Deletion of Selenoprotein P Alters Distribution of Selenium in the Mouse*

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Selenoprotein P (Se-P) contains most of the selenium in plasma. Its function is not known. Mice with the Se-P gene deleted (Sepp−/−) were generated. Two phenotypes were observed: 1) Sepp−/− mice lost weight and developed poor motor coordination when fed diets with selenium below 0.1 mg/kg, and 2) male Sepp−/− mice had sharply reduced fertility. Weaning male Sepp+/+, Sepp−/+ and Sepp−/− were fed diets for 8 weeks containing <0.02–2 mg selenium/kg. Sepp−/+ and Sepp−/− mice had similar selenium concentrations in all tissues except plasma where a gene-dose effect on Se-P was observed. Liver selenium was unaffected by Se-P deletion except that it increased when dietary selenium was below 0.1 mg/kg. Selenium in other tissues exhibited a continuum of responses to Se-P deletion. Testis selenium was depressed to 19% in mice fed an 0.1 mg selenium/kg diet and did not rise to Sepp−/+ levels even with a dietary selenium of 2 mg/kg. Brain selenium was depressed to 43%, but feeding 2 mg selenium/kg diet raised it to Sepp−/+ levels. Kidney was depressed to 76% and reached Sepp−/+ levels on an 0.25 mg selenium/kg diet. Heart selenium was not affected. These results suggest that the Sepp−/− phenotypes were caused by low selenium in testis and brain. They strongly suggest that Se-P from liver provides selenium to several tissues, especially testis and brain. Further, they indicate that transport forms of selenium other than Se-P exist because selenium levels of all tissues except testis responded to increases of dietary selenium in Sepp−/− mice.

Se-P is the most extreme example of the interesting protein class known as selenoproteins. Selenoproteins contain selenocysteine in their primary structures (1). Insertion of this 21st amino acid involves an expansion of the genetic code by redefinition of specific internal UGA stop codons (2). In the majority of selenoproteins there is a single selenocysteine that is at the active site of an enzyme, and it plays a pivotal role in a redox reaction. By contrast, Se-P has many selenocysteines, ranging from 10 in mice and humans to 17 in zebrafish (3, 4). The function of Se-P is not known, and there is no direct evidence that its selenocysteine residues play a chemically active role.

Se-P is an extracellular glycoprotein that contains most of the selenium in plasma (5). Its abundance is such that Se-P in rat plasma accounts for 8% of the selenium in the animal. The liver is the primary source of plasma Se-P (6), although virtually all tissues express it. Substantial amounts of Se-P are synthesized, because the plasma half-life of its selenium is only 3–4 h (7). This indicates that selenium cycles through plasma Se-P at a high rate. Because of its plasma location, Se-P has been postulated to be a selenium transport protein (8). The selenium in Se-P is present in its primary structure as selenocysteine residues, and so delivery of its selenium to a cell would require degradation of the Se-P and catabolism of its selenocysteine. That would make a selenium transport role for the protein costly to the animal. Nevertheless, evidence suggesting a transport function has been presented (7). Other studies have shown that Se-P is a preferred source of selenium for Jurkat cells (9) and embryonic neurons (10).

To facilitate studies on Se-P function we have produced mice that lack Se-P using homologous recombination. We report some of the characteristics of these animals here, along with results that support a selenium transport or distribution function for Se-P.

EXPERIMENTAL PROCEDURES

Reagents—Restriction enzymes and ligases were obtained from Promega (Madison, WI), New England Biolabs (Beverly, MA), andMBI Fermentas (Amherst, NY). Cloning vectors, pBluescript and pBC, were obtained from Stratagene (La Jolla, CA). The loxP-flanked Neo gene, pKT1LoxA, (15), and the TK2 gene (16) were generous gifts of Dr. Kirk R. Thomas, University of Utah. Oligonucleotides were synthesized by core laboratory facilities at Vanderbilt University Medical Center and the University of Utah. [35S]dATP and [32P]dCTP were obtained from PerkinElmer Life Sciences. [35S]SeSelenite (specific activity, 800 mCi/mg selenium) was obtained from the University of Missouri Research Reactor Facility, Columbia, MO. NADPH was purchased from United States Biochemical Corp. (Cleveland, OH). Glutathione reductase was purchased from Sigma. All other chemicals were of reagent grade.
Deletion of Selenoprotein P

