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Selenium Accumulation and Elimination in Mallards

Gary H. Heinz, Grey W. Pendleton, Alexander J. Krynitsky, and Lyn G. Gold

U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland 20708, USA

Abstract. Selenium accumulation and loss were measured in adult mallards (*Anas platyrhynchos*) fed selenomethionine during two experiments. In Experiment 1, both sexes were fed a diet containing 10 ppm selenium for 6 weeks, followed by 6 weeks on untreated feed. Selenium accumulation in liver and muscle of females was described by $C = A(1 - e^{-bt})$. Concentrations of selenium were predicted to reach 95% of equilibrium faster in liver (7.8 days) than in muscle (81 days). The loss of selenium from liver and muscle of females was described by the exponential loss rate equation: $C = Ae^{-bt}$, with half-times of 18.7 and 30.1 days, respectively. Males reached similar levels of selenium in liver and breast muscle as females and declined to similar levels once selenium treatment ended. In Experiment 2, females were fed increasing levels of selenium until some died. Survivors were switched to an untreated diet and selenium was measured in blood, liver, and breast muscle over 64 days. The same equation as in Experiment 1, $C = Ae^{-bt}$, was used to describe the loss of selenium from blood and muscle. Half-times were 9.8 and 23.9 days, respectively. For liver, the equation $C = A_1e^{-b_1t} + A_2e^{-b_2t}$ was used. Selenium initially decreased in liver by one-half in 3.3 days, with subsequent half-times of 3.9, 6.0, and 45.1 days.

Although selenium is an essential trace element, excessive amounts cause reproductive failure and death in animals. In chickens, these effects were reported in the 1930s (Tully and Franke 1935; Poley and Moxon 1938). More recently, high levels of selenium killed adults and impaired the reproduction of aquatic birds in California (Ohlendorf 1986; Ohlendorf *et al.* 1986). In laboratory studies with birds, as little as 25 ppm selenium in the diet caused mortality (Heinz *et al.* 1987) and 5 ppm caused reproductive problems (Ort and Latshaw 1978).

Two factors that determine selenium levels in birds and consequent effects are the rates of accumulation and elimination. Sodium selenite, an inorganic form, quickly reaches equilibrium and is lost rapidly in birds (Arnold *et al.* 1973; Ort and Latshaw 1978). However, in nature selenomethionine, an organic form, may be more dominant than sodium selenite in plants (Olson *et al.* 1970), but little is known about its accumulation and elimination rates in birds. For this reason, our objective was to measure accumulation and elimination of selenomethionine in adult mallards (*Anas platyrhynchos*).

Methods

Experiment 1

On October 10, 1985, two 20-week-old female mallards were randomly assigned to each of 43 1-m^2 pens and were provided with *ad libitum* food and water. Two males of the same age were assigned to each of nine identical pens. Additional birds were kept under identical conditions to fill in as spares as needed.

Pairs were given 12 days to acclimate to their pens. After acclimation, three pens of females and three pens of males were sacrificed to establish pre-treatment levels of selenium in liver and breast muscle. Thirty-six pairs of females and the remaining six pairs of males were switched to a diet containing 10 ppm selenium in the form of seleno-DL-methionine. The remaining four pens of females were maintained as controls. We concentrated our effort on females because of the known effects of selenium on female reproduction; selenium is known to accumulate in eggs and cause teratogenic effects and embryo mortality (Tully and Franke 1935; Heinz *et al.* 1987). In birds, there are no known effects of selenium in males that would affect reproduction. We incorporated a few pens of males into the study to determine if there were marked differences between the sexes in storage and loss of selenium.

Seleno-DL-methionine was dissolved in distilled, deionized water, and mixed into duck developer mash such that 2% treated water had been added to the diet. Two percent untreated water was added to the control diet. One sample of control diet was analyzed and found to contain <0.05 ppm selenium. Three samples of treated feed contained 9 ± 0.2 (mean ± SE) ppm selenium.

The ducks were fed the diet containing 10 ppm selenium for 6 weeks. At weekly intervals, three pens of females were sacrificed. After 6 weeks, three pens of treated males and two pens of female controls also were sacrificed. All remaining birds were switched to an untreated basal diet of duck developer mash. During the next 6 weeks, three pens of females were sacrificed weekly to follow loss of selenium. After 6 weeks post-treatment, the remaining three pens of treated males and two pens of female controls were sacrificed.

