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Selenium Accumulation and Cytotoxicity in Teleosts Following Chronic, Environmental Exposure

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Martin Lake is a 5000 acre reservoir located in Rusk and Panola counties in east Texas. From September 1978 to May 1979, this lake was reported to receive aqueous, selenium-laden effluent from systems used to collect fly ash, scrubber sludge, and bottom ash (R.W. LOWERRE, personal communication). The absence of noteworthy detrimental physico-chemical parameters and pesticide contamination in the lake water was confirmed. Numerous studies show that selenium is accumulated to toxic or lethal levels in teleost species (NIELSEN and NIELSEN 1978, SAKURAYAMA 1960, ELLIS et al. 1937, HALTER et al. 1980, CARDWELL et al. 1976, ADAMS 1976) and that histopathological, hematological, and other characteristic changes can result from selenium exposure (ELLIS et al. 1937, HALTER et al. 1980, CARDWELL et al. 1976). In a continued effort to monitor selenium burdens in critical tissues of Martin Lake fishes and to establish biological correlates to these burdens, this study was undertaken utilizing two members of the Centrarchidae family common to Texas lakes and reservoirs. Fish of the same species were collected from reference lakes for comparison.

MATERIALS AND METHODS

On 13 July 1981, Lepomis cyanellus (green sunfish) and Lepomis microlophus (redear sunfish) were temporarily stunned by electroshocking by Texas Parks and Wildlife personnel. Four sites on Martin Lake (i.e. Sites A, B, C, and D) and one site on a reference lake 8 km upstream (i.e. Site E) were utilized for this study (Figure 1). The absence of green sunfish at Site E necessitated use of a second collection site for this work; these fish were obtained by hook and line from a very small private pond in eastern Harrison County in east Texas (i.e. Site F). Live fish were placed as a group in a polyethylene bag containing water from the collection site; medical grade oxygen was released into the bag directly over the water to maintain adequate dissolved oxygen levels. All specimens from Sites A-E were transported indoors immediately and processed within about 25 hours of collection. Fish were anesthetized with aqueous solutions of MS-222. Total length (in mm) and weight (in g) were determined for calculation of condition factors. For the majority of the fish, the caudal peduncle was severed and from one to five heparinized capillary tubes of blood were collected for blood smears and hematocrit determination. Blood smears were prepared, air dried, fixed in methanol, and stained with Giemsia stain for leukocyte differential counts. The other capillary tubes were spun 3

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min in a microhematocrit centrifuge for hematocrit determinations. The liver (a portion or the entire organ) and occasional skeletal muscle samples were placed individually in labeled, cleaned, preweighed polyethylene vials for neutron activation analysis using previously reported methods (SORENSEN 1976).

The hepatopancreas (i.e. liver and associated, disseminated exocrine pancreas), mesonephros (i.e. teleost kidnay), skeletal muscle, heart, stomach, gonads, gill arches, and spleen for most fish were placed in 10% neutral buffered formalin. These tissues were embedded in paraffin and stained with hematoxylin and eosin using standard procedures for optical microscopy.

RESULTS AND DISCUSSION

Linear regression of selenium concentration in skeletal muscle on selenium concentration in the hepatopancreas for redear sunfish from Sites D and E showed that a highly significant correlation exists between selenium concentrations in the two tissues (Figure 2). Levels of selenium in muscle ranged from 50 to 70% of those in the hepatopancreas. If selenium levels in the hepatopancreas are known, skeletal muscle levels can be computed from the equation $Y = 0.835x - 1.375$, where x is the selenium concentration of the hepatopancreas. Conversely, if selenium levels in the skeletal muscle are known, hepatopancreas values can be computed from the equation $Y = 0.996x + 2.281$, where x represents the selenium level in skeletal muscle.

