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Subchronic Effects of Sodium Selenite and Selenomethionine on Several Immune-Functions in Mallards

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Abstract. The subchronic effects of selenomethionine (SeM) and sodium selenite (SeL) on several immunologic, hematologic, and serologic parameters in mallards were measured, using concentrations in drinking water of 0, 0.5, and 3.5 mg/L selenium (Se) as SeL and 2.2 mg/L (Se) as SeM. Cyclophosphamide (CP) was used as an immunosuppressive control at 20 mg/L. A battery of in *vivo* and in *vitro* immunologic assays was performed on each bird throughout the 12 week study. The SeM-treated birds displayed a significantly impaired delayed-type hypersensitive (DTH) response to tuberculin (M. *bovis*), as measured by the number of positive reactions present 24 hours post PPD challenge ($p \leq 0.05$). The SeM-treated group also exhibited a significantly increased serum alanine aminotransferase (ALT) activity and an increased plasma glutathione peroxidase (GPX) activity ($p \leq 0.05$). Selenium concentration in the liver and breast muscle of SeM-treated birds was significantly elevated 4- and 14-fold, respectively, over controls ($p < 0.05$). Body weight and water consumption of treated birds did not differ from controls. Organ weights were not significantly affected by any Se treatment. Sodium selenite-treated birds displayed no detectable differences in immune-function or Se accumulation in tissues as compared to controls. Serum ALT activity was significantly increased in the 3.5 mg/L group, although to a lesser extent than in SeM-treated birds. Cyclophosphamide significantly depressed white blood cell number, testes weights, and also suppressed the DTH reaction. Concentrations of Se as SeL did not affect the immune system, whereas low concentrations of SeM (2.2 mg/L Se) appeared to suppress certain aspects of the mallard immune response.

Several chemical forms of selenium (Se) have been shown in laboratory settings to be toxic to birds at concentrations comparable to those measured in natural environments. Embryotoxicity and/or teratogenicity occurred in mallard ***(Anus platyrhynchns)*** ducklings when hens were exposed during egg-laying to dietary concentrations as low as 10 μg/g feed (Heinz et al. 1987). In this study, selenomethionine showed more severe effects than sodium selenite. This dietary concentration of Se did not, however, cause increased mortality, alterations in body weight gain, or decreased food intake when administered to mallard ducklings (Heinz *et al.* 1988), indicating that embryotoxicity and/or teratogenicity may be the primary mode of toxicity to waterfowl. Studies at Kesterson Reservoir located in the Kesterson National Wildlife Refuge, Merced County, California, have demonstrated an alarming incidence of teratogenesis in populations of other aquatic birds (Ohlendorf et al. 1986; Hoffman and Heinz 1988). Concentrations of Se in biota taken from the reservoir were reported as high as 440 μg/g dry weight (Saiki and Lowe 1987).

Selenium adequate diets positively affect the immune system (Koller 1980) and specifically play an integral role in proper primary and secondary humoral immune-responses in several species (Spallholz ***et al.*** 1975; Reffett et al. 1980; Sheffy and Schultz 1979). Host disease resistance to coccidiosis in chickens has also been shown to be enhanced by dietary supplementation with Se and/or vitamin E (Colnago et ***al.*** 1984). Increased intracellular killing of ***Staphylococcus aureus*** by human granulocytes has been demonstrated *in* vitro following treatment with supplemental concentrations (200 &day) of organic Se, although antibody titers were not increased (Arvilommi et al. 1983). There has also been recent interest in the potential anticancer properties of Se (Hocman 1988). The usefulness of organic and inorganic Se compounds as anti-carcinogenic agents however, is still under investigation (Ip and White 1987).

The influence of excessive Se intake upon immune-function has been less extensively studied. Selenium has protective qualities when given simultaneously with certain other heavy metals, such as cadmium and mercury, that disturb immune function. For example, Se exposure results in slightly increased antibody synthesis when given to mice in conjunction with methylmercury (Koller et ***al.*** 1979). Also, delayed hypersensitivity, prostaglandin-E2 concentrations,

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and antibody production were suppressed in rats fed 5.0 μg/g dietary Se as sodium selenite while natural killer cell activity was actually enhanced at lower exposures (Koller et al. 1986). Therefore, although dietary Se is necessary for an overall unimpaired immune response, excesses of Se may also lead to undesirable immunological effects.

