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Growth and Behavioral Effects of Early Postnatal Chromium and Manganese Exposure in Herring Gull (*Larus argentatus*) Chicks

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BURGER, J. AND M. GOCHFELD. *Growth and behavioral effects of early postnatal chromium and manganese exposure in herring gull* (Larus argentatus) *chicks*. PHARMACOL BIOCHEM BEHAV **50**(4) 607–612, 1995.—Organisms in marine environments are exposed to chromium and manganese, yet little is known of the effects of these metals on physiology and behavior. In this article we examine the effects of chromium and manganese on early neurobehavioral development in herring gulls, *Larus argentatus*. Each of 36 2-day-old herring gull chicks was randomly assigned to one of three treatment groups to receive either chromium nitrate (25 mg/kg), manganese acetate (50 mg/kg), or a control dose of sterile saline solution. The trios were not siblings, but were matched by age and weight. Behavioral tests examined food-begging, balance, locomotion, righting response, recognition, thermoregulation, and perception. There were significant differences in begging behavior by 5 days postinjection, and there were significant differences in weight gain throughout development until 50 days of age, when the experiment was terminated. Behavioral tests, administered from 18–48 days postinjection, indicated significant differences between control and the exposed groups for time to right themselves; thermoregulation behavior; and performance on a balance beam, inclined plane, actual cliff, and visual cliff (although not all components varied significantly). Of the 14 behavioral measures with significant differences, control birds performed best on 12.

Chromium Manganese Postnatal Behavioral Toxicology Gulls *Larus argentatus*

CHROMIUM and manganese compounds are found in many types of rocks and soil, and in volcanic dust and gases. Although both are essential trace elements, there is concern about excessive exposure through environmental contamination. Manganese is nearly ubiquitous. Most chromium in biota has come from anthropogenic activities involved with industrial waste from mining, refining, electroplating, and alloying with iron and nickel (35). Some industrial and urban complexes release chromium in effluent wastes, and it has been discarded in soils that form the substrate for housing or light industry (22,45). More than two million tons of chromate processing waste contaminate 150 sites in northern New Jersey (18).

Manganese compounds are mined and used to produce manganese metal, which is then mixed with iron to make steel (2). Manganese is also used to produce batteries, and is an ingredient in ceramics, pesticides, fertilizers, and nutritional supplements. However, the potentially greatest source of environment contamination is from the use of additives as antiknock agents in gasoline.

As essential trace elements in the human body, chromium is required to promote the action of insulin in body tissues, and manganese is a co-factor for many enzymes involved in metabolism, normal growth, bone formation, and reproduction (2,3,5,38). Manganese deprivation also is associated with reproductive deficits in animals (2). Based on having low intrapopulation variation and skewness of manganese levels in birds, Walsh (44) concluded that manganese is an essential element that is regulated metabolically, although regulatory ability may not always be sufficient to cope with acute local pollution. Chromium is not so tightly regulated metabolically in birds (44). Harmful effects of chromium and manganese exposure have been noted with a variety of animals (2,3,30).

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Subtle behavioral effects in animals, however, have seldom been examined for either chromium or manganese. Chromium exposure induced marked changes in the swimming and feeding behavior of fish (1). However, Heinz and Haseltine (28) found no differences between control and chromium-exposed young black ducks (*Anas rubripes*).

In this article we examine behavioral effects on young herring gulls (*Larus argentatus*) of low-level neonatal exposure to chromium and manganese. We studied these two metals because gasoline manufactures are considering using organic manganese as an antiknock additive in the United States, and chromium is a common contaminant in many of the landfill soils used for residential, light industrial, and recreational sites in New Jersey and elsewhere (18,40,45).

It is desirable to understand what the potential environmental effects of manganese use in gasoline might be. An organic derivative of manganese has been used as an antiknock agent in unleaded gasoline in Canada since 1977 (20), and it completely replaced lead in gasoline by 1990 (39). Yet, the lack of basic toxicologic data has limited its use throughout the world (43). Because very young animals have increased absorption of manganese and poorly developed blood-brain barriers to metals in general (20), they might be supersensitive to increases in manganese.

We examined the behavioral effects from one exposure at 2 days of age for three exposure groups: chromium, manganese, and controls. We examined balance, locomotion, righting, begging, depth perception, and thermoregulation using a standardized battery of 14 tests. These are behaviors that have relevance to the survival of gulls in nature. We used herring gulls because they are easy to raise in the laboratory, adapt readily to human handling, eat a variety of foods, and have been studied extensively in the field and laboratory (4,7,23, 29,36,37). Herring gulls are the familiar seagull of the Atlantic Coast and Europe; they have increased so dramatically that in many places they displace native colonial birds from nesting areas and pose a threat at airports (4,8). We have been investigating the effects of low-level lead on behavioral development in herring gulls and other species in the laboratory for a number of years (9,10,12,14).

METHODS

Under appropriate federal, state, and local permits we collected 36 1-day-old (4-24 h of age) herring gull chicks from Captree, Long Island, in early June 1993. Only the first hatched chick in any nest was collected to eliminate possible biases due to hatching order, and to reduce any effects on reproductive success. Chicks were marked with numbered leg bands for identification, and randomly allocated to one of three treatment groups. There were no significant differences in initial weights among chicks.

