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Selenium Long-Term Administration and Its Effect on Mercury Toxicity

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An in vivo experiment was conducted to assess selenium bioaccumulation and bioaccessibility through the food chain and its effect on Hg toxicity. For this purpose 72 chickens were fed under different controlled conditions. Chickens were exposed to a common basal diet or a diet supplemented with Hg(II), MeHg, and Se(IV). Enzymatic digestion (feed, chicken muscle, liver, and kidney) as well as simulated human gastric and intestinal digestion (chicken muscle) led to the identification of selenomethionine (SeMet) in all the samples analyzed. Therefore, although chickens have no efficient mechanism for SeMet synthesis they can be considered as a source of SeMet due to its diet and the plant−animal food chain. The kidneys were the target organ for both total Se and SeMet in chickens (1604 ± 136 and 128 ± 6 μg kg^{-1}, respectively), but the greatest body store, among the tissues studied, was the muscle in both cases (84−96% of total Se). Long-term administration of inorganic and organic mercury did not alter SeMet distribution significantly. The antagonistic effect of Se on Hg toxicity by favoring MeHg demethylation is discussed.

KEYWORDS: Selenium; mercury; bioaccumulation; bioavailability; chicken

INTRODUCTION

Selenium (Se) is an essential micronutrient for animals and humans. To date, the major biological functions of selenium are attributed to its antioxidative properties and its roles in the regulation of thyroid hormone metabolism and cell growth (*1*). Although a variety of benefits of Se to human health have been reported (*2*), Se is also considered to be a toxic element at high concentrations.

The most important sources of selenium for human beings in the diet are cereals, meat, and fish. In fact, meat and fish appear to make rather stable contributions of selenium, generally around 40−50% of the total Se ingested (*3*). It has also been established that Se in food occurs in diverse chemical forms with different bioavailabilities (*3*). Therefore, the interest in total selenium and species content in meat samples of high consumption, as well as its bioavailability, are of special concern.

The absorption, distribution, and elimination of selenium in animals and humans can be markedly affected by nutritional and environmental factors (*4*). In fact, there is abundant evidence for a protective effect of selenium against heavy metal action in the body (*4*), which, consequently, can cause a change in its metabolism. Hence, in vivo interaction between dietary Se compounds (or their metabolites) and toxic metals are particularly important from a toxicological point of view.

Interaction of Se with mercury (Hg), one of the most hazardous environmental pollutants in the environment, has long been investigated, yet it is still incompletely understood (*5*). As reported by Parizek and Ostadalova (*6*), and subsequently confirmed by many other researchers, simultaneous administration of selenite counteracts the negative impacts of exposure to inorganic mercury, particularly in regard to neurotoxicity, fetotoxicity, and developmental toxicity (*7*). In addition to the antagonism by selenium of the toxicity of inorganic mercury, its detoxifying effect on methylmercury (MeHg) has attracted the attention of many scientists in heavy metal toxicology (*8*).

Several researchers discussed a few possibilities of the protective role of Se against Hg toxicity, including the redistribution of Hg in the tissues (*9*−*11*), the competition for binding sites (*12*), and the formation of Hg−Se complexes (*13*, *14*). However, there are controversial results on Se involvement in Hg detoxification, which is of prime concern to humans and animals for toxicological reasons.

Most studies have been conducted on the interactions between Hg and Se in mammals and fish systems. In marine mammals and seabirds, mercury is taken up from their diet mainly as methylmercury and then transformed into a less toxic form, inorganic mercury, in their bodies. Consequently, the large fraction of mercury stored at high concentration in the liver is inorganic Hg (*15*). It has been suggested that selenium is involved in the protection of the organism against Hg intoxication (*16*), detoxifying MeHg by forming complexes containing the two elements at an equimolar ratio. In fact, mercuric selenide (HgSe) has been found in the liver of some species of marine mammals and seabirds (*15*). This compound is assumed to be

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Table 1. Experimental Setup

treatment	no. of pens	total no. of chickens
basal diet		
control	1	8
basal diet + Hg(II) [0.2 mg kg^{-1} added to the feed]		
control	2	16
+ Se(IV) (0.2 mg kg^{-1} added to the feed)	2	16
basal diet + MeHg [0.2 mg kg^{-1} added to the feed]		
control	2	16
+ Se(IV) (0.2 mg kg^{-1} added to the feed)	2	16
total	9	72

an inert end product of the detoxification process in these marine animals (*15*).

In this work, as part of an ongoing study of Se bioaccumulation and bioaccessibility through the plant−animal−human food chain, Se quantification and speciation of feed and chicken samples and the subsequent in vitro enzymolyis of the sample were carried out.

In addition, to evaluate the possible antagonistic effect of selenium on mercury toxicity and to obtain insights into the detoxification mechanism of mercury by selenium, some in vivo experiments have been conducted in the presence of inorganic mercury and MeHg.