These data were consistent with those previously observed for teleost tissues. Visceral organs of adult fathead minnows (Pimephales promelas) accumulated from 1.5 to 2.5 ppm selenium compared to from 0.19 to 0.45 ppm for skeletal muscle following a 96 day exposure to aqueous solutions containing 10 or 50 ppb selenium, respectively (ADAMS 1976). Rainbow trout accumulated 19.3 (± 2.4, S.E.M.) ppm in the viscera and 1.0 (± 0.1, S.E.M.) ppm in skeletal muscle when exposed 96 day to 0.31 ppm selenium in the water (ADAMS 1976). Similar results were observed in studies involving livestock. Pigs consuming sodium selenite for 3 to 9 weeks in daily rations of grain accumulated from 0 to 2 ppm selenium in leg muscle compared to from 3 to 10 ppm in the liver (MILLER and SCHOENING 1938). Hereford steers fed sodium selenite for up to 30 weeks accumulated 7.5 (± 0.9, S.E.M.) ppm in the liver and 0.4 (± 0.1, S.E.M.) ppm in muscle, compared to 1.15 (± 0.01, S.E.M.) ppm in liver and 0.09 (± 0.01, S.E.M.) ppm in muscle of control steers (MAAG et al. 1960). Horses given single acute injections of sodium selenite accumulated about 6.5 ppm in the liver compared to about 0.4 ppm in skeletal muscle of the leg (MILLER and WILLIAMS 1940). Ewes treated from 32 to 93 days at 37.5 mg selenium (as selenate)/kg accumulated 14.62 (± 2.36, S.E.M.) ppm in the liver compared to 0.62 (± 0.14, S.E.M.) ppm in skeletal muscle; those treated for similar periods to 50 mg selenium/kg accumulated 24.08 (± 1.11, S.E.M.) ppm in liver and 0.98 (± 0.22, S.E.M.) ppm in skeletal muscle tissue (GLENN et al. 1964). Sheep fed from 632 to 1922 mg selenium as selenite accumulated an average of 23.83 (± 7.45, S.E.M.) ppm in the liver and 0.86 (± 0.06, S.E.M.) ppm in muscle prior to succumbing to selenium exposure (ROSENFELD and BEATH 1945). Above reports provide substantial support for the redear sunfish hepatopancreas and

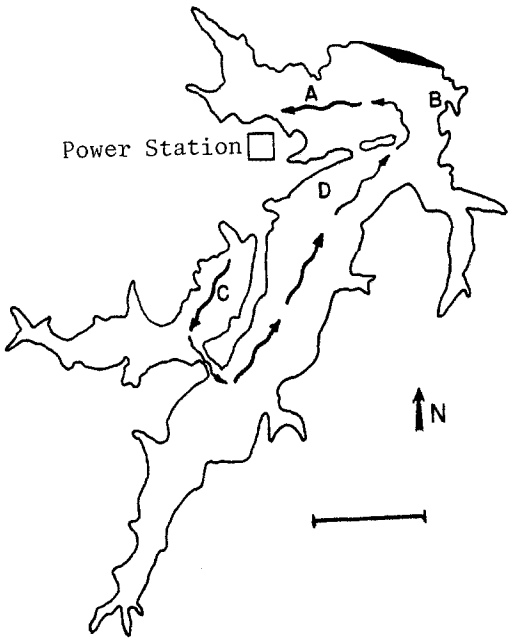

Fig. 1. Map of Martin Lake in east Texas. Collection sites (letters) and current flow (arrows) are indicated. Scale: 600 feet.

