THE ROLE OF SELENIUM IN NUTRITION

Gerald F. Combs, Jr.
Stephanie B. Combs

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Gerald F. Combs, Jr.
Department of Poultry and Avian Sciences
and Division of Nutritional Sciences
Cornell University
Ithaca, New York

Stephanie B. Combs
Department of Poultry and Avian Sciences
Cornell University
Ithaca, New York

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We dedicate this work to

the group of scientists around the world who, having undertaken during the last 40 years to learn about the biological activity of selenium, have produced collectively the information summarized in this volume, and to

those scientists from many countries and backgrounds who, each bringing a new perspective, are extending this information toward the full understanding of the roles of selenium in the nutrition and health of man and other animals, and to

a world that needs many such collaborations.

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Preface

One of the most important discoveries in nutrition in the last 30 years has been the recognition of the essentiality of the element selenium and the elucidation of the biochemical bases of its nutritional interrelationships with vitamin E and other factors. This area of inquiry first involved a new and apparently novel role of the element in the normal nutrition of experimental animals fed purified diets. Subsequently, it has included several applied areas of animal and human health ranging from the prevention of reproductive disorders in cattle to the modification of cancer risk in humans. Thus, despite the economic importance now attached to selenium in agriculture and the potential value now proposed for the element in certain aspects of medicine, our understanding of these roles of selenium is far less applied in nature. The beginning of the selenium story occurred in basic research laboratories and not in field situations; basic research produced answers before practical problems were identified.

Since the mid-1930s, selenium had been recognized as the toxic principle responsible for "alkali disease" and "blind staggers" in grazing livestock on the seleniferous prairies of the northern American Great Plains. Imagine the surprise of the late Klaus Schwarz and his colleagues at the National Institutes of Health 20 years later when they found that this little-known element was also the active principle in brewer's yeast responsible for the protection of vitamin E-deficient rats from necrotic liver degeneration. In his excitement, Schwarz telephoned Milton Scott at Cornell University and said, "Milt, try selenium!" Scott had recently found that brewer's yeast contained a factor (later shown to be niacin) that reduced leg weakness in turkey poults, and had become acquainted with Schwarz through their mutual interest in the nutritional attributes of that feedstuff. Scott had also found that brewer's yeast contained a factor that prevented exudative diathesis (the disease described in the 1930s by Heinrich Dam) in the vitamin E-deficient chick. "Try selenium!" Schwarz said, and Scott did. That year,

1957, Schwarz and Scott announced the discovery of the nutritional essentiality of selenium.

The discovery that exudative diathesis in the chick is a clinical sign of combined selenium and vitamin E deficiency in that species actually preceded the occurrence of that syndrome as a practical problem in the production of poultry. By the time that field cases of exudative diathesis in chicks and gizzard myopathy in turkeys were observed, basic research in several laboratories had generated information applicable to those practical problems. This central role of the basic investigator in selenium-related research has continued over the past three decades. This has resulted in the fortunate present situation wherein the human and veterinary health communities are able to consider the plausibility of apparent associations of selenium and disease in the light of an extensive and growing understanding of the biochemistry, metabolism, and nutrition of the element.

Our purpose in preparing this volume has been to assemble an extensive review of the most pertinent scientific literature dealing with these basic aspects of the present understanding of the roles of selenium in nutrition and health. We have sought, as best we could, to give our own perspectives where we felt that decisions must be made in the face of very limited information (e.g., identification of daily selenium allowances and the upper safe limits of selenium intake for humans). In those cases, we have tried to outline our logic to assist the reader in understanding our recommendations. We have made special efforts to discuss the subjects of selenium bioavailability and the two selenium-related diseases recently reported in China. We see these topics as of central importance to our considerations of the roles of selenium in nutrition and health, yet neither has previously been reviewed as extensively as the information on each deserves. We have also included collations of much experimental data (e.g., the exhaustive summary of the selenium contents of foods), which we believe will be useful to those interested in this field. It is our hope that this volume will serve as a useful reference and guide to the scientific literature for researchers, students, public health officers, and others whose interests relate to selenium in nutrition and health.

We approached the preparation of this volume as an effort of the truest kind of didactic scholarship. But, as Richard Bach wrote,* "You teach best what you most need to learn," we have found that the intellectual rewards of our effort, that is, the understanding that we have gained during the process of poring through the hundreds of papers and of trying to assemble their messages into a coherent story, have given us a perspective that we could not have acquired otherwise. To the extent that we will have been able to share that perspective in a fashion useful to others, our efforts will have been successful. That is our wish.

