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Decreased Levels of Peripheral Leukocytes Following Sodium Selenite Treatment in Female Mice

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Selenium is known to be an essential micronutrient to a number of animal species (Schwarz 1961). Above its trace levels, however, selenium accummulation has long been known to induce deleterious conditions in domestic animals (Smith et al. 1939, Miller and Schoening 1938) and in humans (Smith et al. 1937). The toxicity is manifest in a number of ways depending upon such factors as the route of exposure or administration (Tsuzuki et al. 1960) and form of selenium tested (Moxon et al. 1938). However, irrespective of which compound is employed or what way the selenium is administered many of the adverse effects of selenium are linked either directly or indirectly to erythrocytes and erythrocyte forming tissues. Selenium has been reported to induce a hemolytic anemia (Halverson et al. 1970), alter the configuration of plasma protein (McConnell and Cooper 1950), reduce the synthesis of hemoglobin (Nagai 1959), depress packed red blood cell volume (Goehring 1984), and accummulate in renal and hepatic tissues (Levander and Baumann 1966). There are substantial data in regard to selenium effects on the erythrocyte component of peripheral blood, but there is an obvious deficiency in such information concerning the effects of selenium on the leukocyte component of blood.

The major purpose of this investigation is to focus upon the effects of selenium on formed elements of the blood, and specifically, the leukocytes. Following three separate treatments of selenium, the number and class of peripheral leukocytes were determined as a function of time following administration of the selenium salt.

MATERIALS AND METHODS

The ICR strain of young adult female mice were used in this study (Harlan Sprague Dawley, Inc., Indianapolis, IN 46229). Animal weights ranged from 24 to 30 g with an average weight of 27 g. Standard rodent chow and water were freely available throughout the experimental period. Sodium selenite (Pfaltz and Bauer, Inc., Stanford, CT 06902) was prepared for injection using distilled water as the diluent. On days -4, -2, and 0 one group was injected (i.p.) with 2.0 mg/kg/day of sodium selenite. A second group

was injected on days -2 and 0 and a third group on day 0 using the 2.0 mg/kg/day dosage. The average injection volume was approximately 0.2 ml. Control females were injected with 0.2 ml isotonic saline. Ten mice comprised each of the three experimental groups and the control group.

Mice were lightly anesthetized with ether on days 2, 4, 8, 16, and 24, and blood was collected following tail snips. Blood was smeared on acid-cleaned microscope slides to be used for differential leukocyte scoring employing the criteria for leukocyte cytology described by Bessis (1977). Slides were subsequently stained with buffered Wrights-Giesma stain. The total leukocyte count was made from the blood sample using a Neubauer hemacytometer, and packed red blood cell volume (hematocrit) was determined using a micromethod. The latter measurement was employed to determine any hemodilution effect associated with sodium selenite treatment. Following leukocyte differential scoring, the ratio of agranulocytes (lymphocytes and monocytes) to granulocytes (neutrophils, basophils, and eosinophils) was calculated for each sample of each group. Agranulocyte to granulocyte ratios were used as indices of preferential shifts in a specific class of leukocyte that might occur when the total peripheral leukocyte population (no./mm^3) increased, decreased, or remained the same. Statistical analysis of the data was made using analysis of variance.

RESULTS AND DISCUSSION

Figure 1 shows that on day 8 following sodium selenite, the number of peripheral leukocytes in the highest treatment group is considerably lower than those of the lowest treatment group and controls. The 2 mg/kg x 3-induced reduction represents an approximate 36% decrease below the control count and is statistically significant ($P<0.01$). The trend continues and becomes more exaggerated by day 16 where the mean leukocyte count of the 2 mg/kg x 3 group is about 52% of the mean control value ($P<0.001$). There appears to be a small decline compared to controls on day 16 for the 2 mg/kg x 2 group. This decrease is also statistically significant ($P<0.05$). Recovery from the sodium selenite-induced leukocyte depression is complete by day 24 of the experimental period, at which time the leukocyte counts of all groups mimic those on day 2.