Adverse effects of toxicants upon the immune system occurs at concentrations well below that required for overt signs of toxicity, and can also persist long after other measures of toxicity are no longer present (Kerkvliet *et al.* 1982). There is, therefore, potential for immune-function tests to be useful as sensitive, early-warning and long-lasting indicators of toxicity. It is widely agreed that in order to adequately assess the overall immune response, one needs to employ a battery of assays which, taken together, give a meaningful diagnostic profile of immune-function (Exon *et* al. 1986; Luster *et al.* 1988). This paper describes the development of a battery of immune function tests for the mallard, covering multiple aspects of cell-mediated and humoral immunity, and the results of using the tests to investigate the immunotoxicity of environmentally realistic concentrations of Se. In order to investigate the possibility of sublethal adverse effects upon mallards from exposure to Se at concentrations below those previously recognized as toxic, the mallards were treated with Se at concentrations ranging from typical Se-adequate diets, up to concentrations demonstrated to affect survival, growth, and physiology (Heinz *et* al. 1988). Drinking water was chosen as the vehicle for Se exposure to simulate the oral uptake of Se from natural waters contaminated with Se at concentrations similar to and exceeding those occurring in agricultural drainage water.

Methods

Adult, 9-month old mallard drakes were purchased from Whistling Wings game farm (Hanover, IL) and were housed in individual above-ground cages in a plastic greenhouse at ambient outdoor temperature and photoperiod. Birds were randomly assigned to one of four treatment groups of eight birds each, and one untreated control group of twenty-four. The four treatment groups consisted of two groups receiving sodium selenite (SeL) (Sigma Chemical Company, St. Louis, MO)-treated water at 0.5, and 5.0 mg Se/L (N = 8 and 7, respectively); one group receiving selenomethionine (SeM) (Sigma Chemical Company)-treated water at 2.2 mg Se/L (N = 8); and one receiving the immunosuppressant drug, Cyclophosphamide (CP) (Sigma Chemical Company), at 20 mg/L in their water (N = 8). The control group (N = 24) received untreated reverse-osmotically purified water. The 5.0 mg/L SeL dosage was subsequently modified on day 9 to 3.5 mg/L to avoid complications due to dehydration. Water for each treatment was prepared in a 20 liter quantity. Birds were divided into four blocks of fourteen: each block contained equal numbers of birds for each treatment group plus 6 control birds. Blocks were started on each of four consecutive days. The study lasted 12 weeks. Birds were given Purina@ Maintenance Duck Chow (Se = 0.2–0.45 mg/kg, Zn ≥ 45 mg/kg, Vit E ≥ 32.4 U/kg, and protein = 12.5%) ad *libitum*. Treated or control water was provided in I-L polyethylene water bottles with 45" angle stainless steel sipper tubes (Valentine Animal Supplies, Hinsdale, IL). Birds were acclimatized and adjusted to the water system for two weeks prior to the start of the experiment. All birds were observed to be drinking from the bottles regularly after three days. Small plastic cups containing untreated water were provided to the animals for approximately one hr each week to allow clearance of nasal blockages. Daily water consumption was measured as the average loss from water bottles over two or three day intervals. Food consumption was not measured. All birds were weighed once each week. Se concentrations in the drinking water were verified once before and once during the experiment.