**Illusions. The Adventures of a Reluctant Messiah.* Dell Publ., New York, 1977.

Acknowledgments

A review of this type, by its very nature, depends on the researchers whose ideas, experiments, and reports constitute the body of knowledge addressed. In that respect, we are indebted to literally hundreds of researchers throughout the world whose individual efforts have produced the approximately 1800 reports that we have considered in preparing this volume. To those many, often underheralded, scientists we offer our warmest appreciation.

We owe a special debt to several individuals who generously provided photographs and figures that we have included in this volume. We extend our sincere appreciation for that assistance to the following people: Dr. J. E. Oldfield, Oregon State University; Dr. J. H. Harrison and Dr. H. R. Conrad, Ohio State University; Dr. Liu Jinxu, Institute of Animal Science, Chinese Academy of Agricultural Sciences; Dr. M. L. Scott, Cornell University; Dr. J. F. Van Vleet, Purdue University; Dr. J. A. Marsh, Cornell University; Dr. Tan Jianan, Institute of Geography, Chinese Academy of Agricultural Sciences; and Dr. H. A. Poston, U.S. Fish and Wildlife Service.

We are particularly indebted to several individuals who lent their time and judgment in the review of draft versions of the manuscript. These include Dr. K. R. Mahaffey, National Institute of Occupational Safety and Health; Dr. J. A. Marsh, Cornell University; and Dr. L. C. Clark, Cornell University. Their thoughtful criticisms were valuable to us.

This project was absolutely dependent on two individuals in our research group whose tireless efforts made our tasks manageable. We offer our most sincere gratitude to Ms. Pamela Senter for her highly efficient and enthusiastic handling of the manuscript in its various draft stages and for the layout of the extensive tabular material. Without the dedicated assistance also of Jamie Saroka, we could not have produced this volume. The understanding of the other people in our research group (Lynne Deuschle, Steve Mercurio, Young Sook Kim, Bao-Ji Chen, Johnny Lü, Tom Grant,

and Harlan Redder) and of our children (Jerry, Kiersten, and Matt) during the months in which we engaged in this project is a gift of which we are very much aware and thankful; we will not forget them.

It has been our personal pleasure to participate, over the last dozen or so years, in an intellectual endeavor of global proportions toward the elucidation of the role of selenium and related factors in nutrition and health. This participation has brought us together in various forums (over lecterns, laboratory benches and beer glasses) with many of the scientists whose work constitutes the greater part of the body of information on which our current understanding of the biology and nutrition of selenium now rests. To these scientists, who are too numerous to name, but who may recall some lively conversations into the night in Lubbock, Beijing, Washington, Boston, Helsinki, Chicago, Dallas, St. Louis, Cincinnati, Anaheim, Ithaca, Madison, Davis, Columbia, Saxton's River, or any number of other big and little places, we thank you.

1

CHEMICAL ASPECTS OF SELENIUM

I. FORMS OF SELENIUM

Selenium (Se) is classified in group VIA of the periodic table of elements. This group includes the nonmetals sulfur and oxygen in the periods above Se, and the metals tellurium and polonium in the periods below Se. By period, Se lies between the group VA metal arsenic and the group VIIA nonmetal bromine. Thus, Se is considered a metalloid, having both metallic and nonmetallic properties. Its atomic properties are summarized in Table 1.1.

Elemental Se, like its sister elements, sulfur and tellurium, shows allotropy; that is, it can exist in either an amorphous state or one of three crystalline states. Amorphous Se is a hard, brittle glass at temperatures below 31°C, is vitreous at 31°-230°C, and is a free-flowing liquid above 230°C. The increased viscosity of amorphous Se at temperatures less than 230°C results from the formation of polymeric chains at lower temperatures. Crytalline Se can take the form of flat hexagonal and polygonal crystals (called alpha-monoclinic or "red" Se), of prismatic or needle-like crystals (called beta-monoclinic or "dark red" Se), or of spiral polyatomic chains of the form Se_n (variously called hexagonal, trigonal, metallic, "gray" or "black" Se). The hexagonal crystalline form of Se is the most stable; both monoclinic forms convert to the hexagonal form at temperatures above 110°C, and amorphous Se converts spontaneously to the hexagonal crystalline form at 70°-210°C. The properties of the allotropic forms of Se are discussed in detail elsewhere.[1,2]

