AN ABSTRACT OF THE THESIS OF

Anne Rose Alexander for the degree of Doctor of Philosophy in Foods and Nutrition presented on January 22, 1982

Title: The Effect of Food Processing on the Bioavailability of Selenium in Tuna and Wheat: Human and Rat Studies

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Bioavailability of selenium (Se) in processed tuna and wheat products was studied in humans and rats. The protein source of the rat diets was torula yeast with Se supplied by either raw, precooked or canned tuna, or whole wheat flour, bread or bran. Sodium selenite was used as a control. Each Se source was fed at three levels; 0.05, 0.10 and 0.15 ppm. Using increase in glutathione peroxidase (GSH-Px) activity in various tissues of rats as an indicator of bioavailability, no difference was seen among the three tuna products or among the three wheat products tested. However, significantly lower GSH-Px activity was found in the combined tuna groups as compared to the combined wheat groups, suggesting that the Se in wheat was more available than that in tuna. Se concentration in four rat tissues (liver, kidney, whole

blood and muscle) was also measured. A significant increase in the liver Se content of rats fed canned tuna over those fed raw or precooked tuna was observed. Since this did not correspond with an increase in GSH-Px activity it was concluded that it did not represent increased bioavailability of canned tuna.

In the human experiment, eight young men ate controlled diets where the Se was supplied by either whole wheat bread or canned tuna for two week periods. The Se content of the tuna diet was 331.5 ug/day and the bread diet was 354 ug/day. No difference was observed in whole blood GSH-Px or Se due to the tuna or bread diets but this may be due to the short time period. No significant difference in excretion of Se was observed in the balance study. On the tuna diet, the subjects excreted 72.7% of the Se consumed and on the bread diet they excreted 70.4%.

The Effect of Food Processing on the Bioavailability of Selenium in Tuna and Wheat: Human and Rat Studies

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed January 22, 1982

Commencement June 1982

APPROVED:

Associate Professor of Foods and Nutrition

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Date thesis is presented: January 22, 1982

Typed by Anne Rose Alexander

DEDICATION

I would like to dedicate this dissertation to my parents, Herman and Maxine Hermanson. They made many sacrifices to insure that I received a quality education and demonstrated how one family can successfully support two careers.

ACKNOWLEDGEMENTS

Perhaps one of the most important lessons to come out of all this is how much help is needed to accomplish a major goal. I was helped by many people during my doctoral studies, but a few deserve to be recognized. I wish to thank my committee, Dr. Margy Woodburn, Dr. Frank Conte and Dr. John Smith and especially my two advisors, Dr. Lorraine Miller and Dr. Philip Whanger for supporting, encouraging and educating me. My appreciation is extended as well to Judy Butler for spurring me on to do my assays when it looked like an impossible task, offering her expertise when necessary and most of all for being a good friend. I would like to thank Suean Ott for being most helpful and gracious in assisting me with my animals. But most of all thanks to those on the home front, my husband, Larry, and son, Scott, for encouraging, pushing and prodding, but never letting me forget what is number one in my life.

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I. INTRODUCTION

The study of the bioavailability of selenium (Se) has been a relatively recent undertaking by nutritional scientists. Although Schwarz and Foltz (66) evaluated the Factor 3 activity of various Se compounds in 1958, little research was done in this area until the 1970's. In 1973, Rotruck et al. (57) reported an essential role of Se as an integral component of the enzyme glutathione peroxidase (GSH-Px, EC 1.11.1.9). Following this, much effort was focused on the metabolism of different Se compounds in a variety of animal species.

There is no established standard method for studying Se bioavailability in rats. Early studies used death from liver necrosis as a method to evaluate bioavailabiltiy. Later, after 1973, tissue levels of GSH-Px were employed as a means to determine Se nutriture while Se adequate diets were fed. Human studies on the bioavailability of Se are limited. Robinson et al. (54) reported a human bioavailability study, but it involved only three subjects whose dietary intake was not controlled. In general, it is believed that Se from vegetable sources is better utilized than Se from animal

sources. Presumably this is true in humans as well, but it has never been demonstrated.

Although bioavailability of Se has been studied in several animals with different Se containing foods and compounds, no one has looked at the effects of food processing on the bioavailability of Se. As society increases its dependence on processed foods, this question becomes more important. The possibility of losing nutrients during processing is well established. But how available are those that are left? Reinhold et al. (51) demonstrated that zinc bioavailabiltiy improved in yeast leavened bread. Is there a possibility of a similar enhancement with Se?

In order to answer these questions, two studies were initiated. First the influence of conventional food processing on the bioavailability of Se was determined. For this tuna at three stages of processing (raw, precooked and canned) and wheat as whole wheat flour, bran and whole wheat bread were chosen. Bioavailability of Se in rats was determined by measuring Se concentration of liver, kidney, muscle and whole blood and GSH-Px activity of liver, kidney and whole blood. Second, the bioavailability of Se in men was determined by using two of these foods, canned tuna and whole wheat bread. The bioavailability of these two foods was compared in rats and men by using two biochemical param-

eters from the animal study, whole blood Se and GSH-Px. In addition a controlled Se balance study was conducted in the men.

II. REVIEW OF LITERATURE

SELENIUM ESSENTIALITY

The first evidence for the essentiality of selenium was presented by Schwarz and Foltz in 1957 (65). Previous research by Schwarz, initiated in 1944, revealed that rats fed a torula yeast based diet which is low in sulfur amino acids, and without vitamin E died from severe liver necrosis. Additions of cystine and vitamin E as well as a third constituent, known only as Factor 3, would alleviate the symptoms of this necrosis (64). Further purification of Factor 3 led to the discovery that the active component in the prevention of liver necrosis was selenium. Later sodium selenite was shown to be 500 times as active as vitamin E and 250,000 times as active as L-cystine in preventing liver necrosis. Schwarz and Foltz stated that "It can be inferred from our results that selenium is an essential trace element" (65).

Subsequent research (66) compared the Factor 3 activity of various Se compounds. They were evaluated on the dose required to prevent liver necrosis in rats fed a torula yeast based diet. Schwartz and Foltz found that Factor 3 Se was the most potent; selenite, selenocystine, selenocystathionine and selenomethionine were not significantly diffe-

rent from one another, but less potent than Factor 3. Other inorganic selenium compounds such as sodium selenate, selenic acid and potassium selenocyanate were comparable to selenite. However, elemental selenium was inactive. They suggested that the greater biopotency of Factor 3 could be that it is more closely related to the metabolic form and could be incorporated more readily and efficiently at the active site.

FUNCTION OF SE: A HISTORICAL PERSPECTIVE

In 1951 Schwarz reported that administration of vitamin E to rats on torula yeast-based diets, prevented liver necrosis (62). Subsequently he and Foltz reported that selenite was much more effective than either vitamin E or L-cystine (65). In 1955 Chernick *et al.* (15) reported that liver slices from pre-necrotic rats could not maintain normal respiration *in vitro*. The metabolic defect was prevented by dietary administration of α-tocopherol, selenium and L-cystine. Since the effect of L-cystine was later shown to be partly due to contamination of the amino acid with selenium (17), the relationship of vitamin E and Se was explored.

Diplock (17) noted that in vitamin E-supplemented rats, 36% of the total ^{75}Se in the mitochondrial fraction and 43%

in the microsomal fraction was present as [^{75}Se] selenide. In vitamin E-deficient rats, these figures dropped to 25 and 30%, respectively. When the fractions were dialyzed, the [^{75}Se] selenide was found to be retained in the membrane, suggesting that it was protein-bound. It was concluded that protein-bound selenide is a major component of rat liver subcellular organelles and that its presence depends on dietary vitamin E. Further studies (11) indicated that selenium and particularily selenide were located in the smooth and rough endoplasmic reticulum (ER) of rat liver. These same authors (12,13) later observed that incorporation of ^{75}Se in smooth ER after treatment with phenobarbitone (inducer of liver microsomal drug-metabolizing enzymes) was dependent on dietary vitamin E. Also an oxidant dependent non-heme iron protein was detected in the liver microsomes only in rats fed adequate levels of vitamin E and Se. They suggested that this protein was Se and vitamin E dependent with Se at its active center. They proposed that it acts as an electron carrier between the flavo protein and cytochrome P450 in the NADH-dependent electron transfer chain.

The real breakthrough came from Rotruck et al. (56) in 1972. Using rat erythrocytes in an in vitro system, they demonstrated that the role of Se in preventing oxidative damage to the membrane and hemoglobin of erythrocytes is

different than that of vitamin E and involves the utilization of glutathione in maintaining cell integrity. Further work by Rotruck et al. (57) demonstrated that Se was an integral part of the enzyme glutathione peroxidase. They offered an explanation for the interaction of Se, vitamin E and sulfur amino acids. These three nutrients often influence similar nutritional diseases because they produce a similar biochemical effect; the lowering of the concentration of peroxides or peroxide-induced products in tissues. Vitamin E prevents fatty acid hydroperoxide formation; sulfur amino acids, as precursors of GSH; and Se, as GSH-Px, are involved in peroxide destruction. Se rather than vitamin E would be more likely to protect susceptible non-membrane proteins against oxidation, whereas vitamin E would play a larger role in protecting tissues or subcellular components inherently low in GSH-Px.

Subsequently, Oh et al. (42) purified GSH-Px from ovine erythrocytes and Flohe et al. (21) purified bovine erythrocyte GSH-Px. Both groups of researchers demonstrated that there were four gram atoms of Se per molecule of enzyme. Since the enzyme has four subunits, it was assumed that there was one Se atom per subunit.

SELENIUM AND GLUTATHIONE PEROXIDASE

The function of GSH-Px had been established before Se was found to be associated with it. In the erythrocyte it is involved in the oxidation of glucose and is tied into the hexose monophosphate pathway because of its requirement for NADPH. The reaction sequence is diagrammed in Figure 1.

The relationship of Se and vitamin E is at best complex. In a study by Yang *et al.* (86) both an excess and a deficient level of vitamin E depressed activities of GSH-Px in liver, plasma and uterus. Scott *et al.* (67) varied both vitamin E and Se content of rat diets and discovered that in the presence of vitamin E (100 and 500 mg/Kg diet), a 0.4 ppm supplement of Se in the diet increased the activity of GSH-Px in liver and kidney over non-supplemented groups, but a 4.0 ppm Se supplement decreased the activity of GSH-Px in liver and kidney. This was not seen when vitamin E was not added to the diet. In that instance, the increasing level of Se in the diet increased the GSH-Px activity in liver and kidney. Red blood cell GSH-Px activity increased with each increase of Se supplementation at all levels of vitamin E supplementation (0, 100,and 500mg/Kg diet). It appears that the ability of vitamin E to increase GSH-Px activity occurs within a narrow range of vitamin E and Se supplementation and also varies with respect to the tissue.

