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Time–Dependent Distribution of Sodium Selenite in the Female ICR Mouse

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Experiments probing the distribution of selenium in animal tissues were begun as early as 1913 by Quarelli and co-workers (1913). Since then, numerous studies have shown selenium toxicity to be very complex, being dependent on a number of variables, including the particular selenium compound administered (Schroeder 1967), and species (Cooper and Glover 1974), sex (Schroeder 1967), and age of the test animal (Jacobs and Forst 1981), and the route, concentration, and duration of dosage administered (Cooper and Glover 1974).

There is little information available dealing with the distribution and tissue accumulation of selenium following a single sublethal dosage. Thus, these pilot studies were designed to provide information for future analyses of selenium toxicity by investigating the spatial distribution of sodium selenite in selected tissues of the female ICR mouse as a function of time following a single intraperitoneal injection. By establishing levels of background selenium in these tissues for comparison with the distribution of injected selenium, information was obtained which will be valuable for future studies on this species for toxicity and general biological effects of selenium.

MATERIALS AND METHODS

All tissues were analyzed using a Baird-Atomic SF-1 Spectrofluorometer. Sodium selenite was obtained from Pfatz and Bauer, Stanford, CN 06902. The 2,3-diaminonaphthalene (DAN) was obtained from Lancaster Synthesis, Ltd., Windham, NH 03087. All reagents used were reagent grade or better. Fluorometric analysis involving DAN was chosen because of the sensitivity range and relative simplicity of the method. The analytical procedure used here was derived from a procedure reported by Spallholz and coworkers (1978).

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Female mice of the ICR strain were isolated and randomized into control and experimental groups with five animals in each group. Animal weights ranged from 29 to 40 grams with a standard deviation of 1.8 grams at the time of injection. Food and water were freely available at all times. Sodium selenite solutions were prepared for injection using triple distilled water as the injection vehicle. At time zero, intraperitoneal injections were administered with control mice receiving 0.2 ml physiological saline while selenium-treated animals received 2.5 or 5.0 mg/kg body weight. The higher dosage of 5.0 mg/kg of sodium selenite has been shown to be a maximum tolerable dosage for females of this species within the time course reported here (Hogan and Jackson 1986). The control injection volume of 0.2 ml was not corrected for body weight since weight variations were relatively small. This control volume also represented the average injection volume for the experimental groups.

At 3, 6, 12, 24, and 48 hours following injection, animals were sacrificed by cervical dislocation. Selected tissues were removed, rinsed three times with chilled isotonic saline, blotted dry, and weighed to the nearest 0.1 mg. The mean weights (and standard error of mean) of the experimental tissues were: brain, 126.8 mg (26.4 mg); heart, 100.2 mg (3.95 mg); kidney, 130.6 mg (4.65 mg); liver, 536.4 mg (35.0 mg); lung, 126.6 mg (14.0 mg); and spleen, 74.1 mg (4.19 mg). The brain tissues, however, became extremely hydrated after thawing and could not be analyzed. At the times of sacrifice, animals were examined for necrotic tissue and possible internal damage due to injection. Without exception there were no abnormalities visually observed in either the control or the selenium-treated mice. Tissues were stored in glass sample bottles and frozen until time of analysis. A rapid and efficient digestion mixture was prepared using the method reported by Cummins and co-workers (1964) by dissolving 10 g of sodium molybdate in 150 ml of distilled water, then slowly adding 150 ml of concentrated sulfuric acid, and after cooling, adding 200 ml of 70-72% perchloric acid. This mixture was capable of completely digesting up to 0.7 g of tissue per 5 ml of acid solution. Some tissues from the same test groups were pooled in order to obtain a mass of at least 0.2 g for a single analysis. Each sample was boiled in the digestion mixture for ten minutes in a six-flask Kjeldahl digestion unit, after which the samples turned quite viscous and a lime green color was observed. No charring of tissues was noted. After cooling, the samples were transferred into 35 ml glass vials with screw caps. The digestion flasks were rinsed with 5 ml of distilled water and this rinse was added to the sample vials. At this time the samples were clear and colorless. No undigested material was visible in most cases, although liver tissue tended to be difficult to digest, sometimes requiring the addition of more digestion solution.

