Selenium Metabolites in Urine: A Critical Overview of Past Work and Current Status

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Background: Selenium is an essential trace element that also elicits toxic effects at modest intakes. Investigations of selenium metabolites in urine can help our understanding of the transformations taking place in the body that produce these beneficial and detrimental effects. There is, however, considerable discord in the scientific literature regarding the selenium metabolites thought to play important roles in these biotransformation processes.

Approach: We critically assessed the published reports on selenium urinary metabolites, from the first report in 1969 to the present, in terms of the rigor of the data on which structures have been proposed.

Content: We present and discuss data from ~60 publications reporting a total of 16 identified selenium metabolites in urine of humans or rats, a good model for human selenium metabolism. We assessed the analytical methods used and the validity of the ensuing structural assignments.

Summary: Many of the studies of selenium metabolites in urine appear to have assigned incorrect structures to the compounds. The long-held view that trimethylselenonium ion is a major human urinary metabolite appears unjustified. On the other hand, recent work describing selenosugars as major urinary metabolites looks sound and provides a firm basis for future studies.

Selenium is of considerable interest in human nutrition and health because of its dual role as toxicant and essential trace element. Paracelsus’ maxim “the dose makes the poison” has particular relevance to selenium because its window of beneficial functionality is very narrow. Experiments indicating that selenium was an essential trace element were reported in 1957 (1); its essentiality was confirmed in 1973 following the work of Rotruck et al. (2) demonstrating its role in glutathione peroxidase, and selenium is now known to be part of several important enzyme systems (3). The physiologic requirements of selenium for an adult man have been estimated at ~70 µg/day, whereas the threshold for toxicity may be as low as 700 µg/day (4).

In addition to the scientific interest in selenium, there is also considerable public awareness because of its purported efficacy as a treatment against certain types of cancer. Encouraging results, reported in 1996 from a study carried out in the United States (5) provided the impetus for an ongoing 12-year study involving 32,000 individuals to test the efficacy of selenium intake against prostate cancer (6). Furthermore, although there are no demonstrated health benefits from having selenium intake above physiologic requirements, there is a general perception that increased selenium ingestion is beneficial, which has led to a flourishing market in selenium supplements. These supplements are thought to be more effective when the selenium is ingested in an “organic” form, and many suppliers provide the selenium as selenized yeast, which contains largely selenomethionine bound in proteins in addition to many other unknown selenium species (7). Consumers of such products intent on improved health should be aware, however, of the toxicity of selenium and the possible toxic consequences of overindulgence.

Because urine is a major excretory route for selenium, metabolic changes delineating the boundary between essential and toxic concentrations are likely to be reflected in urinary selenium species. For this reason, investigations into the selenium metabolites in urine are currently a major area of research. This area of research, however, has an untidy past, and perusal of the literature over the last 35 years presents an unclear picture of the important selenium species. Many of the problem arise from loose analytical chemistry and poor interpretation of the ensuing results. This review will endeavor to plot a path...
through the past and present literature describing selenium urinary metabolites and present a “most probable” picture of the current status. The aim is to provide a foundation of solid data on which to base future research into selenium metabolism.

Some Biological Chemistry of Selenium

The names and structures of selenium species of relevance to this review are shown in Fig. 1. We have attempted to limit the use of abbreviations but make an exception for trimethylselenonium ion (Me₃Se⁺), which we will refer to as TMSe.³

Selenium has six stable isotopes, ⁷⁴Se (0.9% natural abundance), ⁷⁶Se (9.0%), ⁷⁷Se (7.6%), ⁷⁸Se (23.5%), ⁸⁰Se (49.8%), and ⁸²Se (9.2%), and many radioisotopes, of which ⁷⁵Se (gamma emitter; half-life, 120 days) is most commonly used as a tracer in biological studies. The isotopes of selenium have been very helpful in experimental studies as well as in the analysis of selenium and its compounds by mass spectrometric (MS) methods.

Selenium lies immediately below sulfur in group 16 of the periodic table, and its chemistry has marked similarities to sulfur chemistry. In general, organoselenium compounds are more reactive than their sulfur counterparts: the C–Se bond (234 KJ/mol) is weaker than the C–S bond (272 KJ/mol). Selenols are more acidic than thiols, and they are more readily oxidized.

The fate of excreted selenium in the body can be described in terms of methylation. Any discussion on methylation of metalloids usually begins with the classic work of Frederick Challenger, carried out in the 1930s and 1940s and reviewed by Challenger in 1945. Because it provided the foundations for the study of biological methylation processes for arsenic, selenium, and tellurium. The end product in Challenger’s biomethylation experiments with microbes was dimethylselenide, and the definitive result showing that dimethylselenide was also formed by animals came in 1952 with the studies of McConnell and Portman, who identified this metabolite in rats. It is at first surprising that Challenger’s work is not quoted more often in reports investigating selenium urinary metabolites: equivalent reports about arsenic species in urine constantly refer to the Challenger pathway because the arsenical intermediates and metabolites (e.g., methylarsonate and dimethylarsinate) he proposed for microbial systems are found in human urine. The equivalent selenium species, such as methylselenite [MeSe(O)O⁻] and methylselenenate [MeSe(O)₂O⁻], however, do not feature strongly in the selenium literature. Indeed, all evidence indicates that selenium is methylated in biological systems after reduction of selenite/selenate to hydrogen selenide, analogous to the reduction of sulfite/sulfate to hydrogen sulfide. In this proposed pathway, the major methylated selenium metabolites are dimethylselenide, which is excreted by respiration, and TMSe, which is excreted in the urine.

The general feeling that methylation processes are a form of detoxification seems to hold for selenium because dimethylselenide has much lower acute toxicity (at least 200-fold) than do the inorganic selenium species selenite/selenate and selenoamino acids (Table 1). The rapid excretion of dimethylselenide, 71–79% by the rat in 6 h, is also suggestive of a metabolically inert detoxification product. Surprisingly, TMSe is at least 20-fold more toxic than dimethylselenide, and thus the further methylation step to give TMSe does not appear to benefit the organism in terms of detoxification.

