

Subcellular Distribution of Selenium-Containing Proteins in the Rat

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ABSTRACT

The subcellular distribution of selenium in rat tissues was studied by measuring ^{75}Se in animals provided for 5 months with [^{75}Se]selenite as the main dietary source of selenium. Equilibration of the animals to a constant specific activity allowed the measurement of ^{75}Se to be used as a specific elemental assay for selenium. Of the whole-body selenium, 51% was in the soluble fractions and 48% was bound to the particulate fractions as follows: 21% in plasma membranes, 11% in microsomes, and 16% in mitochondria. Glutathione peroxidase was primarily a soluble enzyme, but part of the activity was associated with plasma membrane in liver, mitochondria in liver and kidney, and microsomes in testes. Selenium in glutathione peroxidase accounted for about one-third of the particulate-associated selenium. These results indicate that other selenium-containing proteins besides glutathione peroxidase are present in membranes.

INTRODUCTION

Although many bacterial enzymes contain essential selenium [1-4], glutathione peroxidase is the only mammalian enzyme that is known to require selenium. The selenium in glutathione peroxidase is present as selenocysteine [5] inside the peptide backbone [6]. Another report has shown that more than 80% of the selenium in the rat is present as selenocysteine in a group of at least eight proteins, and that glutathione peroxidase accounts for about one-third of the whole-body selenium [7].

The beneficial biological effects of dietary selenium have been rationalized in terms of protection by glutathione peroxidase of cell membranes from lipid peroxidation-induced damage [8]. However, since glutathione peroxidase is primarily a soluble enzyme and accounts for only one-third of the selenium in rats, this hypothesis seems incomplete.

This study was undertaken to measure the abundance of membrane-bound selenium in the major selenium-containing organs of the rat, and to measure the subcellular

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distribution of selenium, glutathione peroxidase activity, and selenium-containing proteins in liver, kidney, and testes. In the rat, these tissues have the highest selenium concentrations. Part of this research was presented previously at a scientific meeting¹.

MATERIALS AND METHODS

⁷⁵Se Equilibration

Five male weanling rats (40–60 g) of the Sprague-Dawley strain (Simonsen Laboratories, Inc., Gilroy, CA) were maintained on a selenium-deficient, Torula yeast-based diet (Teklad Test Diets, Madison, WI) supplemented with 40 I.U. *dl*- α -tocopheryl acetate/kg [9]. The diet contained less than 0.02 ppm selenium. [⁷⁵Se]Selenious acid (New England Nuclear, Boston, MA) was diluted with unlabeled sodium selenite in 0.5 M HCl to a specific activity of 50 mCi/mmol. The radiolabeled selenite stock solution was diluted (1:1000) each week to a concentration of 0.2 ppm selenium in distilled water that was supplied as drinking water for the 5-month equilibration period. The diet and drinking water were supplied ad libitum. That the tissues of the rats were at least 98% equilibrated with ⁷⁵Se could be seen from the weight gain (50–460 g) and from the longest estimates of the biological half-life of selenium in rats of 48–56 days [10, 11]. The percentage equilibration was calculated as % equilibration = $(1 - (50 \text{ g}/460 \text{ g} \times e^{-(0.693 \times 150 \text{ days}/52 \text{ days})}) \times 100\%$. The 98.5% calculated in this manner is a conservative estimate since most of the selenium in the rat has a biological half-life of 7–14 days [10]. The tissues had selenium concentrations [7] similar to those of rats fed selenium-sufficient diets wherein the selenium was provided in the solid food for approximately the same time period and at about the same level [12].

Tissue Sample Preparation

The rats were anesthetized with ether, and cardiac puncture was used to collect blood into syringes that contained 0.6 ml of 1.5% EDTA/0.9% NaCl. The carcass was perfused with 50 ml of ice-cold Hank's balanced salt solution containing 0.02% sodium heparin (w/v). The excised tissues were placed in ice-cold 0.25 M sucrose/10 mM Tris-HCl (pH 7.8)/0.1 mM EDTA (Buffer A). The tissues were minced with scissors and homogenized in 2 volumes of Buffer A with six strokes of a glass-Teflon homogenizer. Heart and hind leg muscles were homogenized by grinding for 90 sec in a microblendor.

Subcellular Fractionation of Homogenates

The total soluble selenium was measured after diluting the tissue homogenates with 3 volumes of Buffer A and centrifuging them for 60 min at 100,000 $\times g$. The amounts of selenium in the various particulate fractions were measured following equilibrium density centrifugation. A 0.6-ml aliquot of homogenate was layered onto 29.4 ml of Percoll™ (Pharmacia Fine Chemicals, Piscataway, NJ), at a starting density of 1.061 g/ml, and centrifuged for 27 min at 66,000 $\times g$. Erythrocyte ghosts were prepared by the method of Dodge et al. [13].

¹ W. C. Hawkes, E. C. Wilhelmsen, and A. L. Tappel, *Fed. Proc.* 42, 928 (1983).