At selected time intervals during the twelve week study, immune-function tests (described below in detail) were performed. Tests were selected such that most aspects of the immune system were monitored. White blood cell counts presented an overview of the cellular components of the immune response; the carbon clearance assay measured in *vivo* macrophage function; the phytohemagglutinin cutaneous reaction, delayed type hypersensitivity test, and lymphocyte migration inhibition assay measured various aspects of cell mediated immunity; and the plaque-forming cell assay and hemagglutinin titers measured the antibody (humoral) response. The order in which the tests were conducted was designed to proceed from the least invasive procedures to the most invasive and, ultimately, lethal tests. This necessitated that the plaque-forming cell assay be done last and the carbon clearance test be done just prior to that. Two weeks were allowed to elapse between these two tests to reduce the interference from carbon present in the spleen. Clinical serum biochemistry tests were determined at the end of the experiment as described at the end of this section using published methods (Fairbrother *et* al. 1989). Selenium concentration in liver and muscle tissues was determined at the end of the experiment.

Peripheral White Blood Cell Counts

Total and differential white blood cell counts were performed on day 27 and day 78 of the study. Blood samples (3 ml) were taken from the right jugular vein and collected in glass tubes containing disodium-EDTA. Total white cell counts were performed using Natt-Herrick's stain with a hemocytometer. Differential white cell counts were performed on blood smears stained with a Wright-Giemsa stain. Cells were stained and classified according to Dein (1984).

Cell Mediated Immunity

The phytohemagglutinin (PHA) cutaneous reaction has been previously described elsewhere for the chicken (Lamont and Smyth 1984). Animals were inoculated intradermally (day 41) in the wing-web with either 0.1 ml (1.0 mg/ml) PHA in phosphate buffered saline (PBS) (right side), or 0.1 ml PBS only (left side). The amount of swelling in the wing-web 24 hr after inoculation was measured by diigital calipers to the nearest 0.1 mm. The PHA index was computed as the width of the PHA-inoculated wing-web minus the width of the opposite wing web. Data were recorded as the average of two readings for each wing web. All measurements were done by one person to increase consistency of measurements.

Delayed type hypersensitivity test. Animals were sensitized to the tuberculin antigens on day 21 with an intradermal inoculation of 0.1 ml of 2-8 x 10^8 CFU/ml BCG (Bacille Calmette-Guerin) (*Mycobacterium bovis*, TB Research Institute, Chicago, IL) in sterile PBS into the right pectoral area under the wing. The purified protein derivative (PPD) (*M. bovis* concentrated stock, Cooper's Animal Health, Billings, MT) was similarly inoculated into the same area two weeks later (day 35). The reaction was measured at 24-hr post PPD inoculation. Preliminary work by our lab has shown that the response to PPD maximizes between 24 and 36 hr in these animals, and begins to dissipate by 48 hr. The response was quantified as percent positive reactors and also as the area (mm^2) of reaction in those animals exhibiting a positive reaction. The area of reaction

was measured with calipers as described above, measuring the longest and shortest diameters for the calculation of an ellipse.

For the leukocyte migration inhibition assay, whole blood samples (5 ml) were collected in heparinized glass tubes five weeks foilowing BCG inoculation as described above. The lymphocyte/monocyte fraction was separated by single-density centrifugation (Histopaque-1077, Sigma Chemical Company) at 400 × g for 30 min, washed three times with PBS, and resuspended in serum-free RPMI-1640 (Gibco). The cell suspensions were placed into 10 μL microhematocrit tubes and centrifuged for 3 min. The microhematocrit tubes were broken at the media/cell interface and the portion of the tubes containing the packed cells were placed into migration chambers. Chambers consisted of two microscope slides held together at longitudinal ends with double-sided tape; the top slide contained two symmetrically placed 1 × 0.5 cm notches where the cut microhematocrit tube was placed. The slide chambers were placed immediately into petri-plates containing serum-free RPMI-1640 media with or without PPD (8 mg/ml). Each plate contained two tubes, and each bird was represented by four plates; two with PPD and two without. The plates were incubated at 37°C in 5% CO_2 for 18 hr. The area of migration of the pelleted cells was measured using a planimeter from photomicrographs taken of the plates at 20 × using an inverted microscope. Percent migration was calculated as: [(average migration area in the presence of PPD)/(average migration area in the absence of PPD)] × 100.

