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SUBCHRONIC EFFECTS OF METHYLMERCURY ON PLASMA AND ORGAN BIOCHEMISTRIES IN GREAT EGRET NESTLINGS

DAVID J. HOFFMAN,*† MARILYN G. SPALDING,‡ and PETER C. FREDERICK§
†U.S. Geological Survey, Patuxent Wildlife Research Center, Beltsville Laboratory, BARC-E, Building 308, 10300 Baltimore Avenue, Beltsville, Maryland 20705
‡Department of Pathobiology, College of Veterinary Medicine, §Department of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida, 32611-0430, USA

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Abstract—In recent years, high concentrations of mercury have been found in wading birds in Florida, USA. Great egret (*Ardea alba*) chicks (2 weeks old) were dosed orally daily with the equivalent of 0, 0.5, or 5 μg/g Hg as methylmercury chloride in the diet for up to 12 weeks. Weakness of the legs or paralysis occurred in all high-dosed birds. Geometric mean blood Hg concentrations were 0.17, 10.3, and 78.5 μg/g (wet wt), respectively. Mercury concentrations for organs (μg/g wet wt), including brain (0.22, 3.4, and 35, respectively), liver (0.34, 15.1, 138, respectively), and kidney (0.28, 8.1, and 120, respectively), increased in a dose-dependent manner. Total glutathione (GSH) peroxidase activity was significantly lower in the plasma, brain, liver, and kidney of the high-dosed group. Plasma aspartate aminotransferase activity increased with mercury treatment, whereas lactate dehydrogenase activity decreased. Four other plasma chemistries were decreased significantly in the high-dosed group and included uric acid, total protein, albumin, and inorganic phosphorus. Lipid peroxidation increased in liver (low and high dose) and brain (high dose). Tissue changes in concentrations of reduced thiols included decreased total thiols and protein-bound thiols in liver, decreased protein-bound thiols in kidney, and increased GSH in kidney and brain. Activities of GSH *S*-transferase and oxidized glutathione reductase increased in liver. In kidney, GSH *S*-transferase and glucose-6-phosphate dehydrogenase activities increased with mercury dose. These findings, including apparent compensatory changes, are compared to other Hg studies where oxidative stress was reported in egrets, herons, and diving ducks in the field and mallards in the laboratory.

Keywords—Great egret Mercury Chicks Subchronic Oxidative stress

INTRODUCTION

Methylmercury has been well-documented as a contaminant that bioaccumulates in aquatic ecosystems and has been linked to adverse effects in top carnivores [1–10]. Methylmercury has been suggested as one of the possible causes for reduced reproduction of long-legged wading birds (Ciconiiformes) in the Florida Everglades, USA, in recent decades [11–16].

Knowledge of the effects of mercury on aquatic birds, including wading birds, is limited. Sublethal effects of methylmercury in controlled studies with mallards (*Anas platyrhynchos*) included lipid peroxidation of tissues and decreased activities of the enzymes' total glutathione (GSH) peroxidase (plasma and liver) and glucose-6-phosphate dehydrogenase ([G-6-PDH]; liver and brain), with further oxidative stress characterized as increased oxidized GSH relative to reduced GSH and loss of thiols [17]. Great egrets were selected for the present study because they feed on fish most prone to mercury contamination and nest colonially in large numbers with relatively constant site conditions. In addition, a recent report has shown GSH metabolism to be affected by mercury in snowy egrets (*Egretta thula*), black-crowned night-herons (*Nycticorax nycticorax*), and double-crested cormorants (*Phalacrocorax auritus*) situated in mercury-contaminated sites along the lower Carson River of Nevada, USA [18].

In the present study, captive great egret nestlings were raised from hatching to 14 weeks old, well after the time that they normally would be independent in the wild (about 9–10 weeks old, [19]), as either controls, or on diets containing 0.5 or 5 μg/g wet weight of methylmercury. Several aspects of this study already have been reported, including effects on survival, growth, and tissue accumulation [20]; histologic, neurologic, and immune function [21]; and behavior [22]. The present report describes the findings with respect to effects on plasma and organ biochemistries, many of which are related to GSH metabolism and oxidative stress.

