms of selenium are apparently less efficacious than inorganic forms. Data also indicate the efficacy of selenium is dependent upon the total tumor mass at the time of treatment. The inhibitory effects of selenium appear to be greater for neoplastic than for normal cells. An intermediate of selenium detoxification, selenodigluthathione, was observed to be more effective than sodium selenite in inhibiting the growth of Ehrlich ascites tumor cells. These studies also indicate that the effect of selenium is not limited to neoplastic cells isolated from mice since human mammary tumors also respond to selenium supplementation.

Selenium is an element of considerable interest. The unique and unusual properties of the element have allowed for its many and diverse industrial uses. However, until 1957 the only known biological action of this element was associated with its toxicity (Schwartz and Foltz, 1957; Wilber, 1980). Proof of the ability of selenium to correct such disorders as exudative diathesis in chicks, hepatosis dietetica in swine, and white muscle disease in young ruminants during the 1950s and 1960s indicated that this trace element must have a role in intermediary metabolism.

Not until 1973 was the role of selenium as a component of glutathione peroxidase described (Rotruck et al., 1973). This enzyme functions to destroy lipid peroxides and thus protects cell membranes against peroxidative damage. Since 1973 selenium has been shown to be a constituent of various enzymes in microorganisms (Stadtman, 1980). The recent identification of selenoproteins other than glutathione peroxidase in rats suggests this nutrient may eventually be shown to have additional importance in mammals (Burk and Gregory, 1982); McConnell et al., 1979).

While selenium is often considered an essential dietary component for mammals, including humans (van Rij et al., 1979), it continues to be considered one of the most toxic elements known. Further concerns about selenium intakes were fostered by an early report suggesting that selenium may be carcinogenic (Nelson et al., 1943). However, a critical review of this study revealed problems in histological identification (Scott, 1973).

Selenium cannot be equated with known classes of carcinogens such as polycyclic aromatic hydrocarbons. While some compounds containing selenium may be shown to be carcinogenic, this evidence should not be construed to mean all selenium compounds are carcinogenic. To date, little data support the notion that selenium is a carcinogen in experimental animals or in humans. Yet, until quite recently, selenium was considered a suspect carcinogen by the National Cancer Institute. Harr et al. (1967) was unable to detect any significant increase in tumor incidence in rats given supplemental selenium. Moreover, their data suggested an increase in longevity in those animals given supplemental selenium.

Selenium as an Anticarcinogen. Increasing evidence does indicate that selenium has anticarcinogenic properties. Epidemiological data have revealed a highly significant inverse correlation between selenium consumption and cancer mortality (Schrauzer et al., 1977a,b). The first experimental evidence that demonstrated selenium could alter the induction of chemically induced tumors came from Clayton and Baumann (1949). Since that time, selenium has been shown to inhibit tumors resulting from administration of a variety of chemical carcinogens (Shamberger, 1970; Harr et al., 1972; Jacobs et al., 1977; Griffin and Jacobs, 1977; Thompson and Becci, 1980; Ip, 1981; Banner et al., 1982). Selenium has also been shown to inhibit the mutagenicity of various carcinogens in the Ames *Salmonella* microsomal mutagen test (Martin et al., 1981; Schillaci et al., 1982).

Selenium thus appears to protect laboratory animals against the induction of cancer caused by various chemical carcinogens under a variety of experimental conditions. The anticarcinogenicity of selenium may be associated with the ability of selenium-dependent glutathione peroxidase to protect cells against peroxidative damage (Benedetti et al., 1974; Little and O'Brien, 1968; Hafeman and Hoekstra, 1977; Omaye et al., 1978) or by alteration of the metabolism of the carcinogenic chemicals or their interaction with tissue macromolecules (Wortzman et al., 1980, Wattenberg, 1978). Recent studies by Daoud and Griffin (1978), Wortzman et al. (1980), and Harbach and Swenberg (1981) suggest the anticarcinogenic properties of selenium, unlike those of other antioxidants, are not associated with a depression in the binding of the carcinogen to DNA.

Few laboratories have addressed the influence of selenium on the metabolism of the proximate carcinogen. Studies of Rasco et al. (1977), Grunau and Milner (1983), Grunau et al. (1983), and Schillaci et al. (1982) support the finding that selenium's anticarcinogenic properties are associated with alterations in the bioactiviation of the parent carcinogen. Additional support for the effects of selenium on the bioactivation of proximate carcinogen are given by recent studies with ultimate carcinogens. The agent 1-methyl-1-nitrosourea has been used extensively as an ultimate carcinogen in biological systems. Sodium selenite was not effective in inhibiting the number of rats developing carcinomas after exposure to 1-methyl-1-nitrosourea (Thompson and Becci, 1979) or methylazoxymethanol (Jacobs et al., 1977), suggesting that selenium may act to inhibit many proximate carcinogens by altering their metabolism to the ultimate carcinogen. The aforementioned epidemiological and experimental evidence clearly suggest that selenium has a preventative role in the etiology of cancer.

