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Comparison of histochemical autometallography (Danscher's stain) to chemical analysis for detection of selenium in tissues

Donal O'Toole, Larry E. Castle, Merl F. Raisbeck

Selenium intoxication induces a variety of clinical syndromes, such as chronic dystrophic digital lesions in horses, cattle, and swine (alkali disease), poliomyelomalacia in pigs, and myocardial necrosis in various species. Diagnosis of selenosis is currently based on the presence of characteristic lesions, combined with chemical analysis (more than 2 and 10 μg/g selenium in blood and hair, respectively).[9]

Autometallographic localization of selenium in fixed and frozen sections of tissue was introduced in 1982[1] as a modification of the intravital Timm sulfide sliver enhancement technique for detecting heavy metals and was used to detect the cellular and subcellular distribution of selenium in the brain,[1] adrenal gland,[15] and anterior pituitary[14] of rats. With the exception of 2 recent reports describing its use in selenium-intoxicated lambs,[12,13] little information exists about the sensitivity and specificity of this technique for detecting exogenous selenium in tissues. The purpose of this study was to establish whether autometallography is a useful diagnostic adjunct to chemical analysis in suspected cases of selenosis. Preliminary studies using intraperitoneal injections of sodium selenite in rats indicated that although positive autometallographic staining occurred following acute intoxication, the intensity of staining in various tissues (brain > kidney > liver) corresponded poorly to concentrations of selenium as detected by fluorometry (kidney > liver > brain). Autometallography of chemically fixed brains from rats with acute selenosis revealed topographically localized, intense punctate staining of boutons and perikarya that was strongest in cerebral cortex, nucleus caudatus-putamen, hippocampus, and nucleus cochlearis dorsalis (Fig. 1), whereas staining in the cerebellum was absent, although selenium accumulation in the brain is essentially diffise.[16] Rats were chosen as subjects because they were used in the original development of autometallographic staining for catalytic selenium bonds.[1] Various forms and concentrations of selenium were fed, because differences may exist in the extent to which forms of selenium accumulate in various tissues and in resultant autometallographic staining patterns[6] An ethyl alcohol-chloroform-acetic acid fixative (Camoy's solution) was used because preliminary studies confirmed that metallographic staining is lost when tissues are fixed in formalin. Following fixation in Camoy's solution, the intensity of staining was comparable to that in unfixed frozen sections, with the advantage of better morphologic preservation.

Twenty-seven male rats with body weights of 215-252 g were housed separately in wire-bottom cages in a room with a mean temperature of 20 C and lights on between 0600 and 1800 hours. Following acclimation, 24 rats were assigned at random to 12 test groups and treated with 3, 5, 7, or 9 ppm selenium in the form of selenocystine,[a] selenomethionine,[a] or sodium selenite[a] in their drinking water for 28 days (Table 1); 3 rats given distilled water (<5 ppb selenium) served as controls. Rats were fed a proprietary rodent laboratory chow[b] containing 0.19 ppm selenium and a declared zinc content of 70 ppm. At the end of the study period, rats were euthanized with diethyl ether and examined postmortem. Body and organ weights (thymus,. spleen, liver, kidney, heart, and brain) were recorded. Samples of liver, kidney, heart, and brain (left cerebrum and brain stem) were fixed by immersion in Carnoy's solution for 2-3 hours, dehydrated in ascending concentrations (70%, 80%, 95%, 100%) of ethyl alcohol over 6 hours, held in toluene overnight, and placed in an automated tissue processor[c] for infiltration under vacuum with paraffin wax. Three coronal levels of the brain were examined histologically: rostra1 cerebrum taken at the head of the caudate nucleus, caudal cerebrum taken through hippocampal areas CA1-CA4, and cerebellum-medulla oblongata taken at nucleus cochlearis dorsalis. Tissues were sectioned at nominal thicknesses of 5 and 14 μm for hematoxylin and eosin staining and autometallographic staining, respectively, and mounted on glass slides coated with 3-aminopropyltriethoxy silane.[a] Autometallographic staining was performed in the dark for 60 minutes using a physical developer (Danscher's

