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THE NATURE AND IMPORTANCE OF SELENIUM METABOLITES IN THE ANIMAL

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Although the toxicity of selenium has been known for several decades and its essentiality has been established for over one decade (1,2), very little is known concerning the organic forms of selenium and their functions within the animal. However, dimethyl selenide has been established as an exhalation product of selenium with rats given selenate (3) or selenite (4) by injection. Recently, the discovery by Byard (5) of Wisconsin and by Palmer et al. (6) of our laboratory of the trimethylselenonium ion as a major urinary form of selenium with rats given selenite appears important, especially since Palmer et al. (7) have also shown other selenium substances to be sources of the metabolite. Studies on the toxicity and metabolism of the trimethylselenonium ion in our laboratory have given data indicating that it is a detoxification product of selenium in the rat. The results showed that the substance was relatively non-toxic when fed or injected (COO-1449-10) and was excreted rapidly and unchanged when injected (8). In addition, it is inactive against the necrogenic syndrome (8).

The aspects of the work on Contract No. AT(11-1)-1449 for 1970 which are reported herein concern further metabolic and toxicological experiments with rats as well as the effort toward isolating a second form of urinary selenium, i.e. U-2. The areas of investigation that are reported on are as follows:

A. **Effect of dietary vitamin E on the urinary excretion of trimethylselenonium ion.**

Selenium metabolism in the rat, as observed by the conversion of injected selenite-Se to the trimethylselenonium ion and U-2 of urine, was not definitely affected by a vitamin E deficiency. Detailed procedures and results are given in Preprint No. COO-1449-12:

**MEASUREMENT OF SELENITE-$^{75}$Se ACTIVITY IN URINARY SUBSTANCES OF RATS FED A TORULA YEAST DIET WITH AND WITHOUT ADDED VITAMIN E**

A. W. Halverson, I. S. Palmer and D.-T. Tsay
B. Relationship of arsenite to trimethylselenonium ion (TMSe) toxicity or vice versa.

In a previous report (COO-1449-10) the acute LD50 of TMSe was shown to be 49.4 mg Se/kg whereas the compound was not toxic when fed at levels of 30 ppm Se. Data were also given which showed the acute toxicity of TMSe was greatly increased in the presence of arsenite. Attempts to publish this information were unsuccessful and therefore the study was expanded. The added data are reported here.

In a complete study of the oral toxicity of TMSe, it was found that a level of at least 240 ppm selenium as TMSe was required to produce significant weight reduction. The presence of arsenite produced a slight increase in the toxicity of selenium (the 120 ppm level showing weight gain reduction). Other data indicate that the apparent synergism between arsenite and TMSe is not due to an interaction between arsenite and the -onium structure since arsenite had no effect on the toxicity of the sulfur analog of TMSe. However, there was an apparent synergistic effect between arsenite and another methylated derivative, dimethyl selenide.

The effects of arsenite on the excretion of TMSe-selenium were also studied in an attempt to explain the apparent synergism in toxicities. Arsenite was found to increase the volatilization of TMSe-selenium, however, it appeared to decrease the urinary excretion of TMSe within a 6-hour observation period. It has not been ascertained whether the decrease in excretion of TMSe in the presence of arsenite was sufficient to explain the increased combined toxicities. Detailed procedures and results are given in Preprint No. COO-1449-13:
C. Mechanisms of selenium methylation.

(a). Rat liver: To gain further knowledge of the synthesis of TMSe, it appeared of interest to determine the tissues responsible for its synthesis. The system developed by Ganther (9) was used to study the in vitro synthesis of TMSe. His original system involved soluble enzymes from mouse liver whereas our studies employed rat liver.

Freshly excised liver was homogenized in nine volumes of 0.25 M sucrose containing 10⁻⁴ M EDTA. The homogenate was centrifuged at 9000 × g for 10 min to remove the cellular debris and the mitochondria. For each assay, an amount of extract equivalent to 0.2 g of liver was added to a Warburg flask containing the following constituents in μmoles: sodium phosphate buffer, 100; reduced glutathione, 60; MgCl₂, 70; ATP, 12; S-adenosylmethionine, 2; EDTA, 3; TPN⁺, 0.4; glucose-6-phosphate, 6; glucose-6-phosphate dehydrogenase, 5 μgrams. A level of 0.15 μmoles of Na₂SeO₃ containing ⁷⁵Se was placed in the side arm (total volume in the flask - 3.0 ml). In experiments where arsenite was used, 0.15 μmoles was added to the medium. In most experiments the flasks were continuously flushed with nitrogen and the effluent nitrogen was bubbled through nitric acid to trap the volatile products. In certain other experiments, the flasks were closed during the duration of the experiment and then flushed with nitrogen into a nitric acid trap at the termination of the study. All flasks were incubated for 40 min. At the termination of the experiment, the medium was removed from the flask and dialyzed 24 h against 100 ml of distilled water. The dialyzate was evaporated and submitted to ion exchange chromatography as described by Palmer et al. (6). A peak of radioactivity was found at the position where TMSe should be eluted. The identification of the peak was further substantiated by paper chromatography and precipitation with ammonium reineckate.