Selenium Metabolites in Urine: The Approach Taken in This Review

We collected information from those studies on selenium in urine that assigned structures to specific selenium metabolites; these data (13–71) are presented in Table 2 in terms of the assigned metabolite and the analytical methods used in that assignment. The early work dealt mostly with rats, whereas human studies became more common in the recent work, reflecting in part the lower detection limits achievable with the new instrumentation. As a general comment, the rat is a good model for studying human selenium metabolism, at least in terms of urinary metabolites, because the patterns of compounds appear quite similar.

Table 2 will serve as our framework as we work through the literature data, more or less chronologically, on identified selenium metabolites. We begin with a general overview of the research from the first report in 1969 of an identified selenium metabolite in urine, up to the present time. We next examine the impact on the identification of selenium species made by a single analytical technique, namely, HPLC-inductively coupled plasma mass spectrometry (ICPMS). We then examine the work on each of the selenium metabolites reported in urine, mostly performed in the last 10 years with HPLC/ICPMS, critically assessing the data on which proposed structures are based. Finally, we present a synopsis of the current state of the art regarding selenium metabolites in urine and touch on some possible future research in the area.

First Selenium Urinary Metabolite: Identification of TMSe Ion

In 1969, Byard (13) reported results from an experiment in which he gave rats sodium selenite containing a trace of H₂⁷⁵SeO₃ in their drinking water. He collected large quantities of the urine and, by tracking the ⁷⁵Se label, he was able to isolate the selenium urine metabolite. Although Byard provided an outline of the isolation procedure, details such as mass balance and increases in concentration achieved at each stage were unfortunately

³ Nonstandard abbreviations: TMSe, trimethylselenonium ion; MS, mass spectrometry; ICPMS, inductively coupled plasma mass spectrometry; NMR, nuclear magnetic resonance; and MS/MS, tandem mass spectrometry.
not reported. But from initial and final $^{75}$Se activity and mass ($\sim 17 \text{ mg}$) of the assumed pure end product, Byard calculated a molecular mass of $193.5 \pm 15$ (for an ionic compound based on a chloride anion). The proton nuclear magnetic resonance ($^1$H NMR) spectrum shown for the natural product indicated that it was pure without a trace.

Fig. 1. Selenium species claimed to be present in urine (rat or human).
of organic impurity, and it matched the spectrum of a synthetic specimen of trimethylselenonium chloride. The electron impact mass spectral data, however, were not so convincing because of, according to Byard, “considerable contamination of the natural compound”. These mass spectral data were not consistent with the NMR, and the discrepancy in the data sets is not readily explained. Nevertheless, on the basis of these NMR and mass spectral data, Byard (13) identified the selenium urinary metabolite as TMSe.

The method of isolation used by Byard involved ion-exchange chromatography followed by precipitation of the selenium metabolite as the reineckate salt. It is surprising that TMSe, a trace constituent of the urine sample, could be obtained pure from such a simple, nonselective procedure, particularly in the light of later work (see below) showing that the reineckate salt of TMSe is appreciably soluble in water and that additional steps must be taken to obtain acceptable yields in the precipitate.

A study by Palmer et al. (14) almost immediately followed that of Byard (13), and similarly identified TMSe in rat urine. These researchers fed rats food containing 15 µg/g Se as selenite with added 75Se selenite; they also used ion-exchange chromatography and reineckate salt precipitation to isolate the compound. Again, the ease with which TMSe was isolated to purity with fairly nonselective procedures was surprising. Nevertheless, the authors provided chromatographic and spectral data (NMR and infrared) of the isolated metabolite, which matched those of a synthesized specimen. Of additional interest was that in a second experiment in which rats were injected with selenite, TMSe constituted ~40% of the urinary Se at both low (2 µg/kg Se) and high (800 µg/kg Se) doses.

In a follow-up report in 1970, Palmer et al. (15) investigated the effect on the selenium excretion products of the type of selenium compound administered to rats. They used 75Se labels for selenate and selenomethionine and cold selenium for Se-methylselenocysteine and selenocystine. They also used 75Se-labeled wheat obtained by growing wheat plants with 75Se selenate. In addition to monitoring the radiolabel, they also monitored selenium by chemical analysis with a fluorometric technique, and the results agreed reasonably well (within 20%). These studies showed that TMSe, originally termed U1, was a common urinary metabolite from all tested selenium sources and constituted 20–50% of the urinary selenium. In addition, for the radiolabeling studies, a second major selenium metabolite, U2, was reported to account for 11–28% of the total urinary selenium. The possible formation of U2 from the nonradiolabeled selenium sources was apparently not checked. Nevertheless, the data on the similarity of urine metabolites were clear enough to lead the authors to suggest that all forms of selenium might be detoxified by a similar mechanism leading to excretion in the urine of metabolites TMSe and U2, which remained unidentified. The authors also mentioned that losses of radiolabeled selenium (up to 33%) occurred during the radiochromatography, which they thought indicated volatile selenium constituents in the urine.

Similar studies with rats and radiolabeled 75Se were also carried out by Kiker and Burk (17) in 1974, and they also reported the presence of TMSe and a second major compound thought to be the same as U2 in the study of Palmer et al. (15).

Byard had earlier suggested (13) that TMSe may be a detoxification end product of selenium metabolism, and this was supported by further work by Palmer’s group (16). They showed that 75Se TMSe given to rats was excreted unchanged in the urine (equivalent to 80% of dose). They also demonstrated that necrogenic syndrome, a syndrome prevented by dietary selenium, was not prevented by addition of TMSe. Together, these two pieces of evidence suggested that TMSe was biologically unavailable to the rat and was excreted without undergoing catabolism.