The pH was raised to about 2 with concentrated ammonia. As the pH approached 2, the solution began to turn a bright lime green, which worked well as an approximate indicator of pH. The final

pH was adjusted to between 2.0 and 2.4, using a pH meter, by adding either ammonia or digestion solution as needed. After cooling, 4 ml of 0.2 M ethylenediaminetetraacetic acid (EDTA), prepared from the disodium salt, was added to mask interfering ions such as iron or copper which may have been present in the biological tissues or in the reagents. A solution of 0.1% DAN was prepared by dissolving 0.1 g of DAN in 100 ml of 0.1 M hydrochloric acid with stirring. This reagent was stored in a dark refrigerator for no more than 48 hours before each analysis. Immediately before each use, the DAN solution was washed in a separatory funnel at least twice with 25 ml portions of cyclohexane to remove any fluorescing impurities. After extraction the cyclohexane wash was routinely checked for fluorescence to determine the purity of the washed reagent. If fluorescence was detected in the wash, the reagent was washed again.

Four milliliters of DAN reagent was added to each sample vial and the vials were immersed in a water bath shaker at 50 C and gently agitated for 20 minutes to allow complete formation of the piazselenol complex. The vials were removed and allowed to cool for five minutes, and 5.00 ml of cyclohexane was added. The screw caps were attached, and the vials were shaken manually for three minutes to extract the piazselenol complex into the organic layer. The vials were centrifuged for three minutes to clarify the cyclohexane layers, which were then transferred into quartz cells using a Pasteur pipet. The samples were excited at 377 nm and fluorescence was measured at 525 nm.

RESULTS AND DISCUSSION

Tables 1 and 2 show the average concentrations of selenium found in each experimental and control group. The concentrations are expressed in parts per million of wet tissue weight. The concentration of injected selenium reached a maximum in all tissues within six hours after injection. Most tissues showed a return to approximately control levels after 48 hours. Tables 3 and 4 represent the percentage of the administered dosage found per gram of tissue for each tissue group. It should be noted that 75 percent of the tissue groups showed an inverse relation of selenium retention to selenium dosage. Table 5 shows the comparison of the results of these studies with concentrations of selenium in rats, pigs, and humans, as reported by others.

A similar study was done (Heinrich and Kelsey 1955) in which mice were injected subcutaneously with much lower dosages (0.1-0.7 mg/kg body weight) of sodium selenite containing Se-75, and distribution was reported as a percentage of the dosage per tissue as a function of time following injection. Our results differ in the time required for maximum concentrations of selenium to occur in some tissues and in the percentage of dosage accumulated in each tissue. It is difficult to compare the two studies, because the authors of the earlier work did not report actual dosage per injection or the actual concentrations of selenium found in each tissue group. Comparison of the percentage of the dosage retained

Table 1. Concentration (ppm) of selenium found in selected tissues after 2.5 mg/kg injection.

	Control	3 hour	6 hour	12 hour	24 hour	48 hour
Kidney	1.72 [0.22](4)	1.55 [0.50](2)	3.87 [0.82](3)	1.70 [0.35](4)	0.69 [0.27](2)	*
Liver	1.05 [0.10](20)	2.05 [0.47](5)	6.59 [1.30](4)	1.56 [0.26](4)	2.10 [0.25](5)	5.63 [1.04](5)
Heart	0.28 [0.02](3)	1.91 [*] (1)	1.13 [0.08](2)	1.12 [0.26](2)	0.64 [0.08](4)	0.82 [0.10](4)
Lung	0.10 [0.02](4)	3.10 [*] (1)	0.28 [0.04](2)	1.42 [*] (1)	1.97 [0.04](2)	0.94 [0.09](4)
Spleen	0.45 [0.11](2)	2.21 [0.21](2)	1.13 [0.01](2)	2.06 [0.20](2)	1.00 [0.37](2)	0.75 [0.18](4)

*Unavailable [Standard error of mean] (Number of analyses)

Table 2. Concentration (ppm) of selenium found in selected tissues after 5.0 mg/kg injection.

