Dosage Window of Selenium on Human Natural Killer Cytotoxicity and Neutrophil-mediated Chemiluminescence

Mary LaBue

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Abstract

DOSAGE WINDOW OF SELENIUM ON HUMAN NATURAL KILLER CYTOTOXICITY AND NEUTROPHIL-MEDIATED CHEMILUMINESCENCE

by

Mary LaBue

Few studies have been conducted on the effect of in vitro selenium (Se) supplementation on cellular immunity. In the present study, the effects of in vitro incubation with sodium selenite on natural killer (NK)-mediated cytotoxicity and phagocyte chemiluminescence (CL) were examined. NK-mediated cytotoxicity and phagocyte CL were observed to be affected by added selenite. These changes generally included augmentation and suppression of both neutrophil CL and NK-mediated cytotoxicity. All dosages of selenite used were shown to be capable of causing augmentation of neutrophil CL. However, 1.0 \( \mu g/ml \) selenite was also observed to occasionally cause depression of neutrophil CL. When Se supplementation enhanced NK cytotoxicity, the effect was always observed at 0.1 \( \mu g/ml \) selenite dosage. Higher dosages of selenite induced both enhancement and depression of activity. The existence of a narrow dosage-window is evident.

The activity of the selenoenzyme glutathione peroxidase (GSH-Px) was also determined in NK cells and phagocytes following selenite supplementation. Dose-dependent augmentation of phagocytic activity by selenite supplementation was accompanied by a corresponding increase in
phagocyte GSH-Px activity. The effect of Se treatment on non-adherent cell GSH-Px activity did not correlate with NK cytotoxicity.
DOSAGE WINDOW OF SELENIUM ON HUMAN NATURAL KILLER
CYTOTOXICITY AND NEUTROPHIL-
MEDIATED CHEMILUMINESCENCE

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Mary LaBue

A Manuscript Submitted in Partial Fulfillment
of the Requirements for the Degree Master of Science
in Microbiology

March 1988
Each person whose signature appears below certifies that this manuscript in his opinion is adequate, in scope and quality, in lieu of a thesis for the degree Master of Science.

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Selenium (Se) is an essential trace element which influences the immune response and host defense mechanisms (1-7). At nutritional levels, Se possesses a broad spectrum of anticarcinogenic (8-12) and antimutagenic activity (13-17). Epidemiological data suggest that adequate dietary levels of Se are associated with a decreased risk of developing cancer while deficiency may be related to an increased cancer risk (18-21). The immune system mediates an array of cytotoxic mechanisms intimately involved in the surveillance against neoplastic disease. Several studies suggest that adequate levels of nutritional Se are required for the optimal functioning of various components of the immune system, such as the humoral immune response (22,23), natural killer (NK) cell-mediated cytotoxicity (3,5,6,7) as well as polymorphonuclear leukocyte (PMN) function (1,2,24). Se deficiency in cows (24) and goats (1,2) is associated with impaired phagocytic function. NK cytotoxicity has been shown to be enhanced by dietary Se supplementation in rats and humans (3,5,7). The role of Se in the microbicidal activity of phagocytes is perhaps the best studied. It has been widely suggested that glutathione peroxidase (GSH-Px), a selenium-containing enzyme, is central in this regard. Se deficiency leads to decreased activity of phagocyte GSH-Px (1,25). This enzyme appears to be involved in protecting phagocytes against deleterious effects of hydrogen peroxide (H$_2$O$_2$) generated in association with superoxide (O$^-_2$), singlet oxygen ($^1$O$_2$) and hydroxyl radicals (OH·) (26-27). These toxic reactive oxygen species mediate the microbicidal activity associated with phagocytosis (28). The involvement of GSH-Px
on Se-modified NK activity has also been suggested but not evaluated (3).

Few of the published studies have been conducted in vitro, where valuable information regarding the cellular mechanisms of Se-modified immunosurveillance might be obtained. Dimitrov et al. (3) failed to demonstrate enhancement of NK activity with the in vitro addition of Se, though enhancement was demonstrated in corresponding in vivo studies. Watson et al. (29) could only demonstrate depression of NK activity at relatively high levels of Se. Virtually all studies of the effect of Se on phagocytic function involve dietary experiments where Se was given orally and phagocytic function and GSH-Px activity were subsequently evaluated in the peripheral blood. The present study examined the effect of in vitro Se supplementation on NK cytotoxicity, neutrophil chemiluminescence (CL) and the intracellular GSH-Px activity of neutrophils and NK cells.
Materials and Methods

Normal Volunteers. This study was carried out using peripheral blood obtained from seven healthy volunteers (24 to 50 years old) of both sexes (four males and three female). Only one had taken selenium supplements. Informed consent was obtained from each volunteer before the beginning of the study. The study was approved by the Institutional Review Board for Human Studies. No volunteer contributed more than 50 ml of blood during any monthly period.