Antipropagation Role of Selenium. Broghamer et al. (1976) examined the association between serum selenium concentrations of 110 patients with various types of carcinomas and the biological behavior pattern of the tumor. Of the 110 patients, the histological types examined were pulmonary (37), orolaryngeal (24), gastrointestinal (18),

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genitourinary (14), and miscellaneous (17), which included primarily skin (basal cell) and epidermoid. Although the serum selenium concentrations of these patients were variable, the majority had levels lower than healthy controls. Lower serum selenium concentrations were also associated with a higher frequency of distant metastases, multiple primary tumors, multiple recurrences, and a shortened survival time. Patients with selenium concentrations approaching or exceeding the mean value of all cancer patients had tumors that remained confined to the region of origin, developed less distant metastasis, had fewer primary neoplasms, and had a decreased frequency of reocurrences.

More recent observations by Broghamer et al. (1978) revealed that the serum selenium concentrations of patients with reticuloendothelial tumors did not correlate with the behavioral patterns of the tumor in terms of the extent of organ involvement, patient survival time, and the incidence of multiple primary neoplasms. It is of interest that considerably greater variability in the serum concentrations was observed in these 59 patients than was found in the 1976 studies. Some of the patients had serum selenium concentrations that were approximately 8 times the normal values for healthy individuals. The reason for the higher selenium concentrations is not readily apparent unless, as the authors suggest, they are a result of the medical treatments being given to these patients.

Schrauzer et al. (1977a) have examined the age-correlated mortalities from cancer. Mortality rates in 27 countries from cancer at 17 major body sites in individuals residing in various countries were correlated with apparent dietary selenium intakes. Their studies reveal a significant inverse correlation of the mortality resulting from cancer of the large intestine, rectum, prostate, breast, ovary, lung, and leukemia with selenium intakes. Similar inverse correlations were observed between the mortalities of patients with the above cancer sites and the blood selenium concentrations of apparently healthy subjects located in the same geographical region. Epidemiological data suggest that selenium may not only serve as a preventative agent but also serve as a naturally occurring therapeutic agent to inhibit tumor proliferation.

Much less attention has been given to selenium as a potential therapeutic agent in the treatment of cancer. However, more than 60 years ago selenium was touted as a cancer therapeutic agent (Dalbert, 1912; Walker and Klein, 1915). Claims, although not well documented, of the beneficial effects of selenium in conjunction with X-ray therapy were made. In 1956, Weisburger and Surhland (1956a,b) reported that selenocystine was effective in reducing the leukocyte count and spleen size of patients suffering from acute leukemia. Unfortunately, at a dose of 50–200 mg/day symptoms of nausea, vomiting, and diarrhea were observed, so the therapy was discontinued. Interestingly, these authors indicated that at this dosage hepatic and renal function were normal and the symptoms described were no worse than those occurring with normally employed therapeutic agents.

Selenium and Neoplasia. Neoplastic tissue, regardless of cellular orgin, is known to concentrate selenium components at significantly higher concentrations than those occurring in normal tissue. In fact, the affinity of tumors for selenium has been the clinical basis for utilizing a radioactive nuclide of selenium as a tumor localizing agent (Cavalieri et al., 1966; Spencer et al., 1967). An outgrowth of these observations has been to examine the in vitro effects of selenium supplementation on cellular propagation. The reason for the localization of selenium is not readily apparent but may reflect enhanced division rates. Considerable evidence has shown that selenium can inhibit the growth of experimentally transplanted tumors. One of the principal cell lines that has been used for many of these studies has been the Ehrlich ascites tumor cell (EATC) (Abdullaev et al., 1973; Poirier and Milner, 1979; Greeder and Milner, 1980). This cell is easily transplanted and transferred into mice to maintain the line and has been used extensively in cancer research. It is a rapidly growing cell that can either form ascites or solid tumors depending on the mode of inoculation. Abdullaev et al. (1973) showed that the parenteral administration of sodium selenite at a dose of 1 μg of selenium/g of body weight of the host retarded the growth of this tumor cell line. Additional studies also revealed that similar quantities of selenium inhibited the growth of Guerin carcinoma and sarcomatous M^{-1} neoplasms (Abdullaev et al., 1973). An enhanced effect of tumor inhibition was observed when selenium was given in combination with X-ray therapy (Abdullaev et al., 1973).