MATERIALS AND METHODS

Birds and treatments

The first-hatched great egret nestlings were collected on March 16, 1996, from 23 different broods in Alley North colony (26°11.25′N, 80°31.05′W) in Water Conservation Area 3 of the central Everglades. Some young were collected as pipped eggs, which took several days to hatch, and others were as old as 5 d. The range in ages was 7 d. The birds were transported to the Florida Field Station of the National Wildlife Research Center (U.S. Department of Agriculture, Gainesville, FL, USA), where they were housed for the rest of the experiment. A more detailed description of the dosing and housing methods is given in Spalding et al. [20]. Briefly, birds were housed individually indoors in plastic boxes and moved outdoors at five weeks. During the last third of the experiment, a shallow, flooded, plastic wading pool was placed in each cage and birds were allowed to catch live fish. All birds received a diet of about 90% thawed Atlantic silversides (*Menidia menidia*) and 10% capelin (*Mallotus villosus*). Food was provided on a modified ad libitum basis, as explained in Spal-

* To whom correspondence may be addressed (david_hoffman@usgs.gov).

ding et al. [20], and dosing was based on daily food offered. Treatment groups included control (6 birds), a low dose (8 birds) of 0.5 μg/g of methylmercury chloride in fish similar to what great egret nestlings in the Everglades currently eat [15], and a high dose (8 birds) of 5 μg/g. Gelatin capsules with the appropriate dose were made by evaporating acetone from a solution of methylmercury chloride in acetone, and these were given orally daily from 8 d of age to the end of the experiment at day 95. Control capsules were made by adding and evaporating acetone only. Average Hg concentration of the control diet was 0.025 μg/g, and Se concentration was 1 μg/g on a wet weight basis. Methylmercury chloride consumed varied with amount of food consumed and ranged from a high of 0.135 μg/g/d during week 3 to a low of 0.048 μg/g/d during week 13 in the low-dose group. The high-dose group initially received 0.5 μg/g of methylmercury chloride in fish for the first four weeks of dosing, but the dose was increased to 5 μg/g for the subsequent duration in order to produce clinical toxicity. Assignment to mercury dose groups was blind to researchers working on the experiment. Blood collection volumes ranged from 0.1 to 0.3% of body weight and varied depending upon the tests run (1.6 ml for weeks 3 and 5 of dosing, 2.6 ml for week 7, and 10 ml just before euthanasia for all birds). Birds in the high-dose group were euthanized by injection of sodium pentobarbital when they could no longer stand following eight weeks of dosing (1 individual), nine weeks (2), and 10 weeks (3), and all remaining birds at the end of the experiment (12 weeks of dosing). Tissues for biochemical assays were immediately frozen and stored at −80°C until assayed.

Measurements of plasma chemistries

Many of the biochemical measurements chosen have been used to indicate mercury toxicity in birds or are known to reflect organ damage and related physiological disturbances. Basic methods and assay conditions are described by Hoffman and Heinz [17]. Indicator assays of potential mercury-related effects are in the following paragraph.

Plasma enzyme activities were measured at weeks 5, 7, 9, and 14 of age (equivalent to weeks 3, 5, 7, and 12 of dosing) on a centrifugal analyzer (Centrifichem 500; Baker Instrument, Allentown, PA, USA) and included glutathione peroxidase (GSH-Px; Enzyme Commission [EC] number 1.11.1.9; coupled reaction at 30°C with GSH reductase using cumene hydroperoxide), oxidized glutathione reductase (GSSG-Red; EC 1.6.4.2), alanine aminotransferase (EC 2.6.1.2), aspartate aminotransferase (EC 2.6.1.1), creatine phosphokinase (EC 2.7.3.2), and lactate dehydrogenase-L (EC 1.1.1.27). Three of these enzymes have been linked to hepatotoxicity in birds (alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase-L), and creatine phosphokinase to neural and muscle tissue alterations. Other plasma constituents measured included albumin, total protein, glucose, uric acid, creatinine, cholesterol, triglycerides, calcium, and inorganic phosphorus [23].

Liver, kidney, and brain biochemistries

Portions of the liver, kidney, and brain were homogenized (1:10 weight/volume [w/v]) in ice-cold 1.15% KCl-0.01 M Na, K-phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 *g* for 20 min at 4°C, and the resultant supernatant was used for assays of enzymes related to glutathione metabolism and antioxidant activity, previously shown to be affected by mercury exposure in mallards [17]. Liver GSH-Px (EC 1.11.1.9; coupled reaction at 30°C with GSSG-Red using cumene hydroperoxide) and GSSG-Red (EC 1.6.4.2) activities were recorded spectrophotometrically by micromethods using a centrifugal analyzer as described by Jaskot et al. [24]. Glutathione-*S*-transferase (GSH-Trans; EC 2.5.1.18) activity was measured using 1-chloro-2,4-dinitrobenzene as the substrate [25] and glucose-6-phosphate dehydrogenase (G-G-PDH; EC 1.1.1.49) activity according to the method of Lohr and Waller [26] using the centrifugal analyzer. Additionally, acetylcholinesterase and creatine phosphokinase in brain tissue were recorded. Reduced GSH as nonprotein sulfhydryl and total hepatic sulfhydryl concentrations (TSH) were measured according to Sedlak and Lindsay [27]. Oxidized glutathione was determined by the method of Tietze [28] as modified by Griffith [29] for GSSG using vinyl pyridine. Protein-bound sulfhydryl (PBSH) concentration was calculated as the difference between TSH and GSH concentrations. Thiobarbituric acid reactive substances (TBARS) were measured as an estimate of hepatic lipid peroxidation using the method described by Aust [30]. Standard curves were generated for the assay using malondialdehyde tetraethyl acetal. Crude homogenate and 10,000 *g* supernatant protein concentrations were determined according to the method of Lowry et al. [31], using bovine serum albumin as a standard.