Selenoprotein P Genomic Clone Selection and Sequence—A P1 plasmid-containing mouse genomic DNA for Sepp was purchased from Genome Systems, Inc. (St. Louis, MO). PCR primers MG5 (5′-GATTTGTCGAAAACATGGGAGAAATC-3′) and MG7 (5′-GAAAGTACGGAGTAGTAGAGAAGCAGGAAG-3′) were submitted to Genome Systems and used to screen their murine 129/Sv P1 library. One clone, MG6138, was shown to contain Sepp by Southern analysis. P1 DNA, prepared using a procedure from Qiagen (Chatsworth, CA), was digested with EcoRI, HindIII, or BamHI. Southern analysis of the digested MG6138 DNA, using [32P]dCTP-labeled 16C1 cDNA (17), showed two HindIII fragments (approximate sizes, 6 and 2 kb), a single EcoRI fragment (10 kb), and a single BamHI fragment (10 kb). The MG6138-HindIII digest was subcloned into pBluescript II KS. Subclones were selected by screening with [32P]dCTP labeled 16C1 cDNA. Double-stranded DNA prepared from these clones was sequenced by the Sanger dideoxy termination method. Intrон-exon boundaries were identified by a comparison of genomic DNA sequences with the sequence of a mouse selenoprotein P cDNA (MB23A). MB23A was obtained by screening a mouse brain cDNA library with the HindIII fragment of MG6138. The cDNA library was a gift from Dr. Thomas Quertermous, Vanderbilt University.

Construction of Knockout Vectors—MG6138P1 DNA was prepared and digested with BamHI. The fragments from the digest were ligated into pBluescript II and transformed into Escherichia coli. Bacterial colonies were screened by PCR using primers SeP1 and SeP2 (5′-GTACCTCT TACGCACTGGCCTG-3′ and 5′-ACCGAGATGTTACGAGCATG-3′) and P1-NeoS (5′-CTCACTGTTACGAGCATG-3′). The inserted P1 genomic DNA was 12 kb. The loxP sites within the Neo cassette was sequenced by the Sanger dideoxy termination method. These results were confirmed by a Southern analysis of the Ultrasensitive HindIII digest of the genomic DNA, using [32P]dCTP-labeled 16C1 cDNA. The expected 10-kb band was present in the lane containing the P1 genomic DNA, and was absent in the lane containing the M13 genomic DNA. The absence of the 10-kb band in the M13 lane demonstrated that the cloned genomic DNA was not digested with HindIII.

Generation of Sepp−/− Mice—ES cell transformation with SePPCKNT and blastocyst injection were carried out in the Mouse Core Facility at the University of Utah using published procedures (18, 19). PCR assays were used to determine the genotype of ES cells and adult mice. DNA was extracted from ES cells and from tail biopsies using established procedures. DNA was resuspended in Tris-EDTA buffer (10 mM Tris-Cl, 1 mM EDTA, pH 7.6). Approximately 1 μg of DNA was dissolved in 50 μl of PCR lysis buffer (50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-Cl (pH 8.5), 0.05% gelatin, 0.05% Nonidet P-40, 0.5% Tween 20), denatured at 95 °C for 5 min and quick-chilled on ice. Two μl of the denatured DNA solution was amplified for 35 cycles (94 °C for 30 s, 66 °C for 20 s, 72 °C for 35 s) in a 10-μl reaction mixture. The primers used to screen for ES-positive cells were MoSePS6 (5′-GAAGACTGTAATGGCATATA CACTGTGTCAC-3′) and ACNNeoS (5′-GGTTGCGGTGTTGGT CCTGATC-3′). The primers used to screen tissue DNA were MoSePS14 (5′-GCCATGCGCTACTGCAG3′), MoSePA16 (5′-GTTCAACGC CAGGGAATGCCAC-3′), and ACNNeoS. PCR product sizes were: wild type (Sepp+/+), 900 bp from MoSePS14 and MoSePA16; homozygous (Sepp−/−), 500 bp from MoSePS14 and ACNNeoS; heterozygous (Sepp+/−), 900 and 500 bp. An additional PCR product of 2.1 kb is predicted for the primers MoSePS14 and MoSePA16 in Sepp+/− and Sepp−/− mice. PCR conditions were optimized for production of this product.