EXPERIMENTAL PROCEDURES

Animals, Diets, and Experimental Setup. Seventy-two 1-day-old Hybro-G female broiler chickens were used in this study. The birds were randomly assigned into nine pens for treatment, each with eight birds. All pens were bedded with a wood-shavings litter and equipped with feeders and waterers in an environmental chamber with 37.5 cm^2 per bird.

The chickens (during a study period of 42 days) were fed either with a common basal diet, formulated to contain all nutrients required, or with a diet supplemented with different compounds (Hg(II), MeHg, Se(IV)) specified in **Table 1**. Ingredients and chemical composition of the basal diet are shown in **Table 2**.

Lights were on 24 h during the first 3 days, after which a lighting schedule was applied consisting of 20 h light and 4 h darkness. The light intensity was reduced gradually during the experiment.

The diets and fresh water were offered ad libitum. The average drinking water consumption was 8.6 L, and the food intake was approximately 4.3 kg of feed.

The chickens were weighed at 0, 21, and 42 days of age to determine gains in body weight and food efficiency (**Table 3**). During the experiment, temperature and humidity were registered. These conditions were in accordance with animal welfare.

The experimental design consisted of five different dietary treatments (**Table 1**) to evaluate the effect of inorganic selenium (sodium selenite) on inorganic and organic mercury bioaccumulation.

For evaluation of mercury and selenium bioaccumulation in the indicated cases, all birds were slaughtered after 42 days. The carcasses were manually eviscerated, and the liver and kidney of each chicken were collected and stored individually at −18 °C.

Instrumentation. An inductively coupled plasma mass spectrometer (ICP-MS, HP-4500 Plus, Tokyo, Japan), fitted with a Babington nebulizer and a Scott double-pass spray chamber cooled by a Peltier system, was used for total selenium determination and selenium detection after chromatographic separation. Before coupling the chromatographic system, the ICP-MS working conditions were optimized by spacing the mass range from beryllium to uranium, at a 10 $\mu g\ L^{-1}$ level.

The chromatographic system, consisting of a PU-2089 HPLC pump (Jasco Corporation, Tokyo, Japan) fitted with a six-port sample injection valve (model 7725i, Rheodyne, Rohner Park, CA) with a 100 μL injection loop was used for chromatographic experiments. Selenium speciation was performed in a Hamilton PRP-X200 (10 μm, 250 mm × 4.1 mm i.d.) (Reno, NV) cation exchange column.

Table 2. Composition of the Basal Diet Used in the Experiments

ingredients	0-42 days (%)
barley	5.00
wheat	30.00
maize	18.57
soybean	35.90
soya oil	6.45
calcium carbonate	0.56
dicalcium phosphate	2.27
sodium chloride	0.30
sodium carbonate	0.19
dl-methionine	0.16
avizyme 1300	0.10
SV-5211-MxMa	0.50
total	100.00
analysis	
true metabolizable energy (kcal/kg)	3075
dry matter (%)	88.55
PB (%)	22.18
EE (%)	8.50
FB (%)	2.63
ash (%)	6.28
carbohydrates (%)	32.57
sugars (%)	4.64
calcium (%)	0.91
phosphorus (%)	0.75
available phosphorus (%)	0.45
Cl (%)	0.22
sodium (%)	0.18
lysine (%)	1.23
methionine (%)	0.55
methionine + cystine (%)	0.93
Thr (%)	0.84
Trp (%)	0.28
lisina av. (%)	1.07
methionine + cystine avg (%)	0.82
Thr avg (%)	0.69
Trp avg (%)	0.24
LI-C18:2	4.60
Na + K − Cl	260.00
unsaturated (%)	6.95
saturated (%)	1.20

The chromatographic system was coupled to the ICP-MS by a 5 cm poly(tetrafluoroethylene) capillary tubing (0.5 mm i.d.) running from the column outlet to the Babington nebulizer inlet.

An atomic fluorescence spectrometer (AFS, Merlin 10.023, P.S. Analytical Ltd., Orpington, Kent, U.K.) was used to determine the total mercury content. Mercury vapor was generated in a flow injection system using a multichannel peristaltic pump (Gilson, Villiers-le-be, France), a six-way injection valve (Omnifit, Cambridge, U.K.), and a U-tube gas−liquid separator. The separator was coupled to a dryer membrane (Perma Pure Products, Farmingdale, NJ) to eliminate the moisture, and both together were used as an interface for CV-AFS.

Ten kilodalton cutoff filters (Millipore, MA) and an Eppendorf (Hamburg, Germany) Centrifuge 5804, F34-6-38, were used as a clean-up method.

For total Hg and Se determination, samples were microwave digested in double-walled advanced composite vessels using a 1000 W MSP (microwave sample preparation system) microwave oven (CEM, Mattheus, NC).