Elemental Se can be reduced to the -2 oxidation state (selenide), or oxidized to the +4 (selenite) or +6 (selenate) oxidation states. Hydrogen

Table 1.1
Atomic Properties of Selenium

Atomic number	34
Atomic weight	78.96
Electronic configuration	$Ar3d^{10}4s^24p^4$
Atomic radius	1.40 Å
Covalent radius	1.16 Å
Ionic radius	1.98 Å
Common oxidation states	-2, 0, +4, +6
Bond energy	
(M—M)	44 kcal/mole
(M—H)	67 kcal/mole
Ionization potential	9.75 eV
Electron affinity	-4.21 eV
Electronegativity	2.55 (Pauling's scale)

selenide (H_2Se) is a fairly strong acid with a pK_a of 3.8 in aqueous systems. The gas is colorless, has an unpleasant odor, and is highly toxic [the LC_{50} for 30-min exposure for guinea pigs is 6 ppm [3]]. It can be produced by heating elemental Se above 400°C in air, but it decomposes in air to form elemental Se and water. Hydrogen selenide is fairly soluble in water (270 ml per 100 ml water at 22.5°C). It reacts directly with most metals to form metal selenides, but these compounds are practically insoluble in water. Organic selenides are ready electron donors to their surrounding environments, thus oxidizing these forms of Se to higher oxidation states.

In the +4 oxidation state, Se can exist as selenium dioxide (SeO_2), as selenious acid (H_2SeO_3) or as selenite (SeO_3^{-2}). Selenium dioxide is formed by burning elemental Se in air or reacting it with nitric acid. It is readily reduced to the elemental state by ammonia, hydroxylamine, sulfur dioxide, and several organic compounds. It is soluble in water (38.4 g per 100 ml water at 14°C) and forms selenious acid (H_2SeO_3) when dissolved in hot water. Selenious acid is weakly dibasic, with a pK_a of 2.6; dissolved selenite salts exist as biselenite ions in aqueous solutions at pH 3.5 to 9. In contrast to the organic selenides, selenites readily accept electrons from their environments, their Se being easily reduced. At low pH, selenite is readily reduced to the elemental state by mild reducing agents such as ascorbic acid and sulfur dioxide. Selenites in soils are strongly bound by hydrous oxides of iron, forming insoluble complexes at moderate pH (4-8.5).

In its most oxidized (+6) state, Se can exist as selenic acid (H_2SeO_4) or as selenate (SeO_4^{-2}) salts. Selenic acid is a strong acid. It is formed by the oxidation of selenious acid or elemental Se by strong oxidizing agents in the presence of water. Selenic acid is very soluble in water; most

selenate salts are soluble in water, in contrast to the corresponding selenite salts and metal selenides. Selenates tend to be rather inert and are very resistant to reduction.

Six stable isotopes of Se exist in nature. These are ^{74}Se (natural abundance 0.815 mass %), ^{76}Se (natural abundance 8.66 mass %), ^{77}Se (natural abundance 7.31 mass %), ^{78}Se (natural abundance 23.21 mass %), ^{80}Se (natural abundance 50.65 mass %), and ^{82}Se (natural abundance 8.35 mass %). These isotopes have been employed in study of the biological utilization of Se in foods, in which their quantitation has been achieved by neutron activation (for ^{74}Se, ^{76}Se, and ^{80}Se),[4] or by mass spectrometry (for ^{76}Se,^{80}Se, and ^{82}Se).[5] More than two dozen radioisotopes of Se can be produced by neutron activation or by radionuclear decay. These include such short-lived species as ^{77m}Se (half-life 17.5 sec) and ^{87}Se (half-life 16 sec), and such long-lived species as ^{75}Se (half-life 120 days). Of these, ^{75}Se and ^{77m}Se have been found to be suitable for the measurement of Se in biological materials by neutron activation analysis.[6,7] Due to its emission of gamma-radiation and to its relatively long half-life, ^{75}Se has been widely employed in biological experimentation and in medical diagnostic work.