When each pen of birds was sacrificed, pooled samples of liver and breast muscle were saved for selenium analysis. Each pool consisted of 5 g of liver or muscle from each of the two birds in the same pen.

Selenium analyses followed the method of Krynitsky (1987). A 0.5-g (wet weight) subsample of tissue was digested for 2 hr in a

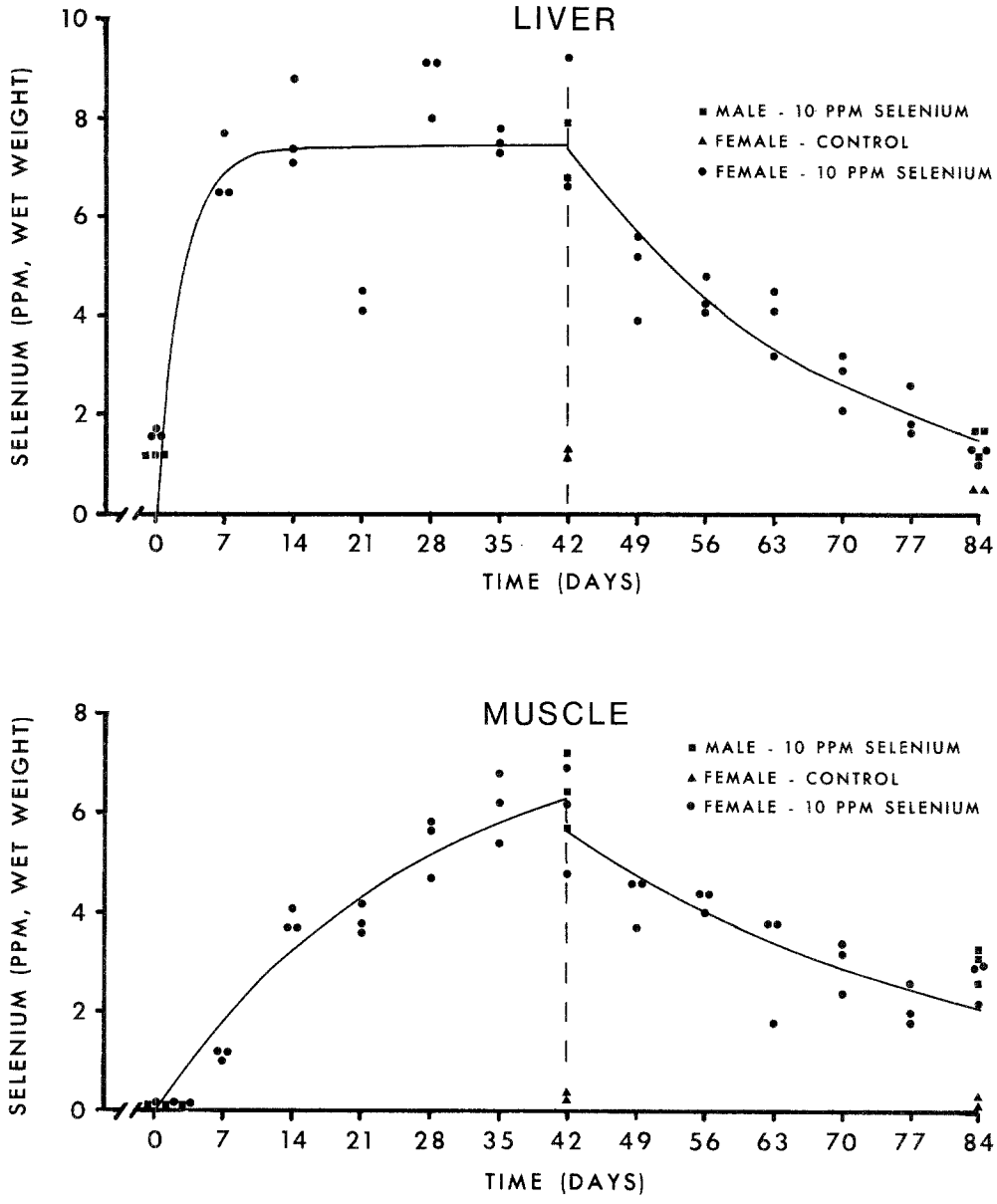

Fig. 1. Accumulation and loss of selenium in mallards fed 10 ppm selenium as selenomethionine. Selenium treatment lasted 42 days, followed by 42 days off treatment. Sample size for selenium-treated birds was 3, except on days 21 (females) and 42 (females and males) for liver when N = 2. N = 2 for control females on days 42 and 84