Animal Husbandry—Adult Sepp−/− and Sepp+/+ mice were transferred to the animal facility at Vanderbilt University. The Vanderbilt University Institutional Animal Care and Use Committee approved animal protocols for studies at Vanderbilt, and the corresponding University of Utah committee approved the protocols used to generate the knockout mice. The mice were housed in plastic cages with wood shavings as bedding material. The light/dark cycle was 12 h:12 h. Mice received pelleted rodent chow (selenium content, 0.29 ± 0.01 mg/kg) and water ad libitum except when fed diets containing specific amounts of selenium. Matings were set up between Sepp−/− males and Sepp−/− females, between Sepp−/− males and Sepp+/− females, between Sepp−/− females, and between Sepp+/− females. Males and females were housed in separate cages.

For experiments in which different amounts of selenium were fed, a Torula yeast-based diet was used (20). The basal form of this experi-mental diet contained <0.02 mg of selenium/kg. Sodium selenite was added to this diet during mixing to give final added selenium concentrations that ranged from 0.05 to 2 mg/kg. Male weaning mice were fed basal or selenium-supplemented diet for 8 weeks. They were weighed weekly, and mice that had lost 20% of their highest body weight were euthanized. At 8 weeks the mice were anesthetized with isoflurane, and blood was removed from the inferior vena cava. The blood was treated with anticoagulant (EDTA 1 mg/ml), centrifuged to prevent coagulation, and plasma was separated by centrifugation. Liver, kidney, heart, testis, and brain were harvested and frozen immediately in liquid nitrogen. Plasma and tissue samples were stored at −80 °C.

Biochemical Measurements—Tissue homogenates (10%) were prepared in 0.1 M potassium phosphate, pH 7.5. Supernatants from centrifugation of the tissue homogenates at 13,000 × g for 30 min were used for measurement of glutathione peroxidase activity. The coupled method was used with 0.25 mM hydrogen peroxide as substrate (21).

Plasma Se−P was measured with a radioimmunoassay (22) that utilized the polyclonal antibody preparation 695. Selenium was measured using a modification of the fluorescent assay of Koh and Benson (23, 24).

The limit of detection of this assay is 1 ng of selenium.

75Se Labeling of Mouse Plasma—Adult mice of all genotypes that were fed the chow diet were each injected intraperitoneally with 15 μCi of [75Se]selenite (in 0.15 M NaCl). Blood was obtained from the mice 24 h after 75Se administration. Plasma was separated by centrifugation and subjected to SDS-PAGE. After being stained with Coomassie Blue, the gel was dried and exposed to Kodak XAR film. In a separate experiment, the 75Se-labeled plasma was subjected to immunoprecipi-tation by polyclonal antibodies raised in rabbits against rat selenoprotein P (25) and by polyclonal antibodies raised in rabbits against human GSHPx-3 (pAb 3495, a generous gift of K. R. Maddipati, Cayman Chemical, Ann Arbor, MI). The immunoprecipitates were separated by SDS-PAGE, and 75Se-labeled proteins were identified by autoradiography.

Metabolic Fate of Selenium Administered by Gavage—Male mice of all three genotypes were fed the experimental diet supplemented with 0.1 mg of selenium/kg for 6–8 weeks from the time of weaning. Then each was administered 15 μCi of [75Se]selenite (in 0.15 M NaCl) by gavage. They were housed individually for 24 h and were then anesthetized and exsanguinated by collection of blood from the inferior vena cava. Liver, kidney, testis, and brain were harvested and weighed. The 75Se content of plasma and tissues was determined in a Compu Gamma 1282 (Amersham Biosciences).

RESULTS

Generation of Sepp-deleted Mice—Se-P knockout mice were produced by homologous recombination using genomic DNA cloned from an Sv-129 P1 library that had been mutated using the strategy shown in Fig. 1. C57Bl/6 blastocysts were injected with ES cells heterozygous for the Sepp mutation and then implanted into pseudopregnant females. A male chimera was identified among the offspring. This male was mated with two C57Bl/6 female mice, and the heterozygote progeny (Sepp+/−) from these matings were used to establish the Se-P knockout mouse colony.