Table I. Effect of Na_2SeO_3 Intraperitoneal Injection (1 μg/g of Body Weight) on Ehrlich Ascites Tumor Cell Development in the Mouse[a]

group	no. of mice per treatment	EATC	solid tumor	no. not developing tumors	av wt gain per animal, g, over test period[c]
control[b]	10	9	0	0	16.1 ± 1.3[a]
Se-IP	10	1	0	9	9.1 ± 2.5[b]

[a] All mice received ip 5×10^5 EAT cells. Selenium (20 μg) was administered by ip injection, as Na_2SeO_3 dissolved in Krebs Ringers phosphate (KRP) buffer (pH 7.4). Control mice received only KRP buffer. All mice received KRP or selenium on days 1, 3, 6, 9, 12, and 15 after tumor inoculation. [b] One animal died for unknown reasons. [c] Means ± standard deviation with unlike superscripts differ at $P < 0.01$. Determined 18 days after tumor inoculation.

Recent studies in various laboratories have extended the observations of the antitumorigenic properties of selenium. Studies in Milner's laboratory have utilized male ICR/Swiss mice (20 g) inoculated with Ehrlich ascites tumor cells to examine the antitumorigenic properties of this trace element. In their studies, mice were injected intraperitoneally with selenium test solutions prepared in Krebs Ringer phosphate (KRP) buffer (pH 7.4). In most of their studies, mice received an intraperitoneal inoculation of 5×10^5 viable tumor cells on day 0. Mice received injections of KRP or test selenium compounds at various time intervals up to 18 days postinoculation. In the first of these studies, the influence of selenium as sodium selenite on the propogation of the Ehrlich ascites tumor cell was examined. Intraperitoneal treatment with 1 μg of selenium/g of body weight resulted in a 90% reduction in tumor incidence (Poirier and Milner, 1979) (Table I). Corresponding to this reduced incidence of ascites tumor was a significant reduction in weight gain of selenium-treated animals. The excessive weight gain that occurred in these control KRP-treated mice resulted from ascites fluid accumulation. This conclusion was easily appreciated by gross examination of the massive abdominal distention of KRP-treated mice (Greeder and Milner, 1980).

Additional studies were conducted to determine whether selenium as sodium selenite, at a dosage of approximately 20 μg of selenium/injection, altered weight gain or had any detectable detrimental influence on the host. Non-tumor-bearing mice, receiving identical treatment, were not significantly influenced by selenium administration (Table II). Therefore, the inhibitory effect of selenium on the

Table II. Effect of Selenium on Tumor and Non Tumor Bearing Mice[a]

treatment	dosage,[b] μg/g of IBW	weight gain, g −EATC[c]	weight gain, g +EATC	EATC-inoculated[c] tumor incidence ascitic	EATC-inoculated[c] tumor incidence solid
KRP		5.4	12.0[a]	5/5	0/5
selenium dioxide	1.0	5.7	6.9[b]	0/5	0/5
sodium selenite	1.0	5.0	5.8[b]	0/5	0/5
sodium selenate	1.0	4.4	4.6[b]	0/5	0/5
selenomethionine	1.0	6.2	12.3[a]	5/5	0/5
selenocystine	1.0	6.1	6.1[b]	0/5	0/5
SEM[d]		±0.8	±1.1		
selenium dioxide[e]	0.25	5.1	7.0[b]	0/4	1/4
sodium selenite[f]	0.25	5.6	7.7[b]	0/3	0/3
sodium selenate	0.25	4.6	5.2[b]	0/5	2/5
selenomethionine	0.25	5.2	12.4[a]	5/5	0/5
selenocystine	0.25	4.6	7.8[b]	2/5	1/5
SEM[b]		±1.0	±0.9		

[a] On day 0, mice weighing 20-22 g were inoculated ip with 5×10^5 EAT cells. All test solutions were prepared in Krebs Ringers phosphate buffer and administered by ip injection on days 0, 1, 3, 5, 7, 9, 12, 15, and 18. [b] Microgram per gram of initial body weight. [c] Means are for three non-tumor-bearing and five tumor-bearing mice per treatment. All means were determined 21 days after initiation of the study. [d] Standard error of the mean. Means not sharing a common superscript letter differ, $P <$ 00.5. [e] One mouse died from tumor-unrelated causes. [f] Two mice died from tumor-unrelated causes.

growth of the tumor was apparently without major effects on the host. The only abnormal symptom that has been observed with this dosage of selenium is a slight increase in irritability and a reduction in intestinal tissue mass in a few of the mice. The hyperirritability appeared to subside once selenium injections were stopped. However, no reappearance of tumor proliferation was observed (Greeder and Milner, 1980).

