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Original article

Urinary selenium excretion in selenite-loaded sheep and subsequent Se dynamics in blood constituents

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Abstract — Renal selenium excretion in sheep was measured during intravenous infusion of sodium selenite, and the post-infusion dynamics of Se levels in whole blood, plasma and red blood cells (RBC) were investigated for the next 5 days. The plasma Se level increased almost twenty fold with the infusion of Na_2SeO_3 (from 0.39 ± 0.02 to 7.83 ± 0.33 μmol·L^{-1}, $P < 0.001$) compared with the baseline value. The selenium concentration in urine (0.07 ± 0.02 vs. 18.53 ± 2.56 μmol·L^{-1}, $P < 0.001$), the amount of Se excreted (0.14 ± 0.07 vs. 21.40 ± 2.31 nmol·min^{-1}, $P < 0.001$) and the renal clearance of Se (0.19 ± 0.03 vs. 3.01 ± 0.34 mL·min^{-1}, $P < 0.001$) were found to be highly significantly elevated during selenite loading. The clearance measurements showed no changes in the urinary flow rate or in the glomerular filtration rate. During and at the end of infusion the highest Se level was attained in plasma, followed by whole blood and RBC. The plasma Se level fell rapidly within 10 min after the end of infusion, but the concentration of Se in RBC was stable up to the fourth hour, when it started to decrease too. On day 5 the Se concentrations in plasma, RBC and whole blood were found to be only slightly but still significantly higher than before the selenite infusion. The large disproportion between the infusion rate of Se (8.76 μg·min^{-1}) and its renal excretion rate (1.69 μg·min^{-1}) found in clearance measurements suggests low glomerular filtration of infused selenium, which might primarily be caused by the binding of selenite metabolites to blood constituents. The presented results confirm the low bioavailability to ruminants of Se from sodium selenite.

sheep / sodium selenite / renal function / selenium excretion

1. INTRODUCTION

Selenium is an essential trace element occurring naturally in inorganic and organic forms. The role of Se as an antioxidant as well as in immunocompetency development, reproduction and performance of animals has been well established [1–4].

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In many European countries the natural selenium content of grain and forages, which consists mainly of the selenoamino acids selenomethionine and selenocysteine in plant proteins, is only 0.03–0.12 mg·kg^{-1} of dry matter (DM) with values more commonly at the lower end of this range. Intake of such feeds can result in serious selenium deficiency and health problems, especially in highly productive animals. For this reason, feedstuffs are routinely supplemented with various selenium sources, usually at the rate of 0.2–0.3 mg of Se per kg of DM [5]. Despite the well-documented advantage of using organic selenium as selenized yeast with 50% of Se in the form of selenomethionine [6], inorganic Se-sources are preferred in many countries of the world to date as a supplement to animal feeds.

Excess inorganic selenium absorbed from the digestive tract and not utilised in selenoprotein synthesis is methylated and excreted [7]. Animals with simple stomachs excrete the surplus of dietary Se of inorganic origin mainly in their urine, but in ruminants a larger proportion is excreted in the faeces than through the kidneys. The reason for this is that inorganic Se compounds are partly reduced by the ruminal microorganisms to unabsorbable elemental Se [8]. A significant portion of selenomethionine from organic sources of Se, however, escapes hydrolysis in the rumen environment, and is evidently absorbed in the lower part of the digestive tract [9]. Selenomethionine which is not reduced to selenide for selenoprotein synthesis has been shown to form significant body deposits of Se by its unspecific incorporation into muscle and other organ proteins of ruminants [10]. The selenium content of milk was also found to be significantly enhanced by addition of Se-yeast into feeds of dairy cows [11].

Renal Se excretion is known to be influenced by the amount and chemical form of Se ingested as well as by the animal's physiological status [12]. Urinary selenium excretion also depends on the glomerular filtration of Se-compounds and on the concentrating/diluting function of the kidneys [13, 14]. The finding of total selenium recovery from urine and faeces for both selenite and selenate (82–95%) in humans indicates that retention of Se of inorganic origin is very low, in contrast to selenomethionine with only 26% of the dose recovered [12].