Following the collective studies of Byard (13), Palmer’s group (14–16), and Kiker and Burk (17), it appeared that TMSe was a major selenium metabolite in rat urine and that at least one other major metabolite (U2) was also present. There was, however, no definitive study demonstrating TMSe in human urine. In 1976, a book chapter written by Burk (18) was published reporting data from one male patient given 75Se selenite, which suggested that TMSe constituted 14–21% of total urinary selenium, depending on the time of collection. The author noted, however, that the presence of TMSe was not conclusively demonstrated. Nevertheless, in the subsequent literature, this report by Burk was often cited as proof that TMSe was a human urinary metabolite.

In summary, in the early work on selenium in rat urine, two separate studies isolated a major metabolite, apparently to purity, and identified it as TMSe by comparison of spectral data (MS, NMR, and infrared spectroscopy) with those of a synthesized specimen. The quantitative aspects of those studies, however, were tenuous, and hence the significance of TMSe in terms of its percentage contribution to excreted selenium metabolites was still open to question.

### Table 1. Acute toxicity of some selenium species to the rat [adapted from Olson (12)]

<table>
<thead>
<tr>
<th>Selenium species</th>
<th>Toxicity to rat</th>
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<tr>
<td>Sodium selenite</td>
<td>Oral; 10-day LD50 = 3.2 mg/kg Se</td>
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<tr>
<td>Sodium selenate</td>
<td>Intraperitoneal; 48-h LD75 = 3.5 mg/kg Se</td>
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<td>d,L-Selenocystine</td>
<td>Intraperitoneal; 48-h LD75 = 5.5 mg/kg Se</td>
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<tr>
<td>d,L-Selenomethionine</td>
<td>Intraperitoneal; 48-h LD75 = 4.0 mg/kg Se</td>
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<tr>
<td>Dimethylselenide</td>
<td>Intraperitoneal; 24-h LD50 = 1600 mg/kg Se</td>
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<tr>
<td>Trimethylselenonium chloride</td>
<td>Intraperitoneal; 4-h LD50 = 49.4 mg/kg Se</td>
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LD50 and LD75, concentrations at which the dose of selenium is lethal in 50% and 75% of animals, respectively.

<table>
<thead>
<tr>
<th>Selenium species</th>
<th>Isolation to (near) purity and identification by spectroscopic means (e.g., NMR/MS/IR)</th>
<th>Two-dimensional paper chromatography</th>
<th>Difference method (see text)</th>
<th>Ion-exchange chromatography (often with reineckate precipitation)</th>
<th>HPLC with offline Se detection</th>
<th>HPLC/online Se detection other than ICPMS</th>
<th>HPLC/ICPMS</th>
<th>MS/MS</th>
<th>Other</th>
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<tr>
<td>Selenite</td>
<td>Rat (17)</td>
<td>Human (26, 28)</td>
<td>Human (35, 37, 59)</td>
<td>Human (44, 52, 61, 62)</td>
<td>Rat (39, 40)</td>
<td>Rat (53)</td>
<td>Human (55, 58)</td>
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<td>Human (60)</td>
<td>Human (60)</td>
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<td>Selenodiglutathionine</td>
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<td>Human (63)</td>
<td>Human (41)</td>
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<td>Selenosugar 3</td>
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*IR, infrared spectroscopy.
Work Carried Out in the 1980s: TMSe Consolidated

There was surprisingly little additional work in the area of selenium urinary metabolites for the remainder of the 1970s. Perhaps the analytical shortcomings of the time precluded further refinement of the reported observations. These problems appear to have been apparent to the group working under Janghorbani, who carried out studies in the 1980s that reexamined much of the previous work after developing an analysis designed to selectively determine TMSe (19,22,37). Their results were interesting because they found that a simple cation-exchange separation, as used in the earlier studies, was not sufficient to separate TMSe from other selenium urine metabolites (19), and consequently, they developed a dual column procedure that was more selective for TMSe. In addition, they reported a quick method for determining TMSe (19) based on its relative resistance to decomposition in nitric acid (when compared with other selenium species). This method, termed the “difference method”, was said to give reliable data, but only when TMSe represented a high proportion of the total selenium. By applying their improved quantitative methods for TMSe analysis, this group was able to show that the chemical form of the ingested selenium species, as well as absolute dose, were factors influencing the quantities of TMSe in rat urine (19,37). Furthermore, one study (22) investigated urine from a man who consumed a modest 109 μg of 75Se and reported values of 15–18% TMSe (for six sample times) expressed as a percentage of total urine selenium. These values were very similar to those (14–21% TMSe) reported earlier by Burk (18). Collectively, the studies of Janghorbani’s group seemed to confirm that TMSe was a major urinary metabolite in rats, and probably also in humans. The authors point out, however, that TMSe was a major metabolite only under conditions of intake greatly in excess of nutritional requirements (37).

At about this time (1984), an excellent review of selenium in human urine was published by Robberecht and Deelstra (72). In regard to selenium species, these authors concluded that the available information was scarce, contradictory, and inconsistent. They commented that much of the reported work was based on very few samples and that there was often no information about the accuracy and reproducibility of the methods used. They also criticized the poor interpretation of the data. Robberecht and Deelstra (72) made a plea for future studies to be performed more carefully and that larger sample populations be used so that definitive conclusions could be drawn. Today, 20 years on, Robberecht and Deelstra might well make the same complaints.

It is clear that other researchers were also unhappy with the data being published about selenium species in urine, particularly in terms of the quantification of TMSe, and analytical procedures claiming further improvements in selectively measuring TMSe were reported. Blotcky and coworkers (25,26,28) were especially conspicuous in this area with several publications focusing on determining TMSe with use of neutron activation analysis for detection. They were also the first to report TMSe in normal human urine (i.e., without selenium supplementation). The data, however, were variable; in one report (25) they claimed that TMSe constituted ~30% of the total selenium in normal urine, whereas in two other very similar studies (26,28), they reported up to 90% TMSe in normal urine.

Foster et al. (23) reported a refinement of the cation-exchange/reineckate precipitation method in which they used buffered cation-exchange chromatography with SP-Sephadex medium and performed the precipitation with trimethylsulfonium ion as carrier to improve the yield of TMSe. The authors stated that without this carrier, the yields of TMSe obtained as the reineckate salt were low, an important result considering the early methods for isolating TMSe. They then used their method in a study of several organoselenium compounds, including selenonium compounds, and reported various degrees of metabolism to TMSe by the different compounds.