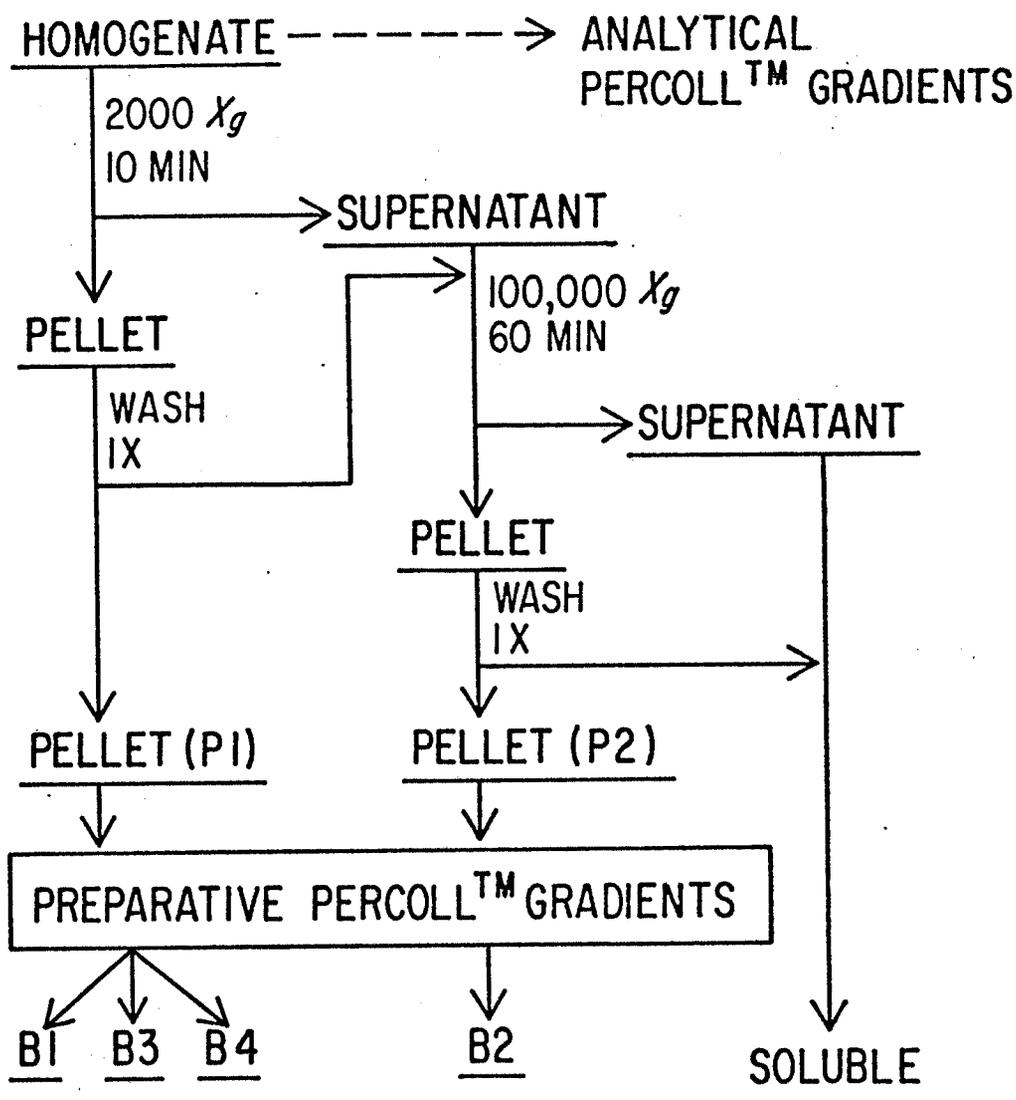


FIGURE 1. Subcellular fractionation of tissues. Tissues were homogenized (1:2) as described in Materials and Methods. Samples from all tissues were separated on analytical Percoll™ gradients (dashed line). The entire homogenates of liver, kidney, and testes were fractionated according to the scheme shown with solid lines. The main bands of ^{75}Se in the gradients are labeled B1–B4, in order of increasing density.

Subcellular Fractionation of Liver, Kidney, and Testes

Liver, kidney, and testes were fractionated by a combination of differential centrifugation and equilibrium density centrifugation on isopycnic gradients of Percoll™, as outlined in Figure 1. All procedures were performed at 0–4°C. Buffer A was used throughout these procedures for resuspending pellets and for making the Percoll™ gradients. The preparative gradients were prepared by mixing 2.5 ml of resuspended pellet with 27.5 ml of Percoll™, at a starting density of 1.086 g/ml, and centrifuging in 30-ml tubes at $66,000 \times g$ for 27 min. The buoyant density was measured by reference to internal standards of density marker beads (Pharmacia).

DEAE Sephacel Chromatography in Triton X-100 and Urea

One-half milliliter samples were mixed with 1 ml 25 mM imidazole-HCl (pH 7.8)/7 M urea/0.1% Triton X-100 (Buffer B), 0.2 ml Triton X-100, 25 mg dithioerythritol, and 0.75 g urea, and heated for 15 min at 50°C. The samples were centrifuged for 10 min at 3600 \times g, and the pellets were extracted twice with 1 ml of Buffer B and recentrifuged. The supernatants were combined and adjusted to pH 7.8 with 1 M imidazole before they were applied to 1.5 \times 30-cm columns of DEAE Sephacel equilibrated in Buffer B. The columns were washed with 60 ml of Buffer B, and then eluted with a linear gradient of 0–0.5 M NaCl in 300 ml of Buffer B. The column was eluted at room temperature at a flow rate of 0.33 ml/min, and 3-ml fractions were collected.

The ^{75}Se -labeled proteins were assigned to one of nine groups based on the fraction numbers and conductivities of the peak ^{75}Se fractions. These groups were designated as chromatographic forms A through I. Chromatographic form I was measured as the ^{75}Se remaining in the top 2 cm of gel after completion of the gradient. Further details on the application of this methodology to tissues and proteins have been published [7, 14].