Ruminants with apparent nutritional Se deficiency are frequently treated with injection preparations containing sodium selenite (Na_2SeO_3). This was a further reason for testing this drug in our experiment. The aim of this experiment was to discover the response to intravenous selenite infusion in renal selenium excretion by sheep, and the dynamics of Se levels in blood constituents for 5 days after loading with Na_2SeO_3.

2. MATERIALS AND METHODS

2.1. Animals and diet

The experiment was performed on young ewes (*Ovis aries*) of the Merino breed weighing 23–26 kg. The sheep were housed individually in cages with free access to water and were fed a normal protein diet for 3 weeks before the selenite infusion. The daily ration for each sheep consisted of 500 g of hay, 300 g of barley and 250 g of wheat bran, providing a total daily intake of 129.3 g of crude protein, 12.03 MJ of digestible energy and 93.2 μg of selenium. The sheep were fed twice daily at 6.00 am and 4.00 pm.

2.2. Experimental procedure

2.2.1. Clearance assay

The renal functions were measured using a standard clearance technique on nine ($n = 9$) conscious sheep held in cages. The right jugular vein was cannulated with a polyethylene capillary (o.d. 1.1 mm; i.d.

0.7 mm) for the infusions of inulin and sodium selenite dissolved in sterile 0.15 mol NaCl. Blood was sampled from the contralateral jugular vein into heparinized tubes at the mid-point of every urine collection period. Each urine collection period lasted 30 min. The urine was quantitatively collected into calibrated glass cylinders through a Foley catheter (French size 14) placed in the urinary bladder.

The clearance procedure started with a priming intravenous dose of 1 g of pyrogen-free inulin (Sigma) in 50 mL of saline, followed by infusion of the same at the rate of 5 $mg \cdot mL^{-1} \cdot min^{-1}$. Two control urine collection periods were carried out 60 min after the start of infusion to allow for the equilibration of inulin in the extracellular space. After this, 3.4 mg of Se in the form of Na_2SeO_3 (Sigma) in 10 mL of saline was administered intravenously as a priming dose of selenite, and the above-mentioned inulin infusion continued enriched with 8.76 $\mu g \cdot mL^{-1} \cdot min^{-1}$ of Se as sodium selenite. Two experimental urine collection periods were applied 60 min after the addition of selenite to the inulin infusion (Fig. 1). The total amount of selenium administered intravenously to each sheep was 4.45 mg within 2 h. The results from consecutive control or selenite-infused urine collection periods in the same animal were averaged.

2.2.2. Blood dynamics assay

The dynamics of Se levels in blood, plasma and red blood cells (RBC) after intravenous selenite loading were investigated in another five sheep (n = 5). The reason for this approach was to avoid the stress due to holding the animals in metabolic cages with Foley catheters inserted in their urinary bladders during clearance measurements, as well as to exclude possible interference with infused inulin. The sheep were fed the same diet as the animals used in the clearance protocol. Both jugular veins were cannulated with polyethylene capillaries (o.d. 1.1 mm; i.d. 0.7 mm). The left cannula served for selenite administration and the contralateral one for blood samplings. To load the sheep with selenite, the same priming dose (3.4 mg of Se in the form of Na_2SeO_3 in 10 mL of saline) followed by infusion lasting 2 h (8.76 $\mu g \cdot mL^{-1} \cdot min^{-1}$ of Se as selenite) were applied.

Blood was collected into heparinized tubes before selenite loading and at the end of selenite infusion. Blood samples for the identification of Se dynamics in blood constituents were taken 10, 20, 30, 40 min and 1, 2, 3, 4, 5, 6, 12, 18, 24, 30, 36, 48, 60, 72, 84, 96 and 108 h after the end of infusion. Plasma was removed after blood centrifugation at 1180 *g* lasting 15 min. The RBC from sediment were separated from the residual leukocytes and all the samples were kept at –20 °C until analyzed.