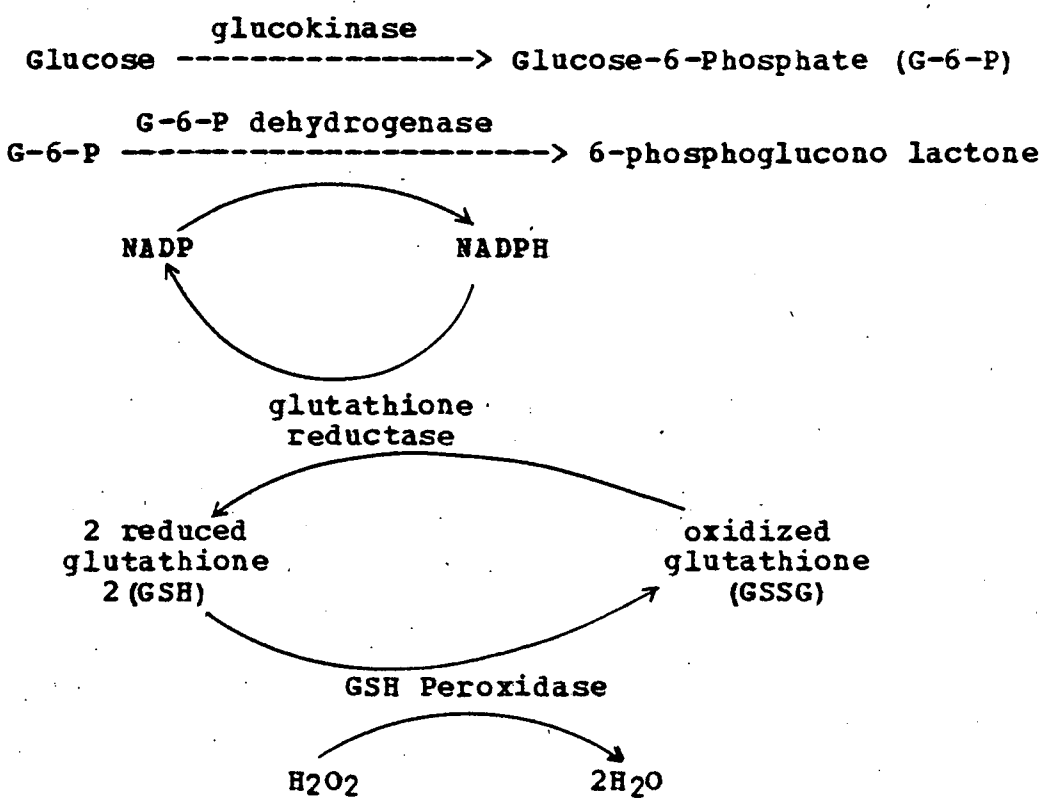

Figure 1: Reaction Pathway of Glutathione Peroxidase

The effect of dietary Se on the activity of GSH-Px was studied by several investigators. Chow and Tappel (16) fed rats a low Se diet for 17 days and then supplemented them with 2 ppm Se as selenomethionine for 11 days. They measured GSH-Px activity and found the most labile tissue for both depletion and repletion was plasma and the most stable was testes. The GSH-Px activity in plasma, kidney, heart, liver, lung and erythrocytes decreased when rats were fed a Se deficient diet and significantly increased when refed selenomethionine in the diet.

Hafeman et al. (28) conducted experiments to quantitate the relationship between dietary Se and activity of GSH-Px in erythrocytes and liver. Increased dietary Se always resulted in an elevated GSH-Px activity compared to any group receiving a smaller supplement except in the livers of rats receiving 5.0 ppm Se. This was attributed to hepatic damage caused by Se toxicity. In a second experiment they supplemented previously depleted rats with 3 levels of Se and found the erythrocyte GSH-Px activity increased with the response being more rapid and higher maxima reached at the higher levels of Se. In all cases this dramatic rise plateaued after approximately 60 days on the diet which suggested that Se is incorporated into GSH-Px only during erythropoeisis. They suggested that GSH-Px is a convenient

assay for Se nutriture, although other factors such as sex, age, vitamin E status, pro- and anti-oxidants should be considered.

The influence of dietary peroxides and Se on GSH-Px activity was studied by Reddy and Tappel (50). They fed rats a torula yeast based diet with either 15% tocopherol-stripped corn oil or 15% autoxidized corn oil with a peroxide value of 692 mEq/Kg. Rats fed these diets were further divided into groups fed either 0 or 2 ppm Se as selenomethionine. In the Se un-supplemented rats, the GSH-Px activity was higher in those fed the autoxidized corn oil than those fed the untreated oil. However, there were no significant differences in GSH-Px activity in Se-supplemented rats with or without peroxides. It would appear, from this study, that peroxides have a significant influence on GSH-Px activity only in rats fed Se-deficient diets.

In order to study the relationship of dietary Se to tissue GSH-Px (72), weanling rats were fed a low selenium torula yeast based diet supplemented with varying amounts of selenomethionine. After five weeks the GSH-Px activity was determined in liver, heart, kidney, erythrocytes, lung and muscle. A significant ($p<0.001$) linear correlation ($r>0.7$) between the GSH-Px activity in these tissues and the log of the dietary selenium was observed.

Depletion and repletion of dietary selenium was studied by Pedersen et al. (47). In the depletion experiment they found the liver tissue was the most responsive. The GSH-Px activity fell to undetectable levels by 4 weeks in the groups unsupplemented with vitamin E and Se, and by 10 weeks in the vitamin E-adequate, Se-deficient group. In the Se repletion experiment, Se-deficient weanling rats were fed either a Se-deficient diet or Se-adequate diet. Within 4 weeks there was a significant increase in GSH-Px activity of erythrocytes, liver, heart and kidney in the Se-supplemented rats. Although the liver was the most sensitive to change in Se status, the erythrocyte was viewed as a better indicator of overall Se status. The enzyme level of the erythrocytes of the rats fed the selenium-depletion diet remained the same over the 10 week period; however, the GSH-Px activity in the erythrocytes of the rats on the selenium repletion experiment increased within 30 days. Apparently the previous level of Se nutriture can affect GSH-Px levels in the red blood cells.

Sunde and Hoekstra (76) examined the rate of Se incorporation into GSH-Px in Se-adequate and Se-deficient rats. Rats were injected with [^{75}Se] selenite at physiological levels and sacrificed at intervals over a 72 hour period. ^{75}Se incorporation into GSH-Px was detected within 30 mi-

nutes in Se-adequate rats, but not until 2-3 hours in Se-deficient rats. After 72 hours, 70% of the supernatant ^{75}Se was incorporated into GSH-Px in the Se-adequate rats, but only 50% in the deficient animals. Injection of cycloheximide prior to injection of ^{75}Se prevented its incorporation, which indicates that Se is incorporated into GSH-Px during protein synthesis.

SELENIUM AND HUMAN STUDIES

Selenium research in humans has been haphazardly accumulated with major areas left unstudied. Some information is available regarding Se levels in disease states, particularly kwashiorkor and cancer. Since Se is associated with the protein components of animal tissues and foods, it is not suprising to see selenium deficiency in children with protein-calorie malnutrition or kwashiorkor. Schwarz (64) described two children with protein-calorie malnutrition who were not gaining weight with apparently adequate treatment. When given 25ug of selenium daily, the children immediately started to gain weight. Burk *et al.* (6) reported Guatemalan children with kwashiorkor had an average blood selenium content of 11 ug/100 ml compared with 23 ug/100 ml in controls.

Many studies correlated blood selenium levels and cancer. This was reviewed in 1972 by Shapiro (70). Schamberger et al. (69) observed that patients with hepatitis, cirrhosis or cancer of the colon, stomach or pancreas had significantly lower blood selenium than the controls or patients with rectal cancer or diabetes. In a New Zealand population, Robinson et al. (55) found that blood selenium levels of elderly controls were lower than those in young or middle aged controls. The blood levels of cancer patients were similar to those of the elderly population and patients with melanomas had the lowest blood Se levels. They suggest that the lower Se levels reported for cancer patients may be caused by the decreased Se consumption due to anorexia. If there was a causal relationship between poor Se status and cancer, New Zealand might be expected to have higher incidence and mortality rates from cancer, but this is not necessarily true.

Two groups of people who are often studied to uncover nutritional deficiencies are pregnant women and infants. At these two times in life, rapid growth can impose a stress that can demonstrate an increased requirement. In one study pregnant women were shown to have a significant decrease in both plasma GSH-Px and Se by the third trimester. This was not skewed by the hemodilution observed during pregnancy (5).

Studies on Se and GSH-Px levels in the blood of infants and children (38,58) have revealed that these parameters are lower in infancy and early childhood in normal children. These levels are even lower in children consuming restricted or synthetic protein diets such as those prescribed for children with phenylketonuria or maple syrup urine disease. The diets of New Zealand residents contain less Se and the blood Se and GSH-Px concentrations of children living there are accordingly lower than those reported for children living in other countries. Clinical examinations of children with low Se concentrations did not reveal any abnormality which could be interpreted as a Se deficiency.

The strongest evidence for a deficiency of Se in humans is Keshan disease (2,14,32,33). It has been described by Chinese researchers as an endemic cardiomyopathy of unknown cause. It is seen mainly in the mountainous regions of China and afflicts primarily children below 10-15 years of age and women of child bearing age. The population in afflicted regions have lower Se levels in blood, hair and urine and lower GSH-Px levels in blood. This is in accordance with lower consumption of Se in their food supply. When target populations were supplemented with sodium selenite, the incidence of the disease and its mortality were dramatically improved. The authors are cautious in designating Se as the

sole factor responsible for the occurrence of Keshan disease because it alone would not explain other epidemiological characteristics. A recent report by Johnson et al. (31) described a patient who had received total parenteral nutrition for two years. The patient eventually died and the autopsy revealed a cardiomyopathy similiar to that described as Keshan Disease. Red blood cell Se and GSH-Px were measured and found to be only 10% of normal values.

Van Rij et al. (81) reported a Se-responsive syndrome of a patient on total parenteral nutrition. The symptoms included pain in the thigh muscles on passive and active movement which inhibited mobility, and tenderness in the involved muscle. The authors compared this to the symptoms of white muscle disease seen in Se-deficient sheep. After one week of intervenous infusion of selenomethionine, the patient was asymptomatic. Numerous anecdotal reports emerge from regions in New Zealand where residents report they improved muscular aches by self-medication with Se. Whether these reports are similar to the muscular conditions of this patient is not known.

Studies describing Se intake and effect of supplementation have been conducted by several investigators. Schrauzer and White (60) reported Se intakes for ten young adult Californians were estimated at 90 to 186 ug/day. The high-

est intakes were observed in individuals selecting diets high in whole grain cereal products and seafood. The Se concentrations of the whole blood correlated significantly with the dietary Se intakes. When the subjects were supplemented with 150 ug Se/day, the whole blood Se increased from 0.15 ug/ml to 0.21 ug/ml within three weeks. GSH-Px activities were not found to be correlated with dietary Se or whole blood levels of Se. In contrast, Robinson et al. (53) found a very high correlation between whole blood Se and GSH-Px in 264 New Zealand residents, but not in New Zealand residents returning from overseas visits or new settlers. Since New Zealand residents have some of the lowest blood levels of Se in the world, it may be that a correlation between Se and GSH-Px is present only in the lower ranges. Valentine et al. (80) studied the relationship between whole blood selenium and erythrocyte GSH-Px activity in the non-deficient state. Their population consisted of 33 residents who had consumed well water for one year or more that contained 0.026 to 1.8 ppm Se. Se concentration in whole blood (0.17 ppm) was comparable to Schrauzer and White's (60) values (0.15 ppm) and neither observed a correlation between Se concentration and GSH-Px. This is also in agreement with Schmidt and Heller (59) who observed no correlation between enzyme activity and Se concentration of hu-

man erythrocytes. In contrast, Rudolph and Wong (58) observed a close positive correlation (r=0.86) in both plasma and red blood cells, in their human maternal and cord blood samples.

BIOAVAILABILITY

After the nutritional value of Se was established, the measurement of this element in foods consumed by various population groups was undertaken (20,29,35,40,41,61). Fruits and vegetables are generally recognized as poor sources; however, some vegetables such as garlic, mushrooms and asparagus can contain appreciable quantities. Dairy products and eggs are variable in Se content depending on the Se content of the grain fed to the animals. Grain products can be quite high or low based on the Se content of the soil where they were grown. Muscle meats have generally good quantities, and organ meats and seafoods have high Se concentrations. The form of Se in these foods is generally unknown; however, Olson *et al.* (44) were able to account for almost half the Se in wheat as selenomethionine.