Poirier and Milner (1979) also examined the efficacy of selenium administration at various times after inoculation of Ehrlich ascites tumor cells. Delaying the injection of selenium to 3 days completely retarded tumor development in 50% of the mice. A significant antitumorigenic effect was observed when the initiation of injections began 72 h after inoculation with tumor cells. However, the degree of inhibition was less than that occurring in animals injected on day 0 or day 1. Initiation of selenium injections 5 days after tumor inoculation did not significantly reduce the percentage of animals developing ascitic tumors. However, the growth of these tumors was significantly reduced, as indicated by the enhanced longevity of these tumor-bearing mice.

Several laboratories have also examined the influence of selenium on the viability and growth patterns of various tumor cell lines in vitro. Addition of selenium in various forms to flasks containing Ehrlichs ascites tumor, HeLa, L1210, canine mammary, or human mammary cells dramatically increased the death rate of these cells as indicated by trypan blue exclusion (Poirier and Milner, 1979; Giasuddin and Diplock, 1979; Gruenwedel and Cruikshank, 1979; Milner and Hsu, 1981; Watrach et al., 1982, 1983). This alteration is not surprising since selenium is known to be toxic when administered in excess. Administration of excess quantities of selenium would surely kill any type of cell. An interesting observation was that Ehrlich ascites tumor cells incubated with selenium apparently lost their ability to propagate before there was any detectable alteration in cell viability as indicated by trypan blue exclusion (Poirier and Milner, 1979). Apparently, the action

Table III. Effect of Sodium Selenite Supplementation to the Growth Media on the Viability of Human Neoplastic and Normal Cells in Culture[a]

	viabilities, % controls, for cell line MCF-7	MDA-MB-231	MRC-5
control	100	100	100
selenium, μM			
2.7	100	62	100
5.4	47	57	100
8.1	39	20	90

[a] The cell cultures were plated (1×10^5 cells) in T25 flasks and incubated in appropriate media at 37 °C in 5% CO_2 in air. After 24 h selenium as sodium selenite was added to the media. Viabilities were determined 72 h after the addition of selenium by the trypan blue exclusion technique.

of selenium is rather rapid and does not correlate with the death of the cell as measured by trypan blue exclusion. Apparently the effect of selenium is a rather dramatic alteration that may be reversible, although all cells would ultimately succumb to excess selenium. Tumor cell lines apparently have differential sensitivity to selenium (Medina and Shepherd, 1980; Medina and Oborn, 1981; Watrach et al., 1982). Recent studies by Watrach et al. (1983) have shown that two human mammary tumor cell lines (MCF-7 and MBA-MD231) have considerably greater sensitivity to selenium than do nonneoplastic human MCR-5 lung cells (Table III). Surely, characterization of differences in these cell lines will add valuable information to the mechanism by which selenium exerts its antitumorigenic properties.

Endeavors have also been aimed at determining the minimum quantity and the most efficacious form of selenium that will completely prevent or significantly retard tumor propagation without significantly affecting the host. Greeder and Milner (1980) examined sodium selenite, sodium selenate, selenium dioxide, selenomethionine, and selenocystine for their antitumorigenic properties. Intraperitoneal administrations of 2 μg of selenium/g of body weight for each of the test selenium compounds completely inhibited the propagation of Ehrlich ascites tumor cells. None of the mice receiving selenium treatment at this dosage developed ascites tumors by 21 days (Table II). Half of the selenium-treated animals continued to be fed a commercial laboratory chow for an additional 21 days after their last selenium injection. Again, there was no indication of tumor proliferation in these animals. Thus, selenium in all of the forms tested was capable of preventing tumor development (Table II). No significant alterations in liver weight, spleen weight, or body weight were detected in selenium-treated mice. However, a significant reduction in intestinal weight was observed (Greeder and Milner, 1980). Selenium administration did not significantly alter the small intestinal content of RNA, DNA, lipid, or protein. Therefore, one must conclude that selenium reduced the total intestinal mass. Since the turnover rate of intestinal cells is known to be rapid compared to that of other tissues, these data may indicate that selenium can alter the growth of rapidly dividing cells, both neoplastic and noneoplastic, if administered in sufficently quantities.