2.3. Sample analysis

The selenium concentrations in the samples of plasma, whole blood, red blood cells, urine and dietary components were analyzed in triplicate using the fluorimetric method of Rodriguez et al. [15]. Inulin was measured in plasma and urine samples fluorimetrically [16]. The osmolality of plasma and urine was determined cryoscopically on a Knauer osmometer.

2.4. Statistical analysis

Statistical analysis of the differences between the control and selenite infusion periods in clearance measurements was carried out using the paired Student t-test. Se level dynamics over the five days following selenite loading was evaluated by ANOVA with the Dunnet post-test, and the differences in selenium contents in blood constituents were assessed by ANOVA with the Tukey post-test. The results are given as means ± S.E.M.

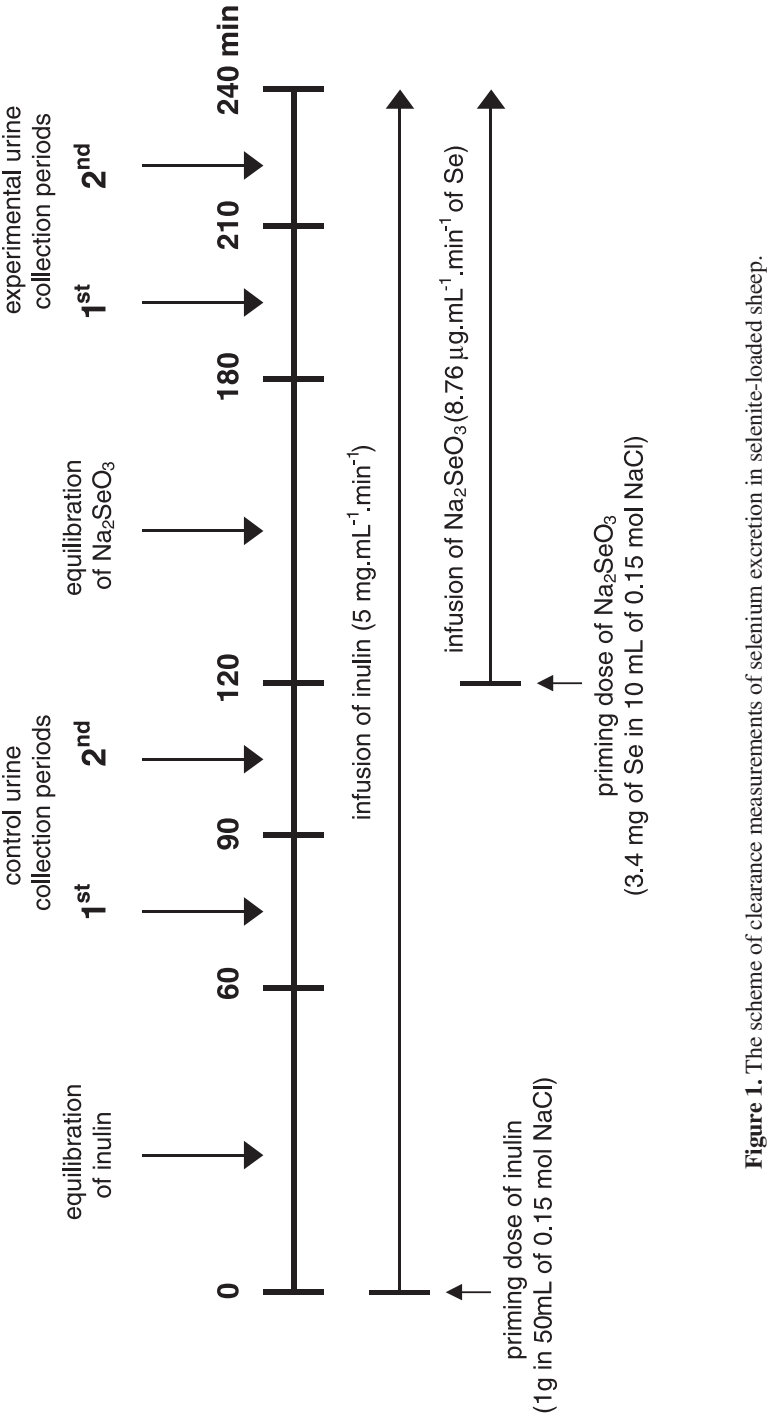

Figure 1. The scheme of clearance measurements of selenium excretion in selenite-loaded sheep.

3. RESULTS

3.1. Renal excretion of Se

Infusion of Na_2SeO_3 into the jugular vein of sheep resulted in an almost twenty fold increase in the average Se level in blood plasma (from 0.39 ± 0.02 to 7.83 ± 0.33 $\mu mol \cdot L^{-1}$, $P < 0.001$). The whole blood concentration of Se was highly elevated (from 0.37 ± 0.02 to 5.87 ± 0.43 $\mu mol \cdot L^{-1}$, $P < 0.001$) too, but the value it ultimately reached was lower than in the plasma ($P < 0.01$). Both values were stable during the whole infusion. The glomerular filtration rate and the urine flow rate were not influenced by the selenite infusion. Neither the plasma and urine osmolalities nor the clearance of osmotically active substances were changed (Tab. I).