nitric acid/hydrogen peroxide solution in a polyethylene tube, partially immersed in a hot water bath at 95°C. The digested samples were quantified directly, using peak area integration, by graphite furnace atomic absorption spectrometry in combination with Zeeman background correction (Slavin *et al.* 1983; Welz *et al.* 1983). The lower limit of detection on a wet-weight basis was 0.05 ppm selenium.

Accumulation and loss of selenium were described, using standard exponential equations (Renwick 1982). The form of equation used for the accumulation of selenium was $C = A(1 - e^{-bt})$, where C is the concentration of selenium on a wet-weight basis, A is the concentration at equilibrium, e is the base of natural logarithms, b is the first-order rate constant, and t is the number of days on the selenium diet. The equation used to describe loss of selenium was a one-compartment model exponential loss function: $C = Ae^{-bt}$, where A is the concentration at time 0 and t is the number of days since selenium treatment ended. Parameter estimates were made using Marquardt's method of nonlinear least squares (Draper and Smith 1981).

Experiment 2

On October 10, 1985, two 20-week-old female mallards were randomly assigned to each of 25 1-m^2 pens. Extra birds were held under identical conditions to replace losses. After a 9-day period of acclimation on untreated feed, 21 pens of ducks plus some extras were switched to a diet containing 10 ppm selenium as selenomethionine. Four pens were maintained on a control diet.

Each week thereafter the dietary concentration of selenium was doubled, first from 10 to 20 ppm, and then to 40, 80, and finally 160

Table 1. Selenium accumulation and loss variables in liver and breast muscle of female mallards

Variable	Liver	Breast muscle
	Accumulation phase	
Equation describing accumulation of selenium[a]	$C = 7.4\ (1 - e^{-0.382t})$	$C = 8.0\ (1 - e^{-0.037t})$
r^2 (P)	0.65 ($P < 0.01$)	0.92 ($P < 0.01$)
Asymptotic level of selenium (ppm)	7.4	8.0
Time (days) to reach 95% of asymptote	7.8	81.0
	Loss phase	
Equation describing loss of selenium[b]	$C = 7.4e^{-0.037t}$	$C = 5.6e^{-0.023t}$
r^2 (P)	0.86 ($P < 0.01$)	0.74 ($P < 0.01$)
Half-time of selenium (days)	18.7	30.1
Time (days) for selenium to return to control level	71.3	143.8

[a] C is the concentration of selenium present in the tissue after t days on selenium treatment
[b] C is the concentration of selenium present in the tissue t days after selenium treatment ended

ppm. Treated diets were prepared as in Experiment 1, except that a 3% addition of treated water to the diet was needed to dissolve enough selenomethionine for the 160-ppm diet. Three feed samples from each of the diets mixed to contain 10, 20, 40, 80, and 160 ppm selenium were analyzed and reported to contain means (±SE) of 9 ± 0.2, 18 ± 1.7, 43 ± 2.4, 109 ± 12.4, and 185 ± 2.9 ppm selenium.

The purpose in gradually increasing the dietary concentration of selenium was to elevate tissue concentrations of selenium to the maximum tolerated by mallards. Two days after being switched to the 160-ppm selenium diet, one bird died. The following day, four more died and others were sick. At that point, we switched all surviving ducks to an untreated diet and began sampling birds for the loss rate data. We felt there would have been very heavy mortality had we kept the birds on the 160-ppm selenium diet any longer. The surviving birds probably carried near maximum body burdens of selenium, enabling us to plot the loss rate curves going from as high as possible to near control levels.

Two pens of controls were sacrificed on the day selenium treatment ended and two pens 64 days later. For the mallards fed selenium, three pens of birds were sacrificed on days 0, 2, 4, 8, 16, 32, and 64 post-treatment. From each pen a pooled sample of blood, liver, and breast muscle was formed by taking 5 g of tissue from each bird. Chemical analyses were conducted as in Experiment 1.