Tissues were analyzed for total mercury concentrations using cold vapor atomic absorption spectroscopy as described by Spalding et al. [20].

Statistical analysis

All plasma and organ clinical and biochemistry measurements were compared among treatment groups using analysis of variance ($p \leq 0.05$). Where homogeneity of variance was lacking, log transformation of data was conducted before analysis. Significant differences from mercury-treated groups and the control group were quantified with Dunnett's multiple comparison test ($p \leq 0.05$). Regression analysis was used to define relationships between residues in tissues and between biochemical measurements.

RESULTS

Birds in the high-dose group exhibited weakness of the legs or paralysis and were euthanized when they could no longer stand following eight (1 individual), nine (2), and 10 (3) weeks of dosing and all remaining birds at the end of the experiment (12 weeks of dosing). Therefore, plasma chemistries are presented for all groups at nine weeks of age, following seven weeks of dosing, and before any euthanization.

Plasma chemistries

After seven weeks of dosing, geometric mean blood concentrations of mercury were 0.17, 10.3, and 78.5 μg/g (wet wt), respectively, for the control, low-dose, and high-dose groups (Table 1), as reported by Spalding et al. [20]. Significant differences in activities of three plasma enzymes were apparent, including GSH-Px, aspartate aminotransferase, and lactate dehydrogenase-L in mercury-treated groups relative to controls (Fig. 1). The GSH-Px activity in the high-dose mercury group was less than 25% of that for the control group and was lower numerically but not significantly different in the low-dose mercury group. A significant negative correlation was found between blood mercury concentration and GSH-Px activity ($r = -0.864$, $p < 0.01$). The aspartate aminotransferase

Table 1. Tissue mercury concentrations in great egret chicks after seven weeks of dosing for blood and 10 weeks for organs[a]

	Control	Low dose	High dose
Blood (μg/g)	0.17 [0.10–0.21][b]	10 [5.6–26]	79 [38–102]
Brain (μg/g)	0.22 [0.20–0.29]	3.4 [2.8–4.3]	35 [30–41]
Liver (μg/g)	0.34 [0.16–0.52]	15 [11–20]	138 (121–160]
Kidney (μg/g)	0.28 [0.12–0.35]	8.1 [5.5–13]	120 [99–140]

[a] Data was derived from Spalding et al. [20].
[b] Values expressed as geometric mean, wet weight [range].

activity was over 1.4-fold higher in the low-dosed mercury group than the control group. In contrast, lactate dehydrogenase-L activity in the high-dose group was less than 50% of that for the control group and appeared lower in the low-dosed group. Four other plasma chemistries were affected significantly by mercury treatment and included uric acid, total protein, albumin, and inorganic phosphorus (Fig. 2). Concentrations for all of these in the high-dose group were less than 75% of those for controls. Calcium appeared lower but did not differ significantly due to variability.

Brain biochemistry

Geometric mean brain Hg concentrations were 0.22, 3.4, and 35 μg/g (wet wt), respectively, in the control, low-dose, and high-dose groups (Table 1). Brain GSH-Px activity in the high-dose mercury group was less than 80% of that for the control group (Fig. 3). Brain acetylcholinesterase appeared higher numerically in the high-dose group, but not significantly so. A significant negative correlation occurred between brain mercury concentration and GSH-Px activity ($r = -0.788$, $p < 0.01$), but positive between brain mercury and acetylcholinesterase activity ($r = 0.502$, $p < 0.05$).

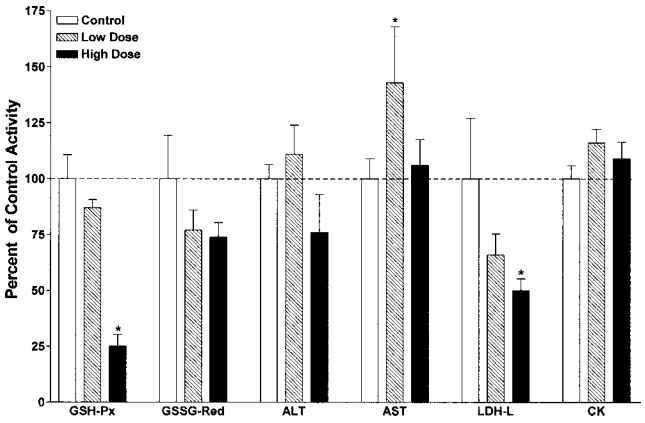

Fig. 1. Effects of methylmercury on plasma enzyme activities of nine-week-old great egret chicks following seven weeks of dosing. Activities are expressed as percent of the control value. Values are means with standard deviation and $n = 6$ to 8 per group. Control values for these enzymes are expressed as international units per L (IU/L): GSH-Px, 13,342 ± 3,186; GSSG-Red, 66.4 ± 28.3; ALT, 59.2 ± 8.2; AST, 177 ± 33; LDH-L, 498 ± 330; and CK, 1,549 ± 198. Bars marked with an asterisk were significantly different ($p < 0.05$) from controls. Abbreviated terms are defined as GSH-Px = glutathione peroxidase; GSSG-Red = glutathione reductase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH-L = lactate dehydrogenase-L; CK = creatine phosphokinase.