Reducing the quantity of selenium administered to 1 μg/g of body weight again revealed that sodium selenite, sodium selenate, selenium dioxide, and selenocystine were completely efficacious in preventing tumor development. However, selenomethionine at this concentration was not

Table IV. Effect of Selenium on Tumor-Bearing Mice[a]

treatment	dosage, μg/g of IBW	weight gain, g/21 days	tumor incidence
KRP		4.7	10/10
selenite	0.25	3.6	0/10
selenite	0.125	3.9	2/10
selenite	0.0625	3.7	4/10
SEM[b]		±0.9	

[a] On day 0, mice weighing 20–22 g were inoculated ip with 5×10^5 EAT cells. All Na_2SeO_3 solutions were prepared in KRP buffer. KRP and selenite were administered by ip injection on days, 0, 1, 3, 5, 7, 9, 12, 15, and 18. Tumor incidence and weight gain were determined 21 days after tumor inoculation. [b] Standard error of the mean.

effective in retarding the growth of ascites tumor cells (Table II). Reducing selenium treatment to 0.25 μg/g of body weight resulted in a similar response for sodium selenite, sodium selenate, and selenium dioxide. All three forms were capable of completely retarding the development of ascites tumors (Table II). Further reductions in the quantity of sodium selenite administered did not allow for complete prevention of ascites propagation. However, administration of as little as 0.0625 μg of selenium/g of body weight resulted in complete inhibition of ascites tumor development in 60% of the tumor-inoculated mice (Table IV). These data reveal that intraperitoneal administration of 2.5–5.0 μg of sodium selenite every third day by injection is extremely effective in preventing ascites tumor development. Furthermore, these data clearly indicate that both the dosage and form of selenium must be considered in the evaluation of this trace element's antitumorigenic properties.

Animal studies have indicated that once selenium is removed from the plasma by tumor cells, it is incorporated into cellular proteins (Awwad et al., 1966; Penner, 1964; Spencer et al., 1967). Spencer et al. (1967) compared the uptake of various forms of selenium in mouse and human lymphomas. In mice inoculated with L5178Y lymphoma cells the movement of selenomethionine was compared with that of sodium selenite and sodium selenate. Their data indicated that selenomethionine uptake by the tumor cell was approximately 4 and 2 times that observed with selenite and selenate, respectively. Assuming some consistency between tumor cells, these data suggest that uptake may not be the primary criteria for determining susceptibility of cells to the form and dose of selenium. Germain and Arneson (1979) reported that selenium as sodium selenite, selenomethionine, and selenocystine was capable of inducing glutathione peroxidase activities in the mouse neuroblastoma. However, the efficacy of selenomethionine on a molar basis was considerably less than that observed with the other two forms of selenium. Recent studies by Poirier et al. (1983) have also shown that differences in susceptibility of two canine mammary cell lines to selenium cannot be explained on the basis of cellular uptake of this trace element. Therefore, the incorporation of selenium into a specific protein or cellular intermediate may be more indicative of the potential antitumorigenic effects of selenium than mere entry into the cell.

The response to selenium is therefore dependent upon the tumor characteristics, plus the quanitity and the form of selenium administered. This is particularly evident since selenomethionine was only efficacious at a dose of 2 μg/g of body weight, yet sodium selenite was completely effective at a dose of 0.25 μg/g. Therfore, more than 8 times as much selenomethionine as sodium selenite is needed to obtain the same response. Weisberger and

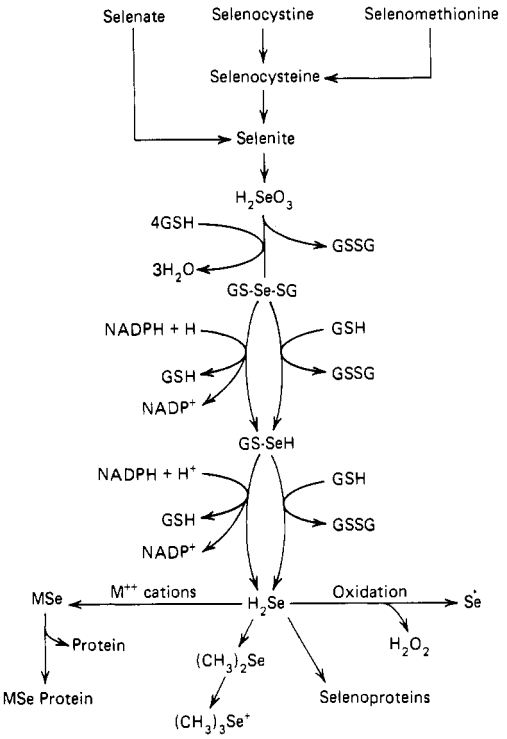

Figure 1. Metabolic interrelationships between both inorganic and organic forms of selenium. Based on articles of Magos and Webb (1980) and Young et al. (1982).