The intravenous infusion of Na_2SeO_3 naturally led to highly-significant increases in Se concentration in the urine (from 0.07 ± 0.02 to 18.53 ± 2.56 $\mu mol \cdot L^{-1}$, $P < 0.001$) and in the amount of Se excreted (from 0.14 ± 0.07 to 21.40 ± 2.31 $nmol \cdot min^{-1}$, $P < 0.001$). Likewise, the renal clearance of Se was significantly elevated (from 0.19 ± 0.03 to 3.01 ± 0.34 $mL \cdot min^{-1}$, $P < 0.001$) during selenite loading (Fig. 2).

3.2. Blood dynamics of Se

The measurements of selenium dynamics in blood constituents showed the same pattern of Se levels in blood constituents at the end of selenite infusion as in the clearance procedure. The intravenous selenite loading induced huge increases in Se concentrations in whole blood (from 0.78 ± 0.11 to 5.62 ± 0.6 $\mu mol \cdot L^{-1}$), plasma (from 0.42 ± 0.09 to 6.6 ± 0.54 $\mu mol \cdot L^{-1}$) and RBC (from 1.5 ± 0.21 to 4.66 ± 0.26 $\mu mol \cdot L^{-1}$), with the highest value attained in plasma. The plasma Se level started to fall within 10 min of the end of the infusion, but the concentration of Se in RBC was found to be stable up to the beginning of the fourth hour, when it started to decrease too. For this reason the plasma Se level (4.73 ± 0.67 $\mu mol \cdot L^{-1}$) was already equilibrated with the RBC selenium concentration 40 min after infusion (4.68 ± 0.45 $\mu mol \cdot L^{-1}$). The plasma Se