Bioavailability studies on Se have been done using a number of animal models and a review of some of these approaches is presented. Se deficiency in the chicken is manifested by exudative diathesis (ED) and pancreatic degenera-

tion even in the presence of dietary vitamin E. In a study by Cantor et al. (8), protection against ED was used as one measure of bioavailability. They found that for the plant products tested, percent bioavailability ranged from 59.8 to 88.6% with the exception of alfalfa meal which was 210%. The biological availability of the products of animal origin were all lower, 8.5 to 24.9%. The bioavailability of organic and inorganic Se compounds ranged from 89.3% for sodium selenate to 7.4% for elemental Se. Since plant products are known to have about 40% selenomethionine, these investigators wanted to compare the bioavailability of selenite versus selenomethionine. Since protection against ED is highly correlated with plasma GSH-Px, they measured plasma levels of GSH-Px and Se in chicks that were fed graded levels of selenite or selenomethione. The regression coefficient for the relation of plasma GSH-Px to dietary Se levels obtained for chicks fed selenite was significantly greater than that for selenomethionine, suggesting a difference in the utilization of these two compounds. They concluded that the biological availability seems to be determined by the ability of the chick to utilize Se for GSH-Px activity and selenite is clearly superior to selenomethionine for this purpose.

In another study, Cantor et al. (9), evaluated the efficacy of Se from selenite, selenomethionine, selenocystine, wheat and tuna meal in preventing pancreatic fibrosis in chicks. Histological examination of the pancreas indicated that wheat and selenomethionine were the most effective sources of Se. When they were unable to show any relationship between enzyme activity in plasma and pancreatic GSH-Px and prevention of the deficiency disease, they suggested that perhaps the role of Se in protecting the pancreas is distinct from its effect on GSH-Px activity. Osman and Latshaw (45) fed selenite, selenomethionine and selenocystine and confirmed that selenomethionine was less effective in preventing ED than selenite or selenocystine. However, more Se was retained in the pancreas and muscle in hens fed selenomethionine than selenite or selenocystine. Latshaw (34) looked further into the differential distribution of Se compounds and fed laying hens a diet that had either 0.10 mg/Kg or 0.42 mg/Kg natural Se or 0.42 mg/Kg selenite Se. The natural selenium diets were formulated from identical ingredients; one obtained from a low Se area and the other from a high Se area. He found that breast muscle, egg white, liver and plasma contain more Se if the hens were fed a natural source of Se than if fed selenite.

Research on the efficacy of different Se compounds in preventing white muscle disease was initiated long before Se was recognized as an integral part of GSH-Px. Ehlig et al. (19) fed selenite and selenomethionine to lambs and reported that those given selenite excreted more Se in their urine than lambs given selenomethionine. Furthermore, Se retention was greater for lambs fed selenomethionine than selenite. According to their measurements, absorption of the two forms was the same, but the excretion varied. Later Jenkins and Hidiroglou (30) fed ^{75}Se labeled selenite and selenomethionine to lactating ewes and compared them as sources of Se for suckling lambs. The source of Se affected its binding to milk proteins in colostrum; however, in mature milk, Se was carried by the caseins, regardless of source. ^{75}Se concentrations were higher in the serum proteins and tissues of the lamb whose dam received selenomethionine, which seems to indicate that selenomethionine is more available to the lamb than selenite. This finding was confirmed by Fuss and Godwin (24) who injected ^{75}Se as selenite and selenomethionine into ewes and noted that the entry of the isotope into milk was rapid and greater when selenomethionine was administered than when selenite was given. In contrast, greater amounts of activity from selenite were found in plasma proteins in both ewes and lambs.

Rats have also been used to study the metabolism of organic and inorganic selenium. Cary et al. (10) measured retention and tissue distribution of different forms and levels of dietary Se fed to Se-depleted rats. As was seen with lambs, rats fed selenomethionine retained more Se than those fed selenite. This was believed to be due to a greater urinary excretion of the selenite-fed group. The concentration of Se in muscle of rats fed selenomethionine was higher than in rats fed selenite. Se concentrations in the other tissues were generally related to the level of dietary Se and not the source of dietary Se.

An examination of the effect of dietary methionine on the bioavailability of selenite and selenomethionine in the rat was recently reported by Sunde et al. (73). They quantitated bioavailability by determining a change in levels of GSH-Px in liver, plasma and heart over a one-week period in Se-deficient rats. They found that sub-optimal dietary methionine decreases the bioavailability of selenomethionine but not selenite, suggesting that selenomethionine is incorporated into protein in lieu of adequate methionine. Another nutrient which affects bioavailability of Se compounds is vitamin B6. Yasumoto et al. (87) fed a diet deficient in Se and vitamin B6 to rats for 2 weeks, then added back vitamin B6 or Se or both for 2 or more weeks. In the presence

of vitamin B6, both selenite and selenomethionine increased GSH-Px activity in liver and erythrocytes. In the absence of vitamin B6, selenomethionine was less effective in the liver and ineffective in increasing GSH-Px above basal levels in the erythrocytes. Selenite performed equally well, regardless of the presence or absence of vitamin B6.

Another way to study bioavailability is through the use of isotopes. Thomson et al. (78) compared the long term fate of an oral dose of [^{75}Se] selenocystine with that of [^{75}Se] selenomethionine in the rat. They found that whole body retention of ^{75}Se was greater with the [^{75}Se] selenomethionine than [^{75}Se] selenocystine for the first week and this difference was maintained throughout the study. Urinary loss of absorbed ^{75}Se derived from selenocystine was greater than that from selenomethionine. This is similar to findings of other researchers using selenite and selenomethionine (10,19). In another study by the New Zealand group (52), ^{75}Se as selenite or selenomethionine incorporated in vivo into fish muscle was compared with the metabolism of [^{75}Se] selenite and [^{75}Se] selenomethionine which were fed to rats with unlabelled fish homogenate. Intestinal absorption of ^{75}Se given as labelled fish homogenate was less complete than that of ^{75}Se mixed with unlabelled fish homogenate. Although there were differences in urinary excretion

of absorbed ^{75}Se, tissue distribution of retained ^{75}Se and long term whole body turnover rate were similar in all groups. This does not confirm other reports (10,19,78) where a difference in retention of various forms of selenium is observed.

Very few studies have been done on the bioavailability of different Se compounds in humans. Robinson et al. (54) supplemented the daily intake of three subjects: one woman with 100 ug Se as selenomethionine, one man with sodium selenite for 10-11 weeks and one woman with 65 ug Se as mackerel for 4 weeks. Only a small attempt was made to control their Se intake over the dietary period. They refrained from liver and kidney ,at least, before urine and fecal collections, and their intake of fish was monitored. Obviously this was only a pilot study so firm conclusions can't be drawn. The subjects consuming the selenomethionine and mackerel had increasing levels of blood Se throughout the study, whereas the subject eating the selenite plateaued after about 7 weeks. The selenite selenium appeared to be less well absorbed and more of the absorbed Se was excreted than in the other two forms. In terms of intake, approximately 0.5 of the intake of selenomethionine and fish Se was retained compared with 0.3 of the intake of the selenite Se. In another study by these researchers (26), the long-term

fate of an oral dose of 20 uCi of [^{75}Se] selenomethionine was studied in four women. Intestinal absorption of the dose was 95.5 to 97.3%. Plasma ^{75}Se concentration reached a maximum within three to four hours and erythrocytes reached a maximum at 8-12 weeks. No radioactivity was detected in expired air.

Levander *et al.* (36) examined the bioavailability of wheat in six young men. Using a depletion/repletion design, they demonstrated a rapid response in urinary and fecal Se to increased dietary intake. Plasma Se responded to increased Se supplementation in 11 days, but erythrocyte Se and GSH-Px, although they steadily decreased during the 44 day depletion, showed no response to supplementation.

Bioavailability of different Se compounds has also been studied *in vitro*. Sunde and Hoekstra (75) used an isolated perfused rat liver to study incorporation of Se into GSH-Px. They found a 9-fold excess of unlabelled selenite or selenide was very effective in reducing incorporation of ^{75}Se from [^{75}Se] selenocystine into GSH-Px, but a 100-fold excess of selenocystine was relatively ineffective compared to selenite and selenide in reducing ^{75}Se incorporation from [^{75}Se] selenite. They concluded that selenide or selenite are more readily available for incorporation into GSH-Px, perhaps because they are more easily metabolized to the im-

mediate Se precursor used for GSH-Px synthesis than seleno-cystine.

III. METHODS

RAT EXPERIMENT

Rats

In order to obtain maximum responses to Se feeding, Se-deficient weanling rats were used. The procedure of Schwarz (63) was followed to produce Se deficient rats. Basically this involved placement of the dam and her pups on a Se-and vitamin E-deficient diet when the young were about seven days old. At about 24 days of age the pups were weaned onto the test diets. OSU Brown rats from the Agricultural Chemistry colony were used with three male and three female rats per treatment.

Diets

The percentage (by weight) composition of the pre-weaning diet was: torula yeast, 40; sucrose, 41.5; Solka Floc, 7.5; tocopherol-stripped lard, 5; mineral mix,[1] 5; vitamin mix,[2] 1; and vitamins A (10 mg/Kg) and D (100 ug/Kg).

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[1] Mineral mix was Hubbell-Mendel-Wakeman Salt mix (J. Nutr. 14, 270 (1937).

[2] The vitamin mix had the following composition: thiamin HCl, 1 gm; riboflavin, 1 gm; pyridoxine HCl, 600 mg; Ca-D pantothenate, 4 gm; choline-dihydrogen citrate, 424 gm; niacin, 20 gm; menadione, 200 mg; folic acid, 400 mg; biotin, 200 mg; B12 0.1% trit., 20 gm; lactose, 1750 gm.

The test diets were the same composition as the pre-weaning diet with the exception of added Se, and the tocopherol-stripped lard was replaced by corn oil which added approximately seven mg α-tocopherol/Kg diet. The test ingredients were analyzed for Se content and added to the diets in place of part of the solka floc depending on the concentration of Se in each ingredient. Each of the Se-containing diets was fed at three levels: 0.05, 0.10 and 0.15 ppm Se. This gave 22 diets including the basal diet (no added Se). Food and water were available to the animals ad libitum. Food consumption was recorded.

The seven Se sources tested were sodium selenite, raw tuna, retorted or precooked tuna, canned tuna (packed in oil), wheat bran, whole wheat flour, and whole wheat bread prepared with water, yeast, salt and sugar. The wheat (North Dakota Hard Red Spring Wheat) was obtained from Dallas, South Dakota and was milled at the Western Quality Laboratory in Pullman, Washington. The wheat products were high in Se (3.5-5.6 ppm) which reflects the high Se content of the soil in South Dakota. The tuna products were provided from Bumble Bee Seafoods, Astoria, Oregon, and were sampled from their usual production. Their Se content ranged from 0.9 to 1.7 ppm Se.

Sample Preparation

The rats were fed the diet for four weeks at which time they were anesthetized with ether and decapitated. Blood was collected in citrated tubes and 0.25 ml of whole blood was immediately frozen on Dry Ice and saved for the GSH-Px assay. The remainder of the blood, liver, kidneys and muscle were saved and frozen at -20°C for Se and GSH-Px assays. Initial and final rat weights were recorded and a record of food consumption for the four-week period was kept. Liver and kidney weights were noted at the time of sacrifice.

The liver and kidneys were prepared for the GSH-Px assays as follows: one kidney or approximately 2 grams of liver tissue were briefly homogenized in 4 or 5 ml, respectively, of 10% sucrose in 0.1M phosphate buffer per gram tissue. After the homogenate was centrifuged at 3°C for 10 minutes at 10,000 x g, the supernatant was filtered through glass wool. GSH-Px activity was determined in the soluble fraction. The 0.25 ml of whole blood was diluted to 1.5 ml with 0.85% NaCl solution. A 0.5 ml aliquot of this hemolysate was mixed with an equal volume of Drabkins solution (39) and diluted to 7 ml with double distilled water. This is the hemolysate on which the enzyme assay was performed.

Laboratory analyses

Glutathione peroxidase activity was determined by the method of Paglia and Valentine (46) using hydrogen peroxide as substrate and added GSH reductase.[3] The hemoglobin concentration in the hemolysate was determined using the method of Shenk et al. (71) and the protein content of the tissue soluble fraction was measured by the Lowry method (37).