Lymphocyte Preparation. Venous blood samples were collected by appropriate venipuncture into vacutainer tubes containing heparin (Becton-Dickinson, Rutherford, NJ). Mononuclear cell were prepared from heparinized blood samples by Ficol-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. The cells were washed twice in Hanks Balanced Salt Solution (HBSS) and suspended in tissue culture medium consisting of RPMI-1640 with 10% fetal calf serum (FCS), 1000 units/ml penicillin and 375 units/ml streptomycin (designated as TC medium henceforth). Phagocytes were removed from the lymphocytes by incubation for 20 minutes at 37°C in an atmosphere of 5% CO₂ in plastic petri dishes (Becton Dickinson Labware, Oxnard, CA). The non-adherent lymphocytes were collected, washed and contaminating erythrocytes lysed with potassium bicarbonate-ammonium chloride buffer (ACK lysing buffer). The resulting erythrocyte-free cells were resuspended to the desired concentration in TC medium. Viability of these cells was greater than 95% by trypan blue exclusion.

Neutrophil Preparation. Polymorphonuclear leukocytes (PMNs) were isolated and purified from heparinized whole blood by density gradient centrifugation using Neutrophil Isolation Medium (United Technologies
Packard, Downers Grove, IL) followed by lysis of contaminating erythrocytes with ACK-lysing buffer. Cell suspensions thus prepared contained greater than 98% PMN and viability assessed by trypan blue exclusion was in excess of 99%. After purification, the PMNs were suspended in Medium 199 without indicator supplemented with 20% heat inactivated FCS, 550 ng/ml heparin, 1000 units/ml penicillin and 375 units/ml streptomycin.

**Natural Killer Cell Activity.** Non-adherent cells were suspended in TC medium and their concentrations were adjusted to ultimately obtain 100:1 and 50:1 effector:target ratios. The target cells (K-562), a human erythroleukemia cell line, were grown as stationary suspension cultures in TC medium supplemented with 1 mM sodium pyruvate. These cells were labeled with 100-200 μCi/ml of chromium-51 as sodium chromate (New England Nuclear Research Products, Boston, MA) per 2-5 x 10^6 cells/ml at 37°C in 5% CO₂ for 2 hours. They were washed four times in TC medium and resuspended to 5 x 10^5 cells/ml. Viability was determined by trypan blue exclusion and found to be greater than 95%. Labeled K-562 cells were added in 100 μl aliquots to the appropriate wells of 96-well flat-bottom microtiter plates (Corning Glass Works, Corning, NY) containing 100 μl of varying concentrations of effector cells. 100 μl of sodium selenite solutions were then added to appropriate wells at concentrations adjusted to obtain final concentrations of 0.1, 0.5 and 1.0 μg/ml, respectively. All tests were performed in triplicate. After centrifugation at 1000 rpm for 2 minutes, the plates were incubated at 37°C in 5% CO₂ for 4 hours. After incubation, supernatants were harvested using a Skatron
Supernatant Collection System (Skatron Inc., Sterling, VA) and radioactivity counted in a TM Analytic Gamma Trac 1193 gamma counter (TM Analytic, Elk Grove Village, IL). Percent cytotoxicity was calculated as follows:

\[
\% \text{ cytotoxicity} = \frac{\text{Experimental Release}}{\text{Maximum Release}} \times 100
\]

where maximum release represents target cells that were lysed with 10% sodium dodecyl sulfate (SDS). The 50:1 ratio was determined to give the most consistent results and subsequently was used throughout this study.