The changes in the type or class of leukocyte with time post-sodium selenite are illustrated in Figure 2. Agranulocyte to granulocyte ratios for both the 2 mg/kg x 2 and 2 mg/kg x 3 groups are significantly different on day 8 ($P<0.01$) and on day 16 ($P<0.001$) from the ratio of control and that of the 2 mg/kg x 1-treated mice. Upon examination of the product of the differential leukocyte percentages and the number of leukocytes, the days 8 and 16 changes in ratios are due to a reduction in the level of neutrophils. By virtue of number, the neutrophil is the most prominent member of the granulocyte class in rodents under normal physiological conditions. The number of agranulocytes appeared to be unchanged as a function of time following the intermediate and

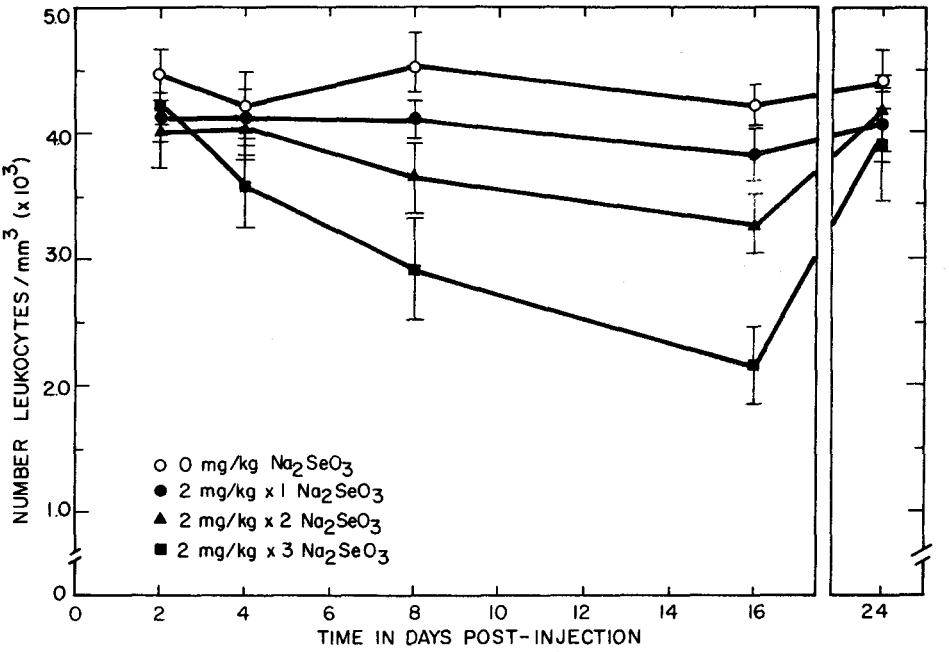

Figure 1. Total leukocyte counts obtained from sodium selenite (Na_2SeO_3)-treated mice. Days represent the times of blood collection following the final or only Na_2SeO_3 injection on day 0. Vertical lines represent standard errors of the means.

highest regimens of sodium selenite tested. The ratio would be elevated if the denominator of the agranulocyte to granulocyte ratio were reduced due to decline in the number of neutrophils without a concurrent reduction in the number of agranulocytes of the numerator. The pattern of ratio differences between the two selenite groups and the control group of days 8 and 16 disappear by day 24, where the ratios of all groups are essentially the same, i.e., a range of 3.9 to 4.5.