Selenium was determined in liver, kidney, muscle and whole blood. The samples were first pre-digested overnight in 10 ml nitric acid and 3 ml perchloric acid, in 50 ml acid-washed erlenmeyer flasks. They were then wet-digested on a hot plate until 15 minutes after the appearance of perchloric acid fumes, at which point 1 ml of concentrated HCl was added and digestion continued for 15 minutes longer. Fifteen ml of 0.027 M EDTA and 2 drops of the combination indicator bromcresol green and cresol red were added to the flasks. The samples were titrated with 5N ammonium hydroxide to pH 2-3. The samples were run on an automated system which extracted the Se into cyclohexane and mixed it with a solution of 2,3 diaminonaphthalene (DAN) (43) and 0.8M hydroxyl amine. The samples were activated at 369 mu and fluoresence was measured at 525 mu. Se concentration was determined by comparisons against a standard curve run along

[3] GSH reductase was obtained from Sigma Chemical Co., St. Louis, MO.

with the samples. The standards were prepared according to Olson (43).

HUMAN EXPERIMENT

Experimental Design

The balance study consisted of a 10-day adjustment period followed by three 14 day experimental periods, in a 3x3 Latin Square design. Three male subjects were initially assigned to one of three diets. Then they rotated to a different diet in each diet period (Table 1). The three dietary treatments were the basal diet plus canned tuna, whole wheat bread or peanut butter. Since subject #6 dropped out before the completion of the first experimental period, no data were collected on him.

The selenium content of the diets ranged from 331.5 to 354.5 ug/day. Since another part of this study involved the bioavailability of vitamin B6,[4] it was not possible to keep the Se constant from one period to another. The peanut butter was also included for the purpose of studying B6 metabolism. Peanut butter, which supplied only 43 ug Se per day, was supplemented with 225 ug/day of selenized yeast during this treatment period to maintain a relatively constant Se intake throughout the three experimental periods. The Se

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[4] Results from this study will be published elsewhere.

TABLE 1

Assignment of Subjects to Diets

Experimental Period	Diets		
	Tuna	Whole Wheat Bread	Peanut Butter
	Subject Number		
I	2,3,5	4,8,9	1,7
II	7,8,9	1,5	2,3,4
III	1,4	2,3,7	5,8,9

balance study data does not include values from the subjects when they were eating the peanut butter diet.

Diets

The menu for the basal diet is presented in Table 2 The Se content of the basal diet was analyzed to be 78.6 ug Se/day. During the adjustment period 70 gm of cheddar cheese and 270 gm (raw weight) of ground beef were added, bringing the Se level to 92.3 ug/day. For the tuna test period, 293 gm of drained, oil packed tuna[5] were added to the basal diet. The Se content of this diet was 331.5 ug/day (275.9 ug from the tuna). For the wheat test period, 415 gm of whole wheat bread[6] providing 252.9 ug Se/day was added to the basal diet for a total Se content of 354.5 ug/day. During the peanut butter test period, 252 gm of smooth peanut butter were consumed along with the basal diet. This diet provided 43 ug Se/day from the peanut butter, 225 ug Se

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[5] The tuna was provided by Bumble Bee Seafoods, Astoria, Oregon. It was from a regular plant run made up of extra small yellow fin. It was brine frozen from a purse seiner, unloaded in Astoria. The fish were from five to eight pounds in size. The cans were packed with 5% vegetable broth and soybean oil to fill and 1.2% salt added.

[6] The bread was made from a blend of whole wheat flours in order to approximate the Se content of the tuna. The flours were the high-Se, North Dakota Hard Red Spring from Dallas, S.D., and a low-Se (0.006 ppm) Soft White and Hard Red blend from Corvallis, OR. The bread was baked in a commercial bakery in Corvallis, OR.

from the selenized yeast and 78.6 from the basal diet, giving a total of 346.6 ug Se/day.

All foods were bought in case lots to insure uniformity. Food composites were made weekly and analyzed for Se content. The diet was supplemented with margarine, hard candy, 7-Up and honey as necessary to maintain the subjects' weight. The diet contained 100% of the recommended dietary allowances (23) for 23+ year old males. The caloric content of the diets was computed to be 2478 Kcal for the adjustment diet, 2245 Kcal for the tuna diet and 2675 Kcal for the wheat diet. The subjects took no drugs or vitamin supplements and did not drink any alcoholic beverages during the study.

TABLE 2

Menu for the basal and experimental diets fed to human male subjects

Breakfast:		
Orange juice, frozen, reconstituted	250 g	
Rice cereal, ready-to-eat	35 g	
Bread, white, enriched	25 g	
Milk (22.5 g dry nonfat)	240 g	reconstituted
Margarine, Honey, 7-Up, Hard candies	variable, available at every meal to maintain weight	
Lunch:		
Bread, white, enriched	25 g	
Carrots, raw	80 g	
Raisins	56 g	
Milk (22.5 g dry nonfat)	240 g	reconstituted
Pears, canned	100 g	drained
Pear syrup	20 g	
Dinner:		
Casserole: Rice	45 g	(raw)
Tomato juice, canned	100 g	
Water	30-40 ml	
NaCl, 10% solution	10 ml	
Green beans	65 g	drained
Liquid from canned beans	10 g	
Corn, whole kernel, canned	30 g	
Peaches, canned	100 g	drained
Peach syrup	20 g	
Ice cream, vanilla	80 g	
Celery, raw	80 g	
Bread, white, enriched	25 g	
Snack:		
Vanilla sandwich cookies	3 cookies	

TEST FOODS ADDED ONE AT A TIME

Adjustment Diet:	Cheddar Cheese, at lunch	70 g
	Ground Beef, at dinner	270 g
Tuna Diet:	Tuna, oil pack, drained	293 g
	Mayonaise-type salad dressing	as desired
Wheat Diet:	Whole Wheat Bread	415 g
Peanut Butter Diet:	Peanut Butter	252 g

The foods were purchased in case lots to insure uniformity. The tuna was supplied to Bumble Bee Seafoods, Astoria, OR from their regular plant run made up of extra small yellow fin. The bread was made from a blend of whole wheat flours. The flours were high Se, North Dakota Hard Red Spring and low Se, Soft White amd Hard Red blend. The bread was baked in a commercial bakery in Corvallis, OR.

Subjects

The subjects were eight young men, 21-30 years old who were students at Oregon State University. Their descriptive data are presented in Table 3. They had no known metabolic disease and exhibited normal xylose absorption in response to a 5 gm dose of L-xylose (7,27). They had normal values for liver function and blood chemistry. The latter analyses were performed at Good Samaritan Hospital, Corvallis, OR (Hycel Super 17 Sequential Multiple Analyzer, Hycel Inc., Houston, TX). Subjects were not confined to the laboratory during the study; however, they were required to consume all their meals in the metabolic facility in the School of Home Economics during supervised time periods. They weighed themselves daily before breakfast, and collected all excreta in designated containers. They refrained from strenuous physical activity and continued their normal daily activities which included a full academic schedule. This investigation was approved by the Human Subjects Committee at Oregon State University. Before participating in this study, the subjects signed an informed consent form approved by this committee.

TABLE 3

Descriptive data of the male subjects

Subject Number	Age yrs	Height cm	Weight Initial kg	Weight Final kg
1	24	17.3	69.0	70.8
2	29	17.5	83.0	85.3
3	28	19.1	89.8	89.8
4	27	17.3	64.0	65.3
5	21	17.0	66.2	66.7
6	21	18.8	92.1	---
7	30	17.8	67.1	69.4
8	21	18.0	70.3	70.3
9	21	17.3	69.0	68.5
Mean ±SD	24.7 ±3.8	17.8 ±0.7	74.5 ±10.8	73.3 ±9.1

SAMPLE COLLECTION

Twenty-four hour urine collections were made every day of the study. The urine was collected under toluene and refrigerated. The following day, the entire sample was mixed, measured and aliquots were frozen for future analyses.

Complete fecal collections were made and stored in the freezer until mixing. The feces were separated by the fecal marker FDC Blue #1 (50 mg FDC Blue #1 plus 200 mg methyl cellulose), which was given to the subjects at breakfast every four or five days. Four or five day fecal collections were mixed and weighed. An aliquot was removed and refrozen until analysis.

Blood was drawn into heparinized Vacutainers by a registered medical technologist before breakfast at regular intervals during each period. Se analysis was done on every whole blood sample, GSH-Px activity was determined only at the beginning and end of each dietary period. For the GSH-Px assay, a 250 ul aliquot was frozen on dry ice as in the rat experiment.

Laboratory Analyses

Selenium assays were performed on composites of each subjects' fecal collections, whole blood samples and every third day for the urine samples. If there was reason to suspect that the urine specimen might not be complete, based

on creatinine content or the subject's report, then the urine specimen from another day was substituted. Selenium was determined by the same method as in the rat experiment.

Glutathione peroxidase activity was measured in the whole blood samples at the beginning and end of each diet period. The same method was used, but two different substrates, hydrogen peroxide and t-butyl hydrogen peroxide, were employed.

Completeness of urine collections was checked by measuring creatinine (Technicon Autoanalyzer, Technicon Corp., Tarrytown, NY) by an automated procedure using alkaline picrate (48). Hemoglobin and hematocrit were determined weekly in each subject by standard procedures. These two indices remained constant over the study.

IV. RESULTS

RAT EXPERIMENT

Tables 4-8 contain the descriptive data collected on the rats: food consumed over the four week period, weight gain, final weight, liver weight and kidney weight. There were no significant differences among tuna treatments or bread treatments at any one level. When the three bread groups and the three tuna groups were combined and compared to each other and the selenite (SeO_3^{2-}) fed groups (Tables 9-13), there were some isolated significant ($p<0.05$) differences and they reflected a general trend of the wheat eating animals eating more and weighing more than the selenite and tuna fed animals. More specifically, the wheat-fed animals consumed more food (Table 9) at all dietary levels than the tuna or selenite-fed animals. This was significantly different ($p<.05$) only at the 0.10 ppm level when the wheat fed animals consumed 445.0±93.3 gms over the four weeks and the selenite-red animals consumed 300.0±52.3 gms. The wheat-fed animals always gained more weight than the tuna- or selenite- fed animals, but this was significant ($p<.05$) only in one comparison, between the wheat-fed rats (119.7±26.4) and the selenite-fed rats (91.7±9.9) at the 0.05 ppm diet level. Consequently the final weight of the rats followed the same pattern (Table 11). The wheat-fed animals were consistently

heavier than the selenite- or tuna-fed animals. This was significant ($p<.05$) only between the wheat- (170±27.3) and selenite-fed rats (137.7±10.8) at the 0.05 ppm dietary level. The rat kidney weights were very similar (Table 13), except for those fed at the 0.05 ppm dietary level. At this level, both the selenite- and tuna-fed rats had kidneys significantly ($p<.05$) smaller (1.2±0.1 and 1.3±0.2 gms respectively) than the wheat-fed animals (1.6±0.2 gms). In this instance this did not represent a trend because five out of the six remaining groups had a mean kidney weight of 1.6 gms. No explanation for this difference can be offered.