Chemiluminescence (CL) Determination of Phagocytosis. Isolated PMNs were divided into four aliquots. The control aliquot was pre-incubated without sodium selenite in siliconized glass tubes for 30 minutes at 37°C in 5% CO₂. The experimental aliquots were handled similarly except sodium selenite dissolved in HBSS was added before pre-incubation to obtain final concentrations of 0.1, 0.5 and 1.0 µg/ml, respectively. The chemiluminescent response of the pretreated cells was used as a measure of reactive oxygen species production and assayed at 37°C in a luminometer (Picolite model 6500, Packard Instrument Co., Downers Grove, IL) interfaced with a PC-compatible computer for data acquisition and processing. All luminometry experiments were performed in triplicate. Briefly, pretreated PMNs were added to 55 x 12 mm plastic luminometry tubes (Los Alamos Diagnostics, Los Alamos, NM) such that each tube contained 5 x 10⁴ cells in a total volume of 50 µl. The tubes were then transferred to the luminometer. The assay was initiated by the addition of 150 µl of an opsonized zymosan A suspension containing
luminol (ZAP™, Los Alamos Diagnostics). The photon emission from each sample was counted every 3 minutes over the course of 30 minutes. Following the assay, the area under each curve representing light emission versus time was calculated and reported as integrated photon emission (30).

**Glutathione Peroxidase Determination.** Isolated neutrophils were prepared and treated with sodium selenite as previously described. Isolated lymphocytes were divided into four aliquots and treated with sodium selenite as described for phagocytes. After incubation, the cells were washed twice with potassium phosphate buffer (31) and resuspended in 2 ml to a concentration between 5 x 10⁶ and 1 x 10⁷ cells/ml. All samples were then sonicated (Branson Sonifier Cell Disrupter 200, Danbury, CT) in the cold with four 10 second pulses, then centrifuged at 10,000 rpm for 20 minutes at 4°C in a Sorvall RC-5C Refrigerated Centrifuge (Sorvall Instruments, Wilmington, DL). The supernatant was removed for GSH-Px and protein determinations.

GSH-Px was measured using the spectrophotometric method developed by Paglia and Valentine (32) as modified by Levander et al. (31). The absorbance was read in a Spectronic 1001 (Baush and Lomb, Rochester, NY) before the reaction was initiated with t-butyl hydroperoxide and at subsequent 1 minute intervals. Absorbance change from 0 to 5 minutes was used to calculate enzyme activity. One enzyme unit is equal to 1 μmole NADPH oxidized per minute and is expressed as μmoles NADPH oxidized/min/mg/ml protein.

**Protein Determination.** Protein concentrations of cell sonicates were determined using a spectrophotometric method (33). Briefly, cell
sonicate was pipetted into a quartz cuvette and its absorbance was read at 280 nm and 260 nm in a Spectronic 1001. Protein concentration was then calculated as follows:

\[
[\text{Protein}] = \frac{A_{280}}{A_{260}} \times F \times \frac{1}{d},
\]

where \( A_{280} \) is absorbance at 280 nm, \( A_{260} \) is absorbance at 260 nm, \( F \) is a conversion factor and \( d \) is the 1 cm pathlength.

**Statistical Analysis.** Data were evaluated using either one-way analysis of variance and Tukey's HSD (honestly significant difference) test for multiple comparisons or Student's two-tailed t-test. All statistical procedures were performed with Statgraphics software version 2.1 (STSC, Inc., Rockville, MD). \( P < 0.05 \) was used for the significance level in Tukey's HSD procedure.
Results

Effect of Selenite on Neutrophil CL. The in vitro addition of sodium selenite to normal human phagocytes caused alterations of phagocytic activity as measured by CL. Table 1 shows the results with samples obtained from seven subjects at various time intervals. All dosages of selenite used induced significant enhancement of CL in samples 2 and 3. 0.1 and 0.5 μg/ml selenite caused significant enhancement of CL in sample 1. However 1.0 μg/ml selenite induced a suppression of activity. Samples 7, 9 and 10 showed no changes in CL with the addition of 0.1 and 0.5 μg/ml selenite but enhancement with the addition of 1.0 μg/ml selenite. Significant suppression of CL upon the addition of 1.0 μg/ml selenite was also observed in sample 4 but the addition of the two lower dosages of selenite used did not induce any change. Samples 5, 6, 8 and 11 exhibited no significant change in CL upon the addition of all selenite dosages used.

Figures 1-5 illustrate the five patterns of CL exhibited in response to added selenite. The five patterns are characterized by: enhancement of CL with all three dosages of sodium selenite (Figure 1), enhancement of CL with 0.1 and 0.5 μg/ml selenite but depression with 1.0 μg/ml selenite (Figure 2), no effect on CL with the addition of 0.1 and 0.5 μg/ml selenite but enhancement with 1.0 μg/ml selenite (Figure 3), no effect with 0.1 and 0.5 μg/ml selenite and depression with 1.0 μg/ml selenite (Figure 4) and finally, no significant effect with all three dosages of selenite (Figure 5).