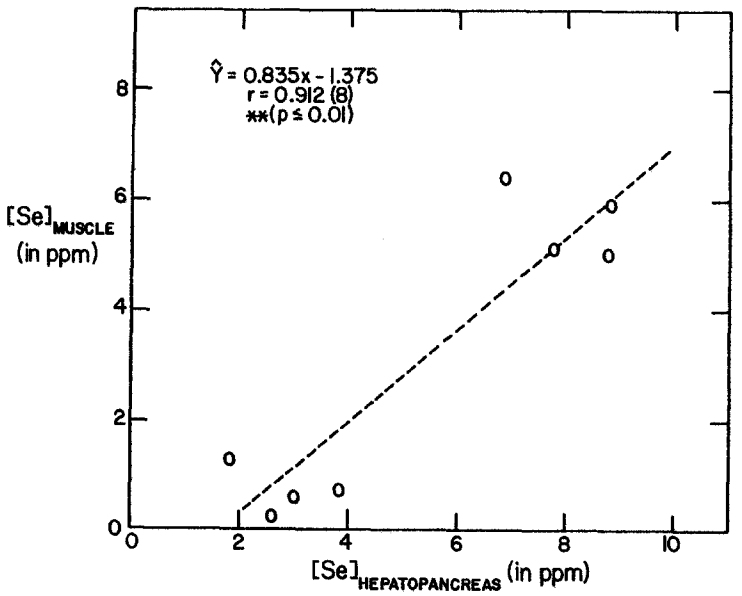

Fig. 2. Linear regression of selenium levels in skeletal muscle onto selenium levels in the liver (or hepatopancreas). The regression is significant to the 99% confidence level.

skeletal muscle data observed here, although a large number of these published studies deal with acute, rather than chronic, exposure levels. It is interesting to note that all redear sunfish muscle samples from Site D (a recognized selenium discharge site) in Martin Lake were well above the 2 ppm levels (set by the National Health and Medical Research Council) for seafoods to be consumed by humans (BEBBINGTON et al. 1977).

Selenium hepatopancreas levels, condition-factors, hematocrit values, and percent hepatopancreas-weight-to-body-weight data were compared for redear and green sunfish collected from various sites within Martin Lake and from the reference lakes (Table 1). Regardless of species, significantly more selenium was accumulated in sunfish collected from Martin Lake than either of the reference areas utilized in this study. With minor exceptions, however, redear sunfish from Martin Lake do not vary significantly from redears from the reference lake with respect to condition-factors, hematocrits, and percent hepatopancreas-weight-to-body weight (Table 1). Only redears at Site D had significantly lower ($p \leq 0.01$) hematocrits than the reference fish. Martin Lake green sunfish at Sites B and C, on the other hand, had significantly higher ($p \leq 0.05$) condition-factors than reference fish. In addition, green sunfish from Martin Lake had reduced hematocrits and percent hepatopancreas-weights-to-body-weights. Green sunfish, therefore, appeared to be edematous from both condition-factor data and organ data (i.e. percentage hepatopancreas-weight-to-body-weight). Edema and/or ascites has been previously reported for a number of studies involving selenium exposure of various teleost species (ELLIS et al. 1937, HALTER et al. 1980, NIIMI and LAHAM 1975), as well as laboratory animals (FRANKE 1934, HALVERSON et al. 1966) and livestock (SMITH et al. 1937, ROSENFELD and BEATH 1947). Redear sunfish exposed to selenium did not show a similar edematous condition.

Histopathological examination of hematoxylin-and-eosin-stained, paraffin-embedded tissues from L. microlophus showed a number of interesting features. Hyperlobulation of the hepatopancreas probably represented a developmental difference unique for this particular population of sunfish. Exocrine pancreas was observed to occupy a greated percentage of the hepatopancreas than normal for the species, although considerable variation existed from section to section; moreover, parasites were common in the hepatopancreas and in other organs examined. Another notable change was hypoxic vacuolation in proximity to central veins (Figure 3b); this condition was absent in reference redear sunfish (Figure 3a). Hypoxic vacuolation could represent selenium-induced, increased capillary permeability resulting in reduced blood volume and hypoxia-induced vacuolar degeneration in those parenchymal cells which are last to receive oxygen supplies in the organ. ELLIS et al. (1937) reported both degeneration of liver cells around central veins and parenchymal cell disruption in Ictalurus punctatus given single 3 mg/kg doses of sodium selenite intraperitoneally.

Proliferative glomerulonephritis in the mesonephros was observed as previously reported (SORENSEN et al. 1982); in this condition marked mesangial cell proliferation and increased matrix production resulted in marked cellularity of the glomerulous compared to that of reference redear sunfish. Mesangial cell proliferation following expo-

Table 1. Comparison of various parameters measured on Martin Lake Lepomis microlophus (R: redears) and L. cyanellus (G: green sunfish) and reference fish of the same species. See Figure 1 for the locations of sites presented in this table. Values are presented as means (± one standard error of the mean).