Tables 14 and 15 present the data on liver Se content. The most significant observation is that rats consuming canned tuna have higher liver Se than rats eating precooked tuna or raw tuna. This is observed at all three levels of Se in the diet and is statistically significant ($p<.05$). When the combined tuna products and combined wheat products were compared with the selenite fed group, there was no consistent significant difference (Table 15). However, those rats fed tuna at 0.10 and 0.15 ppm Se diet levels were lower than the other two groups, but only those fed the 0.10 ppm Se in tuna were significantly different ($p<.05$). Regression equations were calculated for each biochemical parameter; liver selenium, liver GSH-Px, kidney selenium, kidney GSH-Px, whole blood selenium, whole blood GSH-Px and muscle sel-

TABLE 4

Food consumed (gms) by rats over four week period

Level of Se (ppm)	Source of Se						
	SeO_3^{2-}	Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	325 ±25	394 ±57	379 ±33	346 ±17	378 ±80	430 ±123	414 ±117
.10	*300 ±52	375 ±49	407 ±61	367 ±72	446 ±101	440 ±96	450 ±101
.15	410 ±70	407 ±53	391 ±52	459 ±111	424 ±130	467 ±196	428 ±145
0	284 ±33						

Mean±SD
Number of rats per group (N)=6 (*N=5)

TABLE 5

Weight gained (gms) by rats over four week period

Level of Se (ppm)	SeO_3^{2-}	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	91.7 ±9.9	103.3 ±27.4	112.2 ±19.2	99.0 ±13.3	114.7 ±16.6	122.7 ±31.8	121.7 ±32.2
.10	*95.0 ±18.6	119.0 ±28.4	128.5 ±26.9	107.7 ±33.8	120.7 ±29.0	120.0 ±11.3	110.2 ±11.0
.15	119.7 ±28.1	122.3 ±24.2	117.3 ±16.1	127.8 ±51.3	120.7 ±33.0	144.2 ±47.8	118.7 ±35.4
0	73.0 ±6.6						

Mean±SD
N=6 (*N=5)

TABLE 6

Final weight (gms) of rats after four weeks on diets

Level of Se (ppm)	SeO_3^{2-}	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	137.7 ±10.8	150.2 ±25.9	162.0 ±20.6	144.3 ±10.3	165.8 ±22.1	171.2 ±29.0	174.3 ±34.0
.10	*144.4 ±17.1	170.0 ±33.4	181.2 ±33.0	159.3 ±32.4	176.0 ±27.0	177.0 ±17.6	169.7 ±23.0
.15	175.0 ±33.0	175.7 ±23.9	170.8 ±21.3	183.5 ±55.5	175.2 ±36.4	201.7 ±58.7	176.3 ±47.4
0	117.2 ±10.4						

Mean±SD
N=6 (*N=5)

TABLE 7

Liver weight (gms) of rats after four weeks on diets

Level of Se (ppm)	Source of Se						
	SeO_3^{2-}	Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	6.8 ±0.8	6.9 ±1.3	8.0 ±1.3	6.8 ±0.6	7.5 ±0.3	7.9 ±1.1	7.6 ±1.5
.10	*7.1 ±1.1	8.0 ±1.6	8.6 ±1.0	7.8 ±1.3	7.7 ±1.1	8.0 ±0.9	7.0 ±1.0
.15	8.2 ±1.5	7.9 ±1.1	8.4 ±0.8	7.7 ±2.8	7.6 ±1.7	8.2 ±2.1	7.7 ±1.6
0	5.9 ±0.8						

Mean±SD
N=6 (*N=5)

TABLE 8

Kidney weight (gms) of rats after four weeks on diets

Level of Se (ppm)	Source of Se						
	SeO_3^{2-}	Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	1.2 ±0.1	1.4 ±0.2	1.4 ±0.1	1.3 ±0.1	1.5 ±0.2	1.6 ±0.2	1.6 ±0.2
.10	*1.4 ±0.1	1.6 ±0.3	1.7 ±0.1	1.6 ±0.2	1.6 ±0.2	1.7 ±0.2	1.6 ±0.2
.15	1.6 ±0.2	1.6 ±0.3	1.6 ±0.2	1.7 ±0.4	1.6 ±0.2	1.7 ±0.4	1.6 ±0.3
0	1.1 ±0.1						

Mean±SD
N=6 (*N=5)

TABLE 9

Food consumed by rats (gms): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	325.2 ±25.1	373.3 ±42.5	407.2 ±104.1
.10	300.0[1] ±52.3 (N=5)	383.2 ±60.1	445.0[1] ±93.3
.15	409.5 ±70.0	419.1 ±78.4	439.9 ±151.3

Matching superscripts indicate significant differences at $p<.05$.

Mean±SD

TABLE 10

Weight gained (gms) by rats: Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	91.7[a] ±9.9	104.8 ±20.3	119.7[a] ±26.4
.10	95.0 ±18.6 (N=5)	118.4 ±29.4	116.9 ±18.6
.15	119.7 ±28.1	122.5 ±32.3	127.8 ±38.8

Matching superscripts indicate significant differences at $p<.05$.

Mean±SD

TABLE 11

Rat final weight (gms): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	137.7[1] ±10.8	152.2 ±20.2	170.4[1] ±27.3
.10	144.4 ±17.1 (N=5)	170.2 ±32.3	174.2 ±21.7
.15	175.0 ±33.0	176.7 ±35.2	184.4 ±47.2

Matching superscripts indicate significant differences at $p<.05$.

Mean±SD

TABLE 12

Rat liver weight (gms): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Bread Groups (N=18)
.05	6.8 ±0.8	7.2 ±1.2	7.7 ±1.1
.10	7.1 ±1.1 (N=5)	8.1 ±1.3	7.6 ±1.0
.15	8.2 ±1.5	8.0 ±1.7	7.8 ±1.7

Mean±SD

TABLE 13

Rat kidney weight (gms): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	1.2[1] ±0.1	1.3[2] ±0.2	1.6[1,2] ±0.2
.10	1.4 ±0.1 (N=5)	1.6 ±0.2	1.6 ±0.2
.15	1.6 ±0.2	1.6 ±0.3	1.6 ±0.3

Matching superscripts indicate significant differences at $p<.05$.

Mean±SD

enium, regressed on the actual amount of selenium consumed by each rat. This was calculated by multiplying the total food consumed in the four week period by the level of selenium in the diet.

Tables 16-18 delineate the liver GSH-Px activity data. Table 16 indicates that there are no significant differences among the three tuna treatments or three bread treatments, at any dietary level. Table 17 reveals a trend, often significant ($p<.05$), of rats consuming wheat products having higher liver GSH-Px than selenite fed and tuna fed rats. Since there appeared to be a sex difference in GSH-Px activities in the liver, the male and female rats were separated, and the mean and standard deviations recalculated (Table 18). The female rats had higher levels of GSH-Px than the males, in every comparison. Unfortunately due to the small number of animals and high standard deviation, this was significant in only 6 out of 22 comparisons.

Data for kidney Se are presented in Tables 19 and 20 Rats fed canned tuna had significantly more ($p<.05$) kidney Se than rats fed raw tuna or precooked tuna at the two higher levels fed (Table 19). There were no significant differences among the wheat eating animals. However, when the combined tuna and wheat groups were compared with the selenite groups (Table 20), the wheat fed animals always had higher kidney Se than the tuna fed and two out of three

TABLE 14

Selenium content (ppm) of rat liver

Level of Se (ppm)	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	0.15[1,3] ±0.02	0.20[1,2] ±0.01	0.26[2,3] ±0.02	0.20 ±0.07	0.22[9] ±0.08	0.13[9] ±0.04
.10	*0.19[4,6] ±0.03	0.23[4,5] ±0.02	0.45[5,6] ±0.05	0.65 ±0.31	0.69 ±0.30	0.59 ±0.27
.15	0.45[7] ±0.16	0.46[8] ±0.16	1.02[7,8] ±0.10	0.91 ±0.26	0.84 ±0.21	0.72 ±0.09

Mean±SD
Matching superscripts indicate significant differences at $p<.05$
N=6 (*N=5)

Regression equations for rats fed:

Selenite:	$y = 0.01x + 0.01$ ($r=0.87$, $p<0.05$)
Raw Tuna:	$y = 0.007x + 0.006$ ($r=0.86$, $p<0.05$)
Precooked Tuna:	$y = 0.007x + 0.03$ ($r=0.87$, $p<0.05$)
Canned Tuna:	$y = 0.01x + 0.04$ ($r=0.93$, $p<0.05$)
Whole Wheat Flour:	$y = 0.01x - 0.001$ ($r=0.91$, $p<0.05$)
Whole Wheat Bread:	$y = 0.01x + 0.12$ ($r=0.77$, $p<0.05$)
Bran:	$y = 0.01x + 0.02$ ($r=0.85$, $p<0.05$)

TABLE 15

Rat liver Se (ppm): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	0.19 ±0.04	0.20 ±0.05	0.18 ±0.07
.10	0.38 ±0.06 (N=5)	0.29[1] ±0.12	0.65[1] ±0.28
.15	0.81 ±0.33	0.64 ±0.30	0.82 ±0.20

Matching superscripts indicate significant differences at $p<.05$.
Mean±SD

Regression equations for rats fed:
Selenite: $y = 0.01x + 0.01$ ($r=0.87$, $p<0.05$)
Tuna: $y = 0.009x + 0.001$ ($r=0.76$, $p<0.05$)
Wheat: $y = 0.01x + 0.07$ ($r=0.78$, $p<0.05$)

TABLE 16

Rat liver GSH-Px activity (nm NADPH ox/min/mg protein)

Level of Se (ppm)	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	15.97 ±12.36	16.60 ±8.63	11.97 ±7.61	105.50 ±110.34	153.72 ±120.40	51.60 ±44.05
.10	42.78 ±37.37	37.47 ±19.9	46.75 ±27.74	697.97 ±541.22	732.65 ±585.67	717.62 ±582.79
.15	135.45 ±113.95	246.05 ±229.13	236.03 ±166.12	1047.30 ±642.75	957.57 ±358.25	842.18 ±352.13

Mean±SD
N=6

Regression equations for rats fed:

Selenite:	$y = 8.37x - 26.32$ ($r=0.84$, $p<0.05$)
Raw Tuna:	$y = 2.08x - 12.09$ ($r=0.63$, $p<0.05$)
Precooked Tuna:	$y = 3.92x - 40.19$ ($r=0.61$, $p<0.05$)
Canned Tuna:	$y = 2.61x - 5.67$ ($r=0.57$, $p<0.05$)
Whole Wheat Flour:	$y = 18.13x - 112.35$ ($r=0.83$, $p<0.05$)
Whole Wheat Bread:	$y = 11.90x + 58.64$ ($r=0.70$, $p<0.05$)
Bran:	$y = 12.66x - 7.42$ ($r=0.69$, $p<0.05$)

TABLE 17

Rat liver GSH-Px (nmoles NADPH ox/min/mg protein): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	49.6 ±18.9	14.8[1] ±9.4	103.6[1] ±101.3
.10	223.3[2] ±121.7 (N=5)	42.3[3] ±27.7	716.1[2,3] ±535.8
.15	519.7[4] ±211.6	205.8[5] ±173.3	949.0[4,5] ±450.8

Matching superscripts indicate significant differences at $p<.05$.
Mean±SD

Regression equations for rats fed:
Selenite: $y = 8.37x - 26.32$ ($r=0.84$, $p<0.05$)
Tuna: $y = 3.15x - 34.12$ ($r=0.55$, $p<0.05$)
Wheat: $y = 14.22x - 27.32$ ($r=0.68$, $p<0.05$)

TABLE 18

Rat liver GSH-Px (nmoles NADPH ox/min/mg protein): Comparison between males and females

Level of Se (ppm)	Source of Se						
	SeO_3^{2-}	Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
MALES							
.05	42.3[4] ±4.0	7.6[1] ±0.9	9.1 ±1.5	5.5 ±1.3	24.2[2] ±3.9	73.9 ±50.1	35.6 ±32.9
.10	171.3* ±47.9	14.1[5] ±2.9	19.5 ±2.1	22.1 ±3.7	230.4 ±93.9	256.8[3] ±30.0	199.2 ±151.4
.15	333.8 ±46.0	64.1 ±30.3	83.7[6] ±21.5	96.2 ±41.4	540.7 ±221.1	682.1 ±147.9	614.9 ±316.8
0	3.4 ±0.3						
FEMALES							
.05	56.8[4] ±26.9	24.3[1] ±13.2	24.1 ±4.1	18.5 ±4.1	186.8[2] ±102.8	233.6 ±120.9	67.6 ±54.7
.10	258.1 ±154.8	71.5[5] ±31.8	55.5 ±3.7	71.4 ±9.1	1165.5 ±260.0	1208.5[3] ±421.2	1236.0 ±141.5
.15	705.6 ±77.8	206.8 ±127.6	408.4[6] ±227.5	375.8 ±93.0	1553.9 ±462.7	1233.0 ±267.2	1069.4 ±233.9
0	3.2 ±0.4						

Matching superscripts indicate significant differences between males and females at $p<.05$

Mean±SD

N=3 (*N=2)

times had significantly higher kidney Se than the selenite fed controls.