Glutathione Peroxidase, Selenium, and Protein Assays

Glutathione peroxidase was measured by a modification of the coupled assay procedure of Little and O'Brien [15], with cumene hydroperoxide (30 $\mu\text{g}/\text{ml}$) and glutathione (0.21 mM) as substrates and with 10 mM NaCN to inhibit the nonselenium enzyme [16]. Glutathione peroxidase enzyme units (e.u.) were expressed as nanomoles of NADPH oxidized per min.

The whole animals, major organs, and carcasses were assayed for ^{75}Se with a NaI scintillation ratemeter in a shielded enclosure at a distance of 16 in. Samples with less radioactivity were counted for ^{75}Se in a gamma counter (model 5210, Packard Instruments, La Grange, IL). The amount of selenium was calculated by ratio to standards prepared from the same [^{75}Se]selenite solution added to the drinking water.

Protein was estimated by Coomassie Blue binding [17]. The plasma membrane marker, 5'-alkaline phosphodiesterase, was assayed using 5'-(*p*-nitrophenyl) thymidylate as substrate [18]. The mitochondrial marker, succinate-cytochrome *c* reductase, was assayed by the method of Tisdale [19], and the microsomal marker, NADPH-cytochrome *c* reductase, was assayed as described by Masters et al. [20]. The amount of soluble contamination in the pellets was approximated as the ratio of the pellet volume to the resuspended pellet volume: % soluble fraction = $V_p/V_t \times 100\%$, where the pellet volume (V_p) was measured by difference after resuspension.

Multiple Linear Regression Analysis

The amounts of selenium, glutathione peroxidase activity, and individual selenium-containing proteins associated with each of the subcellular fractions in each tissue were estimated by fitting the data to the following equations:

$$\begin{aligned} \text{Se/ml} = & (\text{Se/PM} \times \text{PM/ml}) + (\text{Se/Mt} \times \text{Mt/ml}) + (\text{Se/Mc} \times \text{Mc/ml}) \\ & + (\text{Se/\% sol} \times \% \text{ sol/ml}) \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Gpx/ml} = & (\text{GPx/PM} \times \text{PM/ml}) + (\text{GPx/Mt} \times \text{Mt/ml}) + (\text{GPx/Mc} \times \text{Mc/ml}) \\ & + (\text{GPx/\% sol} \times \% \text{ sol/ml}) \end{aligned} \quad (2)$$

where Se is selenium or an individual selenium-containing protein, GPx is glutathione peroxidase enzyme activity, PM is plasma membrane marker, Mt is mitochondrial

marker, Mc is microsomal marker, and % sol is the estimated percentage of the soluble fraction. These analyses were performed by computer using the method of backwards selection of variables [21]. All variables with negative coefficients were first eliminated, since all the correlations must, necessarily, have been positive. Those variables with a partial F value for deletion of less than 3 were eliminated. Finally, the remaining coefficients that were significant at $p < 0.05$ were used to estimate the contribution from each subcellular fraction.

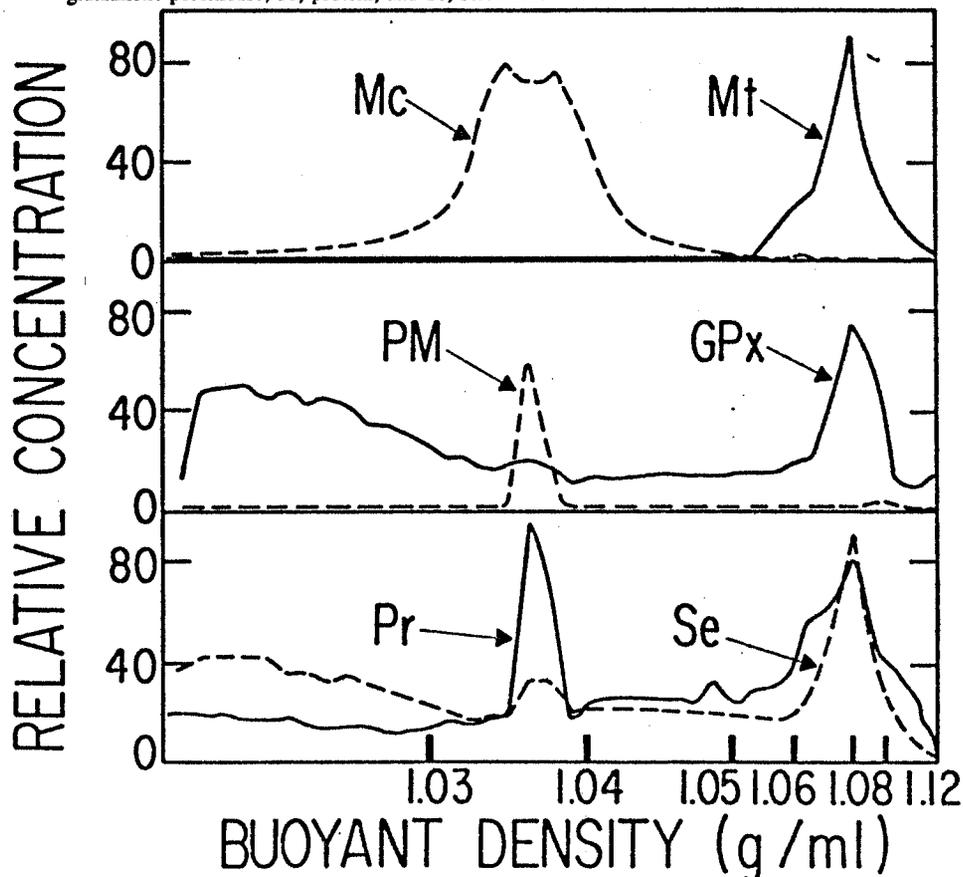
RESULTS

Subcellular Fractionation of Liver

The preparative Percoll™ gradient fractionations described above and shown in Figure 1 produced subcellular fractions for a number of analyses. The fractions were defined for liver, kidney, and testes by their content of marker enzymes, and were analyzed for selenium, glutathione peroxidase, and selenium-containing proteins separated by subsequent chromatography.