In 1988, Ostadalova et al. (53), working with rats injected with 75Se selenite, found that TMSe was the main urinary product in adult rats. Interestingly, they reported that in young rats a selenodiglutathione was the main product. This was the first report of an identified selenium urinary metabolite other than TMSe; unfortunately, however, no data were provided to support the validity of the assignments.

Methods to determine TMSe with greater selectivity continued to be developed. The use of HPLC to separate TMSe was first reported in 1985 with off-line detection by either atomic absorption spectrometry (33) or neutron activation analysis (25). Those studies were the first to describe the use of so-called coupled techniques for the analysis of selenium species in urine. Coupled techniques were the basic tools for the developing analytical field termed speciation analysis, which was given a huge boost from the middle of the 1980s when ICPMS instrumentation became commercially available. ICPMS provided selective and sensitive elemental detection and could be readily connected (coupled) to a chromatographic system such as HPLC. The ensuing technique, HPLC/ICPMS, found great application in selenium metabolic studies because it was able to separate and detect different selenium species at the concentrations found in urine from unexposed humans. In addition, the capability of ICPMS to individually determine the isotopes of selenium simplified those studies attempting to distinguish various possible sources of selenium. Because of the many advantages of HPLC/ICPMS over earlier methods as well as other contemporary methods, essentially all analytical work on selenium species in urine since ~1990 has been carried out with this technique.

With the advent of HPLC/ICPMS came a rash of newly identified selenium urinary species, and we will shortly discuss these compounds. However, we first wish to
make some general comments on the application of HPLC/ICPMS to the analysis of selenium species.

Comments on the Use of HPLC/ICPMS in Selenium Speciation Analysis

The metabolism of selenium, and hence the selenium species found in urine, is complex and is no doubt influenced by a host of chemical and biological factors. One cannot help but suspect, however, that much of the apparent variability and diversity of selenium metabolites appearing in the recent literature has stemmed from poor application of HPLC/ICPMS. We mention here three problem areas.

One problem area is the abuse of ICPMS as a selenium “selective” detector. Because an argon plasma is used as the ionization source, polyatomic species such as $^{40}\text{Ar}^{37}\text{Cl}^+$, $^{38}\text{Ar}^{48}\text{Ar}^+$, and $^{40}\text{Ar}^{40}\text{Ar}^+$, are produced in the plasma and can be abundant when urine samples are analyzed. The major selenium isotopes (m/z 77, 78, 80, and 82) are clustered around these and other polyatomic interferents commonly found in urine samples, with the consequence that selenium selectivity can be severely compromised. These factors have been clearly demonstrated and nicely discussed by Shibata et al. (54) and by others (46, 52). The introduction of collision/reaction cell technology in the last 5 years has improved the situation with regard to some of these polyatomic interferents, but problems still exist, and false selenium peaks (e.g., from $^{1}\text{H}^8\text{Br}^+$/3$^{1}\text{H}^8\text{Br}^+$) can still occur in urine samples. It is quite possible that some of the unknown (and identified) peaks in HPLC/ICPMS chromatograms assigned to selenium species in the past have not contained selenium at all.

The second point concerns the inappropriate use of addition experiments. Ideally, addition experiments are performed by adding an authentic standard of the suspected compound to the sample in an amount approximately equal to the amount in the sample. A single, appropriately enhanced, undistorted HPLC peak provides support for the assignment. When either the standard or the sample compound is in excess, the chances of distinguishing two different but chromatographically similar compounds are greatly diminished. Such addition experiments, with little hope of seeing a negative result (the Admiral Lord Nelson approach: “I see no ships”), are however, remarkably prevalent in the HPLC/ICPMS literature; this practice is particularly troublesome for determining selenium metabolites because of the possible nonselective nature of the detector. Furthermore, an addition experiment performed in only one chromatographic system is generally insufficient to “confirm” the identity of a compound. Many would accept such evidence when assigning a structure to a known compound that has been often reported in a particular type of sample. When a novel or unusual compound is being assigned, however, much more care should be taken, and data showing cochromatography in at least two chromatographic systems should be provided.

The third point deals with the assignment of peaks at or near the void volume of chromatographic systems. There may be instances when other evidence is available for example, in which it is allowable to tentatively assign a structure to a front peak in a chromatogram. In most cases, however, the practice is unacceptable and can lead to gross misrepresentation of the data. These comments are particularly relevant to the identification of selenium species by ICPMS detection because the above-mentioned problems associated with the nonselective nature of the detector are greatly exacerbated with chromatographic peaks eluting at or near the void volume. These comments on the rigor of peak assignments may seem trivial to an analytical audience, but surprisingly, work on selenium metabolites is riddled with poor application of these simple analytical procedures.

Recent Work on Identification and Misidentification of Selenium Species in Urine

Despite the potential problems with HPLC/ICPMS, it is a powerful analytical technique and is now the most commonly used method for determining selenium urinary metabolites. Over the last 10 years, most of the reports of selenium species in urine have used HPLC/ICPMS, sometimes together with molecular MS techniques. In this period, a total of 16 selenium species have been reported in urine, most of them novel human metabolites and some of them completely new compounds. We now wish to individually discuss these metabolites and the data on which their assignments are based.

TMSe

We have already discussed the large body of early data indicating that TMSe is a major urinary metabolite in rats and humans, even without selenium supplementation. Here we focus on those recent studies using modern methods of speciation analysis. Over the last 10 years, there have been an additional 15 reports of TMSe in urine, but the majority of these reports show poor analytical technique in one or more of the three areas discussed above: e.g., poor addition experiments (47, 50, 51, 52) or assignment of a front (void volume) peak (44, 45). The tenuous nature of the data reported by some of these studies is clearly illustrated by Angeles Quijano et al. (45), who commented thus on how they identified TMSe in their urine samples: “This peak was identified as TMSek because of an increase in its area when standard TMSe solutions were added, though it could be other cationic selenium species that also eluted in the dead volume”. We agree with the second part of their data evaluation.