Tables 21 - 23 contain data on kidney GSH-Px activity. As with the liver GSH-Px data, there are no significant differences among the tuna fed groups or the wheat fed groups (Table 21). In Table 22, the combined tuna, combined wheat and selenite-fed groups follow the same pattern as the liver GSH-Px. The wheat-fed groups show the highest activity, followed in decreasing order by the selenite-fed groups, and the tuna-fed groups. Except at the 0.05 ppm level, these are all significantly different from each other ($p<0.05$). When the kidney GSH-Px data for the males and females are examined separately, the females had higher GSH-Px activity in every instance, although this was significant in only 2 out of 22 comparisons (Table 23).

Whole blood Se data are presented in Tables 24 and 25. As was observed with the liver Se data, the rats fed canned tuna had the highest blood Se followed, in decreasing order, by the rats fed the precooked tuna, and raw tuna. This was only seen at the two higher levels and was statistically significant. The rats fed the bran had slightly lower blood Se than the rats fed whole wheat flour or bread. This was significant only at the lowest level of Se fed (0.05 ppm).

TABLE 19

Se content (ppm) of rat kidney

Level of (ppm)	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	0.32 ±0.12	0.31 ±0.07	0.32 ±0.08	0.40 ±0.17	0.54 ±0.19	0.36 ±0.09
.10	0.38[1] ±0.11	0.34[2] ±0.04	0.56[1,2] ±0.15	0.68 ±0.04	0.71 ±0.11	0.64 ±0.10
.15	0.51[3] ±0.18	0.56[4] ±0.09	0.77[3,4] ±0.16	0.91 ±0.14	0.91 ±0.14	0.89 ±0.15

Matching superscripts indicate significant differences at $p<.05$.
Mean±SD
N=6

Regression equations for rats fed:

Selenite:	$y = 0.008x + 0.17$	($r=0.87$, $p<0.05$)
Raw Tuna:	$y = 0.006x + 0.14$	($r=0.76$, $p<0.05$)
Precooked Tuna:	$y = 0.007x + 0.12$	($r=0.86$, $p<0.05$)
Canned Tuna:	$y = 0.008x + 0.18$	($r=0.79$, $p<0.05$)
Whole Wheat Flour:	$y = 0.011x + 0.19$	($r=0.85$, $p<0.05$)
Whole Wheat Bread:	$y = 0.008x + 0.29$	($r=0.74$, $p<0.05$)
Bran:	$y = 0.01x + 0.16$	($r=0.87$, $p<0.05$)

TABLE 20

Rat kidney Se (ppm), comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	0.38 ±0.11 (N=5)	0.32^{1} ±0.09	0.43^{1} ±0.16
.10	0.44^{2} ±0.08 (N=5)	0.43^{3} ±0.14	$0.68^{2,3}$ ±0.09
.15	0.64^{4} ±0.07	0.61^{5} ±0.18	$0.90^{4,5}$ ±0.14

Matching superscripts indicate significant differences at $p<.05$.
Mean±SD

Regression equations for rats fed:

Selenite:	$y = 0.008x + 0.17$	($r=0.87$, $p<0.05$)
Tuna:	$y = 0.006x + 0.19$	($r=0.68$, $p<0.05$)
Wheat:	$y = 0.008x + 0.30$	($r=0.73$, $p<0.05$)

TABLE 21

Rat kidney GSH-Px activity (nm NADPH ox/min/mg protein)

Level of Se (ppm)	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	76.1 ±28.2	67.0 ±17.6	55.1 ±14.4	168.3 ±64.9	297.9 ±260.2	172.4 ±86.3
.10	*85.1 ±15.2	87.2 ±28.2	116.8 ±31.7	448.0 ±206.9	479.6 ±130.4	406.7 ±108.1
.15	160.2 ±44.2	152.9 ±44.9	170.9 ±49.8	669.8 ±123.3	649.1 ±117.2	549.4 ±150.1

Mean±SD
N=6 (*N=5)

Regression equations for rats fed:

Selenite:	$y = 5.50x + 58.07$	($r=0.71$, $p<0.05$)
Raw Tuna:	$y = 2.19x + 18.40$	($r=0.88$, $p<0.05$)
Precooked Tuna:	$y = 2.07x + 18.57$	($r=0.83$, $p<0.05$)
Canned Tuna:	$y = 1.92x + 29.81$	($r=0.77$, $p<0.05$)
Whole Wheat Flour:	$y = 9.61x + 19.28$	($r=0.91$, $p<0.05$)
Whole Wheat Bread:	$y = 7.01x + 122.09$	($r=0.76$, $p<0.05$)
Bran:	$y = 7.35x + 46.47$	($r=0.87$, $p<0.05$)

TABLE 22

Rat kidney GSH-Px (nmoles NADPH ox/min/mg prot.): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	173.8 ±37.7 (N=5)	66.1[1] ±21.5	212.9[1] ±164.8
.10	238.5[2,3] ±87.6 (N=5)	97.0[2,4] ±29.1 (N=17)	444.8[3,4] ±148.2
.15	407.7[5,6] ±222.8	161.4[5,7] ±44.2	622.8[6,7] ±134.4

Matching superscripts indicate significant differences at $p<.05$.
Mean±SD

Regression equations for rats fed:
Selenite: $y = 5.50x + 58.07$ ($r=0.71$, $p<0.05$)
Tuna: $y = 1.89x + 30.47$ ($r=0.74$, $p<0.05$)
Wheat: $y = 7.29x + 99.06$ ($r=0.77$, $p<0.05$)

TABLE 23

Rat kidney GSH-Px (nmoles NADPH ox/min/mg protein): Comparison of males and females

Level of Se (ppm)	Source of Se						
	SeO_3^{2-}	Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
MALES							
.05	156.9 ±14.9	57.4 ±11.0	51.6 ±4.5	42.8 ±7.9	109.5 ±11.1	157.2[2] ±52.5	122.1 ±67.5
.10	294.0* ±123.3	74.7 ±7.2	73.4[1] ±5.0	97.1 ±6.2	440.3 ±273.3	413.3 ±110.3	321.6 ±79.7
.15	293.7 ±68.5	123.5 ±24.6	149.9 ±44.9	137.8 ±40.0	664.7 ±187.0	573.7 ±34.8	536.6 ±111.8
0	10.1 ±1.2						
FEMALES							
.05	199.2* ±55.6	94.8 ±28.6	82.3 ±6.6	67.4 ±1.5	227.2 ±4.3	438.7[2] ±327.2	222.7 ±80.4
.10	201.5 ±51.1	100.7* ±3.9	100.9[1] ±37.4	136.6 ±36.1	455.6 ±179.4	545.9 ±131.0	491.8 ±33.1
.15	521.7 ±283.6	196.9 ±15.4	156.0 ±54.8	204.1 ±36.1	674.9 ±54.6	724.4 ±127.0	562.2 ±208.1
0	13.8 ±2.8						

Matching superscripts indicate significant differences between males and females at $p<.05$.

Mean±SD

N=3 (*N=2)

Table 25 gives the data obtained when the combined tuna groups, wheat groups and selenite-fed group were compared. The tuna-fed group always had the lowest blood Se value and this was significant ($p<.05$) five out of six comparisons. The wheat-fed groups were not significantly different from the selenite fed group.

Data for whole blood GSH-Px are presented in Tables 26-28. There are no significant differences among the tuna-fed groups. However, in the wheat-fed groups, the rats fed bran have lower GSH-Px activity than the whole wheat bread-fed animals at all three diet levels which is significant at the 0.05 ppm level ($p<.05$) (Table 26). When the data for the males and females were separated and compared, the females had higher blood GSH-Px in every instance, although the magnitude of the difference was not as great as that seen in the kidney and liver. There was only one comparison between the males and females that was significantly different, the 0.05 ppm level of canned tuna (Table 27). The whole blood GSH-Px was 34.5±16.6 and 55.9±0.3 nmoles NADPH ox/min/mg Hb for the males and females, respectively. Due to the small number of animals, there were no significant differences among the seven Se sources at any one dietary level of Se that was fed. The comparisons of the com-

TABLE 24

Se content (ppm) of rat whole blood

Level of Se (ppm)	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	0.11 ±0.02	0.11 ±0.02	0.10 ±0.02	0.17 ±0.05	0.17[4] ±0.03	0.12[4] ±0.04
.10	0.14[3,6] ±0.02	0.18[3,1] ±0.02	0.22[1,6] ±0.02	0.30 ±0.06	0.31 ±0.06	0.27 ±0.06
.15	0.21[2] ±0.05	0.23[5] ±0.06	0.30[2,5] ±0.05	0.34 ±0.07	0.35 ±0.02	0.32 ±0.04

Matched superscripts indicate significant differences at $p<.05$
Mean±SD
N=6

Regression equations for rats fed:

Selenite:	$y = 0.005x + 0.06$	($r=0.93$, $p<0.05$)
Raw Tuna:	$y = 0.003x + 0.03$	($r=0.92$, $p<0.05$)
Precooked Tuna:	$y = 0.003x + 0.03$	($r=0.92$, $p<0.05$)
Canned Tuna:	$y = 0.004x + 0.05$	($r=0.90$, $p<0.05$)
Whole Wheat Flour:	$y = 0.005x + 0.05$	($r=0.95$, $p<0.05$)
Whole Wheat Bread:	$y = 0.004x + 0.08$	($r=0.88$, $p<0.05$)
Bran:	$y = 0.004x + 0.04$	($r=0.93$, $p<0.05$)

TABLE 25

Rat whole blood selenium (ppm): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	0.16^{1} ±0.03	$0.10^{1,2}$ ±0.02	0.15^{2} ±0.05
.10	0.24 ±0.02 (N=5)	0.18^{3} ±0.04	0.29^{3} ±0.06
.15	0.33^{4} ±0.05	$0.25^{4,5}$ ±0.06	0.34^{5} ±0.05

Matching superscripts indicate significant differences at $p<.05$.
Mean±SD

Regression equations for rats fed:
Selenite: $y = 0.005x + 0.06$ ($r=0.93$, $p<0.05$)
Tuna: $y = 0.003x + 0.05$ ($r=0.84$, $p<0.05$)
Wheat: $y = 0.004x - 0.009$ ($r=0.87$, $p<0.05$)

bined tuna, combined wheat and selenite-fed groups (Table 28) followed a similar pattern, i.e., the wheat-fed groups were always significantly higher than the tuna-fed groups. The wheat-fed groups were not always higher than the selenite fed groups, however.

Data for muscle Se is in Tables 29 and 30. In Table 29, the rats consuming canned tuna generally had higher muscle Se than those consuming precooked or raw tuna. Rats eating bran, always had lower muscle Se than rats eating whole wheat bread or whole wheat flour, and this was significant ($p<.05$) in two out of three instances. In the comparisons of combined tuna, combined wheat and selenite fed groups (Table 30), no clear pattern emerged. The wheat fed groups had higher muscle Se at the 0.10 and 0.15 ppm diet, but lower at the 0.05 ppm diet level.