Table 1.2
Selenium Compounds Important in Nutrition

Oxidation state of Se	Compound
Se^{-2}	H_2Se
	Na_2Se
	$(CH_3)_2Se$
	$(CH_3)_3Se^+$
	Selenomethionine
	Selenocysteine
	Se-methyl-selenocysteine
	Selenocystathionine
	Selenotaurine
Se^0	Selenodiglutathione
	Amorphous selenium
	Red selenium (alpha-monoclinic)
	Dark red selenium (beta-monoclinic)
	Gray selenium (hexagonal)
Se^{+4}	H_2SeO_3
	Na_2SeO_3
Se^{+6}	Na_2SeO_4

Selenium is a semiconductor with photoconductivity (i.e., excitation with electromagnetic radiation can markedly increase its conductivity).[2] This property has made Se compounds useful in photocells and in xerography. As a result of the utility conferred by these properties, the literature on the chemistry of inorganic and organic Se compounds is large. Because this area is outside of the scope of the present volume, the reader is referred to the comprehensive review edited by Klayman and Gunther[8] and the recent volume of Ihnat[9] for discussions of the chemical properties and analytical chemistry of Se.

The Se compounds of greatest interest in nutrition are presented in Table 1.2. It should be noted that whereas forms of Se available as supplements of foods and feeds are primarily compounds of the higher oxidation states of Se, the metabolites of chief concern in nutrition and biochemistry are compounds in which Se occurs in the reduced state.

II. CHEMISTRY OF SELENIUM

The chemical and physical properties of Se are very similar to those of sulfur (S). The two elements have similar outer-valence-shell electronic configurations and atomic sizes (in both covalent and ionic states), and their bond energies, ionization potentials, electron affinities, electronegativities, and polarizabilities are virtually the same.[9] Despite these similarities, the chemistry of Se and S differ in two respects that distinguish them in biological systems.

The first important difference in the chemistry of Se and sulfur is in the ease of reduction of their oxyanions.[10,11] Quadrivalent selenium in selenite tends to undergo reduction, but quadrivalent S in sulfite tends to undergo oxidation. This difference is demonstrated by the following reaction:

$$H_2SeO_3 + 2\ H_2SO_3 \longrightarrow Se + 2\ H_2SO_4 + H_2O$$

Thus, in biological systems, Se compounds tend to be metabolized to more reduced states, and S compounds tend to be metabolized to more oxidized states.

The second important difference in the chemical behaviors of these elements is in the acid strengths of their hydrides. Although the analogous oxyacids of Se and S have comparable acid strengths [$SeO(OH)_2$: pK_a 2.6 vs. $SO(OH)_2$: pK_a 1.9], the hydride H_2Se (pK_a 3.8) is much more acidic than is H_2S (pK_a 7.0). This difference in acidic strengths is reflected in the dissociation behaviors of the selenohydryl group (-SeH) of selenocysteine (pK_a 5.24) and the sulfhydryl (-SH) group on cysteine (pK_a 8.25).[12] Thus, while thiols such as cysteine are predominantly protonated at physiological

pH, the selenohydryl groups of selenols such as selenocysteine are predominantly dissociated under the same conditions.

Selenite can react with nonprotein thiols and with protein sulfhydryls to undergo reduction of the Se^{+4} to Se^0. This reaction, originally postulated by Painter,[13] was confirmed by Ganther,[14] who demonstrated that selenite can react with either glutathione (reduced form), cysteine, or coenzyme A to form 1,3-dithio-2-selane products of the form RS—Se—SR, which are referred to as "selenotrisulfides." The reaction is represented as follows:

$$4\ RSH + H_2SeO_3 \longrightarrow RS—Se—SR + RS—SR + 3\ H_2O$$

In this reaction, four sulfhydryl sulfurs are oxidized from the -2 oxidation state to the -1 oxidation state of disulfide sulfurs. This is balanced by the concommitant reduction of a single Se atom from the selenite oxidation state of +4 to the zero oxidation state. However, because the electronegativities of Se and S are very similar, Martin[15] has suggested that the -2 charge may be rather evenly distributed across the selenotrisulfide bridge, yielding an effective oxidation number of -2/3 for each of its members.

A similar reaction, indicated by Jenkins[16,17] and confirmed by Ganther and Corcoran[18], can occur between selenite and the free sulfhydryls of proteins to yield selenotrisulfide types of products.

Thus, whereas the chemical and physical properties of Se and S are similar, important differences in their chemistries result in their having very different behaviors in biological systems.

III. ANALYSIS OF SELENIUM

The analysis of Se can be accomplished by a variety of techniques, some of which are applicable to biological materials. Because these methods are reviewed in detail elsewhere,[9] the following discussion is presented to provide the reader with a general orientation concerning the methods and problems of analysis of Se, particularly in foods, feeds, and animal tissues.