Reagents. Inorganic selenium solutions were obtained by dissolving sodium selenite and sodium selenate (Merck, Darmstadt, Germany) in deionized Milli-Q water (18.2 MΩ cm) obtained from a Millipore Milli-Q water purification system (Millipore, OH). Seleno-amino acids ($SeCys_2$ and SeMet) were purchased from Sigma and dissolved in 3% (v/v) HCl and deionized Milli-Q water (Millipore, OH), respectively. Trimethylselenonium chloride was synthesized in our laboratory following the procedure of Palmer et al. (*17*). Stock solutions of 10

Table 3. Effect of Hg, MeHg, and Se on Body Weight Gain and Food Conversion[a]

treatment	bird body wt (g) day 0	bird body wt gain (g) day 21	bird body wt gain (g) day 42	food conversion ratio (g of food g of gain^{-1}) day 21	food conversion ratio (g of food g of gain^{-1}) day 42
basal diet					
control	43 ± 2	733 ± 30	2426 ± 98	1.56 ± 0.05	1.72 ± 0.08
basal diet + Hg(II) [0.2 mg kg^{-1} added to the feed]					
control	43 ± 2	795 ± 29	2451 ± 85	1.54 ± 0.05	1.76 ± 0.08
+ Se(IV) (0.2 mg kg^{-1} added to the feed)	42 ± 2	769 ± 42	2359 ± 101	1.50 ± 0.06	1.72 ± 0.08
basal diet + MeHg [0.2 mg kg^{-1} added to the feed]					
control	41 ± 3	749 ± 32	2472 ± 91	1.52 ± 0.05	1.70 ± 0.06
+ Se(IV) (0.2 mg kg^{-1} added to the feed)	42 ± 2	798 ± 41	2462 ± 98	1.52 ± 0.05	1.74 ± 0.08

[a] Results are expressed as the mean value ± SD, $n = 8$.

mg L^{-1} were stored in the dark at 4 °C, and working standard solutions were prepared daily by dilution.

For HPLC−ICP-MS studies, the mobile phase was 4 mM pyridine formate (Merck) in 3% methanol (SDS, Barcelona, Spain). For the enzymatic hydrolysis procedure, Tris-HCl and the nonspecific protease *Streptomyces griseus* (Pronase E) (Merck) were used to prepare the feed and chicken tissue samples.

Enzymes and bile salts were purchased from Sigma Chemical Co. (St. Louis, MO): pepsin (Porcine), pancreatin (Porcine), and bile salts. α-Amylase was purchased from Merck (Darmstadt, Germany).

Mercury standards solutions were prepared by appropriate dilution of a stock mercury chloride solution [1000 mg Hg(II) L^{-1}] (Merck, Darmstadt, Germany) and methylmercury chloride [1000 mg MeHg L^{-1}] (Alfa Aesar, Karlsruhe, Germany) in deionized Milli-Q water (Millipore, OH). These solutions were stored in amber vials at −18 °C. Standards were prepared daily to reduce mercury losses by volatilization.

Stannous chloride (3% w v^{-1}), used as a reducing agent for Hg(II) in CV-AFS, was prepared by dissolving the appropriate mass of stannous chloride, anhydrous (Merck, Darmstadt, Germany), in 3 M hydrochloric acid that had been prepared by diluting 12 M hydrochloric acid (Merck, Darmstadt, Germany) with ultrapure water.

H_2O_2 (35%) from Panreac and HNO_3 (65%) were used for acid digestion of samples.

Argon (purity 99.999%, Carburos Metálicos, Spain) was used as a make-up gas, sheath gas at the transfer line, and as carrier gas with AFS, respectively.

Measurements. *Total Selenium Quantification.* To determine the total selenium content, the dry samples (50−200 mg) were digested with 1−2 mL of concentrated nitric acid and 0.5 mL of 35% hydrogen peroxide in an analytical microwave oven at 43% power output. The pressure was held at 20 psi for 15 min, at 40 psi for 30 min, and finally at 85 psi for 1 h.

The total selenium concentration was determined by ICP-MS. For this purpose, the isotopes ^{78}Se and ^{82}Se were monitored. ^{103}Rh was used as an internal standard. Total selenium concentration was determined by both external and standard addition calibrations of the signal obtained by ICP-MS.

Selenium Speciation. Portions of 150 mg of the dry samples were enzymatically hydrolyzed following a previously developed method (*18*). The extracts obtained were processed through 10 kDa mass cutoff filters, diluted to 10 mL, and analyzed by cation exchange chromatography coupled to ICP-MS, under the operating conditions given in **Table 4**.

The analytical peaks were evaluated in terms of peak area by a standard addition calibration method at *m*/*z* 78 and 82.

In Vitro Gastrointestinal Digestion Method. The in vitro digestion method used was based on a previously developed method (*19*) and adapted for the chicken sample being studied. About 50 g of sample was placed in a 250 mL Erlenmeyer flask with 150 mL of gastric juice (6% w v^{-1} pepsin in 0.15 M NaCl, acidified with HCl to pH 1.8) and shaken for 1 min for initial degassing. The mixtures were then held in a thermostated water bath for 4 h at 37 °C, shaking periodically.