Effect of Selenite on Natural Killer Activity. The effect of sodium selenite on NK cytotoxicity in vitro is shown in Table 2. Samples were obtained from five subjects at various time intervals.
Seven of the twelve samples exhibited significant enhancement upon addition of 0.1 μg/ml selenite. Four of the samples exhibited enhancement of cytotoxicity upon addition of 0.5 μg/ml selenite, while three samples exhibited depression of activity at this dosage. Addition of 1.0 μg/ml selenite induced enhancement of activity in two samples and depression in three samples. All three concentrations of selenite used were found to be non-toxic to K-562 tumor target cells.

Figure 6 illustrates six patterns of NK cytotoxic responses to selenite. Enhancement of activity with all three dosages of selenite characterized the first response. Enhancement of activity with 0.1 and 0.5 μg/ml of selenite but no significant changes with 1.0 μg/ml of selenite characterized the second response. The third response appeared as enhancement with 0.1 μg/ml of selenite and no changes with 0.5 and 1.0 μg/ml of selenite. The fourth response was characterized by enhancement of activity with 0.1 μg/ml of selenite and suppression with 0.5 and 1.0 μg/ml. The fifth response appeared as no changes in activity with 0.1 μg/ml of selenite but suppression with 0.5 and 1.0 μg/ml. No significant changes in activity with the addition of all dosages of selenite characterized the sixth response.

**Effect of Selenite on GSH-Px Activity.** CL and GSH-Px activity were simultaneously evaluated and the results are shown in Table 3. Selenite supplementation in samples 1-4 induced similar changes in both neutrophil CL and GSH-Px activity. When Se enhanced CL, a concomitant increase in GSH-Px was observed. Reciprocally, when Se had no effect on CL, as in sample 5, no alteration in GSH-Px could be discerned.

GSH-Px activity was evaluated in samples of non-adherent cells used
in the NK cytotoxicity assays. The effect of Se supplementation on GSH-Px activity did not correlate with NK activity. (Data not shown).
Table 1

EFFECT OF SELENITE ON CHEMILUMINESCENCE

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Sample #</th>
<th>Selenite Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control 0.1 µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>3.47 ± 0.24</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1.20 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>2.74 ± 0.19</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>4.82 ± 0.80</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>3.33 ± 0.25</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>1.37 ± 0.16</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>6.63 ± 0.12</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>8.17 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>53.0 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>35.8 ± 0.01</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>73.3 ± 0.04</td>
</tr>
</tbody>
</table>

*Significant increases (p<0.05) in activity; † significant suppression (p<0.05) in activity; determined by Tukey’s HSD test.
Table 2

EFFECT OF SELENITE ON NK CYTOTOXICITY

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Sample #</th>
<th>Control</th>
<th>0.1 µg/ml</th>
<th>0.5 µg/ml</th>
<th>1.0 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Cytotoxicity ± SEMa</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 1 69.8 ± 2.63 81.6 ± 1.45* 74.1 ± 6.10* 71.3 ± 1.00
2 73.5 ± 2.45 82.2 ± 2.70 74.8 ± 3.30 74.0 ± 4.50

2 3 44.8 ± 1.35 57.3 ± 2.90* 59.1 ± 1.70* 56.6 ± 2.85*
4 45.7 ± 1.00 47.2 ± 0.01 39.0 ± 7.10 39.6 ± 0.40
5 49.8 ± 1.55 54.2 ± 2.36* 46.3 ± 0.86 49.3 ± 1.66

3 6 53.2 ± 1.56 67.5 ± 2.00* 61.0 ± 4.25* 58.5 ± 6.50
7 64.7 ± 0.05 69.0 ± 0.32* 63.9 ± 0.20† 64.0 ± 0.10†

4 8 37.2 ± 1.30 52.1 ± 1.15* 49.2 ± 2.75* 50.4 ± 1.15*
9 52.4 ± 0.80 45.6 ± 0.50 43.4 ± 4.25† 39.4 ± 3.00†
10 48.1 ± 1.72 50.9 ± 1.65 47.9 ± 0.47 52.5 ± 0.21