Verification of Se-P Deletion—Twenty-four hours after 75Se had been injected into animals of each genotype, plasma was obtained and subjected to SDS-PAGE. Fig. 2A shows the autoradiograph of the resulting gel. Both the Sepp−/− and Sepp+/− plasma samples yielded bands characteristic for Se-P and GSHPx-3. Sepp−/− plasma exhibited only the band of 75Se radioactivity corresponding to glutathione peroxidase. The Se-P band was not present in the Sepp−/− lane. The identity of each of the radioactive proteins was verified by precipitation using specific antibody preparations (Fig. 2B). Northern analysis of liver RNA was carried out and showed a gene-dose effect with no signal evident in the Sepp−/− lane (Fig. 2C).

Fig. 2D shows the results of measuring Se-P by competitive radioimmunoassay of plasma samples from the three genotypes. A gene-dose effect is evident that mirrors the liver
mRNA results (Fig. 2C). Thus, Se-P has been eliminated from the plasma of the knockout mice, and its concentration is half that of the wild types in the plasma of the heterozygotes.

Breeding of Se-P Knockout Mice—Offspring of Sepp<sup>+/−</sup>/H11001/H11002 and Sepp<sup>+/−</sup>/H11002/H11002 animals fed rodent chow were characterized. The ratio of male to female progeny was 50/50 regardless of the genotype of the parents (data not shown). Mating of Sepp<sup>+/−</sup> males and Sepp<sup>+/−</sup> females resulted in viable pups with the predicted genotype distribution (Table I, top). Those pups survived to weaning (Table I, bottom). Mating of Sepp<sup>+/−</sup> males and Sepp<sup>+/−</sup> females produced fewer Sepp<sup>+/−</sup> pups than predicted, and 31% of these homozygous pups died before weaning. When Sepp<sup>+/−</sup> males were mated with Sepp<sup>+/+</sup> and Sepp<sup>+/−</sup> females over a period of 6 months, only one litter was born from 18 mating pairs (Table II). In contrast, Sepp<sup>+/−</sup> males produced many pregnancies with Sepp<sup>++/−</sup> and Sepp<sup>++/−</sup> females. These results indicate that female homozygotes have difficulties producing and raising homozygote pups and that male homozygotes have sharply reduced fertility.

Biochemical Characterization of Selenium Status in Genetically Altered Mice Fed Different Amounts of Selenium—In the initial experiments with our genetically altered mice (Tables I and II), we fed mouse chow containing 0.29 mg of selenium/kg. The Sepp<sup>+/-</sup> mice grew as well as the Sepp<sup>++/−</sup> mice (Fig. 3A) and appeared healthy. However, when we began feeding a selenium-deficient diet containing &lt;0.02 mg of selenium/kg,
the Pup genotype

<table>
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Pup survival to weaning

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**Table II**

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<tr>
<td>Populations</td>
<td>Breeding pairs</td>
<td>Litters</td>
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Breeding pairs were fed the chow diet and housed together for up to 6 months.

Deletion of Selenoprotein P

![Graphs showing weight changes and liver selenium levels](https://example.com/graph.png)

**Fig. 3.** Weights of Sepp<sup>−/−</sup> (squares) and Sepp<sup>+/+</sup> (circles) mice fed different diets and different amounts of selenium for the 8 weeks after weaning. Values are means ± S.D., n = 4–6 at weaning. A cross indicates death of an animal. D, male (filled symbols) and female (open symbols) animals fed chow diet containing 0.29 mg of selenium/kg. B, male mice fed a selenium-deficient diet. C, male mice fed a diet with 0.05 mg of selenium/kg. D, male mice fed a diet with 0.10 mg of selenium/kg.

The Sepp<sup>−/−</sup> mice did not survive for more than a few weeks, whereas Sepp<sup>+/−</sup> and Sepp<sup>+/+</sup> mice survived. To investigate this sensitivity of Sepp<sup>−/−</sup> mice to selenium deficiency, selenium and glutathione peroxidase were measured in tissues from male mice of all three genotypes that for 8 weeks had been fed diets containing amounts of selenium ranging from deficient to 20 times the dietary requirement. These mice were obtained by mating Sepp<sup>+/+</sup> males with Sepp<sup>−/−</sup> females.