After 1 h the pH was checked and adjusted to 3 with 6 M hydrochloric acid. After gastric digestion, saturated sodium bicarbonate was added to raise the pH to 6.8. Then 100 mL of intestinal juice (1.5%, w v^{-1} pancreatin, 0.5%, w v^{-1} α-amylase, and 0.15%, w v^{-1} bile salts, in 0.15 M NaCl) was added, and the mixture was energetically shaken for 1 min and left in a thermostated water bath for 4 h at 37 °C, shaking periodically. Once gastric/gastrointestinal digestion was completed, a 10 mL aliquot of the suspension was transferred to a polypropylene tube and centrifuged at 1575*g* for 1 h. The supernatant was filtered through a 0.45 μm Millipore filter to reduce any effect from microbial activity, and both supernatants and precipitates were stored in the dark at 4 °C until analysis. Gastric and intestinal digestion blanks were obtained by adding 150 mL of gastric juice to 50 mL of Milli-Q water and 100 mL of intestinal juice, respectively, and the above procedure was applied.

Table 4. Instrument Operating Conditions for Se Determination by HPLC–ICP-MS

HPLC parameters	
analytical column	PRPX-200
eluent	4 mM pyridine formate soln, H_2O:MeOH (97:3)
eluent flow rate	1 mL min^{-1}
elution program	isocratic
injection volume	100 μL
ICP-MS operating conditions	
forward power	1450 W
plasma gas (Ar) flow rate	15 l min^{-1}
auxiliary gas (Ar) flow rate	1.2 l min^{-1}
carrier gas (Ar) flow rate	1.1 l min^{-1}
spray chamber	double pass (Scott type)
nebulizer type	Babington
skimmer cone	nickel, 0.4 mm orifice
sampling cone	nickel, 1.0 mm orifice
acquisition mode	TRA
points per peak	3
integration time	0.7s

Total Mercury Quantification. The samples followed the same acid digestion as mentioned for total selenium quantification.

Total mercury concentration was determined by both external and standard addition calibrations of the signal obtained by continuous mercury cold vapor system connected to AFS equipment. A flow rate of 2.5 mL min^{-1} (3 M hydrochloric acid) and a similar flow rate of the reductant solution (3% stannous chloride in 3 M hydrochloric acid) were used to generate the mercury cold vapor.

Mercury Speciation. Mercury leaching was performed following an acid (HCl) leaching procedure developed previously (*20*).

Afterward, the total organomercury content in the supernatants was determined by difference between total mercury content (after digestion with HNO_3) and inorganic mercury content by using stannous chloride as a selective redundant.

Validation of the Results. In the present work, two certified reference materials were employed for validation of the methodologies used. Method validation for selenium was performed by using a marine tissue reference material (Murst-ISS A2), certified for total selenium (7.37 ± 0.91 μg g^{-1}), from the Institute for Reference Materials and

Table 5. Total Selenium Concentration and Distribution Found in Chicken Feed and Chicken Tissues after Basal Diet Administration[a]

	total Se		SeMet		
sample	total Se (μg kg^{-1})	distribution in chicken (%)	total SeMet (μg kg^{-1})	percentage of total Se (%)	distribution in chicken (%)
chicken feed (basal diet)	750 ± 42	–	680 ± 31	91	–
chicken kidney	1604 ± 136	9	128 ± 6	8	1
chicken liver	450 ± 83	7	63 ± 5	14	2
chicken muscle	225 ± 30	84	119 ± 7	54	96

[a] Results are expressed as the mean value ± SD, $n = 6$ different samples.

Measurement, while for total mercury the reference material CRM-463 (tuna fish), certified for methylmercury (2.85 ± 0.16 μg g^{-1}), from the Community Bureau of Reference of European Commission (BCR) was used.

Because, at the 95% confidence level, no significant differences were detected between the certified value and the experimental one [(2.87 ± 0.07 μg of Hg g^{-1}) and (7.42 ± 0.52 μg of Se g^{-1})], the method used was considered to be accurate for total mercury and selenium determination.

Statistical Analysis. Statistical evaluation of the data was performed with the program Statgraphics Plus version 4.0 (Statistical Graphics). The results are presented as absolute values of arithmetic means and standard deviations. The statistical differences in bioaccessible Se content, feed intake, and growth as well as total mercury and inorganic mercury content between the different treatments studied were analyzed by one-way analysis of variance (ANOVA) (at the level of significance of $P < 0.05$).

RESULTS AND DISCUSSION

Evaluation of Se Bioaccumulation. In an attempt to improve our understanding of the transfer process of selenium along the trophic chain, the uptake of selenium in chickens from feed was evaluated.

During the assays feeding trials, chickens were fed an animal feed of vegetal origin. To assess selenium uptake, total selenium content of the chicken feed and chicken tissues (liver, kidney, and muscle) as well as its speciation was carried out by ICP-MS and LC−ICP-MS, respectively.