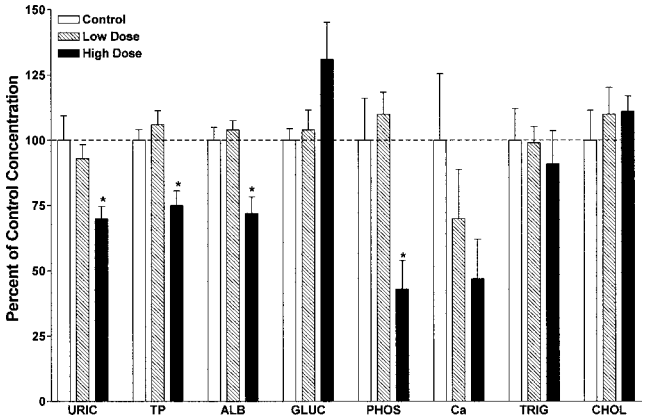

Fig. 2. Effects of methylmercury on nonenzyme plasma chemistries of nine-week-old great egret chicks following seven weeks of dosing. Chemistries are expressed as percent of the control value. Values are means with standard deviation and $n = 6$ to 8 per group. Control values for these chemistries are expressed as mg/dl: URIC, 7.7 ± 1.6; ALB, 13.6 ± 1.5; GLUC, 251 ± 24; PHOS, 9.8 ± 3.5; Ca, 4.6 ± 2.6; TRIG, 33.1 ± 6.9; and CHOL, 181 ± 36; but as g/dl for total protein (TP), 3.1 ± 0.3. Bars marked with an asterisk were significantly different ($p < 0.05$) from controls. Abbreviated terms are defined as URIC = uric acid; ALB = albumin; GLUC = glucose; PHOS = inorganic phosphorus; TRIG =triglycerides; CHOL = cholesterol.

Brain GSH concentration was nearly 1.3-fold higher in the high-dose group, and TBARS exhibited a small but significant increase in this group (Fig. 4). Positive correlations occurred between brain mercury and TBARS ($r = 0.648$, $p < 0.01$) and GSH ($r = 0.710$, $p < 0.01$).

Liver biochemistry

Geometric mean liver Hg concentrations were 0.34, 15.1, and 138 μg/g (wet wt), respectively, for the control, low-dose, and high-dose groups (Table 1). Three different liver enzyme activities were affected significantly by mercury treatment and included GSH-Px, GSSG-Red, and GSH-Trans (Fig. 5). Ac-

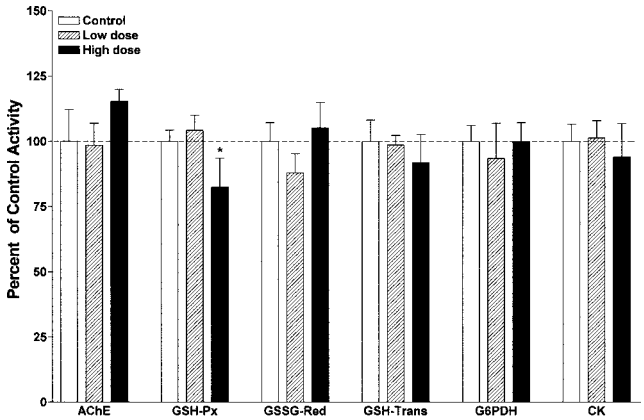

Fig. 3. Effects of methylmercury on brain enzyme activities of 12-week-old great egret chicks following 10 weeks of dosing. Activities are expressed as percent of the control value. Values are means with standard deviation and $n = 6$ to 8 per group. Control values for these enzymes are expressed as nmol/min/mg of 10,000 *g* supernatant protein: GSH-Px, 77 ± 8.7; GSSG-Red, 19.1 ± 3.6; GSH-Trans, 301 ± 65; G-6-PDH, 9.4 ± 1.5; and CK, 5.9 ± 1.0; but as nmol/min/mg for AChE, 59 ± 19. Bars marked with an asterisk were significantly different ($p < 0.05$) from controls. Abbreviated terms are defined in Figure 1 legend. GSH-Trans = glutathione-*S*-transferase; G-6-PDH = glucose-6 phosphate dehydrogenase; AChE = acetylcholinesterase.