Carbon Clearance (Macrophage Function)

Intravenous catheters were used in the jugular vein to administer India Ink carbon (Pelikan, water-based). The carbon was centrifuged in 5 ml polypropylene culture tubes at 2500 × g for IO min. Each tube contained 4-ml, and the top 3 ml were taken and subjected to ultraviolet sterilization. Sodium citrate (1% w/v in PBS) was used as the anticoagulant for the catheters and also for the collection of blood samples. The catheters were always flushed with citrate immediately following any injection or sampling. Immediately preceding the injection of the ink into the catheter, a 0.1 ml sample of blood was taken for a time-zero value for the optical density of the blood. The carbon particle suspension was then injected (1.O ml/kg) through the catheter. A 0.1 ml sample of blood was taken from the catheter every three minutes for 18 minutes. Immediately prior to sampling, a 0.1 ml sample was discarded to exclude any residual sample or citrate remaining in the catheter. The blood samples were placed immediately into 2.0 ml of sodium citrate, with gentle mixing, and placed on ice. Optical density of the samples was measured at 675 nm on a Perkin-Elmer spectrophotometer. The absorbance value of the preinoculation blood sample was subtracted from the values of all subsequent samples. Log$_e$ of absorbance was plotted against time and the slope of the least-squares regression line was used as the phagocytic index (Lamont 1986).

Humoral Immunity

The plaque-forming cell (PFC) assay procedure used is a modification of the assay, developed by Jeme and Nordin (1963), that had been used previously for the mallard (Rocke *et al.* 1984). Complement was prepared by twice absorbing 10 ml pooled, clot-free mallard serum with 1 ml of packed, washed, SRBC on ice for 60 min, and was frozen at -70°C until used. Complement activity was verified by titration with 10% SRBC in PBS, and mallard heat-inactivated (56°C, 30 min) immune sera. Complement was found to be most active in the undiluted serum, and was therefore used as such. Birds were inoculated intravenously (day 78) with 1.0 ml/kg of 20% sheep erythrocytes (SRBC) in PBS. Five days later, birds were exsanguinated and euthanized with CO_2. The spleens were removed and immediately processed into single-cell suspensions in RPMI-1640 media by pressing through a clean nylon mesh with a plastic syringe plunger. Once processed, cells were kept on ice until incubated. Numbers of cells in the clump-free suspensions were counted with a hemocytometer, and cell viability was determined with trypan blue exclusion. Spleen cells were added in several dilutions to a mixture of SRBC (2 x 10^9 cells/ml), RPMI-1640, and mallard complement. The mixtures were loaded into chambers made from two microscope slides, joined and partitioned into two sections by thin double-sided tape. The chambers were then sealed with melted paraffin/Vaseline (1 : 1), and incubated at 37°C for 1 hour. The thin chambers facilitated the desired formation of a monolayer of cells upon loading. Holes in the SRBC monolayer (plaques, representing antibody-forming cells) were observed and counted using an inverted microscope at 16 x . One slide chamber (both sections) was used per dilution of spleen cells per bird. Spleen cell dilutions of 1:10, 1:20, 1:40, and 1:80 were used for each bird. A dilution containing the most plaques without losing distinction of individual plaques was chosen for counting. The PFC response was measured as the number of plaques per spleen, number of plaques per gram of spleen, and number of plaques per 10^6 viable spleen cells.

Antibodies (IgG and IgM) to SRBC antigens in sera collected from exsanguinated birds at the end of the experiment (day 83), were titrated for hemagglutination as adapted from a similar procedure (Tsiagbe *et* al. 1987). Serum was collected from whole blood allowed to clot in a 37°C water bath for 30 min. Serum samples were frozen and stored at −70°C until assayed for antibody titer, at which time they were heat-inactivated (as above). Samples (50 μL of serum) were serially-diluted two-fold in 96-well microtiter U-bottomed plates (Gibco, Grand Island, NY). For each sample, duplicate dilutions received 50 μL of 0.2 M 2-mercaptoethanol (2-ME) in PBS or 50 μL PBS, to determine the relative proportion of 2-ME sensitive antibodies (IgM) to the total antibody titer. Plates were incubated at 37°C for 1 hr after which a 0.5% suspension of SRBC (50 μL) was added to all wells and the plates were left at room temperature overnight. The antibody titer was determined as the inverse of the highest dilution showing hemagglutination (i.e., absence of a "button" of SRBC in the bottom of the well).