TABLE 26

Rat whole blood GSH-Px activity (nmoles NADPH ox/min/mg Hb)

Level of Se (ppm)	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	51.03 ±8.7	48.57 ±7.9	*43.06 ±16.6	78.57 ±18.9	87.65[a] ±22.0	58.10[a] ±18.7
.10	*61.34 ±20.3	57.87 ±8.7	66.80 ±14.3	166.15 ±38.7	191.78 ±46.7	160.15 ±45.6
.15	90.10 ±21.2	104.20 ±16.9	106.93 ±15.0	227.78 ±32.8	236.88 ±69.9	193.75 ±29.4

Matched superscripts indicate significant differences at $p<.05$.
Mean±SD
N=6 (*N=5)

Regression equations for rats fed:

Selenite:	$y = 2.25x + 51.90$	($r=0.88$, $p<0.05$)
Raw Tuna:	$y = 0.96x + 29.78$	($r=0.84$, $p<0.05$)
Precooked Tuna:	$y = 1.10x + 27.24$	($r=0.84$, $p<0.05$)
Canned Tuna:	$y = 0.97x + 31.90$	($r=0.81$, $p<0.05$)
Whole Wheat Flour:	$y = 2.90x + 33.26$	($r=0.94$, $p<0.05$)
Whole Wheat Bread:	$y = 2.41x + 54.62$	($r=0.78$, $p<0.05$)
Bran	$y = 2.44x + 31.03$	($r=0.89$, $p<0.05$)

TABLE 27

Rat whole blood GSH-Px activity (nmoles NADPH ox/min/mg Hb): Comparison between males and females

Level of Se (ppm)	SeO_3^{2-}	Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
MALES							
.05	103.7 ±9.4	45.3 ±4.9	43.0 ±3.5	34.5[1] ±16.6	70.8 ±14.4	73.3 ±15.4	47.6 ±5.6
.10	147.4* ±1.1	43.0* ±9.8	52.0 ±2.5	59.6 ±15.3	134.8 ±24.3	166.3 ±32.1	132.2 ±50.1
.15	183.5 ±30.2	84.9 ±23.5	92.9 ±10.7	97.5 ±10.4	204.8 ±9.6	222.2 ±29.5	193.2 ±38.6
0	30.3 ±6.7						
FEMALES							
.05	127.3* ±5.0	56.8 ±8.0	54.1 ±7.2	*55.9[1] ±0.3	86.3 ±22.5	102.0 ±18.9	68.6 ±22.8
.10	112.3 ±6.1	73.6 ±14.7	63.7 ±9.0	74.0 ±11.2	197.5 ±14.3	217.3 ±49.8	188.1 ±18.6
.15	190.9 ±38.2	95.4 ±22.0	115.5 ±14.6	116.4 ±13.7	250.8 ±31.9	251.6 ±103.5	194.3 ±25.7
0	27.7 ±15.8						

Matched superscripts indicate significant differences between males and females at $p<.05$.
Mean±SD
N=3 (*N=2)

TABLE 28

Rat blood GSH-Px (nmoles NADPH ox/min/mg Hb): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	$113.2^{1,2}$ ±14.7 (N=5)	$47.8^{1,3}$ ±11.1 (N=17)	$74.8^{2,3}$ ±22.7
.10	$126.3^{4,5}$ ±19.7 (N=5)	$62.0^{4,6}$ ±14.4 (N=17)	$172.7^{5,6}$ ±43.5
.15	187.2^{7} ±31.1	$100.4^{7,8}$ ±18.4	219.5^{8} ±48.7

Matched superscripts indicate significant differences at $P<.05$.
Mean±SD

Regression equations for rats fed:
Selenite: $y = 2.25x + 51.90$ ($r=0.88$, $p<0.05$)
Tuna: $y = 1.00x + 30.33$ ($r=0.78$, $p<0.05$)
Wheat: $y = 2.44x + 47.10$ ($r=0.81$, $p<0.05$)

TABLE 29

Se content (ppm) of rat muscle

Level of Se (ppm)	Source of Se: Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
.05	0.07[1] ±0.008	0.08[1] ±0.009	0.08 ±0.01	0.06 ±0.01	0.06 ±0.02	0.05 ±0.01
.10	0.09[2] ±0.02	0.09[3] ±0.004	0.11[2,3] ±0.02	0.12 ±0.02	0.12[6] ±0.02	0.10[6] ±0.02
.15	0.11[4] ±0.01	0.12[5] ±0.02	0.15[4,5] ±0.02	0.15 ±0.02	0.16[7] ±0.02	0.13[7] ±0.02

Matched superscripts indicate significant differences at $p<.05$
Mean±SD
N=6

Regression equations for rats fed:

Selenite:	$y = 0.001x + 0.04$	($r=0.92$, $p<0.05$
Raw Tuna:	$y = 0.001x + 0.04$	($r=0.92$, $p<0.05$)
Precooked Tuna:	$y = 0.001x + 0.04$	($r=0.92$, $p<0.05$)
Canned Tuna:	$y = 0.002x + 0.04$	($r=0.88$, $p<0.05$)
Whole Wheat Flour:	$y = 0.002x + 0.03$	($r=0.96$, $p<0.05$)
Whole Wheat Bread:	$y = 0.002x + 0.04$	($r=0.86$, $p<0.05$)
Bran:	$y = 0.001x + 0.03$	($r=0.92$, $p<0.05$)

TABLE 30

Rat muscle Se (ppm): Comparison of combined tuna, combined wheat and selenite fed groups

Level of Se (ppm)	SeO_3^{2-} Groups (N=6)	Tuna Groups (N=18)	Wheat Groups (N=18)
.05	0.07 ±0.01	0.08[1] ±0.01	0.06[1] ±0.02
.10	0.10 ±0.006 (N=5)	0.09[2] ±0.02	0.12[2] ±0.02
.15	0.12[3] ±0.01	0.13[4] ±0.02	0.15[3,4] ±0.02

Matching superscripts indicate significant differences at $p<.05$.
Mean±SD

Regression equations for rats fed:

Selenite: $y = 0.001x + 0.04$ ($r=0.92$, $p<0.05$)
Tuna: $y = 0.001x + 0.05$ ($r=0.83$, $p<0.05$)
Wheat: $y = 0.002x + 0.04$ ($r=0.86$, $p<0.05$)

HUMAN EXPERIMENT

The Se content of the urine and feces from young adult males receiving tuna and whole wheat bread diets is presented in Tables 31 and 32. There is no difference between the Se excretion on the tuna or bread diets in urine or feces. There is a difference between the adjustment diet and the tuna and bread diets. However, the adjustment diet provides only 27.8% and 26.0%, respectively, of the Se of the tuna and bread diets. This decrease in excretion of Se reflects this decrease in quantity of Se.

The balance study data are found in Table 33. No difference was observed between the two test foods. On the tuna diet, the intake was 331.5 ug Se/day, the excretion was 240.9 ug/day and the subjects were in positive balance, storing 90.6 ug/day. On the bread diet the Se intake was 354.5 ug/day, the total excretion was 249.6 ug/day and the subjects were in positive balance storing 105.0 ug Se/day. Since the subjects were consuming slightly different amounts of Se between these two diets, the total excretion was expressed in terms of percent of intake. On the tuna diet, they excreted 72.7% of the Se consumed and on the bread diet they excreted 70.4%. During the adjustment phase the subjects were consuming only 92.3 ug Se/day. Apparently this was not adequate to cover even their obligatory losses of Se because they were in negative balance losing, 16.2 ug Se/

TABLE 31

Average Se content of urine (ug/day) in male humans fed tuna and bread diets

Subject Number	Adjustment Diet (N=4)	Tuna Diet (N=5)	Bread Diet (N=5)
1	93.7±14.7[1]	199.1± 7.9	165.7±20.3
2	84.6±21.9	169.8±21.3	229.0±16.9
3	69.6±10.9	109.1±11.2	152.0±30.4
4	52.7± 9.8	118.6±14.8	193.6±20.0
5	95.1±19.4	166.4±10.5	181.8± 6.3
7	67.9±11.2	157.3±14.5	142.4± 6.8
8	95.4±40.1	178.2±20.0	146.2±9.5
9	60.4± 4.6	163.1± 4.2	141.8±24.9
Mean±SD	77.4±16.9	157.7±30.0	169.1±30.8

[1] Values represent Mean±SD

TABLE 32

Average Se content of feces (ug/day) in male humans fed tuna and bread diets

Subject Number	Adjustment Diet (N=2)	Tuna Diet (N=3)	Bread Diet (N=3)
1	23.8± 3.6[1]	88.3± 9.3	91.8±51.3
2	29.3± 8.1	86.4±55.2	105.6±19.2
3	41.2±16.0	70.1±59.3 (N=2)	57.8± 9.3
4	19.7± 4.0	84.9±26.9	50.8± 5.9
5	30.2±13.4	67.7±32.6	95.8±50.7
7	35.6±12.3	81.9±25.0	77.4±12.1
8	33.7± 2.9	95.0±45.2	70.1±20.2
9	35.1±17.9	91.1±38.8 (N=2)	94.6±14.0
Mean±SD	31.1±6.9	83.2±9.7	80.5±19.7

[1] Mean±SD

day. Their Se intakes must have been higher before they started to consume the adjustment diets. This would correspond with the estimates of typical Se intakes in the United States which average about 150 ug per day (22).

Table 34 contains the data on the whole blood Se. This remained constant for the entire study. The average Se concentration during the adjustment period was 0.20±.02 ug Se/gm whole blood, for the tuna diet it was 0.23±.03 and for the bread diet it was 0.24±.03.

Likewise there was no difference in the GSH-Px activity among the three diets. The GSH-Px assay was done with two different substrates, hydrogen peroxide (H_2O_2) and t-butyl hydrogen peroxide (t-butyl H_2O_2). Table 35 presents these data. There were no differences among the three diets for either substrate.

TABLE 33

Human Se balance study data, ug Se/day consumed and excreted and per cent of intake that was excreted

	Adjustment Diet		Tuna Diet		Bread Diet	
Intake (ug Se/day)	92.3		331.5		354.5	
Excretion:	ug Se day	% intake	ug Se day	% intake	ug Se day	% intake
Urine	77.4	83.9	157.7	47.6	169.1	47.7
Feces	31.1	33.7	83.2	25.1	80.5	22.7
Total	108.5	117.6	240.9	72.7	249.6	70.4
Balance Mean±SD	-16.2±4.02		+90.6±9.5		+105.0±10.3	

TABLE 34

Average whole blood Se (ug/gm) in male humans fed tuna and bread diets

Subject Number	Adjustment Diet (N=3)	Tuna Diet (N=3)	Bread Diet N=3)
1	0.23±.01[1]	0.28±.02	0.23±.02
2	0.20±.02	0.23±.01 (N=2)	0.27±.01
3	0.19± 0	0.19±.006	0.24±.01
4	0.19±.005	0.27±.04	0.21±.04
5	0.22±.02	0.25±.03	0.29±.02
7	0.19±.02	0.23±.02	0.25±.01
8	0.20±.006	0.23±.04	0.22±.006
9	0.17±.01	0.19±.02	0.19±.02
Mean±SD	0.20±.02	0.23±.03	0.24±.03

[1]Mean±SD

TABLE 35

Average GSH-Px activity (nmoles NADPH ox/min/mg Hb) in human whole blood using two substrates

Adjustment Diet	Tuna Diet	Bread Diet
H_2O_2		
23.67 ±4.9	24.07 ±4.9	26.42 ±5.1
t-butyl H_2O_2		
26.46 ±5.1	25.72 ±5.1	25.74 ±5.1

Mean±SD

N=8

V. DISCUSSION

RAT EXPERIMENT

Although many approaches to the question of bioavailability of Se have been undertaken, there is no real consensus regarding the best method to be employed in its study. Initially, we hoped to compare tissue levels of GSH-Px and Se in the rat to survival time of rats fed a necrosis promoting diet. Unfortunately it was not possible to reproduce this liver necrosis assay even though it had been successfully employed previously in our lab (82, 83), and the descendents of that same strain of rats, OSU Browns, were used. We concluded that the failure was probably due to variability introduced into the strain when it was outbred to increase fertility. In light of this, the measure of Se level and GSH-Px activity in a variety of tissues, as a test of bioavailability, was used to answer our research questions. Another method used to express bioavailability is the slope ratio method reported by Suttle (77). Using this method, the slope of the regression line generated by a dose-response relationship for a test substance is divided by the slope of the regression line generated by the control substance which would be selenite in the present study. The ratio can then be used to quantitatively compare two foods.