It is interesting to note that as the HPLC/ICPMS techniques have improved in the last 4–5 years, the reporting of TMSe in urine has actually decreased. Indeed, the recent literature is almost silent on this purported major urinary metabolite, and importantly, the
very latest reports are stating that TMSe is not detectable in urine samples (detection limit <0.5 µg/L Se) (66). The application of HPLC/ICPMS to the study of selenium species in urine may have been expected to confirm the early work and provide good quantitative data on the distribution of TMSe in human urine samples and its relationship to selenium intake. In contrast, the new analytical methods have failed to produce a single conclusive set of data showing that TMSe is a component of urine, either at endogenous concentrations or after selenium supplementation. The discord between current data and the results from the earlier work remains unexplained.

Not all data collected for TMSe over the last 10 years have been based on HPLC/ICPMS. In 1996, Hasegawa et al. (73) reported an investigation of selenium urinary metabolites. Their experimental animal was the mouse, hence caution may be needed when comparing the data with those from rats and humans. Nevertheless, they claimed that after oral administration of selenocystine, the urine of the mice contained up to 85% TMSe as a percentage of total selenium. Their method for determining TMSe was based on its retention on cation-exchange resins and subsequent elution with strong HCl—a method similar to that used in all the early studies on TMSe in urine.

SELENITE

Few studies, including those in which selenium was administered as selenite, have reported the presence of large amounts of selenite in urine. For example, Gammelgaard and Jøns (62) analyzed 23 urine samples from 11 volunteers (without supplementation) and detected selenite at concentrations generally <5% of total, although for two samples selenite constituted ~13% and 16% of total selenium. There are two exceptions, however. One study (26) reported selenite in 4 of 13 urine samples at up to 95% of total selenium. The method (ion-exchange chromatography with off-line detection), however, seems rather nonselective for selenite, and the data must be questioned. Yang and Jiang (44) reported that for four samples of human urine (from four individuals) with selenium concentrations up to 427 µg/L, selenite constituted >70% of the total in all cases. The authors noted, however, that the retention times between standards and the assigned selenite peaks in the chromatograms were slightly different, and they did not perform any cochromatography (addition) experiments. They probably have misinterpreted their data, and the suggestion that selenite is a major selenium metabolite in human urine can be disregarded. The data (62) showing selenite to be a common minor constituent of urine, however, look sound.

SELENATE

Selenate was also reported as a major metabolite in humans who had ingested small quantities of selenate or selenomethionine (63). No HPLC chromatograms of unadulterated urine samples were displayed; therefore, the rigor of this assignment can not be established. A third study (52) reported selenate in normal human urine, but the displayed chromatograms show large polyatomic interference at the retention time of selenate; thus, that assignment must be considered very doubtful. There are currently no definitive data demonstrating that selenate is a typical constituent of urine.

SELENODIGLUTATHIONE

As mentioned previously, Ostadalova et al. (53) reported a glutathione derivative of selenium together with TMSe and selenite in rat urine. As far as we can ascertain, however, no data were provided in support of this assignment, and there has been no confirmatory report either by this group or any other research groups. Accordingly, we cannot (yet) accept this compound as an urinary selenium metabolite.

METHYLSelenol

In several reports (38–40) from 1995 to 1997, a group led by Suzuki used enriched stable isotopes to investigate the selenium species in rat urine and claimed that methylselenol was a major urine metabolite. Methylselenol had earlier been postulated, for example, by Ganther (11), as a possible intermediate in selenium biochemical pathways leading to dimethylselenide and TMSe. It had never been identified, although a species present in rat urine was shown by Vadhanavikit et al. (35) to generate volatile methylselenol after chemical treatment. The properties of methylselenol (nonpolar volatile molecule) are more similar to those of dimethylselenide, and thus it might be more likely to be detected as a respiratory metabolite rather than as a urinary metabolite. In fact, closer inspection of the series of reports by Suzuki’s group reveals considerable confusion in their reporting and no compelling evidence for the presence of methylselenol in urine. Indeed, in a subsequent report (41), these researchers retracted their earlier work and reported that the methylated selenium metabolite in rat urine “is not monomethylselenol itself but is related to it, and is tentatively called monomethylselenol-related selenium metabolite”. Possibly this was similar to the species that could be converted to methylselenol that was reported previously by Vadhanavikit et al. (35). In summary, the original assignment of methylselenol in urine was ill-based, and this selenium species has no confirmed existence as a urinary metabolite.

SELENOCYSTINE

Selenocystine was first reported as a metabolite of human urine in 1996 by Muñoz Olivas et al. (64) using HPLC/ICPMS. The data, however, are far from convincing; the authors describe a “noisy chromatographic profile” and
note that quantification was not possible because of severe peak overlap. Gómez et al. (63) also reported a major selenium metabolite that “behaved like selenocystine”, but in the absence of any firm data, this assignment also cannot be relied on. No other researchers have reported selenocystine in urine, and we consider its presence unproven.

SELENOCYSTEINE
A technique using HPLC and atomic fluorescence detection was developed by Gómez-Ariza et al. (60) and applied to one sample of human urine. Selenocysteine appears to have been identified on the basis of a peak that showed a retention time similar to, but clearly different from the standard in one chromatographic system; thus, this assignment can be disregarded.

SELENOETHIONINE
The HPLC/atomic fluorescence detection study of Gómez-Ariza et al. (60) also reported a peak in human urine possibly corresponding to selenoethionine, but because this compound had not previously been reported as a natural product, the authors considered that its identity “was not guaranteed”. We concur with this assessment: such an ethylated species would be an unlikely metabolite.

METHYLSelenomethionine
Gammelgaard et al. (49) reported methylselenomethionine in human urine, but they seem uncertain of their assignment. For example, in the abstract they state that one of the selenium peaks coeluted with methylselenomethionine, but in the body of the paper they report “a species apparently co-elutes with methylselenomethionine, or very close to this species”. This is insufficient evidence on which to assign a novel urinary metabolite, and this tentative assignment requires confirmation before being accepted.