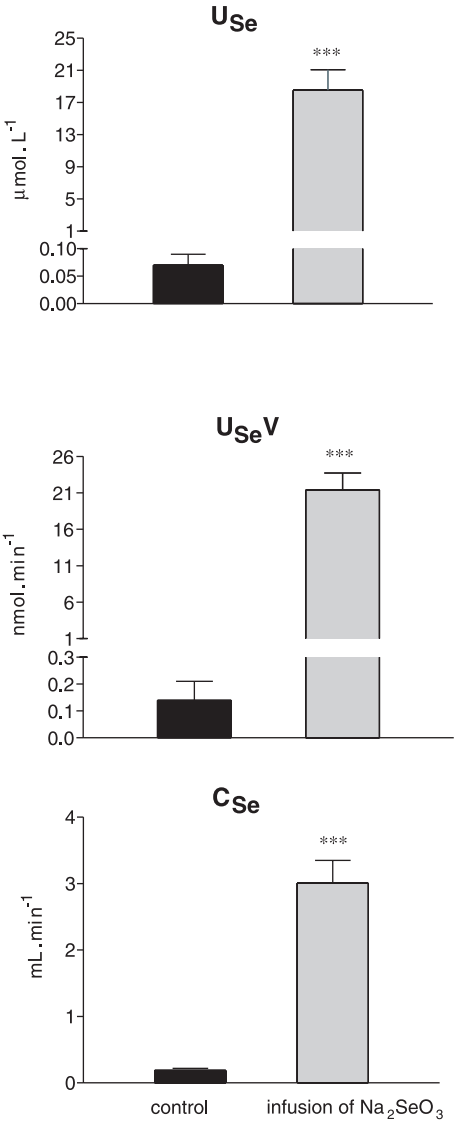

Figure 2. The concentration of Se in urine (U_{Se}), the amount of Se excreted ($U_{Se}V$) and the renal clearance of Se (C_{Se}) in sheep during intravenous infusion of Na_2SeO_3. Values are means ± SEM, $n = 9$. Significance levels: $***P < 0.001$.

Table I. The effects of sodium selenite infusion on urine flow rate (V), glomerular filtration rate (GFR), plasma (P_{osm}) and urine (U_{osm}) osmolality and on osmotic clearance (C_{osm}) in sheep. The values are means ± SEM.

	Baseline values (n = 9)	Selenite infusion (n = 9)	Statistical significance
V ($mL \cdot min^{-1}$)	1.72 ± 0.46	1.55 ± 0.42	NS
GFR ($mL \cdot min^{-1}$)	59.98 ± 5.54	56.67 ± 3.60	NS
P_{osm} ($mosm \cdot kg^{-1}$ H_2O)	296.89 ± 1.77	296.50 ± 1.72	NS
U_{osm} ($mosm \cdot kg^{-1}$ H_2O)	411.11 ± 94.77	470.72 ± 46.71	NS
C_{osm} ($mL \cdot min^{-1}$)	1.52 ± 0.10	2.03 ± 0.24	NS