Serum Chemistry

A series of serological diagnostic tests was performed at the end of the study using a Gilford (SBA-300) automated spectrophotometer (Ciba-Corning Diagnostics, Oberlin, OH), with the exception of the whole-blood glutathione peroxidase assay, which was performed on a Beckman spectrophotometer. Assays included: Calcium (Ca), uric acid (UA), gamma-glutamyl transpeptidase (G-GT), alkaline phosphatase, alanine aminotransferase (ALT), and glutathione peroxidase (GPX). All assays were conducted as previously described for the mallard (Fairbrother et al. 1989). GPX activity was determined by cumene hydroperoxide as the substrate from a previously described procedure (Whanger et a/. 1977). Total protein was quantitated using the method of Lowry et *al.* (1951).

Selenium Analysis

Selenium analyses of drinking water, feed, and tissue (liver and muscle) residues were done by an automated fluorimetric procedure (Brown and Watkinson 1977) by the laboratory of Dr. P. Whanger, Oregon State University, Department of Agricultural Chemistry. The lowest detection limit was 1 μg/L.

Statistical Analyses

The results of all tests except the percentage of reactors in the delayed type hypersensitivity (DTH) reaction, were analyzed by analysis of variance after block effects were determined to be nonsignificant. When significant, means were separated and compared to that of the control group by Dunnett's t-test. The DTH data were compared using Chi-Square analysis. All statistical analyses were performed by SAS statistics software for personal computers (SAS Institute 1986). Values with $p \leq 0.05$ were considered significantly different.

Results

Ducks in the high selenite group initially drank only 25–30% of the volume of water consumed per day as compared to ducks in the other treatment or control groups (Figure 1). On day nine, the concentration of Se in this group's water was lowered from 5.0 mg/L to 3.5 mg/L to avoid significant dehydration. The feces of the animals in this group, throughout the experiment, were distinctly white compared to other groups, although serum from these ducks did not exhibit significantly altered uric acid levels. The ducks in this group started to drink much more regularly and to gain weight immediately following the change. One animal in this group was removed from the study on day 15 due to a foot infection. Body weight data indicated that the remainder of the Se-dosed birds maintained normal weight. The CP group lost weight gradually during the study, despite the relatively low exposure of 3 mg/kg/day (Figure 2). Chemical consumption (mg/kg) was relatively constant for all treatment groups for the duration of the experiment.

Peripheral White Cell (WBC) counts

There were no significantly depressed WBC counts in any of the Se-treated groups as compared with controls (Table 1). On day 27, there was a significant decrease in lymphocytes and total WBCs in the CP group compared to controls, and a decrease in heterophils and total WBCs in the CP group as compared with the high selenite group. The CP group also exhibited significantly decreased lymphocytes compared with the low selenite group. On day 78, the CP-treated group displayed significantly decreased lymphocytes, monocytes, and heterophils from controls, while exhibiting decreased total WBCs from the low selenite group. The absolute difference in WBC numbers with time may be indicative of the cumulative effects of the various assays (*e.g.*, PPD, India Ink, and PHA inoculations) upon the immune systems of the animals, or may be a result of acclimatization of the animals to the stress of handling since the later WBC counts are closer to normal than the early counts.

Cell-Mediated Immunity

Se treatment did not significantly affect the degree of swelling caused by subcutaneous inoculation of PHA as compared to control birds. CP also did not affect the amount of swelling as compared to either control or Se-treated birds.