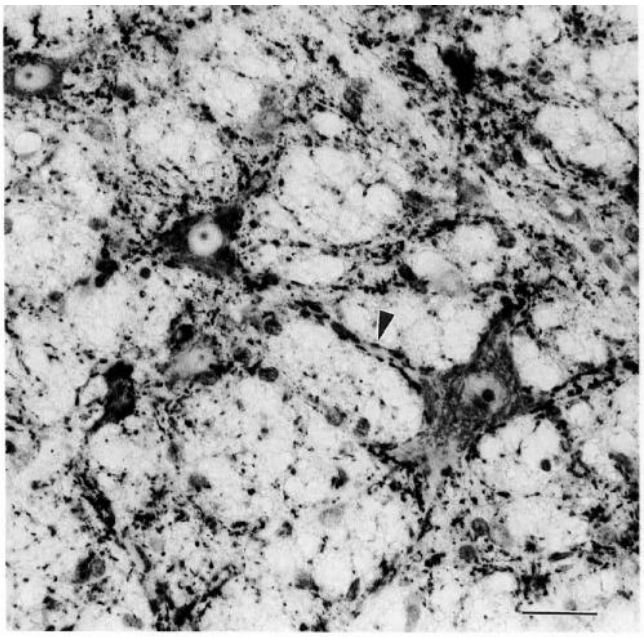

Figure 1. Brain stem; rat. Note intense punctate autometallographic staining on surfaces of perikarya and dendrites of large neurons in facial motor nucleus (arrowhead). This rat was injected intraperitoneally with 20 mg/kg sodium selenite 2 hours earlier; selenium concentration in the liver was 28.3 μg/g. Danscher-TB. Bar = 25 μm.

From the Wyoming State Veterinary Laboratory, 1174 Snowy Range Road, Laramie, WY 82070.

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Table 1. Experimental protocol, selenium concentrations, and semiquantitative autometallographic staining of rats fed selenium for 28 days.

Rat no.	Se form*	Dose (ppm Se)	Tissue Se concentration (μg/g wet weight)				Staining†	
			Kidney	Liver	Brain	Heart	Kidney	Brain
1	Se cys	3	3.5	3.2	0.2	0.3	2	0
2	Se cys	3	2.4	1.2	0.1	0.4	2	0
3	Se cys	5	4.0	2.5	0.1	0.5	2	0
4	Se cys	5	6.2	2.1	0.2	0.6	2	0
5	Se cys	7	8.0	3.4	0.2	0.6	2	0
6	Se cys	7	4.8	2.6	0.2	0.6	2	2
7	Se cys	9	7.9	3.2	0.2	0.5	2	0
8	Se cys	9	12.3	3.5	0.2	0.6	3	0
9	Se met	3	3.4	3.4	0.9	1.4	0	0
10	Se met	3	3.3	2.0	0.7	1.3	0	0
11	Se met	5	5.5	6.1	1.1	1.9	1	0
12	Se met	5	4.5	5.1	0.9	2.5	1	0
13	Se met	7	6.6	8.0	1.0	2.6	2	0
14	Se met	7	6.6	7.3	1.1	2.8	2	0
15	Se met	9	10.6	8.2	1.9	4.0	3	0
16	Se met	9	7.0	9.0	1.7	2.9	3	0
17	SeO_3	3	2.6	1.4	0.2	0.4	0	0
18	SeO_3	3	2.1	1.8	0.3	0.4	0	0
19	SeO_3	5	4.1	3.3	0.3	0.5	2	0
20	SeO_3	5	2.4	1.8	0.3	0.4	0	0
21	SeO_3	7	5.4	4.3	0.2	0.6	1	2
22	SeO_3	7	3.2	2.7	0.3	0.5	2	0
23	SeO_3	9	7.5	5.1	0.3	0.6	3	0
24	SeO_3	9	5.7	3.7	0.3	0.6	3	0
25	control	<1	1.0	0.7	0.2	0.4	0	0
26	control	<1	1.6	1.0	0.2	0.3	0	0
27	control	<1	1.4	1.1	0.2	0.3	0	0

* Se cys = selenocystine; Se met = selenomethionine; SeO_3 = sodium selenite.
† 0 = no stain; 1 = weak; 2 = moderate; 3 = strong. No autometallographic staining was detected in liver or heart from any rat.