Sepp<sup>−/−</sup> mice fed the selenium-deficient diet (no selenium supplementation) gained weight for about a week and then began to suffer from loss of motor coordination and weight (Fig. 3B). When mice had lost 20% of their highest recorded body weight, they were euthanized. Their average survival time from weaning was 15 days. Sepp<sup>−/−</sup> mice fed the diet with 0.05 mg of selenium/kg also had decreased survival times; 2 of 4 mice were sacrificed because of 20% weight loss, one at 3 weeks and the other at 6.5 weeks (Fig. 3C). Thus only 2 Sepp<sup>−/−</sup> mice fed this diet remained alive at the 8-week time point. Animals fed an 0.1 mg of selenium or more/kg diet all survived to 8 weeks without loss of coordination, and weights were similar for all genotypes (Fig. 3D).

Two selenoproteins contribute to plasma selenium: extracellular glutathione peroxidase (GSHPx-3) and Se-P. Fig. 4A shows the selenium content and glutathione peroxidase activity of plasma at different levels of dietary selenium supplementation. The absence of Se-P from the plasma of Sepp<sup>−/−</sup> mice is reflected in the very low plasma selenium concentrations measured at all levels of selenium supplementation (Fig. 4A, left panel). Selenium concentrations in Sepp<sup>−/−</sup> mice were intermediate between the homozygote and wild type concentrations at each supplementation level. This finding is consistent with the gene-dose effect indicated in Fig. 2D by the Se-P concentrations in plasma.

Plasma glutathione peroxidase activity increased as the dietary selenium supplement was increased from 0 to 0.1 mg/kg in wild type (Sepp<sup>+/+</sup>) mice and maintained that level as dietary selenium was increased further (Fig. 4A, right panel). This result is consistent with the established dietary requirement of 0.1 mg of selenium/kg in mice (26, 27). Sepp<sup>−/−</sup> plasma glutathione peroxidase activities were not significantly different from activities in the Sepp<sup>+/+</sup> mice. Sepp<sup>−/−</sup> mice had lower plasma glutathione peroxidase activities than Sepp<sup>+/+</sup> mice at levels of selenium supplementation below 1.0 mg/kg. These results indicate that Se-P is needed to achieve normal plasma activity of GSHPx-3 at dietary selenium concentrations up to 5 times the requirement but not when the dietary selenium concentration is 10 times the required concentration.

Liver selenium levels were not affected by deletion of Se-P, except when dietary selenium was below the selenium requirement (Fig. 4B, left panel). The two Sepp<sup>−/−</sup> animals in the 0.05 mg selenium/kg diet group that survived for 8 weeks had levels that were greater than the averages of the other groups. The liver selenium levels of the two Sepp<sup>−/−</sup> animals that did not survive 8 weeks were also greater than the 8-week averages of the other animals (see legend for Fig. 4). Liver glutathione peroxidase activities had the same pattern (Fig. 4B, right panel) as liver selenium concentrations. These results indicate that the Sepp<sup>−/−</sup> liver retains more selenium than the Sepp<sup>+/+</sup> liver during selenium deficiency and that deletion of Se-P does not cause a decrease in liver selenium content at any dietary selenium level. They also demonstrate that deletion of Se-P does not impair regulation of selenium concentration in liver.
The liver selenium concentrations of the mice that died at 3 and 6.5 weeks were 910 and 1000 ng/g, respectively. In Sepp−/− mice, on the other hand, varied or was similar. The brain was also severely affected with its selenium concentration being 19% of Sepp+/+ when selenium was fed at the dietary requirement. Moreover, increasing dietary selenium 20-fold did not raise the level of testis selenium into the Sepp+/+ range. The brain was also severely affected with its selenium concentration being 43% of control at the dietary requirement. However, brain selenium was raised into the Sepp+/+ range by increasing dietary selenium 20-fold. Kidney selenium was less affected, being 76% of Sepp+/+ when the dietary selenium requirement was fed. Moreover, increasing dietary selenium to

![Image](https://www.jbc.org/)