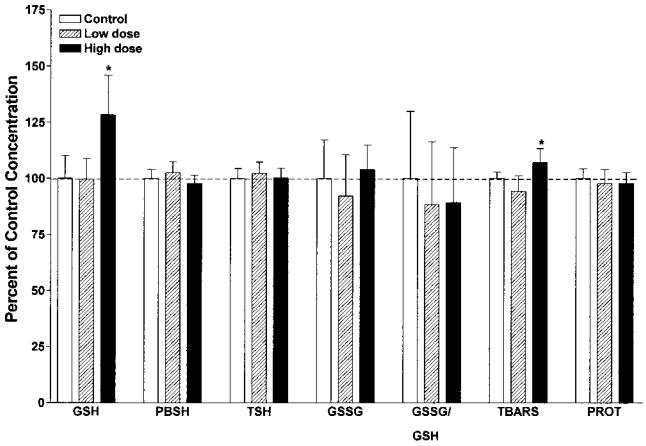

Fig. 4. Effects of methylmercury on nonenzyme brain chemistries of 12-week-old great egret chicks following 10 weeks of dosing. Chemistries are expressed as percent of the control value. Values are means with standard deviation and $n = 6$ to 8 per group. Control values for these chemistries are expressed as μmol/g for GSH, 0.78 ± 0.21; PBSH, 8.4 ± 0.9; TSH, 9.2 ± 1.1; GSSG, 0.077 ± 0.035; as a ratio for GSSG/GSH, 0.10 ± 0.07; as nmol/g for TBARS, 19.5 ± 1.5; and as mg/g for PROT, 99 ± 11. Bars marked with an asterisk were significantly different ($p < 0.05$) from controls. Abbreviated terms are defined as GSH = reduced glutathione; PBSH = protein-bound thiols; TSH = total thiols; GSSG = oxidized glutathione; GSSG/GSH = the ratio of oxidized to reduced glutathione; TBARS = thiobarbituric acid–reactive substances; PROT = protein.

tivity of GSH-Px in the high-dose group was less than 75% of that for the control group. The GSSG-Red activity was approximately 1.25-fold greater in the high-dose group relative to the control group, and GSH-Trans activity was nearly 1.15-fold greater in the high-dose group. A significant negative correlation occurred between liver mercury concentration and total GSH-Px activity ($r = -0.869$, $p < 0.01$), but positive between liver mercury and GSH-Trans activity ($r = 0.559$, $p < 0.01$).

Three other liver biochemistries were altered significantly by high mercury treatment and included PBSH, TSH, and TBARS (Fig. 6). Both PBSH and TSH concentrations were

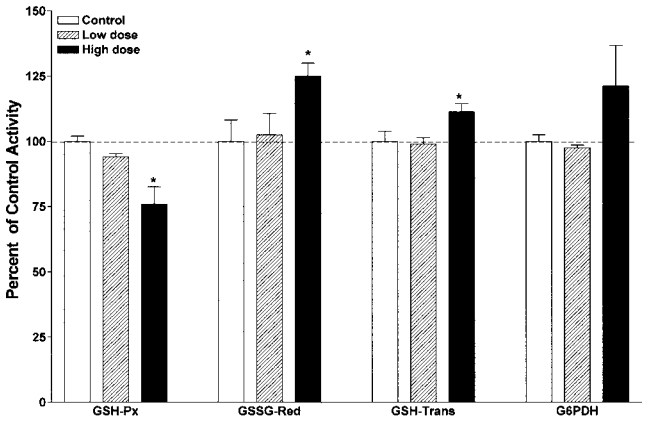

Fig. 5. Effects of methylmercury on liver enzyme activities of 12-week-old great egret chicks following 10 weeks of dosing. Activities are expressed as percent of the control value. Values are means with standard deviation and $n = 6$ to 8 per group. Control values for these enzymes expressed as nmol/min/mg of 10,000 *g* supernatant protein were: GSH-Px, 404 ± 26; GSSG-Red, 39.7 ± 8.5; GSH-Trans, 289 ± 30; and G-6-PDH, 6.1 ± 0.5. Bars marked with an asterisk were significantly different ($p < 0.05$) from controls. Abbreviated terms are defined as in Figure 1 and Figure 3 legends.