Two major pools of particulate selenium were evident in the analytical Percoll™ gradients of liver homogenates (Fig. 2). The lightest band ($\rho 1.04$) contained most of

FIGURE 2. Analytical Percoll™ fractionation of liver. A 0.6-ml aliquot of homogenate was layered on top of 29.4 ml of Percoll™ in 0.25 M sucrose at a mean starting density of 1.061 g/ml. Gradients were developed by centrifuging for 27 min at $66,000 \times g$. Selenium, protein, and enzyme activities were measured as described in Materials and Methods, and are displayed in arbitrary units of relative concentration. Abbreviations used: Mc, microsomes; Mt, mitochondria; PM, plasma membrane; GPx, glutathione peroxidase; Pr, protein; and Se, selenium.



the plasma membrane and microsomal markers, and the heavier band ($\rho 1.09$) contained most of the mitochondrial marker.

Further fractionation of liver showed that significant amounts of selenium copurified with plasma membrane in band 1, microsomes in band 2, mitochondria in bands 3 and 4, and with the soluble fraction (Table 1). Multiple linear regression analysis indicated that the distribution of selenium fit a model of 19% PM, 12% Mt, 9% Mc, and 55% soluble, and that the distribution of glutathione peroxidase activity fit a model of 17% PM, 13% Mt, and 64% soluble. While the percentage distributions of selenium and glutathione peroxidase were similar, the ratio of enzyme activity/selenium varied over a threefold range, reflecting the presence of nonglutathione peroxidase, selenium-containing proteins [7]. Evidence that the ^{75}Se in tissues was protein bound was obtained by release of selenium upon proteolysis, and further verification that the selenium was present as selenocysteine [7].

Except for chromatographic form C, all of the selenium-containing proteins in liver had similar subcellular distributions (Table 2). Although many of these chromatographic forms were enriched up to ninefold in various fractions, only the following associations were significant ($p < 0.05$) in the multiple linear regression analysis: 25% of D (the main chromatographic form of glutathione peroxidase) in mitochondria, and 75% in the soluble fraction; 36% of I in microsomes; and 60% of F in the soluble fraction.

Subcellular Fractionation of Kidney

Fractionation of the kidney homogenates on analytical gradients showed that most of the selenium sedimented with the main mitochondrial band at a density of 1.05 g/ml (Fig. 3). Further purification of the kidney subcellular fractions showed that both selenium and glutathione peroxidase activity copurified threefold with the mitochondrial marker in band 3 (Table 3). Smaller amounts of selenium and glutathione peroxidase activity also copurified with the microsomal marker in band 1. The glutathione peroxidase activity in band 4 (29% of the total) did not copurify with any of the markers. Multiple linear regression analysis showed that the selenium and glutathione peroxidase activity distributions fit a model of 65% of the selenium and 84% of the enzyme activity in mitochondria, and 29% of the selenium and 14% of the enzyme activity in the soluble fraction.

The subcellular distributions of selenium-containing proteins in kidney (Table 4) were very different from those in liver. While many of the chromatographic forms were enriched up to threefold in some fractions, the only significant associations ($p < 0.05$) in the multiple linear regression were 29% of A and 44% of B (glutathione peroxidase) in the soluble fraction, and 63% of E (glutathione peroxidase) and 100% of G in the mitochondria.

Subcellular Fractionation of Testes

Most of the selenium in analytical Percoll™ with the gradients of testes homogenates sedimented in the light membrane fraction ($\rho 1.03$) with the main bands of plasma membrane and microsomes (Fig. 4). There were also two heavier bands of selenium at $\rho 1.06$ and $\rho 1.08$ with the mitochondrial marker. Further purification of the testes subcellular fractions showed that selenium copurified with mitochondria in band 3 and

TABLE 1. Subcellular Fractionation of Liver^a

Fraction	Selenium		Glutathione peroxidase		5'-Alkaline phosphodiesterase		Succinate cytochrome c reductase		NADPH cytochrome c reductase	
	(%)	(pmol/mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b
Homogenate ^c	100	52 ^d	100	350	100	1.1	100	0.7	100	0.48
P1 ^e	28	76	21	250	69	2.6	99	4.0	22	0.54
P2 ^f	13	50	7.5	150	9.6	0.7	1.5	0.1	68	2.7
Soluble	58	160	72	1000	22	0.9	— ^g	—	11	0.30
Band 1 (P1)	5.6	79	2.4	170	41	8.6	5.1	1.2	11	1.4
Band 2 (P2)	8.8	79	1.9	180	4.8	0.6	1.0	0.1	44	3.9
Band 3 (P1)	11	115	2.8	280	4.5	1.6	30	6.8	2.5	0.26
Band 4 (P1)	9.3	95	10	400	7.9	0.8	36	5.6	0.55	0.08

^a Fractionation and assay procedures were as described in Materials and Methods and in Figure 1.