**Fig. 4.** Selenium concentration and glutathione peroxidase activity in plasma (A) and liver (B) of mice with deletion of Se-P (Sepp+/+, circles; Sepp−/−, diamonds; Sepp−/+ squares). Mice were fed, for the 8 weeks following weaning, on experimental diets with selenium added as indicated in the figure. Values are means ± S.D., n = 4–6 except value marked with an asterisk where n = 2. The other two mice died at 3 and 6.5 weeks. All values shown in A, left panel, at each dietary selenium level are different by the Scheffe test (p < 0.05), except Sepp+/+ and Sepp−/− at 0 and 0.05 mg of selenium/kg. In A, right panel, Sepp−/+ values at 0.05, 0.10, 0.25, and 0.50 mg of selenium/kg are different by the Scheffe test (p < 0.05) from the corresponding Sepp−/+ values, but no difference between them is present at 1.0 or 2.0 mg of selenium/kg. The liver selenium concentrations of the mice that died at 3 and 6.5 weeks were 910 and 1000 ng/g, respectively. In B, left panel, the Sepp−/+ values at 0.05 and 0.25 mg of selenium/kg are different from the corresponding Sepp−/+ and Sepp−/− values by the Scheffe test (p < 0.05). In B, right panel, the Sepp−/+ value at 0.05 mg of selenium/kg is different from the corresponding Sepp−/+ and Sepp−/− values by the Scheffe test (p < 0.05). Other values at each dietary selenium level are not significantly different from each other.

**Fig. 5.** Selenium concentrations in the testis (A), brain (B), kidney (C), and heart (D) of mice with deletion of Se-P. The results are for the same groups depicted in Fig. 4. Two of the Sepp−/− mice fed the diet with 0.05 mg of selenium added/kg survived 8 weeks; the average of their values is indicated by the symbol with an asterisk. Tests were not available for analysis for all animals. Values are means ± S.D., n = 4–6. Some S.D. are too small to be displayed on the figure. In A, all Sepp−/− values are different from corresponding Sepp+/+ and Sepp−/+ values by the Scheffe test, p < 0.05. In B, Sepp−/+ values from 0.05 to 1.0 mg of selenium/kg are different from corresponding Sepp+/+ and Sepp−/+ values by the Scheffe test, p < 0.05. The Sepp−/+ value at 2.0 mg of selenium/kg is not significantly different from the Sepp−/+ value. In C, the Sepp−/+ values at 0.05, 0.10, and 0.5 are different from corresponding Sepp+/+ and Sepp−/+ values by the Scheffe test, p < 0.05. In D, none of the values at individual dietary levels are significantly different from one another.

when higher levels of selenium are fed.
just 2.5-fold the requirement raised the selenium content of Sepp$^{-/-}$ kidney to the same level found in Sepp$^{+/+}$ kidney. Heart selenium concentration was not significantly affected by genotype. These results indicate that Se-P facilitates selenium accumulation in testis, brain, and kidney. Heart selenium content does not appear to depend on Se-P. Thus, the tissues studied vary in their dependence on Se-P to maintain their selenium concentrations.

**Tissue Uptake of $^{75}$Se**—To determine the effect of Se-P deletion on the disposition of a single dose of selenium, a tracer dose of $^{75}$Se as sodium selenite was administered by gavage to mice being fed the diet supplemented with 0.1 mg of selenium/kg. Tissues were harvested 24 h after administration, and their $^{75}$Se content was determined; Fig. 6 shows the results. Liver showed a trend of containing more of the $^{75}$Se administered in Sepp$^{-/-}$ mice than it did in Sepp$^{-/-}$ or Sepp$^{+/+}$ mice. Brain and testis contained less $^{75}$Se in Sepp$^{-/-}$ mice than in the other two genotypes. These results show that absorbed selenium is readily taken up by the liver in Sepp$^{-/-}$ mice but is less well taken up and/or retained by the brain and testis. These results are consistent with the tissue selenium levels shown in Figs. 4 and 5.

**DISCUSSION**

Mice with Se-P deleted have been produced by homologous recombination. These mice are viable but exhibit a profound alteration in selenium metabolism, which renders them intolerant of low dietary selenium intake. When intake is low they develop impaired movement and coordination and do not maintain their weight. In addition, males have sharply reduced fertility, even when fed enough selenium to prevent the movement abnormality and weight loss.