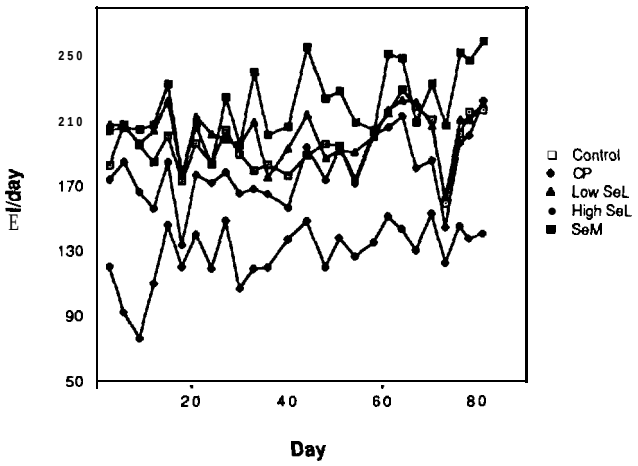

Fig. 1 Average daily consumption of drinking water containing selenium as sodium selenite at 0.5 mg/L (Low SeL) or 3.5 mg/L (High SeL), selenomethionine at 2.2 mg/L (SeM), cyclophosphamide at 20 mg/L (CP), or no additive (Control).

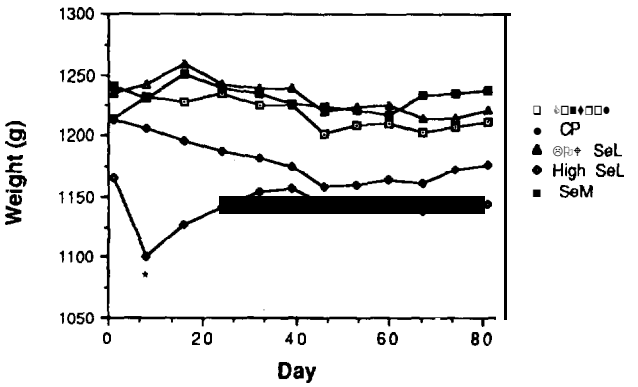

Fig. 2. Body weight of adult male mallards given drinking water containing 0.5 or 3.5 mg/L selenium as sodium selenite, 2.2 mg/L selenium as selenomethionine, 20 mg/L cyclophosphamide, or untreated water (* Significantly different from controls, $p \leq 0.05$, Dunnett's T-test).

The number of birds exhibiting a DTH response was markedly depressed in both the CP and SeM-treated groups as compared to controls (Figure 3). There was no statistically significant difference among treatment groups in the size of the hyperemic area of birds exhibiting a positive response. However, the lack of statistical significance may have been due to small sample size, particularly in the groups receiving higher doses of Se or CP as there were only a few positive reactors in these groups. Figure 3 indicates there was a trend towards decreasing size of the reactive area with increasing Se dose, and that CP also caused a decrease in the percent positive responses nearly to the same degree as SeM treatment.

There was no significant difference in the migration inhibition index from the leucocyte migration inhibition assay among treatments or between treated and control groups. This may have been due to the large amount of variability as

Table 2. Serum diagnostic biochemistry results from mallards at the end of the study (day 83); mean ± SEM

Treatment Test	Control	CP	0.5 SeL*	3.5 SeL	2.2 SeM
Alk. Phos. (IU/L)	38.06 (2.40)[a]	33.57 (4.73)	30.06 (1.98)	37.69 (7.78)[a]	37.94 (4.88)[a]
Calcium (mg/dL)	10.11 (0.14)[a]	10.89 (0.25)[bc]	10.09 (0.29)[ab]	10.18 (0.30)[ab]	10.19 (0.19)[ab]
ALT/GPT (IU/L)	38.08 (2.97)	53.44 (5.86)[abc]	43.21 (6.98)[ab]	61.72 (10.80)[bc]	71.28 (9.37)"
Uric Acid (mg/dL)	2.65 (0.18)	2.95 (0.51)[a]	2.62 (0.37)[a]	3.51 (0.31)"	2.84 (0.19)"
G-GT (IU/L)	2.03 (0.12)[a]	2.41 (0.22)[a]	2.71 (0.75)"	2.31 (0.32)"	2.39 (0.34)[a]

* SeL = sodium selenite; SeM = selenomethionine. Concentrations are in mg/L
(abc) Exclusively different superscripts indicate a significant difference between groups ($p \leq 0.05$, Dunnett's T-test)