5 11 43.5 ± 0.45 54.9 ± 1.95* 38.6 ± 1.95† 37.4 ± 1.10†
12 35.3 ± 2.75 41.2 ± 0.35 38.5 ± 1.75 33.7 ± 0.65

a50:1 cell ratio used.
*Significant increase (p<0.5) in activity as determined by Tukey's HSD test.
†Significant decrease (p<0.5) in activity as determined by Tukey's HSD test.
Table 3
EFFECT OF SELENITE ON CHEMILUMINESCENCE AND GSH-Px ACTIVITY

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Selenite Dose (µg/ml)</th>
<th>Cl&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tukey’s HSD Intervals</th>
<th>GSH-Px&lt;sup&gt;b&lt;/sup&gt; Activity (x 10&lt;sup&gt;-2&lt;/sup&gt;)</th>
<th>Tukey’s HSD Intervals</th>
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<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1.20 ± 0.14</td>
<td>*</td>
<td>1.91 ± 0.02</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.71 ± 0.03</td>
<td>*</td>
<td>2.49 ± 0.08</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.56 ± 0.08</td>
<td>*</td>
<td>2.44 ± 0.02</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.61 ± 0.03</td>
<td>*</td>
<td>2.29 ± 0.01</td>
<td>*</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2.74 ± 0.19</td>
<td>*</td>
<td>1.44 ± 0.01</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3.40 ± 0.16</td>
<td>**</td>
<td>1.47 ± 0.01</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>3.73 ± 0.17</td>
<td>*</td>
<td>1.54 ± 0.02</td>
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<tr>
<td></td>
<td>1.0</td>
<td>3.24 ± 0.04</td>
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<td>1.49 ± 0.03</td>
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<tr>
<td>3</td>
<td>0</td>
<td>3.47 ± 0.24</td>
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<td>1.60 ± 0.05</td>
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<tr>
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<td>0.1</td>
<td>4.63 ± 0.38</td>
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<tr>
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<td>4.17 ± 0.17</td>
<td>*</td>
<td>2.1 ± 0.11</td>
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</tr>
<tr>
<td></td>
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<tr>
<td>4</td>
<td>0</td>
<td>4.82 ± 0.80</td>
<td>*</td>
<td>3.36 ± 0.01</td>
<td>p&lt;0.01&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>4.55 ± 0.41</td>
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<tr>
<td></td>
<td>0.5</td>
<td>5.49 ± 0.30</td>
<td>*</td>
<td>4.41 ± 0.04</td>
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<td></td>
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<tr>
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<td>1.43 ± 0.01</td>
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<tr>
<td></td>
<td>1.0</td>
<td>3.26 ± 0.02</td>
<td>1.50 ± 0.08</td>
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</table>

<sup>a</sup>Chemiluminescence expressed as mean integrated photon emission ± SEM (x 10<sup>7</sup>).

<sup>b</sup>µmole NADPH oxidized/min/mg/ml protein ± SEM.

<sup>†</sup>Determined by Student’s two-tailed t-test.
Figure 1. The effect of selenite on chemiluminescence exhibiting significant (p<0.05) enhancement of chemiluminescence with all dosages of selenite used. Chemiluminescence is expressed as integrated photon emission X 10^7.
Figure 2. The effect of selenite on chemiluminescence exhibiting significant (p<0.05) enhancement of chemiluminescence with 0.1 and 0.5 μg/ml selenite and significant (p<0.05) depression with 1.0 μg/ml selenite. Chemiluminescence is expressed as integrated photon emission X 10^7.
Integrated Photon Emission $\times 10^7$

Selenite Dosage (µg/ml)
Figure 3. The effect of selenite on chemiluminescence exhibiting no significant changes with the addition of 0.1 and 0.5 µg/ml selenite but significant enhancement with 1.0 µg/ml selenite. Chemiluminescence is expressed as integrated photon emission $X 10^7$. 
Figure 4. The effect of selenite on chemiluminescence exhibiting significant (p<0.05) depression of chemiluminescence with 1.0 μg/ml selenite. 0.1 and 0.5 μg/ml selenite had no affect on chemiluminescence. Chemiluminescence is expressed as integrated photon emission X 10^7.
Figure 5. The effect of selenite on chemiluminescence exhibiting no significant changes with the addition of all dosages of selenite used. Chemiluminescence is expressed as integrated photon emission $X \times 10^7$. 
Figure 6. The effect of selenite on NK cytotoxicity demonstrating six different responses to in vitro selenite supplementation.

* represents significant (p<0.05) enhancement of activity.