The mean selenium concentration in chicken fed with the basal diet (no supplementation) ranged from 0.23 mg kg^{-1} in muscle to 1.6 mg kg^{-1} in kidney, the skeletal muscle being the largest body pool of Se (**Table 5**).

The enzymatic digestion specified in the procedure section followed by ultrafiltration with 10 kDa cutoff filters was applied to the chicken feed and chicken samples in order to identify and quantify the seleno-amino acids and inorganic Se species.

For chicken feed, no selenium losses were detected in this step (Se recoveries 93−97%), which indicates that the molecular weight of most of the selenium species extracted during the hydrolysis was lower than 10 kDa. On the other hand, the selenium recoveries for chicken kidney, liver, and muscle were not quantitative, which means that some selenium may remain in peptide form. This explanation stems from the knowledge that, during the enzymatic hydrolysis of proteins, some peptide bonds can remain intact, depending on the cleavage specificity of the enzyme. This could be the reason a selenium fraction with a molecular weight higher than 10 kDa remained after the enzymatic hydrolysis step.

To ensure that no selenium compounds lower than 10 kDa were retained in the filters, the total selenium content in the extracts (spiked with the standards) after filtration was determined. The results, with an average recovery of 97 ± 3% of total selenium, showed that significant selenium losses did not occur with this sample treatment.

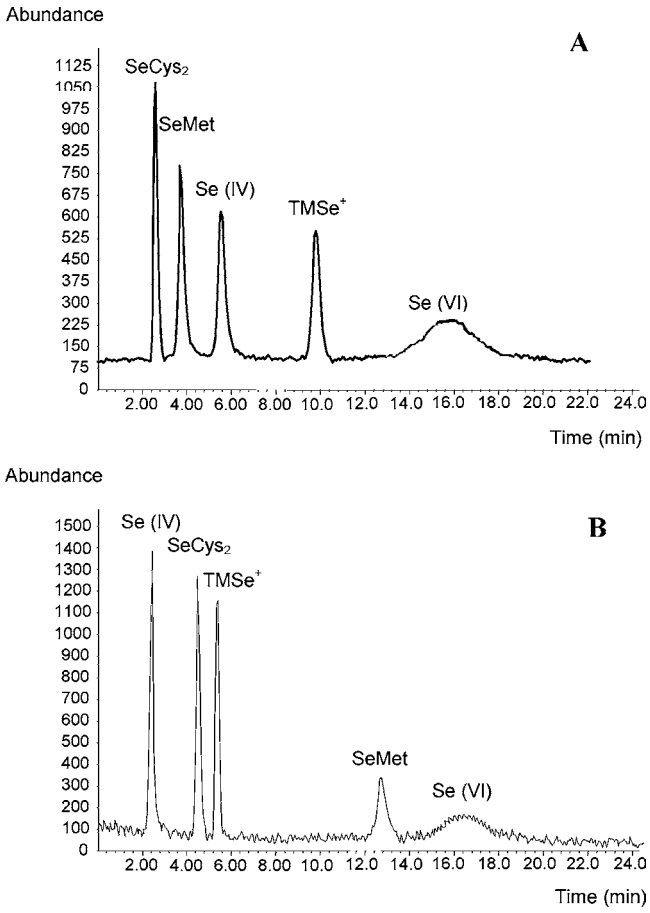

Figure 1. Chromatograms of 10 μg L^{-1} of Se species obtained using cationic exchange chromatography at two pH values: (**A**) 4.7 and (**B**) 2.8.

In this study, five standard Se compounds were tested for Se separation in a cationic exchange column. If a species is identified under two different chromatographic conditions, its identity can be stated with more certainty. Because of this, the identification and quantification were performed by the standard addition method using two different chromatographic conditions (pH 2.8 and 4.7). Standard chromatograms for both pHs are shown in **Figure 1**.

Chromatographic analyses were performed on the chicken feed and chicken samples. **Figure 2** shows the chromatograms obtained for these samples. Two peaks could be differentiated in each of the evaluated samples. The first peak was unidentified; it could not be attributed to any of the selenium species tested ($SeCys_2$, Se(IV), Se(VI), and $TMSe^+$), so it could correspond to any of the selenium species that elutes in the dead volume. The second peak was identified as SeMet, the only seleno-amino acid found in all samples. The identification of the peaks was carried out by the spiking procedure. The same chromatographic profiles were obtained by the two chromatographic methods used (at pH 2.8 and 4.7).