	Control	3 hour	6 hour	12 hour	24 hour	48 hour
Kidney	1.72 [0.22](4)	2.65 [0.53](3)	4.25 [0.92](4)	1.84 [0.14](4)	1.93 [0.21](5)	1.80 [0.18](5)
Liver	1.05 [0.10](20)	5.23 [0.90](5)	11.91 [3.61](5)	1.27 [0.60](4)	2.30 [0.36](4)	1.43 [0.17](5)
Heart	0.28 [0.02](3)	2.13 [0.61](4)	2.35 [0.43](2)	2.29 [0.67](3)	1.11 [0.26](2)	0.68 [0.14](5)
Lung	0.10 [0.02](4)	3.24 [0.77](2)	0.35 [0.21](3)	2.29 [0.61](3)	1.22 [0.39](2)	1.13 [0.31](5)
Spleen	0.45 [0.11](2)	5.80 [0.23](2)	3.42 [0.47](3)	1.52 [0.62](3)	1.63 [0.25](2)	1.19 [0.37](5)

Table 3. Percentage of administered dosage found per gram of whole tissue: 2.5 mg/kg dosage (average dosage = 82.5 µg)

	3 hour	6 hour	12 hour	24 hour	48 hour
Kidney	1.88 (0.61)	4.69 (0.99)	2.06 (0.42)	0.84 (0.33)	*
Liver	2.48 (0.57)	7.99 (1.58)	1.89 (0.32)	2.55 (0.30)	6.82 (1.26)
Heart	2.32 (*)	1.37 (0.10)	1.36 (0.32)	0.78 (0.10)	0.99 (0.12)
Lung	3.78 (*)	0.34 (0.05)	1.72 (*)	2.39 (0.05)	1.14 (0.11)
Spleen	2.68 (0.25)	1.37 (0.01)	2.50 (0.24)	1.21 (0.45)	0.91 (0.22)

*Unavailable (Standard error of mean)

Table 4. Percentage of administered dosage found per gram of whole tissue: 5.0 mg/kg dosage (average dosage = 165 µg)

	3 hour	6 hour	12 hour	24 hour	48 hour
Kidney	1.61 (0.32)	2.58 (0.56)	1.12 (0.08)	1.17 (0.13)	1.09 (0.11)
Liver	3.17 (0.55)	7.22 (2.19)	0.77 (0.36)	1.39 (0.22)	0.87 (0.10)
Heart	1.29 (0.37)	1.42 (0.26)	1.39 (0.41)	0.67 (0.16)	0.50 (0.08)
Lung	1.96 (0.47)	0.21 (0.13)	1.39 (0.37)	0.74 (0.24)	0.68 (0.19)
Spleen	2.49 (0.14)	2.07 (0.28)	0.92 (0.38)	0.99 (0.15)	0.72 (0.22)

(Standard error of mean)

Table 5. Selenium concentrations in selected tissues of certain species (ppm whole tissue)

	Kidney	Liver	Spleen	Heart	Lung
Rat (1)	1.45	1.29	0.45	0.37	0.38
Pig (2)	1.27	0.72	*	0.21	*
Man (3)	0.63	0.39	0.27	0.22	0.21
Sheep (4)	1.57	0.99	*	0.32	*
Mouse (5)	1.72	1.05	0.45	0.28	0.10

*(ppm whole tissue), [1]Behne and Wolters 1983, [2]Leibetseder et al. 1974, [3]Dickson and Tomlinson 1967, [4]Dye et al. 1963, [5]This study.