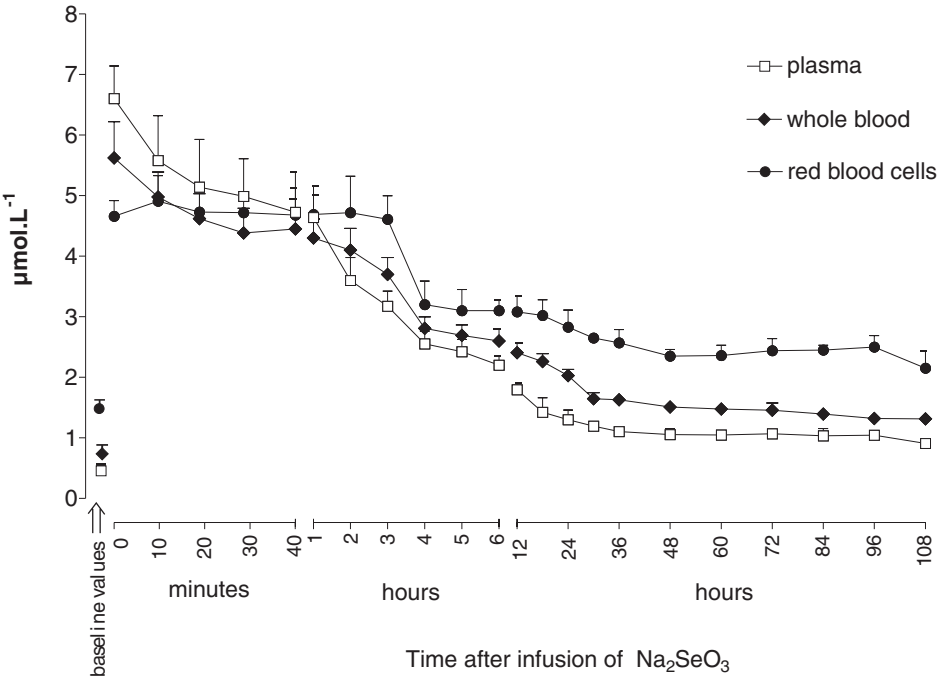

Figure 3. The dynamics of Se levels in whole blood, plasma and red blood cells during a period of 5 days after the intravenous loading of sheep with sodium selenite. Values are means ± SEM, n = 5.

concentrations in all samples collected later than 1 h after the end of infusion were lower than in RBC. The post-infusion Se levels in plasma and whole blood were already reduced by half after the fourth hour. This pattern of the decreases in Se levels was followed in all further samples collected up to the 30th hour. The selenium concentrations in the blood constituents from samples collected after this time were found to be almost stable (Fig. 3).

The final Se concentrations in whole blood (1.31 ± 0.06 vs. 0.78 ± 0.11 $\mu mol \cdot L^{-1}$, $P < 0.01$), plasma (0.91 ± 0.07 vs. 0.42 ± 0.09 $\mu mol \cdot L^{-1}$, $P < 0.01$) and RBC (2.15 ±

0.29 vs. 1.5 ± 0.21 μmol·L^{-1}, $P < 0.01$) were found to be significantly higher than their baseline values before selenite loading.

The packed cell volume (30.78 ± 0.99,%) was stable during the whole experiment.

4. DISCUSSION

The twenty fold rise in plasma Se level in sheep induced by selenite infusion increased the amount of Se excreted during the clearance procedure by a factor of 150. The most striking finding was that of a large disproportion between the infusion rate of Se (8.76 μg·min^{-1}) and its renal excretion rate (1.69 μg·min^{-1}). This means that only 19.3% of Se from the infused amount of selenite appeared in the urine during the infusion period. Almost the same proportion of excreted selenium (23.0%) was found in steers given an intravenous injection of 5 mg of labelled Se in the form of selenite [17].

Surprisingly, the sheep used in the clearance protocol did not show any statistical difference between the control values of Se levels in plasma and blood. Now we can only speculate that this finding could be the result of a low Se-status of sheep brought from a farm with a poor dietary regimen and short transitional period of 3 weeks with our diet before the clearance measurements. This was a further reason to use other sheep in the experiment with measurements of blood dynamics of Se.

The low selenium status of sheep presented by the baseline blood Se level (0.37 ± 0.02 μmol·L^{-1}) did not seem to play any significant role in Se excretion, because the huge amount of selenium was infused within a relatively short time. We did not measure the Se uptake by organs or tissues during the infusion period, but some immediate and excessive synthesis of specific selenoproteins does not seem to be a probable reason for the small proportion of excreted Se compared with the amount of loaded Se.

The explanation for the discrepancy between the infused and the excreted amounts of Se in our experiment is based on the metabolism of sodium selenite already occurring in the blood stream. It has been shown that sodium selenite injected intravenously is taken up rapidly by RBC, where it is reduced by glutathione into selenide within a few minutes [18]. After efflux into plasma, selenide is bound to albumin via 17 intramolecular disulfide bonds and transported to the liver for the synthesis of selenoprotein P, and the surplus of H_2Se is methylated for subsequent excretion [19, 20].

This might explain why the portion of selenium infused and bound to albumin in the form of H_2Se is apparently not filtered in the kidney glomeruli, and might account for the considerably smaller amount of Se being excreted in our sheep than the amount infused. Under conditions of normal selenium intake, Se bound to albumin in the form of H_2Se is a minor selenium source in plasma compared with selenoprotein P and plasmatic glutathione peroxidase [21]. Selenide is a key intermediate product of the metabolism of both inorganic and organic Se dietary forms, before selenium is used in the synthesis of selenoproteins [6, 22].