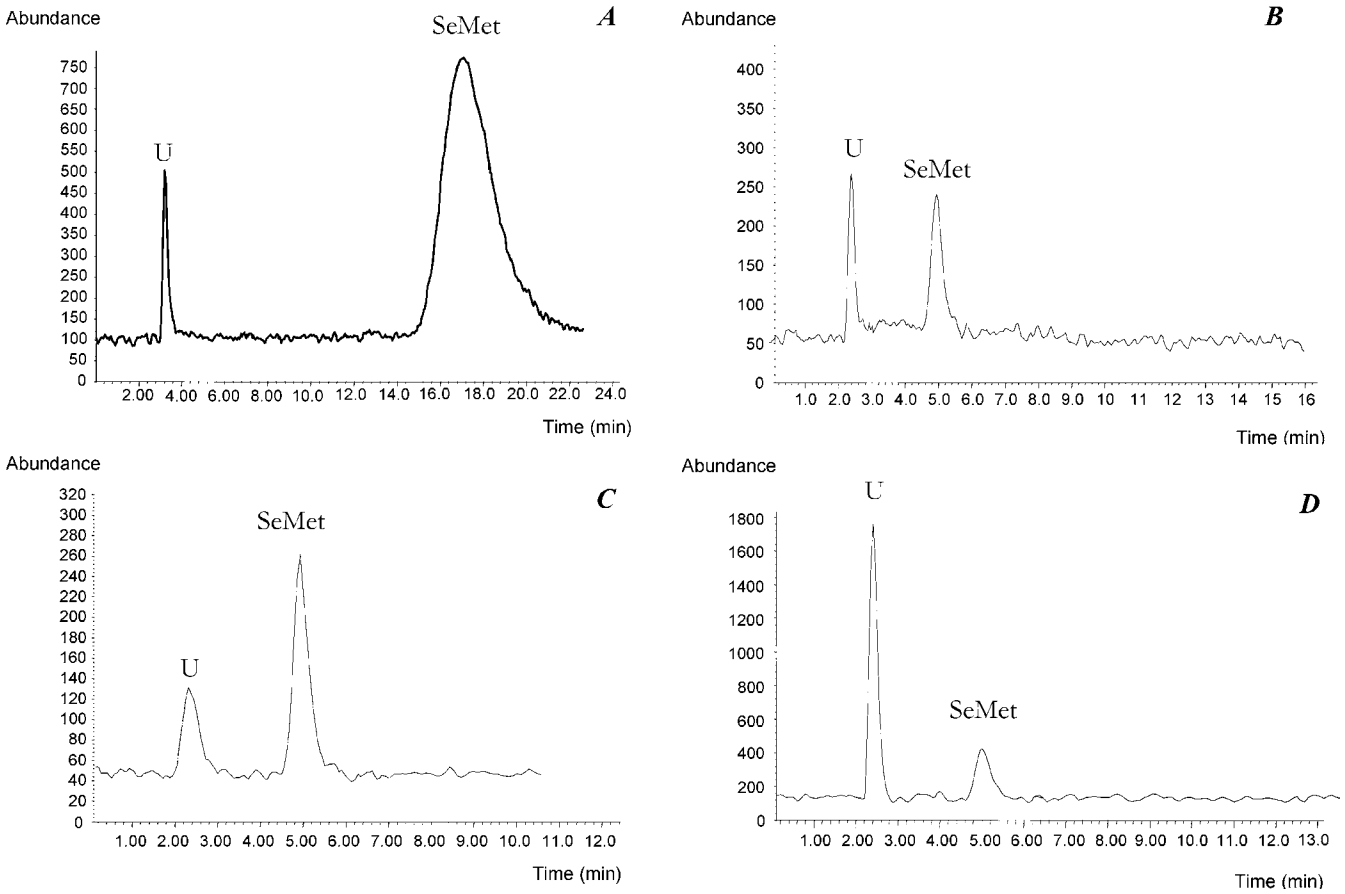

Figure 2. Chromatograms of Se species found after probe sonication extraction of (**A**) chicken feed at pH 2.8 and (**B**) chicken liver, (**C**) muscle, and (**D**) kidney at pH 4.7. U = unidentified Se species.

The results from the speciation analyses of the samples are shown in **Table 5**. The amount of SeMet varied depending on the type of sample.

In the chicken feed the main selenium species found was SeMet (91% of total Se). Therefore we can conclude that the chemical form of Se consumed by the animals used in this study was mostly SeMet.

According to the literature, plants such as cereals and forage crops convert Se predominantly into SeMet (*21*) and incorporate it into protein in place of methionine (*22*). Therefore, in nature animals receive Se mainly in the form of selenomethionine (*3*). Taking into account that the main components of the feed were corn, wheat, and soybeans, the value found is comparable with other SeMet data found in this type of cereal (*21*).

Each chicken consumed approximately 4.3 kg of feed throughout the feeding period. Therefore, 3.24 mg of total Se and 2.94 mg of SeMet were ingested.

Assuming that 55.6% of carcass weight is muscle, 2.3% is liver, and 0.7% is kidney and Se is evenly distributed throughout these tissues, 365 μg of total Se and 168 μg of total SeMet were bioaccumulated in the chickens analyzed. Therefore, this experimental animal study showed that 11% and 5.7% of the ingested Se was accumulated in the evaluated tissues as total Se and as SeMet, respectively. It was also noted that the greatest body store of Se and SeMet in chicken was the muscle, kidney being the target organ of total Se and SeMet.