Rats fed canned tuna as a source of Se consistently had

significantly higher tissue Se levels than rats fed the other two tuna diets. However, there was no difference in GSH-Px activity among these three groups. This observation was confirmed by the slope ratios presented in Table 36. The bioavailability of the canned tuna, for example, appeared to be 143% greater than either the raw or precooked tuna when liver Se levels were used. However, when liver GSH-Px activity was the measure of bioavailability, the slope ratios demonstrated that the canned tuna had only 68% of the availability of the precooked tuna and 128% of the raw tuna. But does this mean that the Se in canned tuna was more available to the rat? If so, what is the rat doing with the additional Se if more GSH-Px is not produced?

One of the more significant observations of this study was the great difference in GSH-Px activity between the combined tuna fed groups versus the combined wheat fed groups. This was seen in the liver, kidney and blood (Tables 17, 22, 28). The wheat fed groups clearly had much greater activity. The slope ratios in Table 36 confirm this observation and this also agrees with data collected on chicks by Cantor et al. (8,9) and on rats by Douglass et al. (18). An examination of the tissue levels of Se in liver, kidney and blood (Tables 15, 20, 25) reveals that, although the wheat fed groups are usually higher, and sometimes significantly so, the magnitude of the difference is less than the differ-

TABLE 36

Slope ratios (slope from regression line of test food divided by that of selenite)

Biochemical Parameter	Diets					
	Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
Liver Se	0.70	0.70	1.00	1.00	1.00	1.00
Kidney Se	0.75	0.88	1.00	1.38	1.00	1.25
Blood Se	0.60	0.60	0.80	1.00	0.80	0.80
Muscle Se	1.00	1.00	2.00	2.00	2.00	1.00
Liver GSH-Px	0.22	0.47	0.32	2.17	1.42	1.51
Kidney GSH-Px	0.40	0.38	0.35	1.75	1.28	1.34
Blood GSH-Px	0.43	0.49	0.43	1.29	1.07	1.08

	Combined Tuna	Combined Wheat
Liver Se	0.90	1.00
Kidney Se	0.75	1.00
Blood Se	0.60	0.80
Muscle Se	1.00	2.00
Liver GSH-Px	0.38	1.70
Kidney GSH-Px	0.34	1.33
Blood GSH-Px	0.44	1.08

ence in GSH-Px activity. Again this is illustrated in the slope ratios (Table 36). The ratios of the wheat-fed animals were 1.1 to 2.0 times larger than the tuna-fed animals when tissue Se levels were considered. When tissue GSH-Px activity was used, the slope ratios of the wheat-fed rats were 2.5 to 4.5 times larger than the tuna-fed rats. When a high Se level is observed, as in the animals fed canned tuna versus precooked or raw, but not a correspondingly higher GSH-Px activity, then the question arises as to which assay is the better method to determine bioavailability. It would appear that the GSH-Px activity is a more reliable indicator of bioavailability, since this represents biologically active selenium. High levels of Se do not necessarily indicate that the mineral is participating in a metabolic reaction. It may be in a storage form or it may be in an elimination or detoxification form. Thus it appears that the canned tuna promoted the deposition of more non-GSH-Px Se than the other tuna sources. The presence of Se in GSH-Px, however, indicates that it is in a form ready to carry out a particular metabolic role in the tissue.

Using GSH-Px activity as a criteria for bioavailability, it can be seen that no differences were found in the bioavailability of Se due to food processing in either tuna or wheat products. Since this test can distinguish between levels of Se fed, source of Se (i.e. tuna versus wheat) and

sex, its failure to distinguish between different forms of the same food is probably real. Another possibility is that the high variability of these samples masked any differences other than the most obvious. The variability was quite large partly due to the mixing of sexes in each group and partly because of the poorly controlled outbreeding which had been done previously with these animals. If there is a difference due to processing, then it must be a subtle difference.

Animals consuming bran often had lower tissue levels of Se and occasionally it was significantly lower. Since this was accompanied by a trend of lower GSH-Px activity, it suggests that less Se was available to these animals. The possibility must be considered that the bran was not as well digested and, therefore, less Se was absorbed. This seems likely in view of the fact that small decreases were found.

Previous studies (8,9,10,54) have compared the availability of different Se compounds. In general, they agree that Se from wheat and selenomethionine were more available than Se from selenite, selenocystine and tuna. This corresponds well with the data presented here, which demonstrates that Se in the wheat, which is approximately 40% selenomethionine (44), is more available than tuna.

Thrower and Andrewartha (79) fed a diet to rats in which the protein source and Se content varied. They found

that feeding 10 to 20% fish protein enhanced the GSH-Px response over that in rats fed 20% protein as torula yeast, even when supplemented with methionine. This does not agree with our findings of a decreased availability of Se in tuna, but perhaps this is due to the greater amount of fish that they fed and the higher sulfur amino acid content of their diets. A relationship between suboptimal dietary methionine resulting in low bioavailability of selenomethionine was noted by Sunde et al. (73). It is possible that the high sulfur amino acid content of their diet could enhance the availability of Se in the fish protein concentrate.

Douglass et al. (18) depleted rats for four weeks and repleted for four additional weeks. They found that selenite (0.2ppm) was more effective than tuna (0.2ppm), beef kidney (0.2ppm) or wheat (0.2ppm) in raising liver Se, but only tuna was less effective than selenite in increasing liver GSH-Px. Liver GSH-Px was lower in the rats fed tuna than in the rats fed wheat and selenite. This was indicated by a slope ratio of 0.54 of the tuna relative to selenite in inducing liver GSH-Px. We do not observe a superiority of selenite over wheat and canned tuna in increasing liver Se, but we did agree on the inferiority of tuna Se in raising liver GSH-Px activity. Our slope ratio of tuna relative to selenite for liver GSH-Px was 0.38, which, in view of the differences in experimental design, is comparable. Ga-

brielsen and Opstvedt (25) compared the availability of Se in fish meal with soybean meal, corn gluten and selenomethionine. They found in chicks that Se in soy meal and corn gluten were less available than Se in mackerel and capelin fish meal for induction of plasma GSH-Px with respect to selenite. Slope ratios for the two fish products were 0.34 and 0.48. This corresponds well with our values for whole blood GSH-Px in rats.

Another observation which has not been stressed previously is the sex difference in GSH-Px response. Pinto and Bartley (49) noted that female rats had higher tissue GSH-Px activity, but this was before any association with Se and the enzyme was discovered. We saw the greatest difference between male and female rats in liver GSH-Px, less in kidney and whole blood. This leads to the speculation that GSH-Px activity may be under some hormonal influence. Behne et al. (3,4) noted a decrease in serum Se and plasma GSH-Px in pregnant and lactating female rats. Although they suspected this decrease was due to the high level of circulating hormones, they were unable to duplicate it in progesterone treated ovarectomized rats.

In the present study, tissue levels of Se correlate very highly with tissue GSH-Px activities (Table 37). Correlation coefficients for each Se source as well as for the combined tuna and wheat products are presented. This is in

agreement with data from New Zealand researchers (38, 53) who saw a significant correlation in their human populations, and Smith et al. (72) and Hafeman et al. (28) in rats. Whanger et al. (84) noted that a good correlation between whole blood Se and GSH-Px was observed in sheep only at the lower blood Se levels. Valentine et al. (80) and Schrauzer and White (60) observed no correlation in their human populations, possibly indicating that this correlation is influenced by the level of Se consumed. The New Zealanders, as well as many of the animals studied, were consuming dietary levels close to minimum requirements; whereas, the two American populations (60, 80) were consuming generous amounts. It is possible that if an excess of Se is being consumed, then when all the necesary GSH-Px is synthesized, the excess is stored as some otner compound; however, when marginal amounts of Se are available, little Se is stored and most of it is used in GSH-Px.

In summary, it appears that GSH-Px activity is the better measurement of bioavailability of Se. Using that criteria, we found that the Se in wheat products tested was more available than Se in the tuna products and sodium selenite at all levels fed. There was no difference due to food processing in the availability of Se from the tuna or wheat products tested. However, rats fed canned tuna deposited more Se in their tissues than rats fed the other two tuna

TABLE 37

Correlation coefficients (r) for Se content and GSH-Px activity of rat tissues for each diet

Tissue	SeO_3^{2-}	Raw Tuna	Precooked Tuna	Canned Tuna	Whole Wheat Flour	Whole Wheat Bread	Bran
Liver	0.96	0.84	0.94	0.78	0.96	0.96	0.94
Kidney	0.73	0.70	0.62	0.82	0.79	0.50	0.88
Blood	0.79	0.85	0.81	0.86	0.92	0.82	0.96

All r values significant at $p<0.05$

	Combined Tuna	Combined Wheat
Liver	0.67	0.95
Kidney	0.71	0.73
Blood	0.82	0.90

All r values significant at $p<0.05$

products.

HUMAN STUDY

Using whole blood Se and GSH-Px activity as a measure of bioavailability in the men, no differences between tuna or bread were revealed. It is possible that the subjects were not on either diet long enough to see an effect. This would agree with the findings of Levander et al. (36) who after feeding a Se depletion diet for 44 days to 6 young men, found the erythrocyte Se did decrease during depletion but did not show any relationship to Se supplementation upon repletion. Likewise, erythrocyte GSH-Px did not respond to Se supplementation. The life span of the erythrocyte is 120 days, therefore, in 14 days only 5.8% of the erythrocytes will turnover. According to Hafeman et al. (28), Se is incorporated into RBCs only during erythropoeisis. Perhaps one reason why more differences were seen in the rat than human is because the rats were fed diets for 28-30 days and the average life span of their erythrocytes is 60 days. This accounts for almost 50% turnover. The human blood Se and GSH-Px activity were also unresponsive to a change in amount of Se fed. The adjustment diet provided only 92.3 ug Se/day and the tuna and bread diets provided 331.5 and 354.5 ug Se/day, respectively. The lack of response, even in light of the three fold increase in dietary Se, adds cre-

dence to the explanation that inadequate time was allowed to permit equilibration.

The whole blood Se values (0.20 ppm) on the adjustment diet are in agreement with other researchers who looked at normal American populations (1, 60, 80). Unfortunately the whole blood GSH-Px activities were not comparable because different methods were used and the activity was expressed in different units.

There was enough time to observe differences in the balance study. The subjects quickly adapted to the change in Se levels from the adjustment diet to the test diets. During the adjustment diet, however, they were in negative balance with a loss of 16.2 ug/day. This was not expected in view of the fact that they were consuming 92.3 ug Se/day, which is more than Schroeder et al. (61) found in a standard two day hospital diet (62 ug) and much more than the intake of New Zealanders, reported to be 6-35 ug Se/day (54). It is possible that our subjects had been consuming more Se than the average American, and the 92 ug/day was not enough to allow for their obligatory losses.Levander et al. (36), even after a 44 day Se depletion in their male subjects, were unable to reduce the Se excretion in urine to that observed in New Zealand subjects. They found that their plasma Se levels during depletion were double the levels common in New Zealand. It appears that it takes a long

time to adapt to low levels of intake.

There was no difference in the Se retained between the tuna and bread diets. Since the subjects were eating slightly different amounts of Se, the per cent of Se excreted expressed in terms of Se intake is the most convincing: 72.7% for tuna and 70.4% for bread. This is somewhat surprising in view of the results in other species. Rats and chicks definitely show a superiority of plant Se over animal Se in increasing tissue levels of Se and GSH-Px and prevention of deficiency diseases (8,9,10,54,18), although retention of Se does not always correspond with the different measures of bioavailability. This was observed with lambs and ewes (24,30), chicks (45) and rats (10). It is possible that is also the case with the human subjects. A disadvantage of using human subjects is that the only tissue available to study metabolism is blood. It was apparent from the rat study that the blood was one of the least responsive of the tissues. The slope ratios for the blood GSH-Px ranged from 0.43 to 1.29, contrasted with liver GSH-Px which ranged from 0.25 to 2.17. We did not collect urine and feces on the rats so we were unable to compare the human retention data with our animal model. Since we apparently did not allow sufficient time to see a change in the whole blood Se and GSH-Px, it is premature to conclude on retention data alone, that there is no difference in the availability of Se

in wheat and tuna for humans.

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