The loss of selenium from blood and muscle were described by the same equation, $C = Ae^{-bt}$, used in Experiment 1. For liver, the addition of a second exponential term gave a visually better fit to the data (although not a statistically significant improvement), resulting in a two-compartment model: $c = A_1e^{-b_1t} + A_2{}^{-b_2t}$; A_1 and A_2 are analogous to the concentration of selenium at time 0 in each compartment and b_1 and b_2 are the first- and second-order rate constants. Estimates of parameters were made as in Experiment 1.

Results

Experiment 1

Curves for accumulation and loss of selenium in liver and muscle are shown in Figure 1. The equation fitted to the data for accumulation of selenium predicts asymptotic concentrations of 7.4 and 8.0 ppm selenium for liver and muscle (Table 1). The estimated time to reach 95% of the projected asymptotic level was 7.8 days for liver and 81.0 days for muscle. Because it would take infinity according to the uptake equation to actually reach the peak concentrations, the time to reach 95% of the projected peak level is commonly used (Hayes 1975).

Loss of selenium from liver was faster than loss from muscle; the calculated half-times (time for selenium concentrations to decrease by one-half) were 18.7 and 30.1 days, respectively (Table 1). For selenium to decrease to control levels would take 71.3 days for liver and 143.8 days for muscle.

Males were not sacrificed each week; however, at the start, week 6 of treatment, and week 6 post-treatment selenium concentrations in the liver and breast muscle of males were similar to those in females (Figure 1).

Experiment 2

Loss of selenium from liver, muscle, and blood are shown for mallards with near-maximum selenium burdens (Figure 2). Mean selenium concentrations on day 0 were 22, 6.3, and 12 ppm, respectively. Half-times for muscle and blood were 23.9 and 9.8 days (Table 2). The value for muscle was similar to the value in Experiment 1 (30.1 days). A single half-time could not be calculated for liver, because there were two exponential terms in the loss rate equation. The effect of this two-compartment model of loss from liver was a rapid initial loss of selenium followed by a gradual slowing of loss. The liver initially lost one-half of its selenium in only 3.3 days; a second 50% decrease took 3.9 days; a third, 6.0 days; and a fourth, 45.1 days.

The predicted times for selenium concentrations to decrease to control levels in liver, breast muscle, and blood were 161.8, 120.4, and 58.4 days (Table 2).

Discussion

We conclude that mallards feeding at a selenium-contaminated site will quickly accumulate high levels of selenium. Liver was calculated to take only 7.8 days to reach 95% of its peak concentration. Breast muscle took 81 days to reach 95% of its peak selenium concentration; however, after only