Table V. Longevity of Mice Inoculated with Ehrlich Ascites Tumor Cells Incubated for 15 min with Different Selenium Compounds

treatment compound[a]	supplemental selenium, ppm	tumor incidence	longevity, h[b]	longevity range, h
control		10/10	622 ± 30[a]	480-768
Na_2SeO_3		10/10	626 ± 33[a]	480-792
	3	10/10	607 ± 35[a]	480-840
GSSeSG	1	10/10	789 ± 52[b]	600-1032
	3	10/10	1069 ± 145[c]	672-1656
$(CH_3)_2Se$		10/10	605 ± 24[a]	528-768
	3	10/10	607 ± 14[a]	552-768

[a] All tumor cells were incubated for 15 min in the presence or absence of selenium before inoculation into 20–25-g mice. All mice were inoculated with 5×10^5 viable EAT cells. No further selenium treatment was given to the mice except that present in the commercial laboratory feed. [b] Vertical means ± SEM not sharing common superscripts differ, $P < 0.05$.

Suhrland (1956a,b) also reported the form of selenium administered influenced the inhibitory properties of this trace element on the growth of Murphy lymphosarcoma.

Ganther (1971) has proposed a sequence of metabolic events leading to the detoxification of selenium in tissue (Figure 1). Poirier and Milner (1983) have compared the efficiency of selenite, selenodigluthathione, and dimethyl selenide for their ability to inhibit tumor proliferation (Table V). These studies suggest that an intermediate in the pathway for selenium detoxification may be responsible for this trace elements antitumorigenic properties. Vernie et al. (1979) have shown that selenodiglutathionine is an effective inhibitor of protein biosynthesis in some cells. Previous studies have indicated that sodium selenite can act as a strong inhibitor of amino acid uptake and utilization (Everett and Holley, 1961; Vernie et al., 1974; Hogberg and Kristoferson, 1979). Whether this property accounts for the benefical effects of seleno-

Table VI. Effect of Supplemental Selenium on Macromolecule Biosynthesis in SV 40-3T3 Cells in Culture[a]

	protein, dpm/μg	DNA, dpm/μg	RNA, dpm/μg
control	425 ± 16[a]	19.3 ± 1.6[a]	2.09 ± 0.07[a]
selenium	379 ± 84[a]	18.8 ± 0.5[a]	1.59 ± 0.01[b]

[a] Values are means ± SEM for five replicates per treatment per assay. Cells were incubated for 30 min for determination of label in specific macromolecules. RNA, DNA, and protein were analyzed as previously described (Greeder and Milner, 1980). Determined by the incorporation of [^{3}H]thymidine, [^{3}H]uridine, and [^{3}H]leucine for DNA, RNA, and protein, respectively. Cells were grown with 0 or 0.6 μg/mL supplemental selenium as sodium selenite for 48 h prior to the incorporation studies.

digluthathione in inhibiting tumor growth in the studies of Poirier and Milner (1983) and Vernie et al. (1981) is unknown.

Recent studies by Poirier et al. (1983) have shown that one of the first macromolecules to change after selenium exposure is RNA (Table VI). This response was not associated with a decrease in RNA polymerase or increase in RNase activity (Milner and Hsu, 1983).

Other Tumor Cell Lines. Various investigators have examined the effectiveness of selenium in inhibiting the propagation of various transplantable tumor cell lines. Studies of Milner and Hsu (1981) using mice inoculated with 10^5 L1210 cells and treated with either 0, 20, 30, 40, or 50 μg of selenium as sodium selenite per day for 6 days were recently reported. The aggressive L1210 leukemic cell line leads to the death of mice within 8–10 days postinoculation. Selenium administration was begun at the same time the mice received the tumor cell inoculation and effectively retarded propagation of this tumor cell line as indicated by longevity of the tumor-bearing mice (Table VII). No significant alterations in the growth of nontumor bearing mice occurred until quantities as great as 40 μg of selenium per day were administered. A 20% increase in longevity of mice inoculated with L1210 leukemic cells is considered highly significant and may be indicative of the possible importance of the test compound as a therapeutic agent in the treatment of cancer. Selenium administration of 20 μg/day resulted in a highly significant 43% increase in longevity of tumor-bearing mice. Similar increases in the lifespan of tumor-bearing mice were also observed when greater quantities of selenium were administered. These data clearly indicate that selenium, if administered in sufficient quantities, may also be effective in inhibiting very agressive tumor cells.