Since higher animals are unable to synthesize SeMet (*21*), any detectable amount found in the organs and tissues must arise only from dietary sources. As a result, SeMet is incorporated into chicken tissue proteins in place of Met. This allows Se to be stored in the organism and reversibly released by normal metabolic processes, thus offering an advantage over other Se compounds. Any SeMet that is not immediately metabolized is incorporated into organs with high rates of protein synthesis such as the skeletal muscles, liver, and kidney.

In this case, intake of dietary SeMet is reflected in the SeMet content of the chicken. SeMet is incorporated into tissue proteins especially in the skeletal muscles (96%) and the liver, according to previously published data (*21*−*23*).

Selenium Bioaccessibility. The role of Se in human nutrition is an important topic of recent research, and it is well recognized that adequate dietary Se is an important determinant of human health (*24*). In some countries (e.g. the USA), 50% of the total Se in the typical diet is provided by beef, pork, chicken, white bread, and eggs. This means that meat is among the major Se sources for humans (*25*).

Se status relies on the dietary selenium intake and the element bioavailability. Therefore, total selenium and species determination in chicken meat (muscle) is of special concern because of its high consumption and its important contribution to the Se status of humans.

Unfortunately the total concentration of Se in food does not provide information about its bioavailability. The extent of the toxic or beneficial effects caused by Se is not governed by their total concentration, but rather regulated by the forms of the metal that can interact efficiently with sites on the biological ligands. Consequently, total determination and speciation of Se in the gastrointestinal tract is essential to understand and predict its availability for absorption (*26*).

To study Se bioaccessibility from human diet an in vitro

Table 6. Total Mercury Concentration Found in Chicken Tissues after Hg(II), MeHg, Hg(II) + Se(IV), and MeHg + Se(IV) Supplementation[a]

	total Hg content (μg kg^{-1})			percentage of inorganic mercury (%)		
treatment	kidney	liver	muscle	kidney	liver	muscle
Hg(II) (control)	85 ± 24	22 ± 4	6 ± 3	100	100	100
Hg(II) + Se(IV)	6 ± 3*	5 ± 3*	3 ± 1	100	100	100
MeHg (control)	190 ± 70	304 ± 36	113 ± 27	5 ±1	5.3 ± 0.2	nd
MeHg + Se(IV)	160 ± 10	841 ± 108**	206 ± 4**	14 ± 2**	7.7 ± 0.8**	1.3 ± 0.5**

[a] Results are presented as mean value ± S. D. n=6 different samples. * Statistically significant difference ($P < 0.05$) for planned comparisons: Hg(II) (control) vs Hg(II) + Se(IV). **Statistically significant difference ($P < 0.05$) for planned comparisons: MeHg (control) vs MeHg + Se(IV).

enzymolysis simulating the human gastrointestinal digestion of chickens fed with the basal diet (no supplementation) was carried out.

The recoveries of endogenous Se from chicken muscle in the gastric supernatant (pH 2.0) did not differ significantly ($P < 0.05$) from the gastrointestinal supernatant (pH 6.8). The amount of total Se found to be bioaccessible in the simulated stomach and intestinal digestion was 43 ± 3% and 40 ± 5%, respectively.

The percentage of ultrafilterable (<10 kDa) selenium (23% of total Se) following simulated gastrointestinal digestion of chicken muscle showed an important decrease in the bioaccessible fraction, which means that the in vitro digestion was not completely effective in breaking down the peptides or proteins in smaller fractions, so some selenium may have remained in peptide form.

A mass balance was performed after application of the in vitro digestion method. Both the soluble fraction and nonsoluble fraction resulting from application of the in vitro digestion method were analyzed. The mass balance result for Se was 96 ± 8%.

To evaluate whether the in vitro digestion method employed keeps the integrity of the selenium species present in the initial product, or brings some transformation, selenium speciation of the gastrointestinal extracts was carried out.

The identification of the peaks was carried out by the spiking procedure. The first peak was unidentified and could not be attributed to any selenium species tested. SeMet was found (second peak) to be the dominant Se species, being the only seleno-amino acid found in both extracts (gastric and gastrointestinal). The chromatographic profiles obtained by either chromatographic method were identical.

The results of speciation analysis of the gastrointestinal digestion extract shows that 21 ± 2% of the bioaccessible Se was found as SeMet.

Although chickens have no efficient mechanism for methionine synthesis and consequently are unable to synthesize SeMet, they are a source of SeMet due to their diet and the plant−animal food chain.

Effect of Mercury Long-Term Administration on Selenium Speciation. The effect of Hg on natural levels of Se (*27*) has been described previously; however, no speciation studies have been reported.

To evaluate the effect of mercury on selenium metabolism, the speciation of several chicken tissues (kidney, liver, and muscle) of chickens fed with inorganic and organic mercury has been carried out.