SELENOMETHIONINE
Selenomethionine has been identified as a major form (protein-bound) of selenium in selenized yeast (7), and it seems to be generally accepted as a common urine metabolite as well. The urine data should be scrutinized carefully, however. In the first report of its presence in human urine, by Gammelgaard et al. (47), it was determined by HPLC/ICPMS and electrophoresis/ICPMS. In a later study from the same group (49), selenomethionine was identified in only some of the urine samples, although the study participants received selenomethionine supplements.

The possible inadequacy of assignments made on the basis of cochromatography in a single system has recently been clearly demonstrated in the study of Chatterjee et al. (52). A peak initially assigned to selenomethionine on the basis of cochromatography with standard compound in one system was shown to be another compound when tested in a different chromatographic system.

Selenomethionine was also reported in urine by Wrobel et al. (51), again on the basis of cochromatography in a single system. Collectively, the reported data (47, 51) might be considered as fair evidence that selenomethionine is a natural constituent of human urine. We make the point, however, that although one might expect to find selenomethionine in normal human urine, no study to date has conclusively demonstrated its presence. We note that the report of selenomethionine in human urine by Cao et al. (65) was also from an individual who had ingested this compound; thus, the sample cannot be considered as normal. We now discuss further the work of Cao et al. (65), but with the focus on selenocystamine, the major selenium species in that study.

SELENOCystamine
In 2001, Cao et al. (65) used HPLC/ICPMS and tandem mass spectrometry (MS/MS) with multiple-reaction monitoring (also known as selected reaction monitoring) to identify and quantify selenocystamine and selenomethionine in human urine. This combination of techniques is considered to provide rigorous identification of compounds, well above that provided by HPLC/ICPMS alone, and the authors claimed the first positive identification of these two selenium species in human urine. The report, however, has some unusual aspects, which we expand on here.

The urine sample under investigation was collected from an adult male for 4 consecutive days after he had ingested 400 µg of selenomethionine supplement (presumably this was 400 µg of Se as selenomethionine). Because the authors expected low selenium concentrations (they estimated 20–200 µg/L), they concentrated their sample fourfold, by evaporation, before direct analysis by HPLC/ICPMS. The ensuing chromatogram contained several small peaks and a huge front peak, which for some (unstated) reason the authors thought might be selenocystamine. A standard selenocystamine solution, reported as 500 ppm but presumably it was 500 µg/L Se, was used to provide retention time matching and data for quantification of this major urine metabolite. Many other selenium species could elute in this front peak; thus, the HPLC/ICPMS data provide no firm evidence for the presence of selenocystamine in the sample.

The authors appeared to have been aware of this; they therefore collected the front HPLC fraction and examined it by MS/MS. Although source mass spectra for authentic selenocystamine were displayed, no such confirmatory data were provided for the urine metabolite. The authors state that the data they report meet the acceptance criteria for positive MS/MS identification of compounds, but this is not totally correct. One of the criteria stated is that HPLC retention times of standard and sample analyte should be within 2%; but the MS/MS data reported were obtained with direct injection, bypassing the HPLC col-
umn. All compounds in the urine fraction would therefore be introduced to the ionization source at the same time.

The quantitative aspects of the study by Cao et al. (65) also appear to contain a substantial error. The authors claim that selenocystamine was present at 40 µg/L Se in the original urine sample (equivalent to 160 µg/L Se after concentration), and the detection limit of their HPLC/ICPMS system was 10 µg/L Se. The front chromatographic peak for the urine sample in HPLC/ICPMS, however, is enormous; in fact, it is off-scale (>80 000 counts/s for 77Se; natural abundance, 7.6%). This peak thus could not result from such low quantities of selenium species in the urine from an analytical system with a detection limit of 10 µg/L. Clearly there are analytical anomalies in these reported data that require an explanation before we can accept that selenocystamine is a major selenium urinary metabolite. We note also that, subsequent to the results reported by Cao et al. (65) in 2001, Gammelgaard et al. (70) stated that they have never detected a selenium species in urine with the chromatographic properties of selenocystamine.

**SELENOADENOSYL METHIONINE**

One of the more interesting selenium urinary metabolites to be recently identified was selenoadenosylmethionine. Wrobel et al. (74) had previously seen traces of this compound in selenized yeast after a sample preparation procedure designed to capture this unstable species. It was subsequently identified by the same group (51) by HPLC/ICPMS in one human urine sample based on cochromatography in a single HPLC system. Surprisingly, the peak assigned to selenoadenosylmethionine in the urine sample was one of the largest peaks in the chromatogram. For the reasons discussed above for other purported selenium metabolites, we believe that this assignment requires confirmation before being accepted as fact. The reported instability of this species must raise some additional concerns.

**SELENOSUGARS 1, 2, AND 3**

The first reports of a selenosugar in urine were by Ogra et al. (42) and Kobayashi et al. (68). This group had for some time been investigating the selenium metabolites in urine from rats given selenite and had shown that a major metabolite (not TMSe) was produced. As discussed above, they mistakenly thought that the metabolite was methylselenol, but later referred to it as a methylselenol-related compound (41), presumably to indicate that the metabolite contained a CH₃-Se moiety. In a later report (42), however, these researchers isolated the rat urine metabolite to a sufficient purity to enable MS/MS analysis after electrospray ionization. The characteristic cluster of signals reflecting the isotopic pattern of selenium facilitated their search for selenium-containing ions. The fragmentation pattern of the compound provided clues to its structure, on the basis of which they synthesized a model compound, selenosugar 1 (methyl 2-acetamido-2-deoxy-1-seleno-β-d-glucopyranoside). Although this model did not match the chromatographic properties of the natural compound and thus could be ruled out as a possible candidate, the mass spectral data of the two compounds were sufficiently similar to suggest that they were structurally similar. On the basis of their collective data, Ogra et al. (42) proposed that the natural compound was not selenosugar 1, a β-glucopyranoside, but its α-galactopyranoside isomer. One might assume here that the assignment as the α-isomer was a typographical error (α-isomers of 2-acetamide sugars are sterically hindered and hence do not feature as natural products) and that the authors meant to propose the β-galactopyranoside isomer, namely selenosugar 2 (methyl 2-acetamido-2-deoxy-1-seleno-β-d-galactopyranoside).