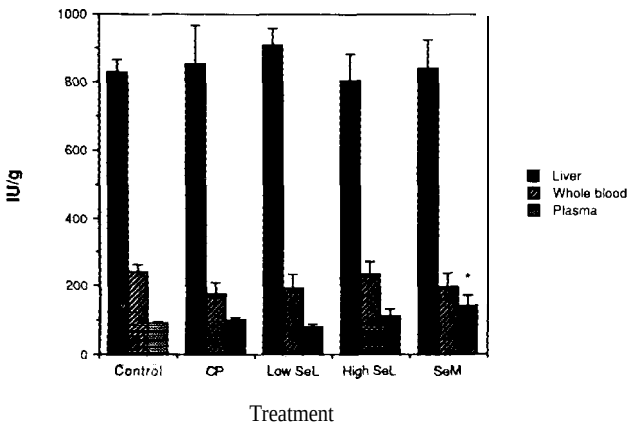

Fig. 6. Glutathione peroxidase activity in liver, whole blood, and plasma of mallards following 83 days of treatment. Liver and plasma activities are expressed per gram of protein, and whole blood activity is expressed per gram of hemoglobin; mean ± SEM. * Significantly different from controls ($p \leq 0.05$, Dunnett's T-test).

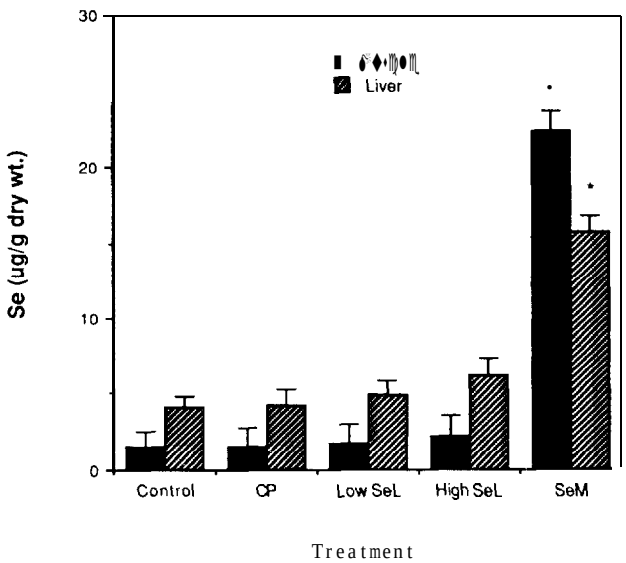

Fig. 7. Selenium concentrations in mallard pectoral muscle or liver following 12 weeks treatment; mean ± SEM. * Significantly different from controls ($p \leq 0.05$, Dunnett's T-test).

There were no significant differences from the control group in the ability of circulatory leukocytes to inhibit their migration in the presence of PPD. This indicates that there was not a reduction in production or response to migration inhibition factor (MIF) at the site of PPD challenge. Impaired migration inhibition does not, therefore, appear to be responsible for the absence of a DTH reaction in the SeM or CP-treated birds, although the high variability inherent in these tests makes it difficult to reach definitive conclusions.

Reticuloendothelial function as measured by carbon clearance was not impaired in any group. Macrophage phagocytosis is typically more resistant to chemical suppression than many other immune-functions, thus it is not surprising that the relatively low concentrations of Se in this study did not produce significant differences from controls. Similarly, CP, although a proven positive control for many immunological reactions involving both B and T cell functions (Dean et *al.* 1979; Douvas and Crowle 1981; Mansour and Nelson 1979; Renoux and Renoux 1980), is not an effective control for suppression of phagocytosis. SeM treatment induced plasma GPX activity without a corresponding induction in the whole blood, indicating that the non-plasma fraction of the blood (i.e., RBC's) probably contained less GPX activity than in control birds. We expected to see a more pronounced, dose-dependent increase in liver and whole blood GPX, since this enzyme is inducible through Se supplementation in most species. The enzyme induction reaches a plateau at some concentration of Se before toxicitiy occurs, and it may be that GPX activity had already achieved a maximum with the relatively high Se content of the feed (0.2-0.45 mg/kg). Correlations between GPX activities and erythrocyte or whole-blood Se concentrations have been ineffective when Se concentrations exceed 90-120 µg/kg in these tissues (Combs and Combs 1986).