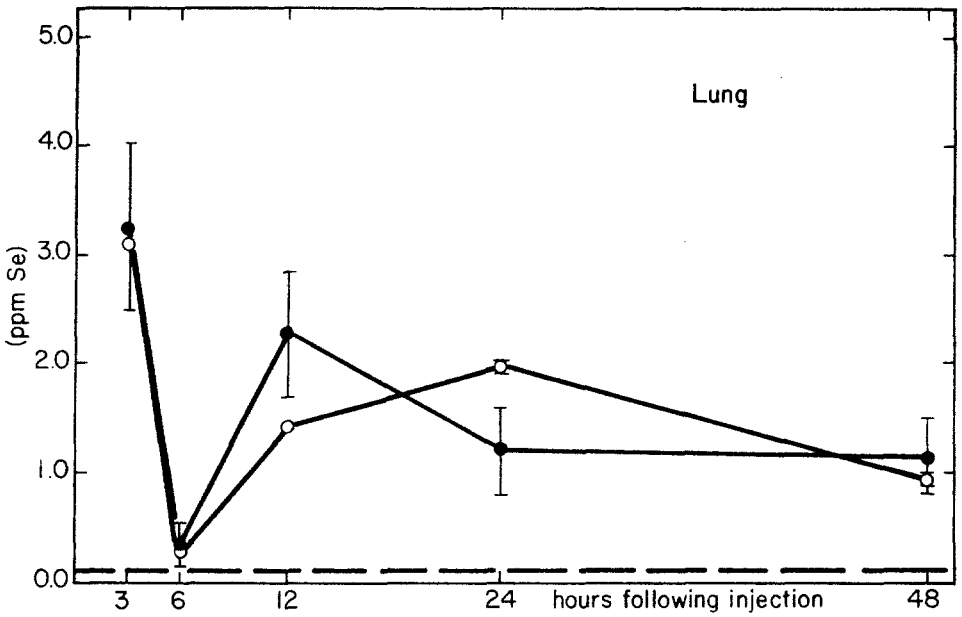

Figure 1. Selenium concentration in the lung as a function to time following injection of 2.5 mg/kg (o), or 5.0 mg/kg (•) sodium selenite. Dashed line denotes average concentration of selenium in control lungs.

per gram of tissue reveals a steady decrease in dosage accumulation per gram of tissue as the dosage increases. Since the rate of elimination of sodium selenite is apparently a function of the dosage size, it is even more important to report the exact dosage level administered in each distribution study.

The lung (Figure 1) reached maximum concentration after three hours and then appeared to return immediately to near the control concentrations, followed by another broad concentration peak. This paradoxical response may be explained by assuming that the injected selenium is capable of existing in several forms which arrive at the lungs at various times. The first peak may represent the expected accumulation of the injected selenite, while the later peak may represent the arrival of organoselenium compounds that had been rapidly manufactured in other tissues, as reported by McConnel and Portman (1952). After forty-eight hours the lungs still retained over ten times the concentration of selenium in the control tissues.

The relatively high standard errors in the tissue concentrations, particularly in the liver, were possibly due to differences in the metabolic rates of individual mice. Small differences in individual metabolic rates might have caused large differences in observed selenium in the liver after three hours, as can be seen in earlier data. Heinrich and Kelsey (1955) reported substantial changes in selenium concentration within a thirty minute to two hour period following injection, particularly in the liver.

It was observed that in most cases tissues accumulated selenium at a rate that was affected by the dosage level. Future studies are needed to probe this relationship further, perhaps providing insight into the body's mechanism of detoxification and/or elimination routes. It would be of interest to learn how this change in accumulation or elimination rate might be affected by the presence of elements that are known to be toxicologically related to selenium, such as cadmium, arsenic, or mercury.

These results indicate that while the kidney concentrated more selenium than other tissues in the control group, the liver generally concentrated more of the injected selenium. The role of the liver in detoxification is also pronounced by its large mass, making it capable of accumulating up to thirty percent of the injected dosage as reported in previous studies (Heinrich and Kelsey 1955). The lung appears to maintain relatively high levels of selenium throughout the 48 hour period following injection. Expiration of dimethyl selenide and other organoselenium compounds is known to be an important route of elimination of selenium (Ganther 1965). This is particularly true between 24 and 48 hours following injection, when expiration of selenium through the lungs becomes comparable to elimination through the feces and urine (Heinrich and Kelsey 1955). It would be of interest to determine the selenium species present in the first and second peaks, thereby gaining more exact knowledge of the detoxification mechanism.

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