Several procedures for Se analysis that have been employed for industrial purposes do not lend themselves to biological applications due to low sensitivity. These methods have been discussed by Nazarenko and Ermakov[19] and include

(i) gravimetric measurement of Se after reduction and quantitative precipitation with acid
(ii) gravimetric measurement of Se after electrolytic deposition with Cu

(iii) colorimetric titration with oxidizing agents after reduction with thiocyanate or other reducing agents
(iv) colorimetric measurement of Se hydrosols after reduction by hydrazine, $SnCl_2$, or ascorbic acid, and stabilization of the hydrosols with hydroxylamine hydrochloride, gum arabic, or gelatin
(v) colorimetric measurement of azo compounds formed by the reaction of aromatic amines with diazonium salts, the latter being produced by the oxidation of organic compounds by Se^{+4}
(vi) colorimetric measurement of complexes of Se^{-2} with phenyl-substituted thiocarbazides or semicarbazide after reduction of Se to the Se^{-2} state.

The limits of detection of Se by these procedures are generally in excess of 0.5 ppm and, for the gravimetric methods, can be several ppm. These procedures are not free of interferences by elements that can co-precipitate in the case of gravimetric methods, or by oxidizing agents in the case of the chemical methods. Therefore, they are generally not suitable for analysis of Se in biological materials.

Other methods have been found to be useful for the determination of Se in plant and animal specimens. These are presented in Table 1.3.[20-67]

Of these procedures, the fluorometric method using diaminonaphthalene (DAN) has been the most popular. This method involves oxidation of sample selenium to Se^{+4}, and reaction with DAN to form benzopiazselenol. The product fluoresces intensely at 520 nm when excited at 390 nm and can be quantified using a fluorometer. The chief advantages of the DAN procedure are its good sensitivity (ca. 0.002 ppm) and its relatively low cost. Nevertheless, the method has two potential pitfalls that must be avoided.

The first involves the loss of Se during the acid digestion of samples containing large amounts of organic materials. Adequate acid digestion of selenium in biological materials requires the complete conversion of the native forms of the mineral to Se^{+4} and/or Se^{+6}, and the subsequent reduction of any Se^{+6} formed in the process to Se^{+4} without loss of total Se. Inorganic Se can be volatilized to an appreciable extent under the conditions of acidic digestion in the presence of such large amounts of organic materials that charring occurs, especially when sulfuric acid is used as an oxidant.[53,68] The volatilized Se, probably in the form of H_2Se, can result in significant errors in the analysis of fatty materials, such as egg yolks or adipose tissues. Because Se is volatilized from acid solutions by reducing agents,[69] this loss can be avoided by maintaining strongly oxidizing conditions during digestion and by using low heat such that the oxidation of Se^{+4} to Se^{+6} proceeds relatively slowly by gradually raising the temperature of the perchloric acid solution to 210° C.[55] When the nitric-perchloric acid digestion is controlled and carefully attended, it produces satisfactory conversion to Se^{+4} even

Table 1.3

Methods of Analysis of Selenium in Biological Materials

Method	Detection limit (ppm)	Sample preparation	Known interferences	References
Polarigraphic determination of piazselenol after reaction with diaminobenzidine	0.1	Perchloric-nitric acid digestion	—	20
Cathodic stripping voltametric determination following ion-exchange separation of Se	0.001	Nitric-sulfuric acid digestion	Preconcentration of Se on anion-exchange resin	21
Inductively coupled plasma atomic emission spectrometry with hydride generation	0.0005	HCl digestion	Hydride generation matrix effects	22
Direct current plasma atomic emission spectrometry	0.02	Acid digestion	Matrix effects	23
Atom-trapping atomic absorption spectrometry	0.025	O_2 combustion	Mineral cations	24
Electrothermal atomic absorption spectrometry	0.003	Thermal stabilization with Ni	Matrix effects	25-32
Atomic absorption spectrometry with hydride generation	0.01	Acid digestion; hydride generation	Matrix effects (particularly Cu, As, Sb)	33-37
Proton-induced X-ray emission analysis	0.01	Lyophilization: pelletization	—	38-41
X-ray fluorescence spectrometry	0.04	Lyophilization, pelletization	—	42, 43
Isotope dilution with detection by combined gas-liquid chromatography/mass spectrometry	0.0005	Nitric-phosphoric acid digestion; chelation with 4-nitro-*o*-phenylenediamine	—	5
Neutron activation analysis using ^{75}Se	0.02	—	—	44-46
"Fast" neutron activation analysis using ^{77m}Se	0.05	—	—	6,7,44,47-53
Fluorimetric determination of piazselenol after reaction of Se^{+4} with 3,3′-diaminobenzidine	0.01	Nitric-perchloric acid digestion, or O_2 combustion	Loss of volatilized Se if digests char	54-57
Fluorimetric determination of piazselenol after reaction of Se^{+4} with 2,3-diaminonaphthalene	0.002	Nitric-perchloric acid digestion, or O_2 combustion	Loss of volatilized Se if digests char	57-67