^b The specific concentration of selenium and specific activities of enzymes are per milligram of protein.

^c One hundred percent values are the sum of P1 + P2 + soluble.

^d Data are the averages for two rats.

^e 2000 × g pellet.

^f 100,000 × g pellet.

^g Not detectable.

TABLE 2. Subcellular Distribution of Selenium-Containing Proteins in Liver^a

Fraction	Percentage distribution of selenium-containing protein ^b						
	A	B	C	D	E	F	I
Homogenate ^c	100	100	100	100	100	100	100
P1 ^d	32	31	79	28	33	35	31
P2 ^e	16	7.1	—	7.9	17	18	27
Soluble	51	62	21	64	50	47	42
Band 1 (P1)	3.6	6.2	—	2.8	—	14	4.6
Band 3 (P1) ^f	8.9	5.1	78	3.9	15	16	14
Band 4 (P1)	2.5	6.3	—	10	9.4	5.1	12

^a The selenium-containing proteins were assayed by chromatography on DEAE Sephacel in denaturing conditions as described in Materials and Methods, and were expressed as a percentage of the total amount of each form in liver.

^b Data shown are the averages of the percentage of each selenium-containing protein in each subcellular fraction, in two rats.

^c One hundred percent values taken as the sum of P1 + P2 + soluble.

^d 2000 × g pellet.

^e 100,000 × g pellet.

^f Not detectable.

^g Data not available for band 2.

FIGURE 3. Analytical Percoll™ fractionation of kidney. Conditions, procedures, and abbreviations were the same as described in Figure 2.

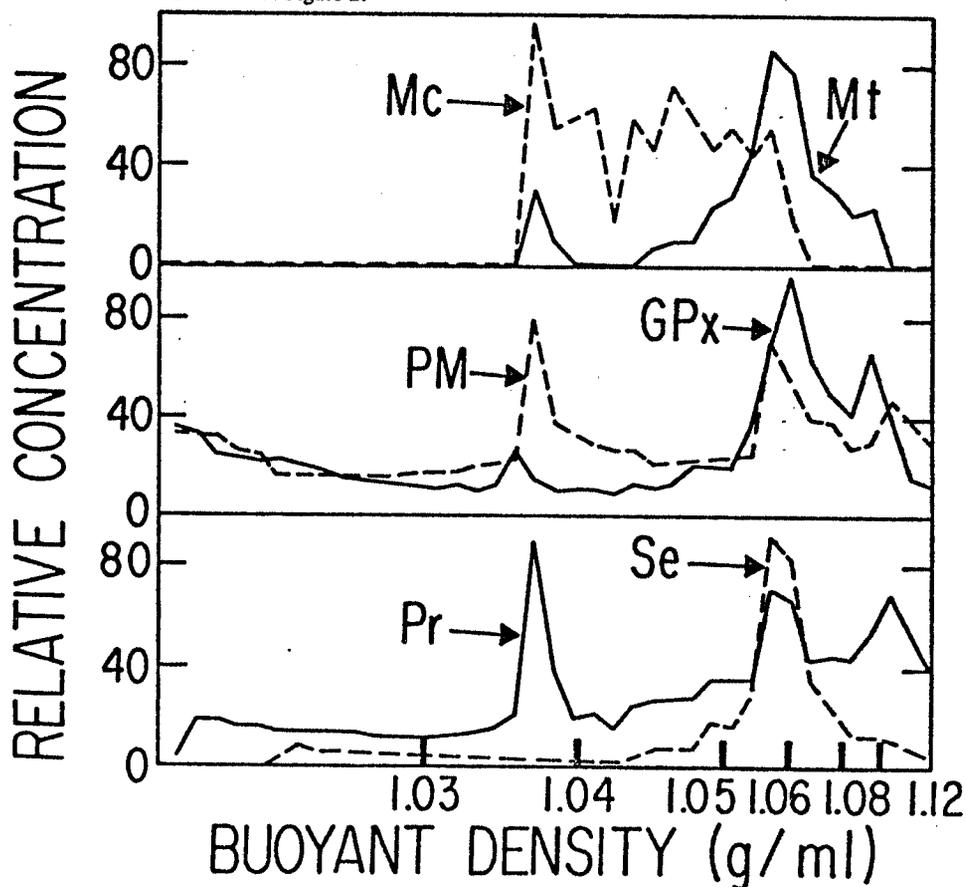


TABLE 3. Subcellular Fractionation of Kidney^a

Fraction	Selenium		Glutathione peroxidase		5'-Alkaline phosphodiesterase		Succinate cytochrome c reductase		NADPH cytochrome c reductase	
	(%)	(pmol/mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b
Homogenate ^c	100	280 ^d	100	300	100	9.6	100	1.2	100	0.10
P1 ^e	75	650	59	600	15	2.5	90	4.2	26	0.07
P2 ^f	5.7	95	3.7	68	85	16	10	0.64	69	0.35
Soluble	19	180	37	390	— ^g	—	0.21	0.01	5.2	0.04
Band 1 (P1) ^h	2.2	330	2.4	490	0.09	0.09	2.5	1.6	21	1.2
Band 2 (P2) ^h	1.4	120	0.46	43	4.4	3.1	10	3.7	20	0.66
Band 3 (P1) ^h	23	830	22	870	0.27	0.08	35	3.0	7.6	0.11
Band 4 (P1) ^h	4.5	74	29	530	0.001	0.0002	7.1	0.40	—	—

^a Fractionation and assay procedures were as described in Materials and Methods and in Figure 1.