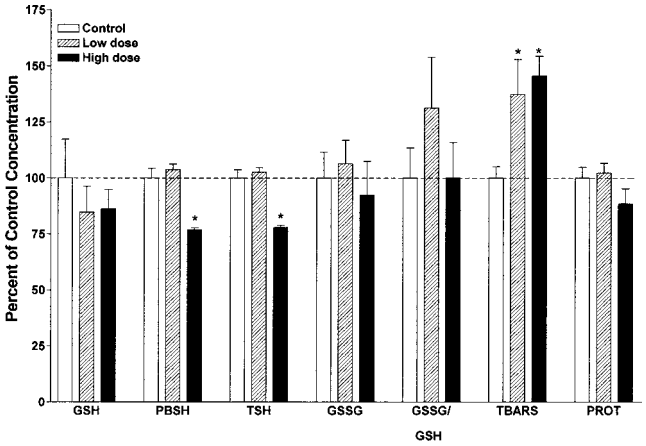

Fig. 6. Effects of methylmercury on nonenzyme liver chemistries of 12-week-old great egret chicks following 10 weeks of dosing. Chemistries are expressed as percent of the control value. Values are means with standard deviation and $n = 6$ to 8 per group. Control values for these chemistries are expressed as μmol/g for GSH, 1.52 ± 0.69; PBSH, 16.3 ± 2.0; TSH, 17.8 ± 1.7; GSSG, 0.171 ± 0.051; as a ratio for GSSG/GSH, 0.126 ± 0.045; as nmol/g for TBARS, 15.5 ± 2.1; and as mg/g for PROT, 148 ± 19. Bars marked with an asterisk were significantly different ($p < 0.05$) from controls. Abbreviated terms are as defined in Figure 4 legend.

less than 80% of those for controls. The TBARS concentrations were significantly greater (1.35- and 1.45-fold, respectively) in the low- and high-mercury groups relative to the control group. Significant negative correlations occurred between liver mercury concentration and hepatic PBSH concentration ($r = -0.739$, $p < 0.01$) and hepatic TSH ($r = -0.770$, $p < 0.01$). A positive correlation occurred between liver mercury and TBARS ($r = 0.492$, $p < 0.03$).

Kidney biochemistry

Geometric mean kidney Hg concentrations were 0.28, 8.1, and 120 μg/g (wet wt), respectively, for the control, low-dose, and high-dose groups (Table 1). Three different kidney enzyme activities were affected significantly by mercury treatment and included GSH-Px, GSH-Trans, and G-6-PDH (Fig. 7). Activity of GSH-Px in the high-dose group was less than 60% of that for the control group. The GSH-Trans activity was approximately 1.3-fold greater in the high-dose group relative to the control group, and G-6-PDH activity was nearly 1.15-fold greater in the high-dose group. A significant negative correlation occurred between kidney mercury concentration and kidney total GSH-Px activity ($r = -0.907$, $p < 0.01$). Positive correlations occurred between kidney mercury and GSH-Trans ($r = 0.850$, $p < 0.01$) and G6PDH ($r = 0.566$, $p < 0.01$).

Three other kidney biochemistries were affected significantly by mercury treatment in the high-dose group (Fig. 8). The GSH concentration was nearly 1.25-fold greater than controls, whereas PBSH was less than 75% of the control concentration, and GSSG was less than 70% of the control concentration. The ratio of GSSG to GSH was 61% of the control value. Significant negative correlations occurred between kidney mercury concentration and kidney PBSH ($r = -0.513$, $p < 0.05$), GSSG ($r = -0.931$, $p < 0.01$), and ratio of GSSG to GSH ($r = -0.857$, $p < 0.01$). Positive correlations occurred between kidney mercury and GSH ($r = 0.676$, $p < 0.01$).

DISCUSSION

Several aspects of this study already have been reported on, including effects on survival, growth, and tissue accu-

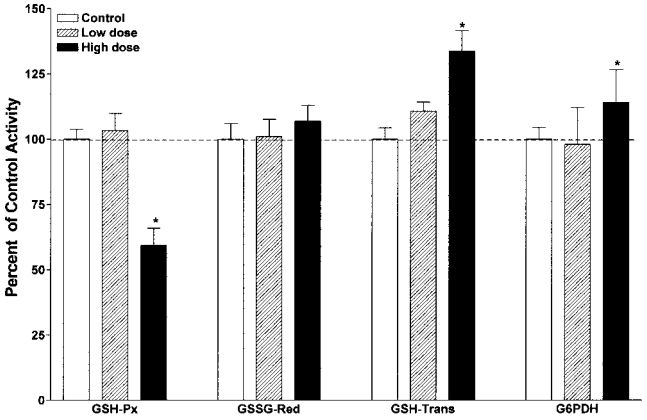

Fig. 7. Effects of methylmercury on kidney enzyme activities of 12-week-old great egret chicks following 10 weeks of dosing. Activities are expressed as percent of the control value. Values are means with standard deviation and $n = 6$ to 8 per group. Control values for these enzymes are expressed as nmol/min/mg of 10,000 *g* supernatant protein: GSH-Px, 463 ± 47; GSSG-Red, 202 ± 33; GSH-Trans, 405 ± 48; and G-6-PDH, 5.1 ± 0.6. Bars marked with an asterisk were significantly different ($p < 0.05$) from controls. Abbreviated terms are defined in Figure 1 and Figure 3 legends.