Recent studies (Poirier and Milner, 1983; Watrach et al, 1982, 1983) have also shown that selenium is effective in inhibiting the growth of solid tumors transplanted into mice. Again, the intraperitoneal administration of selenium resulted in the sufficient depression of the growth rate of solid tumors induced by the inoculation of EATC, various canine mammary tumors, and two human breast tumor cell lines. In these studies (Poirier and Milner, 1983; Watrach et al., 1982, 1983), selenium was given by intraperitoneal injections to mice with tumor cells previously inoculated subcutaneously on the shoulder. In studies utilizing solid tumors, selenium did not completely eliminate the tumor but did result in a 75–90% reduction in the growth rate of the particular tumor. Apparently, selenium is transported to the site of the tumor development where it exerts its effect.

Medina and Shepard (1980, 1981) have examined the influence of supplemental selenium on the growth of various preneoplatic outgrowth lines from BALB/c mice and breast cancer cell lines obtained from mice treated with 7,12-dimethylbenzanthracene. These authors suggest that differences in sensitivity to selenium do exist between a particular tumor type. Support for this contention comes from recent studies of Poirer et al. (1983) showing that some canine mammary cell lines and human mammary cell lines are extremely sensitive to selenium, yet others are somewhat resistant. Further characterization of these various cell lines may shed some insight into the exact mechanism by which selenium can alter tumor growth.

Table VII. Effect of Daily Injections of Graded Quantities of Na_2SeO_3 upon Mice Inoculated with L1210 Cells[a]

treatment	growth, g/7 days	liver wt, mg	mean survival time, h	increase in mean survival time above controls, %
controls				
KRP	0.6 ± 0.2[aa]	881 ± 35[a]	174 ± 15[a]	
Na_2SeO_3				
20 μg/day	0.4 ± 0.3[a]	910 ± 40[a]	250 ± 12[b]	43
30 μg/day	0.2 ± 0.2[a]	890 ± 43[a]	268 ± 8[b,c]	54
40 μg/day	0.3 ± 0.3[a]	1080 ± 75[a]	286 ± 15[c]	65
50 μg/day	−1.6 ± 0.5[b]	900 ± 25[a]	290 ± 35[c]	67

[a] All mice received 5×10^5 L1210 cells. KRP with or without Na_2SeO_3 was 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 20 tumor-bearing mice per treatment, except for the 50 μg/day Na_2SeO_3 therapy, where means are for four non-tumor-bearing and 7 tumor-bearing mice per treatment.

Tumor Cell Mass. The tumor cell mass occurring in the host may affect the efficacy of therapy. While the therapy may be of benefit in reducing tumor cell division in very pronounced cases of cancer, it may be impossible to detect because of the aggressiveness and degree of metastasis occurring. Milner and Hsu (1981) conducted experiments designed to examine the influence of tumor mass on the efficacy of selenium administration. Mice were inoculated with either 10^4, 10^3, or 10^2 L1210 cells. Selenium as sodium selenite was also administered at 30 μg/g of body weight for 6 days. In their studies, selenium administration retarded the growth of the tumor as indicated by longevity of the animals. However, not until the tumor cell mass was reduced sufficiently was their any indication of cure rates. Of considerable interest was the observation that in the ten mice receiving selenium and inoculated with 10^2 cells, only one mouse died 410 h postinoculation. The remaining animals were alive and apparently in good health 7 months after selenium therapy was discontinued. Clearly, these data indicate that selenium can retard the growth of L1210 tumors and that the efficacy of selenium is highly dependent upon the initial size of the inoculation of tumor cells. These observations are extremely interesting in light of the virulent tumor cell line used. These data indicate that similar types of neoplastic cells may show little response to selenium if it is administered long after the tumor has undergone proliferation.

Mode of Selenium Administration. Selenium effectively inhibits the growth of various types of tumors when administered by intraperitoneal injection. However, other routes of administration have only recently been examined. The effect of oral consumption of selenium on tumor development has been examined indirectly in studies examining chemical carcinogens. In most studies where prox-

Table VIII. Effect of Selenium Supplementation on Mice Inoculated with L1210 Cells[a]

water treatment	non tumor bearing	tumor bearing	
	growth, g/10 day	mean survival time, hr	% of increase above controls
controls	4.0 ± 0.4[aa]	248 ± 8[a]	
selenium			
1 ppm	4.1 ± 0.5[a]	264 ± 7[b]	6.5
3 3 ppm	2.5 ± 0.3[b]	323 ± 10[c]	30.3
5 ppm	2.5 ± 0.5[b]	294 ± 10[b,c]	18.7
10 ppm	0.1 ± 0.3[c]	278 ± 8[b]	10.0

[a] All mice were acclimated to their respective water treatments for 2 weeks prior to tumor inoculation. Selenium was provided in the water as sodium selenite. All mice were treated with 10^4 cells. Means not sharing a common superscript letter differ by $P < 0.05$. Mean ± SE for 5 non-tumor-bearing and 10 tumor-bearing mice per treatment.

imate carcinogens have been utilized, one generally observes a reduction in tumor number and mass. However, this general observation may result directly from inhibiting the binding of the test carcinogen employed or by stimulating cellular repair processes and may, therefore, not be indicative of selenium's direct effect on tumorous tissue.