Se speciation of the chicken tissues revealed the same chromatographic profiles in all the cases. The first peak was unidentified, and the second one was identified as SeMet. Furthermore, the addition of Hg(II) or MeHg to the control diet did not change the SeMet distribution (**Figure 3**). Therefore, Hg exposure did not significantly alter SeMet uptake.

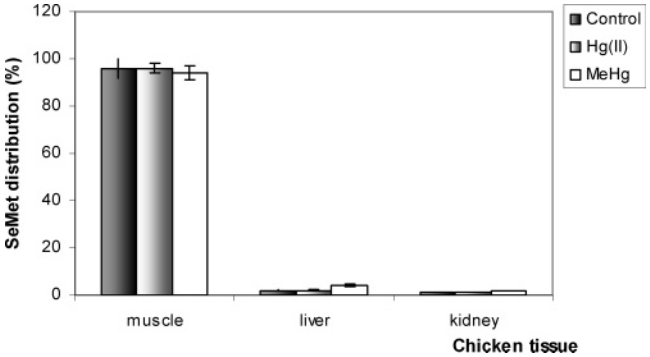

Figure 3. SeMet distribution in chicken tissues after exposition to a common basal diet (control) or a diet supplemented with Hg(II) or MeHg.

Effect of Selenium Long-Term Administration on Mercury Toxicity. *A. Feed Intake and Growth.* Data presented in **Table 3** show the effect of the five dietary treatments on body weight gain and food conversion ratios of broiler at 0, 21, and 42 days of age. Neither mercury nor mercury + selenium added to the basal diet had a significant effect on food conversion. Furthermore, the results show that the chickens fed with a mercury concentration of 0.2 mg kg^{-1} with or without Se had similar weights gains up to 42 days.

No significant differences were found in the food conversion among treatments and between groups on the five treatments ($P < 0.05$). Therefore, the inclusion of Hg(II), MeHg, and Se(IV) did not affect food conversion.

B. Hg−Se Antagonistic Mechanism. To understand the molecular basis of the Hg−Se antagonistic mechanism involved in the accumulation process of mercury in chicken, we designed a feeding experiment to test the effect of dietary selenite on dietary mercury poisoning. Since we were also interested in the molecular specificity of the mercury−selenium interaction, mercury was fed in two forms, mercuric chloride and methylmercury chloride.

To assess mercury uptake, total mercury content of the chicken tissues (liver, kidney, and muscle) from chickens fed with inorganic and organic mercury as well as speciation analyses were carried out by CV-AFS.

The mean mercury concentration in chicken ranged from 0.006 mg kg^{-1} in muscle to 0.304 mg kg^{-1} in liver (**Table 6**); however muscle represented the largest body pool of mercury.

As it has been reported previously (*27*), the addition of Se to a Hg(II) containing diet significantly alleviated the adverse accumulation of Hg in chicken. However, the addition of Se to the MeHg containing diet did not affect the mercury accumulation in kidney, but Hg concentration in liver and muscle was highly affected by Se administration.

As a consequence mercury speciation has been carried out to clarify whether Se enhances MeHg accumulation or MeHg conversion into less toxic forms.

The inorganic and total mercury determination was achieved by selective reduction with $SnCl_2$ followed by measurement with CV-AFS.

The results (**Table 6**) show that inorganic mercury does not undergo a biotransformation process because it remains unalterable as inorganic mercury. However, some of the MeHg which enters the body is partly demethylated to inorganic mercury (a less toxic form). It seems likely that most of this demethylation process takes place in the liver, with subsequent accumulation of Hg(II) in the kidneys (*28*). In fact, according to the literature a large fraction of mercury stored at high concentration in the liver of some animals (marine mammals and seabirds) is found as Hg(II) despite the fact that mercury is taken up from their diet mainly as methylmercury, performing a toxicologically less damaging alternative to the accumulation of MeHg.

Some authors suggest that Se is directly involved in the demethylation process. In fact, recently, Urano et al. (*29*) suggested that MeHg might act as a methyl donor for methylating selenide to the volatile (exhable) form. The net result of this would be the demethylation of MeHg by selenide, which then could lead to other interactions between selenide molecules and newly formed inorganic mercury (*30*).

In our case, the addition of Se to the MeHg-containing diet (**Table 6**) meant promotion of MeHg conversion into a less toxic form (inorganic Hg), because an increase, up to 145−280%, of MeHg demethylation in chicken liver and kidneys was observed.

It has been suggested previously that the process of demethylation of MeHg and inorganic mercury transformation by reaction with selenium to form mercuric selenide would be an effective mechanism for counteracting the potentially damaging action of mercury (*31*). Se, in addition to its role as micronutrient, could exert an antidotal action on the toxic effects of mercury through the formation of highly insoluble complexes consisting of mercury selenide HgSe (*31*). This compound is assumed to be an inert product of the detoxification process in marine animals (*32*).

In conclusion, this study shows that the liver is the main site of MeHg biotransformation in chickens. In addition, an antagonic effect of Se favoring a MeHg detoxification pathway involving the liver is observed.

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