of such forms as trimethylselenonium (a major urinary metabolite) which are resistant to oxidation by nitric acid alone.[63,67,70] Comparisons of the nitric-perchloric digestion method with direct combustion in an oxygen environment have shown that both yield comparable results.[57]

The second potential problem involves interfering fluoresence due to apparent degradation products of DAN itself, which can produce fluoresence.[71] These can be avoided by purifying the DAN reagent by recrystallization from water in the presence of sodium sulfite and activated charcoal,[72] or by stabilizing the DAN reagent with HCl and extracting with hexane.[62] Several investigators have incorporated these procedures into methods using DAN that are very convenient for use in the routine analysis of Se in biological materials.[64-66]

Conventional atomic absorption spectroscopy (AAS) has not been suitable for the determination of Se in biological samples due to the generally high limit of detection (ca. 0.1 ppm) by that procedure. Variant AAS methods, however, have been developed with sensitivities adequate for biological use. One such method involves hydride generation of sample Se followed by quantitative detection by AAS.[36,37] This method requires only small sample sizes (e.g., 0.1 ml of serum), has adequate sensitivity (ca. 0.01 ppm), and the hydride generation step has been automated. However, it suffers from possible interferences due to other elements that can form hydrides (e.g., Cu, As, Sb). Of these, the most serious interference is due to Cu; steps must be taken to remove Cu by the use of HCl,[34] tellurite,[73] or thiourea.[37] Better sensitivity has been obtained using electrothermal AAS. This method avoids the problems associated with wet digestion by employing high temperature oxidation in a graphite furnace. Use of high temperature (e.g., atomization at 2400° C) reduces interferences due to nonspecific absorption of organic compounds and non-Se salts, but introduces the problem of volatility of Se under such conditions. This problem can be avoided by the use of salts for the thermal stabilization.[74] In practice, electrothermal AAS has sensitivity for Se at ca. 0.003 ppm[30,31]; with the use of a Zeeman-effect background correction system, sensitivities approaching 0.001 ppm have been reported.[32]

Plasma atomic emission spectrometry (PAES) has not been used widely for the analysis of Se in biological materials. Although very good sensitivity (ca. 0.001 ppm) has been reported using inductively coupled PAES,[22] matrix effects present such a great amount of interference that most laboratories are not able to obtain reasonable sensitivity by this method. Direct current PAES has not had adequate sensitivity for biological use.

Instrumental neutron activation analysis (INAA) of Se offers the advantages of applicability to small sample sizes and relative ease of sample preparation. Although the greatest sensitivity (ca. 0.02 ppm) by this method

is obtained by measuring ^{75}Se, its use necessitates lengthy irradiation (100 hrs), and long periods of post-irradiation holding (60 days) and counting (2 hrs). Greater economy by increased sample throughput has been achieved, at the expense of sensitivity, by the use of the short-lived (17.38 sec half-life) ^{77m}Se. This isotope can be irradiated (5 sec), decayed (15 sec), and counted (25 sec) very quickly in an automated system.[51] Due to the ease of this procedure as well as to its nondestructive nature, some investigators with access to research reactors have found instrumental neutron activation analysis useful for the measurement of Se. Nevertheless, the utility of the "fast" method is limited at the present time by its relatively low sensitiviy, rendering it unsuitable for accurate quantitation of such low concentrations of Se as are found in tissues of animals chronically deficient in the element.