Experiments were conducted to determine the effect of adding selenium to the drinking water of mice inoculated with L1210 leukemic cells (Milner and Hsu, 1981). These studies revealed that this method of selenium administration was less effective in inhibiting tumor propagation than intraperitoneal injections but did increase the longevity of the tumer-bearing mice. No significant reduction in the growth of mice occurred until the drinking water contained 3 ppm of selenium, since 3 μg of selenium/mL was used. Water containing selenium resulted in an approximate 40% reduction of growth of the mice. While this is a significant reduction, it represents a difference of 1.5 g over a 10-day study. Thus, the overall significance of such a small reduction in body weight is unknown. Selenium supplementation did result in a clear increase in the longevity of L1210 tumor-bearing mice (Table VIII). Mice consuming drinking water containing 1 ppm of Se had a 6% increase in longevity. Mice receiving water supplemented with 3 ppm of Se had a 30% increase in longevity compared to that of controls. A decline in longevity at concentrations of selenium above 3 ppm also occurred. These results may have occurred as a result of a voluntary decrease in food or water consumption by the mice. Milner and Hsu (1981) reported that fluid intake of mice given water supplemented with 10 ppm of selenium was approximately 10% of that consumed by mice given water containing 1 ppm of selenium. Selenium alters the growth of tumors when it is administered by gastrointestinal routes, such as water supplementation. Obviously, water supplementation is not as efficacious as injection of selenium. In these studies a relatively large tumor mass was used. Since the initial tumor burden has been shown to alter the efficacy of selenium, more dramatic effects may have been expected in mice inoculated with fewer tumor cells.

Poirier and Milner (1983) have compared selenium administration by intraperitoneal injection, gastric gavage, or dietary preparation. In their studies, all modes of administration were found to significantly increase the longevity of tumor-bearing mice. Again, the efficacy of selenium in inhibiting the growth of Ehrlich ascites tumor cells was considerably less by gastric gavage or dietary administration than by intraperitoneal injections. These studies suggest that the liver may be sequestering supplemental selenium and reducing its ability to travel to the site of tumor proliferation. A similar reduced response to dietary selenium supplementation was reported by Ip et al. (1981) using a transplantable tumor inoculated into rats. Although selenium supplementation resulted in a significant retardation in the growth of the tumor, the influence on survival times was not evaluated.

Selenium may have a wide application as a therapeutic agent against numerous types of tumors. The data available to date clearly show that selenium must be considered a nutrient that can retard tumor development. The efficacy of selenium appears to depend on the form and dosage of selenium administered, the tumor cell line being examined, as well as the total tumor cell mass. Selenium administration at less than 40 μg/day did not result in any detectable detrimental effects in studies with mice except for a slight reduction in weight gain. In light of the known adverse effects of any chemotherapeutic agent used in man, one must question the meaning of slight weight reduction. The mechanism by which selenium can retard and completely inhibit tumor growth certainly deserves considerable attention. The interesting studies conducted by Weisburger and Suhrland (1956a,b) showed that selenocystine (50–200 mg/day) was effective in the treatment of leukemia patients. Whether other types of cancers in humans would respond to selenium supplementation clearly deserves added attention. Recent studies suggest that selenium in an inorganic form may yield the same degree of efficacy as the organic terms, when given in considerably smaller quantities.

Selenium in Combination with Other Treatments. Recent studies suggest that selenium may also function at least additively and perhaps synergistically with known chemotherapeutic agents (Milner and Hsu, 1981). The mechanism by which selenium may enhance the effectiveness of chemotherapeutic agents is unknown. However, it may well be related to the detoxification of foreign compounds. It may therefore be possible to reduce the quantity of selenium administered even further when used in combination with more classic therapy, such as methotrexate or 5-fluorouracil.

The data presented in experimental models definitely show that selenium should be considered as a therapeutic agent in the treatment of cancer. However, data are not available on the effects of long-term therapy in man. Caution must be exercised in the indiscriminate use of selenium by humans until well-designed clinical trials are completed.

Registry No. Se, 7782-49-2; Na_2SeO_3, 10102-18-8; Na_2SeO_4, 13410-01-0; SeO_2, 7446-08-4; glutathione peroxidase, 9013-66-5; selenomethionine, 1464-42-2; selenocystine, 1464-43-3; selenodiglutathione, 33944-90-0; dimethyl selenide, 593-79-3.

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