^b The specific concentration of selenium and specific activities of enzymes are per milligram of protein.

^c One hundred percent values taken as the sum of P1 + P2 + soluble.

^d Data are the averages of measurements, in two animals, except as shown.

^e 2000 × g pellet.

^f 100,000 × g pellet.

^g Not detectable.

^h Single measurements.

TABLE 4. Subcellular Distribution of Selenium-Containing Proteins in Kidney^a

Fraction	Percentage distribution of selenium-containing protein ^b							
	A	B	D	E	F	G	H	I
Homogenate ^c	100	100	100	100	100	100	100	100
P1 ^d	53	40	58	86	85	81	81	82
P2 ^e	9.3	5.6	5.4	3.7	1.7	15	3.6	5.3
Soluble	37	53	37	10	13	4.3	16	14
Band 1 (P1) ^f	4.4	12	1.0	2.6	— ^g	—	2.8	3.2
Band 3 (P1) ^{f,h}	16	24	15	84	—	38	—	21
Band 4 (P1) ^f	14	5.2	16	—	—	—	—	2.9

^a The selenium-containing proteins were assayed by chromatography on DEAE Sephacel in denaturing conditions as described in Materials and Methods and were expressed as a percentage of the total amount of each form in kidney.

^b Data shown are the averages of the percentage of each selenium-containing protein in each subcellular fraction, in two animals, except as shown.

^c One hundred percent value taken as the sum of P1 + P2 + soluble.

^d 2000 × g pellet.

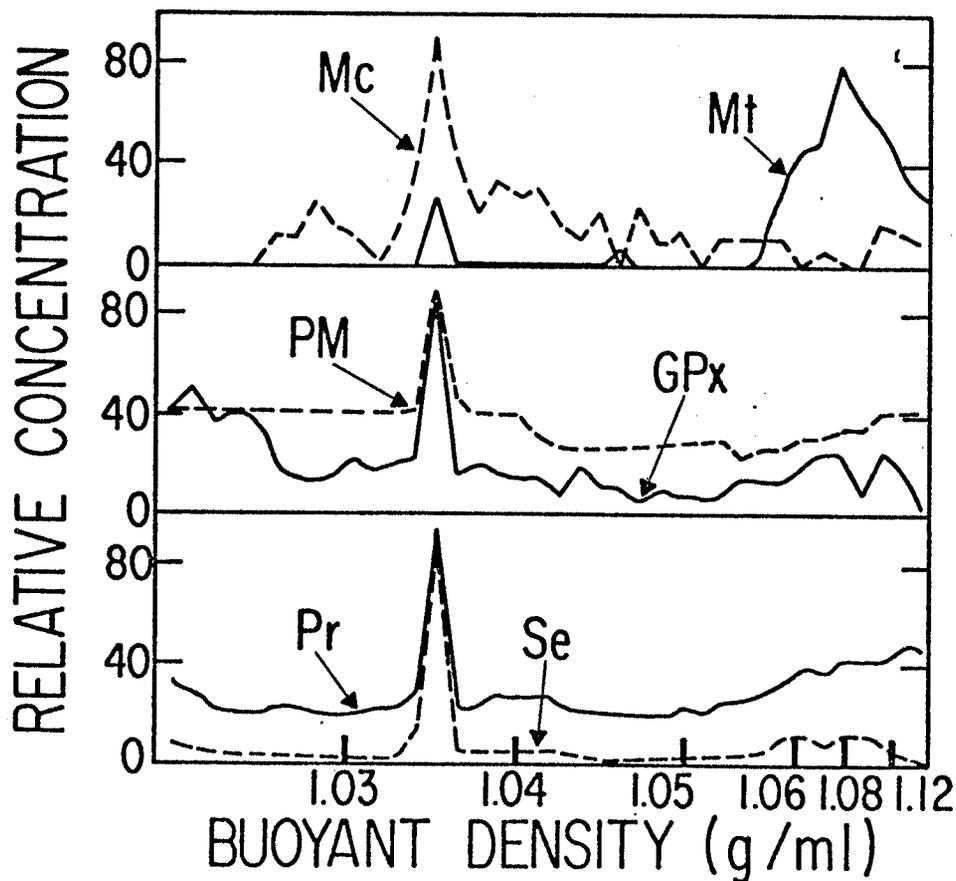
^e 100,000 × g pellet.

^f Single measurements.

^g Not detectable.

^h Data not available for band 2.

FIGURE 4. Analytical Percoll™ fractionation of testes. Conditions, procedures, and abbreviations were the same as described in Figure 2.



with plasma membrane in the soluble fraction (Table 5). Most of the particulate selenium was recovered in band 1 and did not copurify with any of the markers. Multiple linear regression analysis indicated the distribution of selenium fit a model where up to 60% was associated with the plasma membrane and up to 31% was associated with the mitochondria. A similar analysis of the glutathione peroxidase distribution indicated that 59% of the activity was associated with the soluble fraction and up to 32% was associated with the microsomes.

The distributions of the chromatographic forms of selenium-containing proteins were more heterogeneous in testes than in kidney or liver (Table 6). While several of the selenium-containing proteins were enriched up to ninefold in some fractions, the only associations that were significant ($p < 0.05$) in the multiple linear regression analysis were 98% of H in plasma membrane; 38% of B in mitochondria; and 87% of E, 94% of F, and 84% of G in the soluble fraction.