Site	Species	Se_h [a]	K_{TL} [b]	Hct [c]	$\frac{\text{h wt}}{\text{body wt}}$ [d]
A	R	10.28 ± .87***	1.47 ± .04	31.5 ± 1.3	.756 ± .077
	G	6.50 ± .27***	1.45 ± .09	30.5 ± .6**	.996 ± .176*
B	R	8.38 ± .67***	1.44 ± .02	30.8 ± 1.6	.825 ± .091
	G	6.05 ± .25***	1.64 ± .08*	36.7 ± 1.5[e]	1.056 ± .027**
C	R	11.03 ± 1.43**	1.47 ± .02	32.2 ± 1.5	—
	G	6.98 ± 1.75*	1.46 ± .02*	38.0 ± 2.0[f]	—
D	R	10.03 ± 1.33**	1.44 ± .06	27.0 ± 1.0**	.664 ± .069
	G	9.30 ± 1.20***	1.47 ± .06	32.5 ± 2.2*	1.013 ± .162*
E	R	2.80 ± .43	1.49 ± .04	33.2 ± 1.4	.815 ± .049[g]
F	G	1.31 ± .17	1.36 ± .03	39.5 ± 2.0	1.729 ± .216[h]

[a]Selenium concentration in the hepatopancreas in ppm on a wet weight basis. [b]K- or condition factor. [c]Hematocrit or packed red blood cell volume. [d]Percent hepatopancreas weight to body weight. [e]Differs from the Hct of fish from Site A ($p \leq 0.05$). [f]Differs from the Hct of fish from Site A ($p \leq 0.01$). [g]Data collected from November 1980 redear sunfish. [h]Previously reported (Sorensen 1974). * $p \leq 0.05$ compared to reference lake fish of the same species. ** $p \leq 0.01$ compared to reference lake fish of the same species. *** $p \leq 0.001$ compared to reference lake fish of the same species.