mulation [20]; histologic, neurologic, and immune function [21]; and behavior [22]. Effects related to health and immune function caused by methylmercury consisted of lower packed cell volumes, dingy feathers, increased lymphocytic cuffing in a skin test, increased bone marrow cellularity, decreased bursal wall thickness, decreased thymic lobule size, fewer lymphoid aggregates in lung, increased perivascular edema in lung, and increased phagocytized carbon in lung of the low-dosed birds [20,21]. The most severe histologic lesions were in immune and nervous system tissues where the high-dosed birds became severely ataxic. Bouton et al. [22] recorded activity levels, maintenance behavior, and foraging efficiency, and determined that mercury affected activity and maintenance behavior. During the postfledging period, there were no differences between low-dose and control birds in time required to capture live fish

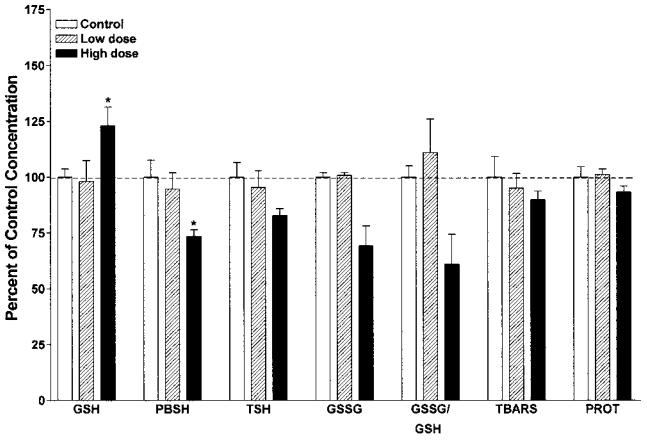

Fig. 8. Effects of methylmercury on nonenzyme kidney chemistries of 12-week-old great egret chicks following 10 weeks of dosing. Chemistries are expressed as percent of the control value. Values are means with standard deviation and $n = 6$ to 8 per group. Control values for these chemistries are expressed as: μmol/g for GSH, 3.2 ± 0.3; PBSH, 13.8 ± 2.8; TSH, 17.0 ± 3.0; and GSSG, 0.078 ± 0.003; as a ratio for GSSG/GSH, 0.0246 ± 0.0033; as nmol/g for TBARS, 16.2 ± 4.0; and as mg/g for PROT, 128 ± 15. Bars marked with an asterisk were significantly different ($p < 0.05$) from controls. Abbreviated terms are defined as in Figure 4 legend.

or in efficiency of capture, but the low-dose birds were less active, were less likely to hunt fish, and had a greater tendency to seek shade. For the low-dose group, Spalding et al. [21] did not find any neurohistologic changes that would explain the subtle behavioral changes noted by Bouton et al. [22], nor in the present report were any of our measurements of brain oxidative stress in the low-dose group significantly different from controls.

In the present report, oxidative stress occurred in brain tissue of egrets in the high-dose group that became ataxic. Effects included decreased brain total GSH-Px activity, increased lipid peroxidation recorded as TBARS, and increased GSH concentration. In a previous study, manifestations of oxidative stress also were reported in the brains of mallards that became ataxic with approximately 18 μg/g wet weight mercury in the brain [17]. These included increased TBARS concentration and decreased G-6-PDH activity. In keeping with these findings, cellular mitochondrial damage from oxidative stress and lipid peroxidation are reported to be among the earliest signs of neurotoxicity with respect to methylmercury exposure [32,33]. Ataxia, progressing to paralysis, also has been reported in other species of birds dosed with methylmercury [34–37]. The most severe lesions in the high-dosed great egret chicks were in peripheral nerves and spinal cord, followed by midbrain, cerebellum, and cerebrum [21]. These changes occurred at a mean of 35 μg/g mercury in the brain, which generally is higher than reported in other animals and humans [6]. The lesions in both peripheral and central nervous tissue generally were similar to those described in goshawks (*Accipiter gentilis*), with about 40 μg/g mercury in brain [34]. Heinz and Locke [38] reported demyelination, neuron shrinkage, necrosis, and cerebellar meningeal hemorrhage in offspring of mallards fed methylmercury. In peripheral nerves of the great egrets, there was a marked difference between the lesions in brachial and sciatic nerves. In the sciatic nerves, very little inflammation was observed, and the lesions consisted of neuronal loss, atrophy, and demyelination. The brachial nerves had marked Wallerian degeneration, indicating a more acute process. This may explain the loss of hind limb function first in birds, as has been reported also in humans [39] and guinea pigs [40]. Although ataxia frequently is reported in birds with methylmercury toxicosis, the effects on flight rarely are noted, in keeping with the above observations.