The data in Table I indicate that the rat liver system was active in producing dimethyl selenide (DMSe) with over 50% of the added selenite being converted to DMSe within 40 min. The liver system was also shown to be capable of producing TMSe although the actual amount was rather small (0.58%). The soluble enzyme fraction from kidney tissue was also capable
TABLE I

In vitro Synthesis of TMSe and DMSe

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Addition to Medium</th>
<th>DMSe Produced</th>
<th>TMSe Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selenite</td>
<td>Arsenite</td>
<td>% of Added</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Selenite</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>-</td>
<td>53.8 ± 2.1</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>+</td>
<td>2.22 ± 0.37</td>
</tr>
<tr>
<td>Kidney</td>
<td>+</td>
<td>-</td>
<td>40.0 ± 3.7</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>-</td>
<td>38.5 ± 6.1</td>
</tr>
</tbody>
</table>

Vessels Constantly Flushed with (N2)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Addition to Medium</th>
<th>DMSe Produced</th>
<th>TMSe Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Selenite</td>
<td>Arsenite</td>
<td>% of Added</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Selenite</td>
</tr>
<tr>
<td>Liver</td>
<td>+</td>
<td>-</td>
<td>38.5 ± 6.1</td>
</tr>
</tbody>
</table>

a Each constituent was added at the level of 0.15 umole/3 ml of medium.
b Values are means ± SE for 3 rats.
c Values are means ± SE for 4 rats.

of producing both DMSe and TMSe. It seems likely that both tissues are sites of the synthesis of TMSe within the animal.

The addition of arsenite to the in vitro system resulted in a dramatic reduction in the production of DMSe as reported previously (Ganther, 9). However, the production of TMSe was not significantly altered. These data are consistent with the hypothesis that DMSe and TMSe are not produced by the same biosynthetic pathway. When the experiment was repeated with the vessel closed so that DMSe was not continuously removed, the amount of TMSe formed was increased 4-fold. These data can be interpreted to mean that TMSe is a product of the direct methylation of DMSe and is synthesized in larger amounts in the closed vessel because of the higher concentration of DMSe. However, an alternative explanation is that DMSe accumulation
results in the accumulation of an earlier intermediate which is then converted to TMSe. Further data are required to fully understand the pathway of TMSe synthesis and certainly some worthwhile information could be obtained by adding radioactive DMSe to the reaction vessel and then analyzing for the presence of radioactive TMSe.

(b). Bacteria: Studies were also undertaken to examine the methylation of selenium by bacteria. Challenger (10) has shown that molds produce DMSe and Ganje and Whitehead (11) and Abu-Erreish et al. (12) have shown that volatile selenium products are released by microbiological action in the soil.

Selenium containing soil samples were obtained and used as a source for isolation of bacteria. Isolations were carried out on nutrient agar plates containing 20 ppm Se as sodium arsenite. Surviving colonies were plated out and screened for detectable odor. When a bacterial species was detected which produced a selenide-type odor, it was subjected to pure culture techniques. After obtaining a pure culture, the bacterium was used to inoculate nutrient broth containing 20 ppm and an air train was set up to constantly bubble through the medium and into various traps including HNO₃.

The bacterium was not completely identified but according to the usual identifying procedures, it appeared to be a Pseudomonas species. Pure cultures of the organism were used to inoculate nutrient broth containing 20 ppm Se as sodium selenite. The culture flask was placed in an air train in series with several traps. In a 72 hour period, 30% of the total selenium in the medium was volatilized. Most of the remainder appeared to be converted to elemental selenium. In an attempt to identify the volatile product, various selective traps were inserted into the train. It was found that Hg(CN)₂ would trap a major portion of the selenium. According to Challenger (10), diselenides would be trapped in this system while selenides would pass on through. Gas chromatographic analysis by the
procedure of Evans and Johnson (13) indicated that the main volatile product of the bacterial metabolism was dimethyl diselenide.

D. Isolation and characterization of U-2 in rat urine.

Time did not permit a great deal of work on the isolation of U-2. However, a second purifying step was developed. Rat urine was extracted once with 1.5 volume of ether which was discarded. The urine was then acidified, extracted two times with 2 volumes of ether, and the extracts were evaporated in a flash evaporator. The extract residue from 15 liters of urine was taken up in 50 ml ethanol and allowed to stand several days in the refrigerator. This solution was filtered and 10 ml of the filtrate was mixed with 3 g of silicic acid (100 mesh) and allowed to dry. This material was then placed on the top of a column 1.3 cm in diameter containing 12 g of silicic acid, which had been poured in ether. The column was eluted successively with ether, 95% ether-5% ethanol, 1:1 ether-methanol, and methanol. The methanol fraction containing most of the U-2 was combined and evaporated. The column step gave a 6-fold purification of the ether extract.
BIBLIOGRAPHY


(9) Biochemistry 5, 1089 (1966).