The data reported here indicate that sodium selenite is capable of causing a rather dramatic decline in the number of circulating leukocytes 8 to 16 days following treatment. Others (Jacobs and Forst, 1981) have implied that, upon chronic exposure, selenium promotes a leukocyte decrease in mice. The decline in the total number of leukocytes reported here is apparently due to a decrease in the total number of neutrophilic granulocytes. There are many postulates that may be stated in regard to the mode of action(s) of selenite on the peripheral neutrophil titer. For example selenite might exert a cytolytic action on circulating neutrophils. This notion, however, does not seem to be a feasible one because of the protracted time course for the decline. If a total dosage of

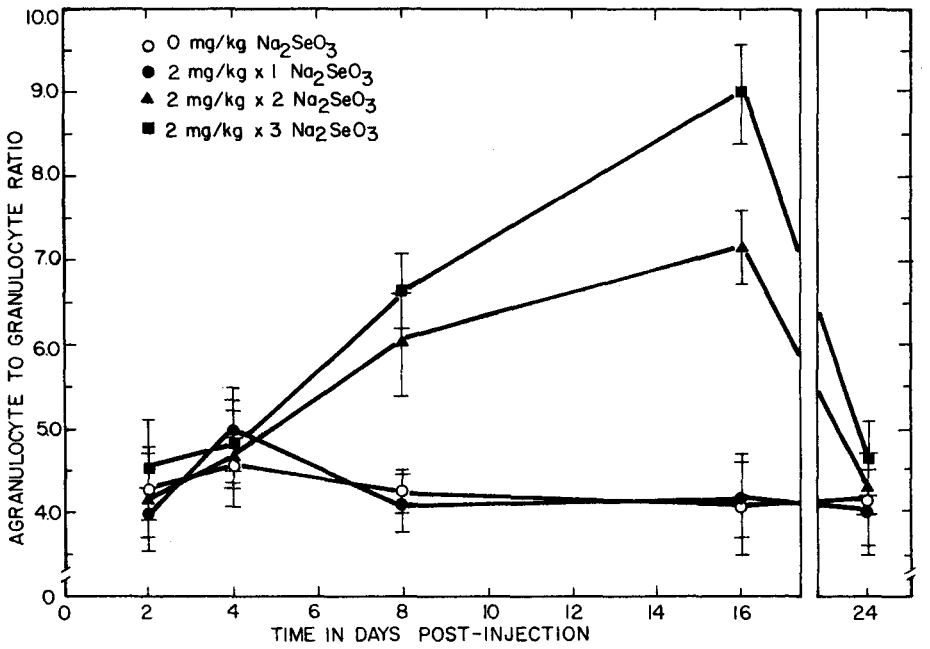

Figure 2. Effect of sodium selenite (Na_2SeO_3) treatment on the agranulocyte to granulocyte ratios of female mice. Days represent the times of blood collection following the final or only Na_2SeO_3 injection of three treatment groups on day 0. Vertical lines represent standard errors of the means.

6 mgs/kg were destructive to circulating neutrophils, an abrupt decrease would be expected shortly after, i.e., day 2 or 4, the accummulation of a critical plasma level of selenite were reached. In relation to this effect, it would be difficult to explain why there would be a high degree of selectivity of selenite for only the neutrophilic granulocytes. It is clear that the leukocyte depression effect was not due to hemodilution, i.e., the plasma component increases, thus, reducing the number of cells per unit volume observed. This is the case since there were no significant differences in the percentages of hematocrit among the experimental and control groups. It is tempting to postulate that the selenite effect is due to depressed neutrophil production. This postulate seems to be an attractive one. The presence of a decrease in the number of cytoplasmic granules and the increase in the number of pyknocytic nuclei in many neutrophils observed on days 8 and 16, suggest a greater number of "aging" neutrophils were present in the two effective selenite dosage groups. Such changes are characteristic of dying granulocytes (Bessis 1977). Also the neutrophil is a short-lived type of cell (Bessis 1977), and if

the neutrophil production were reduced, as their level declined due to senescence without replacement cells, the total neutrophil number and, thus, the total leukocyte count would decline in synchrony. Whatever the specific mode(s) of action of sodium selenite in this system, it is apparent that this compound induces a depressed number of peripheral leukocytes that is allied closely with a drop in the number of circulating neutrophils.

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