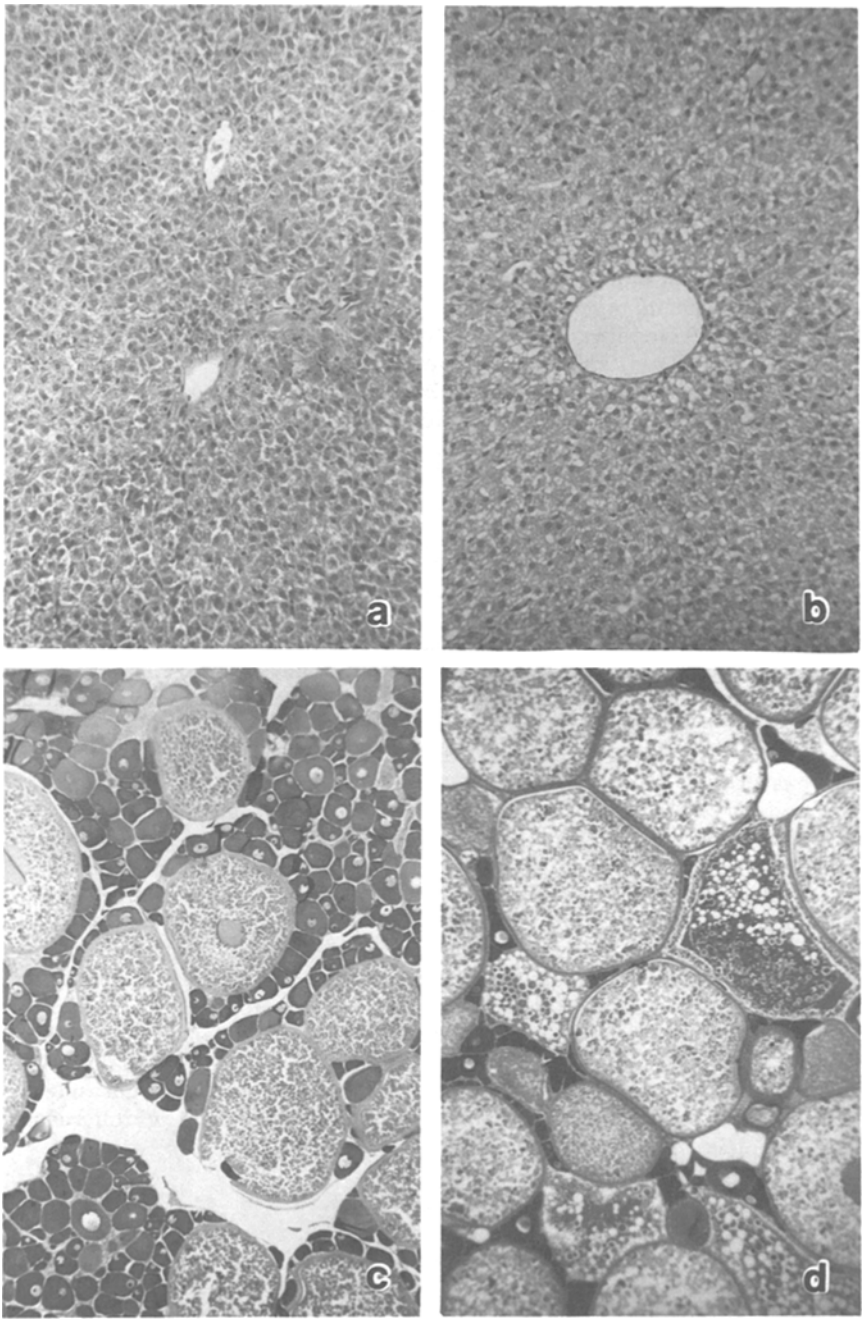

Fig. 3. a) Reference redear sunfish liver showing two normal central veins, 170X. b) Martin Lake redear sunfish liver showing hypoxic vacuolation of the central vein, 170X. c) Reference ovary with normal maturing follicles (circular profiles), 34X. d) Martin Lake ovary with abnormal numbers of degenerating follicles, 34X. Hematoxylin and eosin stain.

sure of vertebrates to various chemicals is believed to result in permeability changes within the renal corpuscle--possibly leading to the loss of blood (uremia) and/or amino acids (aminoaciduria) to the urine.

All ovaries of four female redear sunfish from Martin Lake showed marked increases in the numbers of atretic follicles (i.e. maturing follicles which degenerate in situ). The necrotic process was advanced in some follicles; the zona radiata appeared markedly indistinct, yolk vacuole coalescence and leakage from the zona radiata occurred, and the numbers of fat vacuoles appeared to be increased in some instances (Figure 3d) compared to the ovaries of reference redear sunfish (Figure 3c). Testicular abnormalities were not observed, nor were pronounced changes noted for the heart, gill, stomach, or spleen of Martin Lake redear sunfish. Gonadal tissue from over thirty-six redear and green sunfish was examined.

Green sunfish from Martin Lake, likewise, showed no pronounced abnormalities in the testes, stomach, and spleen. Changes in the hepatopancreas, mesonephros, and ovaries were similar for both redear and green sunfish. Striking changes occurred, however, in myocardium and gill lamellae (i.e. the actual site of gaseous exchange). Lamellae of reference green sunfish appeared somewhat "spiny" and vacuoles were rarely observed; gill lamellae of Martin Lake green sunfish, however, were markedly vacuolated and three to six times thicker than those of reference fish (Fig. 4a, b). These changes were similar to those observed in green sunfish from another selenium--contaminated lake (data unpublished). Such lamellar changes might reflect an adaptation towards reduced ion or water influx into the gill vesculature and/or an edematous reaction.

The ventricle of the heart of reference green sunfish collected from east Texas was similar to that of other vertebrates; a pericardial sac lined by a thin serous membrane was present and few cells were located within the pericardial sac (Fig. 4c). Martin Lake green sunfish, however, exhibited a condition noted in another selenium-contaminated lake, that is, myocarditis and epicarditis in which marked increases in the numbers of inflammatory cells were observed between myocardial cells and within the pericardial space, respectively (Fig. 4d). These changes may have occurred through a number of possible mechanisms, for example a) a direct or indirect action of selenium exposure akin to cobalt cardiomyopathy in humans resulting from consumption of beer containing cobalt sulfate added as a foam stabilizer (BRAUNWALD 1980, MCKINNEY 1974) or b) a direct action of selenium on the mesonephros (i.e. glomerulonephritis) leading to uremia which results in myocarditis and epicarditis.