Kobayashi et al. (68) subsequently synthesized selenosugar 2 and reported that its NMR and mass spectra matched those of the rat urine metabolite. This correct assignment resulted from several years of concerted and persistent research, and the group deserves credit for the satisfactory resolution of this difficult problem. It appears likely that the methylselenol-related compound referred to earlier by Shibara et al. (41) was in fact selenosugar 2, although the authors do not specifically state this (68).

Gammelgaard et al. (70) quickly used the results of Suzuki’s group, and they reported the presence of a selenosugar (likely to be selenosugar 2) in urine from humans who had been given selenized yeast. The structural assignment was based on MS/MS data and comparison with the data reported by Suzuki’s group (42). Subsequently, Gammelgaard and Bendahl (66) synthesized the target selenosugar 2, in addition to its isomer 1, and confirmed their earlier assignment by chromatographic comparison (HPLC/ICPMS) of the major selenium metabolite and authentic selenosugar 2. The compound appeared to be a natural constituent of urine, and its concentration was greatly increased after selenium dietary supplementation. Gammelgaard and Bendahl (66) claimed that selenosugar 1 was also present in their samples (2% of selenosugar 2) on the basis of data from HPLC/ICPMS and capillary electrophoresis/ICPMS, and these researchers subsequently provided MS/MS data confirming the assignment (67). It is interesting to note that this newly reported minor metabolite, selenosugar 1, was the selenosugar that Ogra et al. (42) first thought they had.

A third selenosugar (selenosugar 3, methyl 2-amino-2-deoxy-1-seleno-β-d-galactopyranoside) has also recently been identified by capillary electrophoresis/MS/MS in human urine after a volunteer had consumed selenized yeast (67). Significantly, selenosugar 3 (identified by HPLC/ICPMS) was also present in urine from the volunteer before ingestion of the selenium supplement; its concentrations did not increase after ingestion of the supplement despite the formation of large quantities of...
the related selenosugar 2. An explanation for this unusual observation is not readily apparent.

It was interesting that Gammelgaard’s group, working with humans and selenized yeast, identified the same major selenium metabolite as did Suzuki’s group, which was investigating rats and selenite. With some assumptions, these results suggest that the type of selenium compound ingested does not influence the major metabolite and that humans and rats produce similar selenium metabolites, which has been the accepted view since the early studies. Kobayashi et al. (68) actually stated that the selenium metabolite in human urine, after selenite ingestion, was the same as that in rat urine, but their experimental data were insufficient to make such a claim. A later study by Diaz Huerta et al. (69), however, detected selenosugar 2 by MS/MS in rat urine after ingestion of selenomethionine and thus provides further evidence for a selenium metabolism common to rats and humans.

METHYLSELENITE

Methylselenite was identified in rat urine by Ogra et al. (71) and was shown to be produced from selenosugar 2 (presumably the other selenosugars would also serve as precursors to methylselenite). These researchers refer to methylselenite as an selenosugar artifact and consider that it should not be regarded as a naturally occurring metabolite of urine.

OTHER SELENIUM SPECIES

Kresimon et al. (75) detected selenium species in human urine by gas chromatography/ICPMS after converting the original species present in the urine to volatile derivatives with sodium borohydride. The volatile analytes produced were said to be (CH₃)₂Se, (CH₃)₂SSe, and (CH₃)₂Se₂ (no supporting evidence was presented). The precise structures for the species originally in the urine, however, remain unknown.

Zheng et al. (50) specifically looked for selenoureia in nine samples of human urine, but could find no evidence for the presence of this species.

Summary of Selenium Metabolites in Urine

The foregoing information has been summarized in Table 3. To be regarded as a urinary metabolite, the selenium species must be produced in the body; we therefore do not consider those species detected in urine after their administration because such urinary species may simply represent unchanged administered compound rather than a metabolic product. The data fall cleanly into two groups, before and after the application of HPLC/ICPMS, and it seems prudent to discuss the two data sets separately.

Before the advent of HPLC/ICPMS, the only selenium species regularly reported in urine was TMSe. It was first reported (13) in the urine of rats administered selenite, but was later claimed (18) to also be in human urine after increased selenium intake, and later still to be a constituent of normal human urine (25). Although the qualitative and quantitative aspects of many of these studies are questionable, the data displayed in support of the original structural assignment appear sound. Those data consisted of NMR, mass spectra, and/or infrared spectra (13, 14), which matched (sometimes precisely) the spectra for authentic synthesized TMSe. It is surprising, however, that pure TMSe could be obtained from urine with the fairly nonselective isolation procedure used. Despite this anomaly, TMSe was regarded as a major selenium urinary

| Table 3. Summary of reported selenium urinary metabolites: confirmed, unconfirmed, and retracted. |