These two phenotypes correlate with the effect of Se-P deletion on the selenium content of the brain and testis (Fig. 5, B and A, respectively). Low selenium in the brain correlates with the apparent neurological impairment, and low selenium in testis appears to underlie the impaired male fertility. Selenium is essential for spermatogenesis (28, 29). Brain and testis have been identified as tissues that retain selenium well under conditions of extreme selenium deficiency (30, 31). Therefore, a decade ago we compared the ability of these two tissues to take up selenium administered in the form of $^{75}$Se-labeled Se-P (7). Both tissues took up the $^{75}$Se avidly. The brain was able to increase its uptake more than 4-fold when selenium deficiency was imposed. In contrast, testis uptake, although brisk, was unaffected by selenium deficiency. These results and the present ones suggest that the brain and testis remove Se-P from plasma to acquire its selenium. Moreover, the brain would appear to be able to up-regulate this uptake mechanism in selenium deficiency. In addition, the neurological phenotype could be prevented by feeding selenium to Sepp$^{-/-}$ mice at the level of dietary requirement or higher. This suggests that the brain can take up other plasma forms of selenium than Se-P and therefore utilizes two or more mechanisms to obtain the selenium it needs. No evidence was found that the testis is able to utilize a form of selenium other than Se-P.

The severity of the effect of Se-P deletion on selenium concentration forms a continuum in the tissues we examined. Although there were severe effects on the testis and brain, the kidney was only moderately affected and the heart was not significantly affected. This indicates that other transport forms of selenium than Se-P exist and that each tissue may have preferred forms of plasma selenium from which it obtains the element.

In the mice studied here, plasma selenium was present as Se-P, GSHPx-3, and small molecule forms. The origin of plasma Se-P is mostly liver (6) and that of GSHPx-3 is kidney (32). The intestine is known to release a small molecule form of selenium that is taken up by the liver (6), but additional small molecule forms of selenium, other than excretory metabolites, have not been detected. It can be inferred that the kidney can also take up a small molecule form of selenium, because kidney is the source of plasma GSHPx-3 and the selenium concentration of kidney responds to dietary selenium in animals lacking Se-P (Fig. 5C).

There is no direct evidence that GSHPx-3 serves to supply tissues with selenium. However, one finding in this study is consistent with such a function. Plasma glutathione peroxidase activity did not rise to its control level until 10 times the dietary requirement of selenium was fed (Fig. 4A), whereas kidney selenium level rose to control at only 2.5 times the requirement (Fig. 5C). If it is assumed that synthesis and secretion of GSHPx-3 was normalized when kidney selenium was at control levels, then increased removal of GSHPx-3 from plasma must be invoked to explain the low glutathione peroxidase activity in plasma at that point. Mice with deletion of GSHPx-3 will facilitate study of its putative transport role.

The liver has a central role in selenium metabolism. It receives small molecule selenium directly from the intestine and is the major organ for removal of selenium from the dietary form, selenomethionine, via the trans-sulfuration pathway (33). The liver also is the principal organ producing excretory metabolites of selenium to prevent the accumulation of toxic levels of the element. Thus, the liver is the portal through which selenium enters the body and the organ that maintains its homeostasis through excretion of the element.

Secretion of Se-P into the plasma is another important function of the liver. The increase of liver selenium concentration in selenium-deficient mice with deletion of Se-P is therefore not surprising (Fig. 4B). Selenium entering the liver in Sepp$^{-/-}$ mice is presumably diverted from export as Se-P to hepatic selenoproteins such as glutathione peroxidase. Se-P deletion did not interfere with the regulation of liver selenium content when levels above the dietary requirement for selenium were fed. Whether the liver selenium concentration was maintained by release of selenium in a form that could be taken up by other tissues or by production of excretory metabolites was not addressed in these experiments. Determination of this will be important in the elucidation of selenium homeostasis.

The studies reported here address the selenium transport function of Se-P. They indicate that the testis and brain have mechanisms for acquiring selenium from plasma Se-P. The characterization of those mechanisms will require additional research, as will assessment of the putative oxidant defense role of Se-P.
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Addendum—During final preparation of this manuscript, a report on Se-P gene deletion in mice was published by Schomburg et al. (34).

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Deletion of Selenoprotein P
Deletion of Selenoprotein P Alters Distribution of Selenium in the Mouse

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