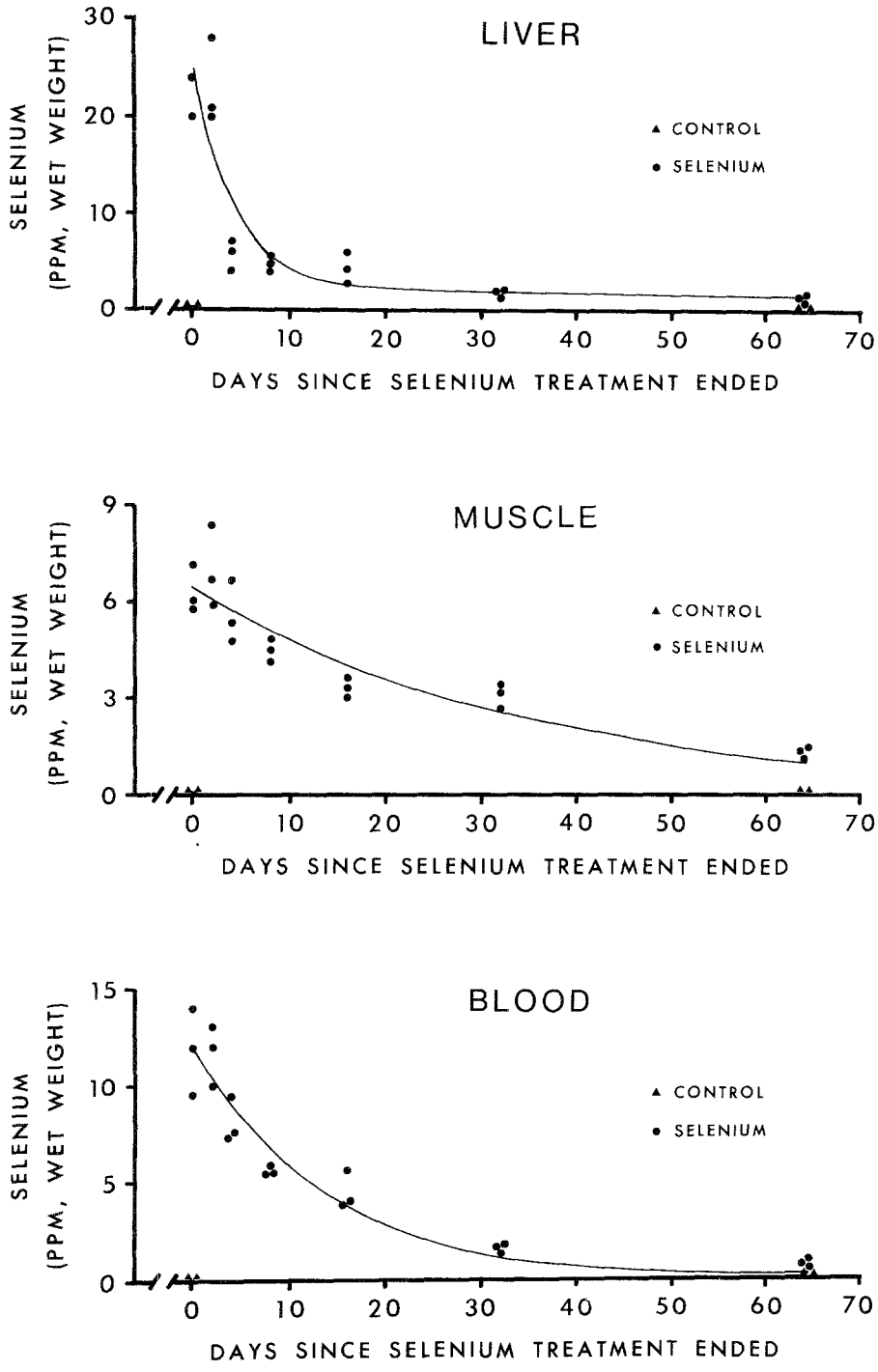

Fig. 2. Loss of selenium by female mallards carrying maximum body burdens of selenium. Sample size for selenium-treated birds was 3, except on day 0 for liver when N = 2. N = 2 for controls on days 0 and 64

6 weeks selenium in muscle reached about 6.3 ppm or 79% of the predicted peak. Within 1 week, even in muscle, selenium had increased to several times the control level. If ducks entered an area where their food contained enough selenium to impair reproduction or threaten adult survival, these harmful effects could be expected to begin quickly, probably within a few weeks.

We also conclude that ducks would quickly lose selenium once they left a selenium-contaminated site. Loss from liver is especially fast; but even with breast muscle in Experiment 1, where loss was comparatively slow, selenium decreased by 62% in 6 weeks.

Although accumulation and loss curves were calculated only for females, the small number of samples taken for males leads us to believe that the curves would be similar for males. During the reproductive season, selenium is eliminated through eggs, resulting in higher levels in males than females (Heinz *et al.* 1987). The results suggest that, except for the reproductive season, selenium accumulation and loss is the same for both sexes.

There are no comparable data for selenium accumulation and loss for waterfowl, but results with chickens agree with our findings. Moksnes (1983) fed chickens 6 ppm selenium as selenomethionine and measured selenium in eggs from

Table 2. Selenium loss variables in liver, breast muscle, and blood of female mallards

Variable	Liver	Breast muscle	Blood
Equation describing loss of selenium[a]	$C = 22.6e^{-0.246t} + 2.5e^{-0.008t}$	$C = 6.4e^{-0.029t}$	$C = 12.0e^{-0.071t}$
r^2 (P)	0.79 ($P < 0.01$)	0.84 ($P < 0.01$)	0.91 ($P < 0.01$)
Half-time of selenium (days)	NA[b]	23.9	9.8
Time (days) for selenium to return to control level	161.8	120.4	58.4

[a] C is the concentration of selenium present in the tissue t days after selenium treatment ended