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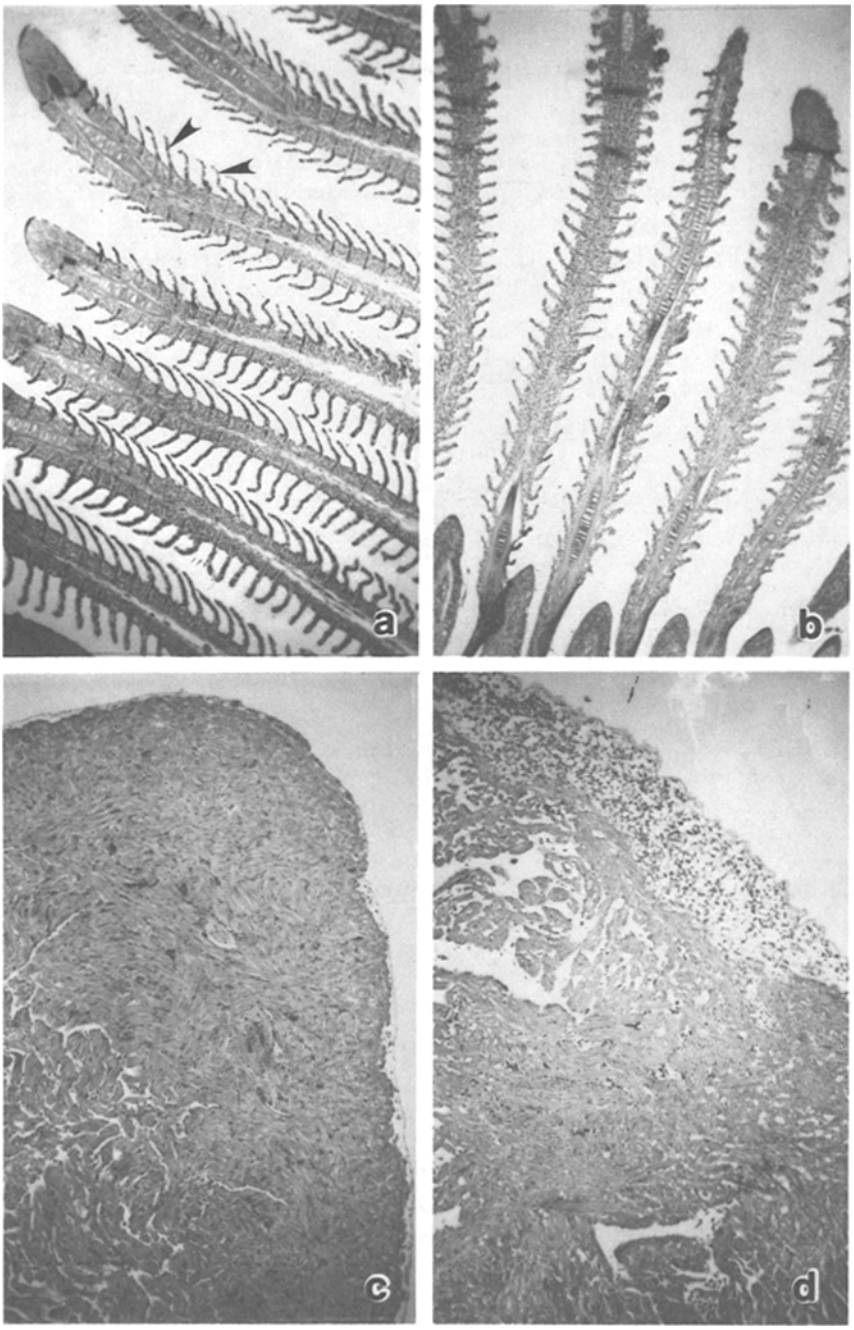

Fig. 4. a) Reference green sunfish gill filaments with normal lamellae (arrows). b) Martin Lake green sunfish gill filaments showing proliferation of lamellar cells. c) Apex of the heart of a reference green sunfish with a normal pericardial sac. d) Pericardial sac filled with inflammatory cells from a green sunfish collected from Martin Lake, Hematoxylin and eosin, 100X.

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