We did not measure the speciation of Se-compounds in sheep urine. To date there are three known methylated selenium metabolites excreted into urine (monomethylselenol and trimethylselenonium ions-TMSe) or into breath (dimethylselenide-DMSe) Their formation is dependent on the dose of Se. Monomethylselenol as a major urinary Se metabolite in animals with normal Se intake was recently assigned as selenosugar (Se-methyl-*N*-acetylseleno-hexosamine) [23]. TMSe in urine and/or DMSe in expirated air are reported to appear only with excessively large doses of selenium [22], which was the case of the infusion in our sheep. Several other minor Se metabolites have been detected in urine but without being identified [7]. In

contrast to selenite, a considerable portion of selenate given intravenously to rats was found to be directly excreted in urine without being metabolised [24]. The dose of selenite we infused in our sheep was extremely large, as was the renal Se excretion. For this reason we cannot exclude the possibility that some small part of the infused selenite could escape reduction in RBC, and that after rapid filtration of its small molecules in glomeruli and intrarenal recycling, some Na_2SeO_3 was excreted in the urine too.

Although the glomerular filtration of selenide seems to be limited due to its albumin bonds, its methylated products are very rapidly excreted. We did not measure the selenium excretion in urine, faeces or exhalation by breath during the post-infusion periods, but the 50% decline in the plasma and whole blood Se levels within 4 h suggests its rapid elimination from the ovine body. With regards to dietary organic selenium, it should be stressed that all amino acids including selenomethionine (Se-Met) are almost completely reabsorbed from kidney glomerular filtrate as early as in the first tubular segment of the nephron-proximal tubule. Se-Met shares the active transport processes with common sulphur-containing methionine in that tubule. Reabsorbed Se-Met is captured by the blood in the kidney capillaries and re-enters the whole body metabolism via the blood stream [25]. This is a further reason for preferring preparations based on Se-yeast in ruminants' nutrition.

On the 5th day after selenite infusion, the Se levels in whole blood and its constituents fell to almost the same values as before infusion. Despite significant differences between the blood baseline levels and the values 5 days after infusion, this finding corroborates previous data on low retention of Se from selenite in ruminants [9]. It should be stressed at this point that feed supplementation with a selenite dose equivalent to the one we infused in our sheep will result in a considerably smaller amount of selenium absorbed and entering the blood stream due to the reduction of this compound to elemental Se in the rumen [8]. Our results show that in addition to limited absorption of inorganic selenium from the digestive tract, the rapid urinary excretion of its metabolic products is another significant disadvantage of selenite compared with the exploitation of organic selenium in ruminant nutrition.

While the plasma Se level began to fall immediately after the end of infusion, the RBC concentration of selenium was found to be stable for a further 3 h. We can only speculate about this, but the explanation for the delay might be based on oversaturation of the selenite reduction process with subsequent cumulation and binding of some intermediary Se product(s) to erythrocyte proteins. After the concentration gradient of selenium metabolites from plasma to RBC disappeared, the Se level in erythrocytes also started to fall some 2 h later.

In conclusion, the dramatic increase in the rate of selenium excretion during selenite infusion and the subsequent rapid fall of its concentrations in blood constituents indicate the low potential of Se from this inorganic compound for retention in the bodies of sheep. The disproportion between the infusion rate of Se (8.76 $\mu g \cdot min^{-1}$) and its renal excretion rate (1.69 $\mu g \cdot min^{-1}$) found in clearance measurements suggests reduced glomerular filtration of infused selenium, which might primarily be caused by the binding of selenite metabolite(s) to blood constituents. The presented results corroborate the low bioavailability to ruminants of Se from sodium selenite.

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