† represents significant (p<0.05) suppression of activity.
Discussion

This study demonstrates that the activities of human NK cells and neutrophils were affected by in vitro incubation with sodium selenite. These effects included augmentation and suppression of neutrophil CL and NK-mediated cytotoxicity. All dosages of selenite used were shown to be capable of inducing augmentation of CL activity. In some samples of phagocytic cells, the addition of 1.0 μg/ml selenite caused a depression of phagocytic activity. When Se supplementation enhanced NK cytotoxicity, the effect was always observed at 0.1 μg/ml selenite. At the two higher dosages used, selenite induced both enhancement and depression of activity. These results are in agreement with those obtained from dietary Se supplementation of humans and animals. Aziz et al. (1) and Gyang et al (24) demonstrated a dose-dependent increase in PMN function in goats and cows receiving Se supplementation. Talcott et al. (7) and Koller et al. (5) both observed a dose-dependent enhancement of NK activity in rats after in vivo supplementation with Se. Dimitrov et al. (3) demonstrated a similar dose-dependent enhancement of NK activity in humans receiving a selenium supplement.

The findings of this study contradict those of Watson (29) and Dimitrov (3). Neither investigator observed significant augmentation of NK activity with in vitro supplementation of sodium selenite. These authors did, however, observe significant depression of NK activity when cells were incubated in vitro at selenite dosages of 1.0 μg/ml or greater. The differences between these reports and the present study may be due to the fact that Dimitrov’s group employed an 18 hour preincubation of mononuclear cells in selenite prior to performing the NK cytotoxicity assay while Watson preincubated cells for 48 hours.
prior to the assay. These long preincubations are in contrast to the procedure employed in this study wherein selenite was added directly to the effector-target wells at the start of the actual NK cytotoxicity assay. Moreover, Nabel (34) reported that NK cells undergo a loss of activity in as few as 6 hours when maintained in vitro at 37°C.

During the course of this investigation, there were samples which demonstrated no significant augmentation of phagocytic or NK activity as a function of Se supplementation. This perhaps reflects the presence of ideal intracellular Se levels before treatment. Depression of NK cytotoxicity and neutrophil-mediated CL upon Se supplementation may indicate Se toxicity resulting from an increase in intracellular Se levels over ideal intracellular concentrations. Though intracellular and plasma Se levels were not evaluated in this study, this view finds support in the literature. Severe systemic Se toxicity has been reported in the People’s Republic of China in individuals whose whole blood Se levels averaged 3200 ng/ml (35). Severe Se toxicity was also reported in a New York woman taking Se supplements containing 27.3 mg per tablet (36). Her serum Se level was reported as 528 ng/ml, approximately four times the normal levels for the U.S. population. The proposed adequate and safe daily dosage of Se is 50-200 μg per day. Serum Se levels much lower than those reported in individuals with systemic Se toxicity have been found to cause immunotoxicity. Dimitrov (3) demonstrated increases in plasma Se levels from 0.11 μg/ml to 0.12 μg/ml were associated with increases in NK cytotoxicity. However, increases in plasma Se levels beyond 0.12 μg/ml began to depress activity. A narrow dosage-window appears to be evident. Therefore
caution should be exercised in recommending Se supplementation. Tests evaluating immune function or intracellular Se levels may be valuable in deciding whether supplementation is warranted.

Previous investigators have demonstrated that Se deficient animals exhibited a marked reduction in phagocytic intracellular killing with a corresponding reduction in the activity of the selenoenzyme GSH-Px (1,2,4,25). In animals, the level of phagocyte GSH-Px activity appears to be directly dependent upon the Se concentration in the phagocyte’s environment (1). The present study of human neutrophils is in agreement with the results obtained in animals. Dose-dependent augmentation of CL by in vitro selenite supplementation was accompanied by a corresponding increase in phagocyte GSH-Px activity. Conversely, no dose-related changes in GSH-Px levels were observed in neutrophils which exhibited no change in CL as a function of selenite treatment. The view that selenium-enhanced phagocytic activity is mediated via GSH-Px (1) is sustained by these data.

In addition to the anti-oxidant role of GSH-Px, this enzyme has been demonstrated to be closely coupled to arachidonic acid metabolism (37-40). Since NK activity is dependent upon arachidonic acid metabolism (41), a correlation between selenium-enhanced NK activity and intracellular GSH-Px levels was sought. Addition of selenite to non-adherent cells had effects on GSH-Px activity which did not correlate with previously determined NK activity. This suggests that GSH-Px may not be involved in selenite-induced enhancement of NK cytotoxicity. Further investigation is therefore warranted.
Literature Cited


