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Subcellular distribution of mercury in liver of lake trout *(Salvelinus namaycush)*[1]

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Summary. Mercury was found primarily (80%) in the submicroscopic material (magnification $\times < 7700$) of environmentally exposed adult lake trout from Lake Michigan, USA.

Determination of subcellular distribution of contaminants in fish holds promise as a means of predicting metabolic pathways most likely affected by toxicants and of predicting possible modes of detoxification. Heavy metals have been reported to be sequestered in the cytosol of fish liver as protein-bound metallothioneins. Studies thus far have involved short-term laboratory exposure[3,4]. The objective of our work was to determine the subcellular distribution of mercury in liver of environmentally exposed lake trout, *Salvelinus namaycush* (Walbaum), from Lake Michigan (USA). Lake trout contain high levels of common Great Lakes contaminants[5].

Two adult lake trouts were captured by gill net in Lake Michigan near Saugatuck, Michigan (42°41′N, 86°18′W). A male (2640 g, 643 mm, 5 years old) collected in October 1975 was killed and the liver was immediately removed, frozen, and stored at −20 °C. A female (4400 g, 732 mm, 7 years old) collected in September 1977 was transported alive to the laboratory, where it was killed by a blow to the head and the fresh liver sampled. Subcellular fractions of liver were prepared by differential centrifugation in sucrose as described by Hogeboom[6] except that frozen liver was homogenized in room-temperature sucrose to quick-thaw the tissue[7]. Subcellular fractions for electron microscopy were preserved in 2% phosphate buffered glutaraldehyde and fractions for contaminant analysis were frozen and stored at −20 °C. Electron microscopy preparation[8] and examination of fractions were performed by Clinical Laboratories, Veterans Administration Medical Center, Ann Arbor. They determined the average percent volume of each cell organelle in each fraction using 100 points in 10–15 electron micrographs of each fraction[9]. We measured total mercury (inorganic and organic) of subcellular fractions and whole fresh liver by a combustion-amalgamation technique[10].

Of the total mercury in fresh liver, 69% was present in the soluble fraction (table 1). The value of 1.2 µg total mercury/g (table 1) was confirmed by analysis of samples of whole liver. In frozen liver, 58% of the mercury was in the soluble fraction, 19% in the nuclear fraction, and 14% in the microsomal fraction; however, data from frozen liver were not used for further calculations because electron microscopy revealed that the mitochondria were ruptured. Of the polychlorinated biphenyls and *p,p′*DDE (saponified *p,p′*DDT plus *p,p′*DDE), 96 to 97% were in the soluble fraction of this frozen lake trout liver, but these data should be considered preliminary since we have no data for organochlorine distribution in fresh liver.

We calculated the proportion of mercury in each cell organelle in fresh liver by multiplying the proportion of mercury in each fraction (table 1) by the proportion of each organelle in the same fraction (table 2) and summing across all fractions for a particular organelle (table 3). The SE of the proportion was calculated according to Cochran[11]. These calculations assume that the distribution of mercury is proportional to the volume representation of the different organelles. The highest proportion of mercury (0.799) was in the submicroscopic material, i.e., material not visible at magnification × 7700.

Our determination of subcellular distribution of mercury in liver is the only study of fish exposed environmentally for several years – assuredly long enough for the distribution of the metal in tissues and subcellular organelles to reach equilibrium. After exposure of rainbow trout *(Salmo gairdneri)* for 24 h to methyl mercury chloride in water, Olson et al.[4] reported that mercury was highest in the cytosol

Table 1. Total mercury (wet wt) present in subcellular fractions of fresh liver of Lake Michigan lake trout

Subcellular fraction	Total mercury µg/g[a]	Proportion
Nuclear fraction	0.110	0.0917
Heavy mitochondria	0.064	0.0534
Light mitochondria	0.052	0.0434
Microsomal fraction	0.149	0.1243
Soluble fraction	0.824	0.6872
Total	1.199	1.0000

[a]Calculated as µg/g of whole liver (wet wt).

Table 2. Proportions of organelles in subcellular fractions of fresh liver of Lake Michigan lake trout

Organelle	Subcellular fraction				
	Nuclear	Mitochondrial		Microsomal[a]	Soluble
		Heavy	Light		
Nucleus	0.271				
Mitochondria	0.019	0.422	0.652	0.022	
Lipid droplets	0.052	0.075	0.078	0.007	0.003
Membranes	0.027				
Rough endoplasmic reticulum		0.087	0.083	0.335	
Ribosomes				0.171	
Cytoplasmic debris	0.088				0.008
Particulate cytoplasmic debris		0.148	0.169		
Non-specific cytoplasmic debris				0.024	
Submicroscopic material	0.543	0.269	0.018	0.441	0.989

[a]The proportions of organelles in the microsomal fraction were calculated on an area basis since the few mitochondria present were swollen and their volume was disproportionately large.

Table 3. Calculated proportion of mercury by volume in subcellular organelles of fresh liver of Lake Michigan lake trout

Organelle	Proportion of mercury (p ± SE)
Nuclei	0.025 ± 0.0012
Mitochondria	0.055 ± 0.0012
Lipid droplets	0.015 ± 0.0014
Membranes	0.002 ± 0.0004
Rough endoplasmic reticulum	0.050 ± 0.0018
Ribosomes	0.021 ± 0.0014
Cytoplasmic debris	0.014 ± 0.0019
Particulate cytoplasmic debris	0.015 ± 0.0007
Nonspecific cytoplasmic debris	0.003 ± 0.0006
Submicroscopic material	0.799 ± 0.0031

fractions and made up 50–80% of the total tissue mercury. They identified a metallothionein-like protein in the liver cytosol that bound about 40% of the mercury. Marafante[3] also reported the presence of a cytosol protein that bound mercury in liver of goldfish *(Carassius auratus)*. These metallothionein-like proteins, first discovered in horse kidney by Margoshes and Vallee[12], serve as a protective mechanism for animals[13]. Hence, we suggest that lake trout are partly protected from the toxic effects of mercury by the binding of the metal to a metallothionein-like protein in the submicroscopic material of the liver.

1 Contribution 582 of the Great Lakes Fishery Laboratory, U.S. Fish and Wildlife Service, Ann Arbor, Michigan 48105. We thank Dr T.F. Beals (Veterans Administration Medical Center, Ann Arbor) for helpful discussions of electron micrographs, P.M. Haack for statistical analysis, and W.A. Willford for critical review of the manuscript.
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Rat sternothyroid muscle: Dissection and preparation for electrophysiologic and electronmicrographic studies[1]

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Summary. The sternothyroid muscle of the rat is described. The ease of dissection and localization of end-plate regions within the sternothyroid muscle by direct visualization of nerve supply provides a convenient mammalian muscle preparation for electrophysiological and ultrastructural research.

In a recent publication, Dreyer et al.[5] characterized the omohyoideus muscle of the mouse as a convenient mammalian muscle preparation for use with Nomarski interference optics in electrophysiological investigations of the neuromuscular junction. Because of its insertion onto the scapula, we have found dissection of the omohyoideus muscle cumbersome. As a result, an alternative preparation, the rat sternothyroid muscle, has been evaluated.

Materials and methods. More than 50 female Lewis rats weighing 150–220 g were studied. The dissection consisted of reflecting the sternal heads of the sternomastoid muscles from the sternum, thereby freeing the sternohyoid muscles from the sternum; dissecting the sternohyoid muscles free of each other in the midline; bisecting the trachea transversely; splitting the larynx and proximal trachea in the midline; and finally dissecting the sternohyoid muscle free