Activity of serum ALT was increased in the SeM-treated mallards. This enzyme is indicative of renal or, to a lesser degree, hepatic dysfunction in birds (Lewandowski *et al.* 1986). However, no overt signs of toxicity (weight loss, lowered activity, or altered organ weights) were apparent in these birds, indicating that there may be significant adverse physiological changes occurring in adult mallards exposed via drinking water to concentration of SeM below that previously thought to be toxic. Selenomethionine in the diet over a 14-week period affected other enzymes in adult mallards indicative of hepatoxicity, although ALT was not affected (Hoffman *et al.* 1988). The kidney is typically high in

Se concentration, probably because it is the primary route of excretion. Renal concentrations however, typically represent less than 10% of the total body burden of Se, while liver and muscle may represent 30% and 40% respectively (Combs and Combs 1986). In view of the elevated ALT activity, it would have been of interest to examine Se concentration in the kidney.

The low dose of sodium selenite caused no adverse effects throughout the study. This was not surprising since the actual difference in Se exposure of this group and the control group was small due to the relatively high Se content of the feed. The high dose of selenite, despite having the greatest Se concentration, did not affect immune function significantly, but did increase serum ALT activity similar to that seen in the SeM-treated birds. At slightly higher selenite concentrations, the birds stopped drinking and eating. Therefore, it does not seem likely that the primary route of toxicity of sodium selenite, if any at these concentrations, is directed at the immune system.

As expected, CP provided a useful positive control for several of the immune-function tests. We had hoped that the CP group would display a decreased PFC response to SRBC, but the dose used was probably too low. CP is typically used in much higher acute doses (i.e., 25–100 mg/kg) at specific times in the response rather than the chronic, low-level exposure used in this experiment. CP is an alkylating agent, which is most potent against mitotic cells. It was not surprising therefore, to find that CP caused decreased WBC counts and significantly lower testes weights.

Tissue accumulation of Se and induction of glutathione peroxidase activity in rats has been shown to vary according to the form of Se given, with the magnitude of variation dependent upon the amount fed and the type of tissue analyzed (Whanger and Butler 1988). Typically, SeM exposure results in the preferential deposition of excess Se in liver with muscle tissue accumulations present in lesser amounts (Heinz et al. 1987; Whanger and Butler 1988). From this study, it appears that subchronic exposure favors the preferential deposition of Se from SeM in muscle tissue. Exposure to sodium selenite results in a smaller amount of Se sequestered in these tissues, and this accumulation is saturable at a relatively lower exposure of Se. This pattern of tissue accumulation of Se was seen in the present experiment (Figure 7) and is consistent with experiments done on rats, where the Se content in muscle tissue from SeM-treated rats was increased 26-fold over selenite-treated rats (Whanger and Butler 1988). These researchers also found that GPX activity was not a reliable indicator of Se bioavailability, and that the proportion of Se associated with GPX activity was generally greater in rats fed selenite than in rats fed SeM.

The present experiment with mallards has shown several effects of Se exposure: (1) certain aspects of the immune system were suppressed, as measured by the delayed hypersensitivity reaction to tuberculin; (2) renal and/or hepatic function were altered, resulting in increased serum ALT activity; (3) GPX activity was induced; and (4) excess Se was deposited in muscle and liver. Collectively, the above results indicate that there may be adverse effects from exposure to Se (especially as SeM) at concentrations previously thought to be insignificant. The fact that most plants accumulate Se as SeM, makes this compound easily incorporated into the higher levels of the food chain. Considering that suppression of the immune system was evident at 2.2 mg/L Se in the drinking water, selenium-induced immunosuppression could be a contributing factor in the continuing epidemics of avian cholera and other diseases affecting free-ranging waterfowl and shorebird populations exposed to considerably greater Se concentrations in their food, such as has been occurring in the agricultural drainwater basins in California.

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