The hepatic biochemical parameters measured in this study included ones known to be indicative of mercury exposure in birds and mammals. Mercury exposure has been shown to result in decreased total GSH-Px activity in plasma and liver of at least four species of birds, including mallards [17], diving ducks [41], snowy egrets, and double-crested cormorants [18]. Similarly in mammals, methylmercury is known to decrease hepatic GSH-Px activity by as much as 60% in rats relative to untreated controls [42]. In the present study, total GSH-Px activity was significantly lower in the plasma, brain, liver, and kidney of the high-dosed group. In contrast, another form of GSH-Px that is selenium-dependent, but was not measured in the present study, increased rather than decreased in activity in the plasma and tissues of snowy egrets and double-crested cormorants in response to mercury [18].

In the present study, further evidence of oxidative stress was apparent as increased lipid peroxidation (TBARS) in the liver (low- and high-dose mercury groups) and brain (high-dose). Increased lipid peroxidation in response to mercury also has been reported in mallards, double-crested cormorants, and

black-crowned night-herons [17,18]. Other manifestations of oxidative stress related to mercury exposure in the great egrets included tissue changes in concentrations of reduced thiols. These included decreased TSH and PBSH in liver, decreased PBSH in kidney, but increased GSH in kidney and brain. Thiol depletion related to mercury exposure has been reported in mallards, surf scoters (*Melanitta perspicillata*), greater scaup (*Aythya marila marila*), ruddy ducks (*Oxyura jamaicensis*), great blue herons (*Ardea herodias*), cormorants, and snowy egrets. The liver is a major site of reduced GSH synthesis in mammals, and also apparently so in birds. Because methylmercury is a sulfhydryl-binding toxicant, GSH and other thiols are important factors in hepatobiliary excretion of methylmercury in mammals, accounting for diminished hepatic GSH concentrations with increasing concentrations of mercury [33,43,44]. This process appears to involve the formation of a mercury-glutathione complex in liver cells, followed by the secretion of the complex through a process closely linked to GSH secretion [45,46].

In the present study with great egret chicks, the liver total mercury concentration in the high-dose group was comparable to that found in adult cormorants at a mercury-contaminated site on the Carson River, Nevada [18]. The concentration in the low-dose group was comparable to that in fledgling cormorants at the same site. Yet, the extent of hepatic oxidative stress appeared to be greater in cormorants than in the great egrets, as reflected by amount of increase in TBARS concentration and depression of G-6-PDH activity in adults and multiple manifestations of oxidative stress in the fledgling cormorants. This, in part, may be due to a difference in species response where protective enzymes, including GSSG-Red, GSH-Trans, and G-6-PDH, exhibited increased activity in liver and kidneys of the great egrets, but not in mallards, cormorants, or diving ducks. Activity of these enzymes, particularly G6PDH, was lower with mercury exposure in ducks and cormorants than in great egrets. Increased activity of these protective hepatic enzymes also was noted in snowy egrets and herons along the Carson River, but with lower concentrations of mercury than in the cormorants. However, brain concentrations of total mercury were high enough in the great egrets and mallards to cause ataxia and paralysis, but considerably lower in the brains of cormorants, snowy egrets, and black-crowned night-herons found alive along the Carson River of Nevada. This, in part, may have been due to predation of any severely weakened or ataxic birds in the field.

CONCLUSION

Methylmercury resulted in accumulation of mercury (35 μg/g wet wt total Hg) and oxidative stress in the brain of high-dosed great egret chicks. The oxidative stress was linked to weakness, ataxia, and paralysis of the legs. Manifestations of oxidative stress included decreased brain total GSH-Px activity, increased lipid peroxidation as TBARS, and increased GSH concentration. In a previous study, oxidative stress also was reported in the brains of mallards that became ataxic with approximately 18 μg/g wet weight mercury in the brain [17]. Lower dosed great egrets (3.4 μg/g wet wt total brain mercury) did not exhibit ataxia or oxidative stress of the brain. Total GSH peroxidase activity was significantly lower in the plasma, brain, liver, and kidney of the high-dosed group, similar to mercury effects reported in plasma or organs of at least four species of birds including mallards [17], diving ducks [41], snowy egrets, and double-crested cormorants [18].

Other evidence of oxidative stress also was apparent in liver and kidney. Significant negative correlations occurred between liver and kidney mercury concentrations and between reduced thiols, whereas positive correlations occurred between liver and kidney mercury and TBARS. Tissue changes in concentrations of reduced thiols included decreased total thiols and protein-bound thiols in liver, decreased protein-bound thiols in kidney, but increased GSH. In liver, activities of GSH *S*-transferase and GSSG reductase increased, and, in kidney, GSH transferase and glucose-6-phosphate dehydrogenase activities increased with mercury concentration. These increased enzyme activities appear to be compensatory mechanisms in liver and kidney, and may be species-related, because they were found in egrets and herons but not in other species such as mallards, cormorants, and diving ducks. Such compensatory changes would tend to stabilize the ratio between GSSG and GSH with mercury exposure, which was the case in the present study and also for snowy egrets and herons [18].

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