Subcellular Fractionation of Erythrocytes

The soluble fraction of erythrocytes contained 96% of the selenium and 98% of the glutathione peroxidase activity (Table 7). This is consistent with a previous report that soluble glutathione peroxidase accounts for 82% of the selenium in erythrocytes [22]. The erythrocyte ghosts contained much less selenium than did the membranes of liver, kidney, or testes. While most of the chromatographic forms of selenium-containing proteins were concentrated in the soluble fraction, chromatographic forms G, I, A, and E were enriched in the erythrocyte ghosts.

Whole-Body Subcellular Distribution of Selenium

Since the selenium in the tissues studied accounted for most of the whole-body selenium (except for plasma selenium), average values for the selenium distribution calculated by weighting the distribution in each tissue (Table 8) by the amount of selenium in each tissue and then normalizing by the total selenium are estimates of the subcellular distribution of selenium in the whole animal. Based on the detailed fractionations of liver, kidney, and testes (Tables 1, 3, and 5), and the Percoll™ gradients of the homogenates (Table 8), the following generalizations can be made: 48% of the whole-body selenium was bound to the particulate fractions. The selenium in band 1 was associated mainly with plasma membrane, and it accounted for 21% of the whole-body selenium. The selenium in band 2 was associated mainly with microsomes, and it accounted for 11% of the whole-body selenium. The selenium in bands 3 and 4 was associated with mitochondria, and it accounted for 16% of the whole-body selenium.

DISCUSSION

Subcellular Localization of Selenium

Although many of the subcellular fractions in liver, kidney, and testes were well separated from each other, the techniques used in these experiments did not allow complete purification of the individual particulate components. It is possible, therefore, that some of the selenium associated with a given subcellular marker may actually have been contained in a particulate component that was not assayed, e.g.,

TABLE 5. Subcellular Fractionation of Testes^a

Fraction	Selenium		Glutathione peroxidase		5'-Alkaline phosphodiesterase		Succinate cytochrome <i>c</i> reductase		NADPH cytochrome <i>c</i> reductase	
	(%)	(pmol/mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b	(%)	(e.u./mg) ^b
Homogenate ^c	100	258 ^d	100	43	100	2.3	100	0.18	100	0.18
P1 ^e	69	336	26	26	26	1.1	99	0.60	42	0.14
P2 ^f	10	258	12	62	2.7	0.59	0.80	0.03	44	0.74
Soluble	21	324	63	206	71	9.4	— ^g	—	14	0.14
Band 1 (P1)	33	333	5.8	22	—	—	4.1	0.08	—	—
Band 2 (P2)	4.8	66	3.6	11	1.3	0.24	0.92	0.01	9.5	0.20
Band 3 (P1)	14	260	4.4	29	—	—	25	0.82	—	—
Band 4 (P1)	3.7	95	2.1	20	17	6.5	3.0	0.14	—	—

^a Fractionation and assay procedures were as described in Materials and Methods and in Figure 1.

^b The specific concentration of selenium and specific activities of enzymes are per milligram of protein.

^c One hundred percent values taken as the sum of P1 + P2 + soluble.

^d Data shown are from measurements in one animal.

^e 2000 × g pellet.

^f 100,000 × g pellet.

^g Not detectable.

TABLE 6. Subcellular Distribution of Selenium-Containing Proteins in Testes^a

Fraction	Percentage distribution of selenium-containing protein ^b								
	A	B	C	D	E	F	G	H	I
Homogenate ^c	100	100	100	100	100	100	100	100	100
P1 ^d	85	91	76	77	10	12	21	29	78
P2 ^e	5.6	1.6	23	17	— ^f	6.9	—	—	10
Soluble	8.4	6.6	1.4	6.7	90	81	79	71	12
Band 1 (P1)	50	41	28	52	—	3.1	—	29	23
Band 3 (P1) ^g	34	15	5.8	9.8	10	6.3	2.9	—	7.4
Band 4 (P1)	1.5	6.7	1.4	11	—	2.5	—	—	3.8

^a The selenium-containing proteins were assayed by chromatography on DEAE Sephacel in denaturing conditions as described in Materials and Methods, and were expressed as a percentage of the total amount of each form in testes.

^b Data shown are the percentage of each selenium-containing protein in each subcellular fraction from one animal.

^c One hundred percent values taken as the sum of P1 + P2 + soluble.

^d 2000 × g pellet.

^e 100,000 × g pellet.

^f Not detectable.

^g Data not available for band 2.

TABLE 7. Distribution of Selenium-Containing Proteins in Erythrocytes^a

Fraction	Percentage distribution of selenium and selenium-containing proteins ^b							
	Selenium	Glutathione peroxidase	A	C	D	E	G	I
Soluble	96	98	95	99	99	95	— ^c	90
Ghosts	3.8	1.9	4.7	0.9	0.9	4.7	100	90

^a The selenium-containing proteins were assayed by chromatography on DEAE Sephacel in denaturing conditions as described in Materials and Methods, and were expressed as a percentage of the total amount of each form in erythrocytes.

^b The data are the averages for two animals.

^c Not detectable.