The measurement of Se by proton-induced X-ray emission (PIXE) offers the potential advantage of simultaneous elemental analysis of biological materials. This method involves proton bombardment of target atoms (the sample) to cause loss of inner shell electrons and their consequent replacement by electrons from the outer shell. The X-rays emitted during that transition are characteristic of the energy differences between electron shells and are, therefore, identifiable and quantifiable. At the present time, the sensitivity of this procedure for the determination of Se (ca. 0.01 ppm)[40] makes it useful for many biological purposes, especially when simultaneous elemental analysis may be needed; however, it is not sensitive enough for the accurate determination of very low tissue levels.

X-ray fluorescence spectrometry offers another nondestructive technique for multi-element analysis[42,43]; however, its sensitivity for Se does not compare favorably with other methods available for biological use.

A procedure for determining Se by double isotope dilution has been developed.[5] This method involves the use of two stable isotopes of Se as tracer (^{76}Se) and internal standard (^{82}Se). Samples spiked with a known quantity of the internal standard are digested in nitric-phosphoric acid, undigested lipids are removed with chloroform, and hydrochloric acid is used to reduce any Se^{+6} to Se^{+4}. Selenite is reacted with 4-nitro-*o*-phenylenediamine to form 5-nitropiazselenol, and the nitropiazselenonium ion cluster is determined by combined gas-liquid chromatography/mass spectrometry. The native Se in the sample is calculated from the measured isotope ratios, using the ^{80}Se naturally present in the sample. Reamer and Veillon[5] have carefully developed this technique and have reported a sensitivity of less than 0.001 ppm. Their method employs a rapid digestion, which avoids several of the problems associated with the use of perchloric acid, and is capable of fully oxidizing the often problematic trimethylselenonium.[5] It thus appears to be suitable for biologic measurements and has been put to such use already.[75]

A IUPAC interlaboratory (12 sites) comparison of the more widely used methods for the determination of Se in clinical materials[76] found statistically significant differences among the mean concentrations reported for Se in lyophilized human serum analyzed by either (a) acid-digestion/DAN-fluorometry, (b) electrothermal AAS, (c) acid-digestion/hydride generation AAS, or (d) acid-digestion/isotope dilution mass spectrometry, with slightly higher values reported by the first procedure. The four methods compared very favorably for the analysis of pooled lyophilized urine samples. However, only the fluorometric method showed homogeneity of variance among laboratories.

The DAN fluorometric procedure remains the most widely used method for the analysis of Se in biological materials due to its good sensitivity and good reliability. Despite the tedious nitric-perchloric acid digestion which it entails, the operating costs of the method are not great because the only instrumentation required is a good quality fluorometer. Therefore, this procedure is the method of choice for many biomedical laboratories. Newer methods, including electrothermal AAS, AAS with hydride generation, PIXE, "fast" INAA, and isotope dilution with gas-liquid chromatography/mass spectrometry, offer good options for biological investigations, but these methods generally require large amounts of background development with large start-up costs. As these methods improve, future investigations will probably employ them more extensively.

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SELENIUM IN THE ENVIRONMENT

I. SELENIUM IN MINERAL DEPOSITS

Selenium is widely distributed in the earth's crust with an abundance that has been estimated to be approximately 0.09 ppm.[1,2] It is most frequently found in greatest abundance in igneous rock, particularly in mineral sulfide ores, where the Se contents may be in excess of 1000 ppm.[3-7] Selenium forms selenides or sulfoselenides with Cu, Ag, Pb, and Hg; in fact, the sulfidic copper ores of North America and the Soviet Union (some of which have been reported to contain as much as 24% Se[3]) are the principal commercial source of Se, which is obtained as a by-product of the electrolytic refining of copper.[8] More than 80% of the world's annual production of almost 1100 tons of Se comes from this source. Whereas copper sulfide ores represent point sources of Se in the environment, sources of greater agronomic significance are the sedimentary rocks, which comprise the principal parent material for cultivated soils. Sedimentary rocks generally contain lower levels of Se than do igneous formations, which obtained Se from volcanic discharges during earlier geological ages; however, the Se provided by sedimentary rocks is important in determining the concentrations of Se in soils. Shales, which comprise over half of all sedimentary rocks, have been found to contain in general a few ppm of Se. However, some, such as the Permian shales of Wyoming, USA, have been found to contain several hundred ppm of Se, and the Pierre and Niobrara shales of the seleniferous area of the United States Great Plains have been found to contain approximately 2 ppm.[5] Limestones and sandstones generally contain lower and more variable amounts of Se; however, some carbonaceous deposits of these materials may have as much