solution) composed of silver lactate, hydroquinone, and gum arabic in a titrated buffer.[1] Sections were then washed, exposed to 5% thiosulfate in water, and counterstained with toluidine blue (Danscher-TB stain). Slides were coded and scored for intensity of reaction product as follows: 0 = no stain, 1 = weak, 2 = moderate, 3 = strong. Samples of liver, kidney, heart, and brain (right cerebrum) were collected fresh at necropsy, stored at -70 C, and analyzed for selenium concentration μg/g wet weight) using a fluorometer.[d,8] Quantitative data (weekly weight gain, water and food intake, organ weights at necropsy, liver: brain weight ratios, concentrations of selenium in tissue) were analyzed statistically by correlation and regression analysis" and considered significant at $P \leq 0.05$.

No abnormal clinical signs developed during the study. Rats fed sodium selenite and the higher doses (7 and 9 ppm) of selenomethionine and selenocystine had a small (8-15%) significant decrease in food consumption with respect to controls. All 24 treated rats demonstrated a significant decrease (25-30%) in total water intake with respect to controls. Weight gain, body condition, organ weights at necropsy, and liver: brain weight ratios were not significantly affected by treatment. No microscopic lesions, such as hepatocellular necrosis or regeneration,[7,10] were detected. Concentrations of selenium in liver, kidney, and heart but not in brain had a significant positive correlation with drinking water concentration for any given form of selenium. Selenomethionine resulted in significantly greater concentrations of selenium in tissues than did equivalent concentrations of selenocystine or sodium selenite (Table 1).

No autometallographic staining (Table 1) was evident in tissues from control rats. Positive staining occurred in 1 or more tissues from 19/24 treated rats. Staining was most commonly found in the kidney, with reaction product demonstrable in proximal convoluted tubules where it was concentrated at apical margins (Fig. 2). In the 2 treated rats that had reaction product in the brain, staining was restricted to either nucleus cochlearis dorsalis or the hippocampal dentate gyrus (Fig. 3). The livers of treated rats contained concentrations of selenium comparable to those in the kidneys and were unstained by autometallography. No detectable staining was found in myocardium.

This study indicates that a poor correlation exists between selenium concentration in various organs and the intensity of autometallographic staining. The highest concentrations of selenium were found in liver and kidney, and considerably lower concentrations occurred in heart and brain, as anticipated from earlier studies.[12,13] No autometallographic staining was detected in any organ from the control rats. Moderate to intense staining was most consistently found in the kidneys of treated rats, probably because a primary route of selenium excretion in monogastric animals is via the urine. The ab-

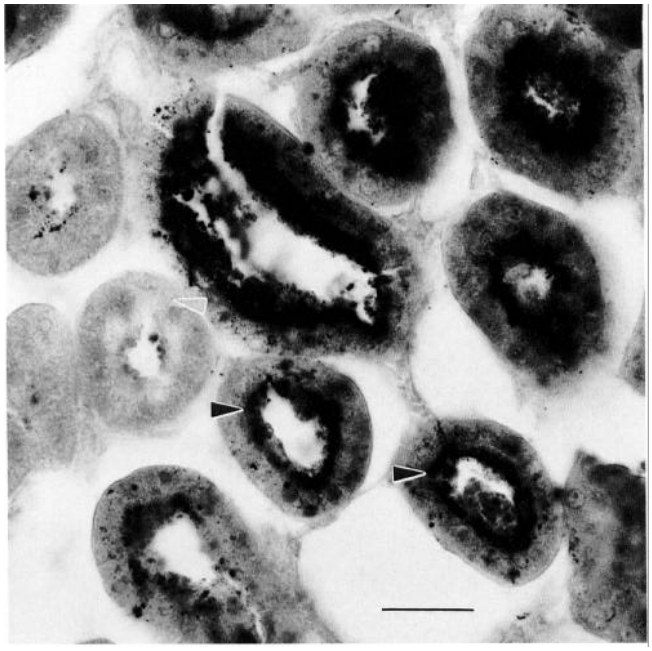

Figure 2. Kidney; rat. Note intense autometallographic staining of apical portion of epithelium in proximal convoluted tubules (arrowheads). The rat (no. 8, Table 1) was fed 9 ppm selenium as selenocystine for 28 days; selenium concentration in the kidney was 12.3 μg/g. Danscher-TB. Bar = 25 μm.