TABLE 8. Whole-Body Subcellular Distribution of Selenium^a

Tissue	Whole-body selenium (%)	Percentage of tissue selenium ^b								
		Soluble (%)	Band 1		Band 2		Band 3		Band 4	
			(%)	(ρ)	(%)	(ρ)	(%)	(ρ)	(%)	(ρ)
Leg muscle	36	34	38	1.041	17	1.048	11	1.052	— ^c	—
Liver	16	55	8.6	1.036	13	1.041	—	—	24	1.088
Erythrocytes	12	96	2.3	1.031	—	—	—	—	1.5	>1.14
Kidney	6.0	19	8.3	1.029	9.3	1.042	53	1.050	11	1.071
Testes ^d	4.4	17	41	1.033	9.7	1.040	18	1.055	15	1.077
Lung	3.3	46	22	1.041	17	1.051	12	1.060	3.0	1.135
Heart	1.2	44	25	1.039	13	1.050	—	—	17	1.090
Average Percentage Abundance ^e		51	21		11		9.5		6.3	

^a Soluble selenium was measured after centrifugation of homogenates at $100,000 \times g$ for 60 min, and selenium in the particulate fractions was measured after separation of the homogenates on isopycnic gradients of Percoll™ (1.061 g/ml mean starting density, 27 min at $66,000 \times g$).

^b Data shown are averages of measurements in two animals, except as shown.

^c Not detectable.

^d Single measurement.

^e Weighted by the percentage of whole-body selenium in each tissue.

nuclei, lysosomes, peroxisomes, golgi, etc. This may have been the case for band 4 in kidney and for band 1 in testes, neither of which copurified with any of the markers. On the other hand, the fact that certain fractions were not significantly correlated with selenium or selenium-containing proteins should not be interpreted to mean that those fractions did not contain selenium.

While the identification of bands 1 and 2 as plasma membrane and microsomes in the whole body is subject to some uncertainty, the very light density of these bands does indicate that they were composed primarily of membranes. Similarly, the heavy densities of bands 3 and 4 in all tissues indicate that these bands were composed primarily of intact organelles. Because of the large amounts of selenium in the muscle membrane fractions, further detailed fractionation studies would seem justified.

Subcellular Distribution of Glutathione Peroxidase

As reported by others [23, 24], glutathione peroxidase activity in liver, kidney, and testes was associated primarily with the soluble fraction. In addition, significant amounts of the activity were associated with the mitochondrial fraction in liver and kidney, a finding in agreement with results obtained with rat liver [25]. The apparent association of glutathione peroxidase activity with the plasma membrane in liver appears to be the first indication of a membrane-bound form of the enzyme in liver. Although the correlation of glutathione peroxidase activity with the microsomes in testes was only evident in the multiple linear regression analysis, other workers have reported the occurrence of a nonmitochondrial, particulate form of this enzyme in epididymal fat pad and seminal vesicle of rats [23].

Association of glutathione peroxidase activity with the membranes of liver and testes is reflected in the distributions of the chromatographic forms that are known to arise from the enzyme (B, D, and E). When the selenium in these three forms was summed, glutathione peroxidase was calculated to account for about one-third of the selenium in the particulate fractions in liver, kidney, and testes. This estimate is in agreement with that obtained by alternative calculations, based on the apparent specific activities of the various fractions—fraction of selenium in glutathione peroxidase = (e.u./pmol selenium)/8.1; where 8.1 is the specific activity of pure glutathione peroxidase [5]. The membrane-bound forms of glutathione peroxidase might provide an insight as to how this enzyme may act on lipid hydroperoxide substrates in membranes.

Membrane-Bound, Non-Glutathione-Peroxidase Selenium-Containing Proteins

Selenoproteins could be labile, therefore, there is the potential that artifacts were produced during the chromatographic fractionations used in this study. When the chromatographic fractionation procedure was applied previously [7, 14], the known selenoproteins, glutathione peroxidase and sperm selenoprotein, were recognized, thus, lending confidence that major artifacts were not introduced. Using the same methods described above, approximately two-thirds of the particulate-associated selenium in liver, kidney, and testes was calculated to be in selenium-containing proteins other than glutathione peroxidase. Several of these proteins were enriched in the membrane fractions; e.g., F in band 1 from liver, A and G in the 100,000 X g pellet from kidney, and A in band 1 from testes. The ratio of glutathione peroxidase

e.u./pmol selenium varied from 7.2 in kidney band 4 to 0.066 in testes band 1. Since the specific activity in kidney band 4 was nearly as high as in pure glutathione peroxidase, it is difficult to account for the low specific activities of the other fractions on the basis of enzyme latency. The low glutathione peroxidase activities and the low abundances of forms B, D, and E, therefore, must have been due to the presence of selenium-containing proteins other than glutathione peroxidase. Since the non-glutathione-peroxidase, selenium-containing proteins accounted for the majority of the particulate selenium, it seems reasonable to expect that they have important roles in membrane biochemistry.

Except for previous studies of rat liver mitochondrial matrix glutathione peroxidase [25], there is no information available as to the mechanism of binding of selenium-containing proteins to the various particulate fractions in which they occur. Since the selenium was not removed by repeated washings of the resuspended pellets, it seems likely that membrane-bound selenium-containing proteins reflect physiologically significant associations with the membranes. Further work needs to be done to define the mode and significance of this membrane binding.

Even though many important aspects of membrane-bound forms of selenium remain unexplained by this work, several key points have been established. Approximately one-half of the whole-body selenium in the rat is associated with the particulate fractions of the solid tissues; up to one-third of the whole-body selenium is membrane-bound; and most of the selenium associated with particulate fractions is not in glutathione peroxidase.

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