<table>
<thead>
<tr>
<th>Selenium species</th>
<th>No of reports</th>
<th>Quality of data*</th>
<th>Accept as a normal urinary metabolite?</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMSite</td>
<td>46</td>
<td>⭐️ to ⭐️⭐️⭐️⭐️⭐️</td>
<td>Yes for now, but the early work urgently needs confirming with modern analytical methods</td>
</tr>
<tr>
<td>Selenite</td>
<td>16</td>
<td>⭐️ to ⭐️⭐️⭐️</td>
<td>Yes, but a minor one</td>
</tr>
<tr>
<td>Selenate</td>
<td>5</td>
<td>⭐️</td>
<td>No</td>
</tr>
<tr>
<td>Selenodiglutathione</td>
<td>1</td>
<td>None given</td>
<td>No</td>
</tr>
<tr>
<td>Methylselenol</td>
<td>3</td>
<td>⭐️</td>
<td>No; the original discovers have retracted this assignment</td>
</tr>
<tr>
<td>Selenocystine</td>
<td>2</td>
<td>⭐️</td>
<td>No</td>
</tr>
<tr>
<td>Selenocysteine</td>
<td>1</td>
<td>⭐️</td>
<td>No</td>
</tr>
<tr>
<td>Selenoethionine</td>
<td>1</td>
<td>⭐️</td>
<td>No</td>
</tr>
<tr>
<td>Methylselenomethionine</td>
<td>1</td>
<td>⭐️</td>
<td>No; reported in the abstract but appears to have been retracted in the text of the same report</td>
</tr>
<tr>
<td>Selenomethionine</td>
<td>4</td>
<td>⭐️ to ⭐️⭐️⭐️⭐️⭐️</td>
<td>Not just yet; several studies report it after ingestion of selenized yeast</td>
</tr>
<tr>
<td>Selenocystammine</td>
<td>1</td>
<td>⭐️⭐️</td>
<td>Not just yet; the HPLC/ICPMS data do not match the MS/MS data</td>
</tr>
<tr>
<td>Selenoadenosylmethylamine</td>
<td>1</td>
<td>⭐️⭐️</td>
<td>Not just yet; the species is reported as being very unstable</td>
</tr>
<tr>
<td>Selenosugar 1</td>
<td>2</td>
<td>⭐️⭐️⭐️⭐️⭐️⭐️</td>
<td>Yes, a minor constituent</td>
</tr>
<tr>
<td>Selenosugar 2</td>
<td>4</td>
<td>⭐️⭐️⭐️⭐️⭐️⭐️</td>
<td>Yes; firmly established as a major selenium metabolite after supplementation with selenite or selenized yeast; also data showing it is present in urine without selenium supplementation</td>
</tr>
<tr>
<td>Selenosugar 3</td>
<td>1</td>
<td>⭐️⭐️⭐️⭐️⭐️⭐️</td>
<td>Yes, a minor constituent</td>
</tr>
<tr>
<td>Methylselenite</td>
<td>1</td>
<td>⭐️⭐️</td>
<td>Thought to be formed in urine from oxidation of arsenosugar 2</td>
</tr>
</tbody>
</table>

*⭐️, ??: ⭐️⭐️, ??: ⭐️⭐️⭐️, ??: ⭐️⭐️⭐️⭐️, ??.
metabolite and was generally thought to increase when selenium exposure was above nutritional requirements. Unknown selenium species were also reported in urine, but the only other known selenium species claimed to be in urine were selenite \( (26, 28, 53) \) and selenodiglutathione \( (53) \).

After the introduction and application of HPLC/ICPMS for the determination of selenium urinary species, one may have anticipated that TMSe would be regularly reported as a major metabolite. Interestingly, however, this has not occurred. In fact, although early HPLC/ICPMS work reported TMSe in urine, usually at low concentrations, the more recent studies do not find it at all, even after selenium supplementation. For example, Gammelgaard and Bendahl \( (66) \), categorically state that TMSe was not detected \( (<0.5 \mu g/L \text{ Se}) \) in urine from humans receiving 1000 or 2000 \( \mu g \) of selenium as selenized yeast. For balance, we note that Suzuki’s group \( (41, 42, 68) \) commonly reported that TMSe was in their rat urine samples, but this result was ancillary to their goal (identification of the major metabolite), and they seldom provided data. In summary, although there is a large body of early data showing that TMSe is a major selenium urinary metabolite, both at high selenium exposure and at no dosed exposure (i.e., a typical constituent of urine), the recent data with sophisticated instrumentation suggest that TMSe is, at most, a minor or trace constituent. The discord between the old and new data is such that a repeat of those early experiments with rats and high selenium exposure together with quantitative HPLC/ICPMS analysis is urgently needed.

In addition to TMSe, 15 selenium metabolites have been identified in urine, mostly with HPLC/ICPMS (Table 3). For many of these, the assignments have been made on very little evidence and require confirmation before the compounds can be accepted as typical urine metabolites. Some, such as methylselenol, have already been retracted \( (41) \) by their discoverers. Selenosugar 2 is now firmly established as a major urinary metabolite when selenium is administered, and it is also a constituent of natural urine \( (66, 68) \). There have also been reports of selenosugar 1 \( (66, 67) \) and selenosugar 3 \( (67) \) as minor constituents. Selenite appears to be a common minor metabolite (generally \(<5\% \) of total selenium) in normal urine \( (62) \).

**Future Research**

The recent work on the identification of selenosugars in rat urine by Suzuki’s group \( (68) \) and in human urine by Gammelgaard’s group \( (66, 67, 70) \) has provided a firm basis for further investigations into selenium metabolism. The outcome from these studies may well be a completely new metabolic pathway describing how humans deal with exposure to excess selenium. Future work should use HPLC/ICPMS in combination with MS/MS to provide good quantitative data and reliable assignments for the metabolites. A priority investigation should be to repeat the early experiments with rats exposed to different quantities of selenium to try to establish the significance or otherwise of TMSe as a urinary metabolite (the investigations of Suzuki’s group are unclear on this aspect).

The selenium species present in normal (unsupplemented) urine require further study. Available data indicate that selenite and selenosugars 2 and 3 constitute three of the typical urinary species, but there are several other species that remain unknown. Investigations into the factors influencing the pattern of selenium urinary metabolites (type and concentration), such as biological effects, nutritional status, and chemical effects, are also likely to produce interesting and useful data.

Finally, the potential health information contained in the pattern of selenium metabolites—a selenium species profile—should also be investigated by determining individual variability in humans with regard to selenium metabolism under various selenium regimens. Possibly this selenium species profile may be a useful indicator of selenium status in terms of the element’s essential and toxic roles and may reveal correlations with health effects not apparent from the total selenium concentration data.

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**References**


50. Zheng J, Ohata M, Furuta N. Reversed-phase liquid chromatography with mixed ion-pair reagents coupled with ICP-MS for the direct