[b] NA = not applicable; a single half-time does not exist because the loss equation has two exponential terms. The loss of selenium from liver slows down with time. To lose the first one-half of selenium would take 3.3 days. To decrease by one-half again would take 3.9 additional days, and the third and fourth reductions by one-half would take 6.0 and 45.1 additional days

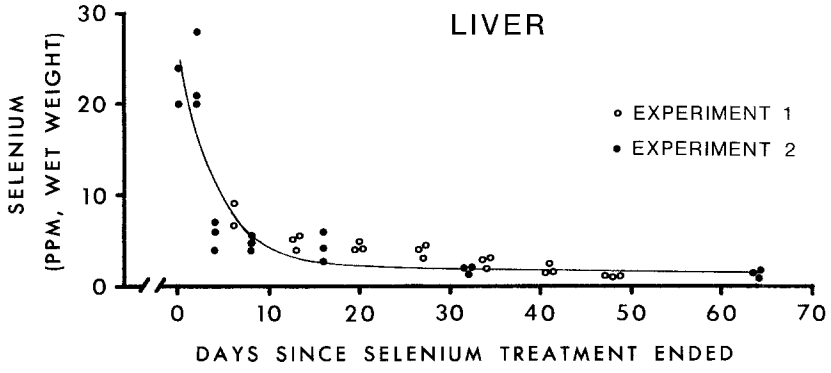

Fig. 3. Comparison of loss rate data for female livers from Experiments 1 and 2. Data from Experiment 1 were superimposed on the curve generated for Experiment 2. The x-axis values for Experiment 1 were shifted to the right until the mean selenium residue at the first sampling time fell on the curve predicted in Experiment 2 (what originally was day 0 for Experiment 1 was relocated to about day 6 on the time scale for Experiment 2). This shifting of x-axis values from Experiment 1 was necessary to bring the data into alignment with those of Experiment 2

weeks 4 through 18. There was no trend toward increasing concentrations after week 4 on treatment. In another study, chickens were fed 0.67 ppm selenium derived from seleniferous grains and fish meal (Scott and Thompson 1971). Selenium reached 0.327 ppm in blood after 4 weeks and 0.338 ppm after 8 weeks, indicating little, if any, real increase beyond 4 weeks. When chickens were fed 0.5 ppm selenium as selenomethionine for 10 weeks followed by 9 weeks off treatment, the half-time of selenium in blood was 27 days (Moksnes and Norheim 1986). After the 10 weeks on 0.5 ppm selenium treatment, eggs from the chickens contained an average of 0.99 ppm selenium in yolk and 0.65 ppm in white. Four weeks later, yolks contained 0.38 ppm selenium and whites 0.10 ppm. After 9 weeks, only a small additional decline was seen—down to 0.35 ppm in the yolk and 0.09 ppm in the white.

In the present study, liver reached a peak concentration of selenium faster than muscle and also lost selenium faster than muscle. Liver is an organ of active metabolism and excretion of selenium, whereas muscle is not. In muscle, selenomethionine competes with methionine for incorporation into proteins (Beilstein and Whanger 1987). Therefore, rate of turnover of protein may be an important factor in buildup and loss of selenium in muscle. Blood may have been intermediate in its loss of selenium because it is the tissue in which selenium is carried to and from other tissues, some of which would have a faster and others a slower loss of selenium.

When selenium reached a high level in liver in Experiment 2 the loss rate was fast at first and slower later. Hayes (1975) described this type of loss function as one in which excretion is more efficient at higher storage levels due to the greater stimulation of detoxifying enzymes. Another possible explanation may be that above certain levels, selenium is stored in the liver in a more easily excreted form. The results for loss rate of selenium from liver in Experiments 1 and 2 are not contradictory. In fact, if one superimposes the data points from Experiment 1 on the loss rate curve from Experiment 2, the results are seen to be quite similar (Figure 3). This suggests that a completely different loss phenomenon is not operating at low versus high starting levels of selenium; but rather that at lower starting levels, the initial rapid drop seen with higher levels does not take place.

For muscle, the equations for loss of selenium were similar for both experiments. Unlike liver, selenium in muscle did not reach a much higher level in Experiment 2 than in Experiment 1, possibly because muscle is not as metabolically active as liver and may not accumulate selenium above a given level.

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