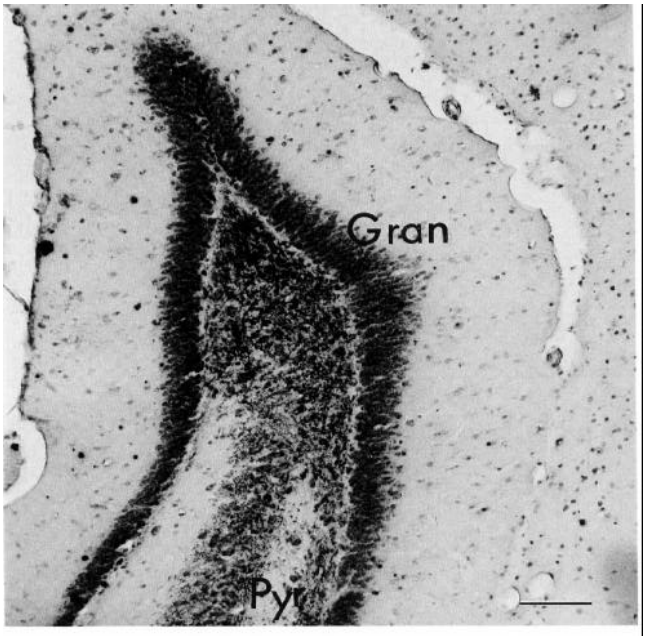

Figure 3. Hippocampus; rat. Note moderate autometallographic staining of hippocampal dentate gyrus between stratum granulosum (gran) and the pyramidal neuronal layer (pyr). This rat (no. 6, Table 1) was fed 7 ppm selenium as selenocystine for 28 days; selenium concentration in cerebrum was 0.15 μg/g. Danscher-TB. Bar = 100 μm.

sence of detectable staining in hepatic tissues with concentrations of selenium that were comparable to those in the kidney (up to 9 μg/g) and the presence of staining in brains of 2 treated rats with low (10.2 μg/g) levels of brain selenium indicate that successful staining is affected by factors other than selenium concentration. Two recent experimental studies of yearling calves and mice fed selenium in several chemical forms for various periods (cattle: 120 days; mice: 42 days) also found a poor correlation between autometallographic staining intensity and the concentration of selenium in brain, liver, kidney, and heart (D. O'Toole, M. F. Raisbeck, unpublished data).

Since autometallography for catalytic selenium bonds was introduced as a technique in 1982, the bulk of histochemical studies detailing its use concern the distribution of zinc-containing neural pathways in various mammalian, avian, and reptilian species.[3,4,11] Suggestions that positive staining was due to the formation of zinc selenide crystal lattices[2] are corroborated by the detection in the brains of healthy animals of similar cytoarchitectonic staining patterns using zinc-specific histochemical methods, such as chelation with dithizone and fluorescence with N-(6-methoxy-8-quinolyl)-para-toluene sulfonamide.[5] Localized autometallographic staining of hippocampus in the present study is consistent with the high concentration of histochemically reactive zinc (bouton zinc) at this site in normal rats.[4] Caution is required in interpreting positive staining as specific for zinc selenide because autometallographic staining also detects silver and selenides of other heavy metals such as mercury and gold.[3]

This study indicates that, unlike control rats, some tissues from rats with elevated selenium concentrations stained positively with the autometallographic technique. There was no absolute correlation of the intensity and distribution of staining with the concentration of selenium in the 4 organs examined, probably because the concurrent presence of endogenous heavy metals such as zinc is essential to generate the heavy metal selenides that catalyze precipitation of metallic silver from silver ions in Danschers' solution. Autometallography should be used with caution in studies of suspected selenosis.

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Sources and manufacturers

a. Sigma Chemical Co., St. Louis, MO.
b. Purina Mills, St. Louis, MO.
c. Shandon, Pittsburgh, PA.
d, Shimadzu, Tokyo, Japan.
e. Minitab, State College, PA.

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