Chicks were caged in groups of three (one from each group), and maintained in a warm laboratory (22-25°C) with a natural light-dark cycle. Three to five times daily, they were fed a diet of commercial dog and cat food, supplemented with canned fish. In nature, gull chicks are fed a diversity of foods, and chicks in the laboratory respond best to variation. Chicks were weighed at 19-20, 26-28, 33-34, and 46-48 days of age. Chicks were maintained until 50 days of age, when they were sacrificed.

At 2 days of age, chicks were given a single intraperitoneal injection of chromium nitrate (50 mg/kg), manganese acetate (25 mg/kg), or normal saline (0.9%). We used injection rather than providing it in their food to ensure equal doses. We used a single dose because birds in the wild can receive a single dose in food (often from garbage dumps); the levels achieved are similar to levels that can occur in wild birds (10), and use of injection ensures a consistent dose. Because birds can eat different amounts, giving a toxicant in the food does not result in equal dosing. This dose was based on previous work with lead (12,13), and with a pilot study of eight birds to ensure that these levels did not cause mortality or acute toxicity.

At 50 days of age, when the chicks were sacrificed, blood levels were analyzed using a Perkin Elmer 5000 graphite furnace AA with autoanalyzer (14,16). The mean blood level of manganese for manganese chicks was 15.1 ± 3.7 μg/dl; in chromium-exposed birds blood levels of chromium averaged 38.5 ± 1.6 μg/dl; and for controls, mean chromium levels were 35.2 ± 1.1 μg/dl, and mean manganese levels were 5.5 ± 0.2 μg/dl.

Testing

Behavioral tests (except for begging) were performed at ages 18-20, 26-28, 33-34, and 46-48. All groups were tested at the same ages, on the same days. There were not many age-related changes in behavior, and thus we present behavioral data for the initial tests (at 18-20 days of age), as well as the mean for all tests (see Table 2). Normally, chicks were fed at least 2 h before tests were performed. Although several assistants performed the tests, they were blinded as to treatment, and the same assistant performed the same tests to avoid interobserver variability. The chicks were maintained in visual and vocal isolation while tested.

Begging was examined by offering each chick food on a spoon and recording the time lag to initiate pecking and the number of pecks in the first 15 s. Chicks had been deprived of food for 2 h. These observations were made only at 5-7 days postinjection to avoid habituation, and because begging behavior varies dramatically by age.

Righting response was measured by putting the chick on its back and recording the time required to return itself to a standing position. We tested balance by placing chicks on a narrow level board (6 cm wide and 35 cm long) and allowing them to walk; we timed the length of time they remained on the board without falling and recorded the distance they walked. If a chick failed to walk on the balance beam, it was scored as 0. The test was 2 min long, and if a chick did not fall off, its time was 120 s. The incline test involved placing chicks on a level board and slowly elevating it to determine the angle at which they fell off.

Thermoregulation was tested by placing chicks in the center of an apparatus that offered choices between full sun, a raised object that provided no shade (but appeared to provide cover), and a shaded area without cover. We scored their behavior as: 1 = went to vertical object and stayed there; 2 = wandered but did not reach the shade; 3 = no response; 4 = moved in and out of the shade; and 5 = moved in and stayed in the shade. In nature, chicks can be exposed to very high thermal stress from warm temperatures and radiation from the sand; it is adaptive for chicks to seek shade.

Depth perception was tested on an actual cliff, and on a visual cliff apparatus [after Burger (9)], where the chicks could move about on a solid opaque surface, cross onto a transparent surface (falling off the cliff), or jump or fall off the sides. Chicks were placed in the center and given 2 min to respond. Their behavior was scored as follows: 1 = moved onto the interior clear surface (fell off cliff); 2 = fell off outer cliff edge; 3 = moved onto both clear surfaces (would have fallen

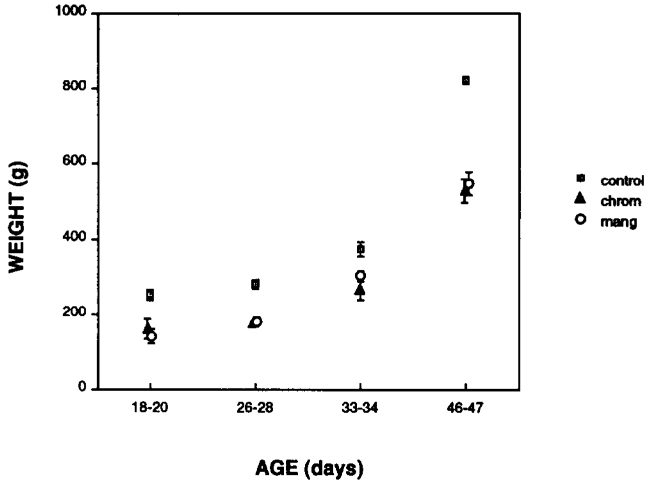

FIG. 1. Mean ± SE weight of control herring gulls chicks and chicks exposed to chromium (▲) or manganese (○).

off the cliff twice); 4 = did not move; 5 = moved onto the opaque surface (safe surface); 6 = moved onto the opaque surface, moved to the edge, and peered over the edge but did not fall.

Chicks were tested on an actual cliff by placing them at the edge of a 1-m-high table, providing them with food 10 cm from the table edge, and recording their behavior (number of peers at cliff edge, time to fall off). Chicks had been deprived of food for at least 2 h. If they fell, they landed on a pillow on the floor.

Statistical Tests

We used ANOVA to examine differences between each exposure group and controls, and we used Duncan's Multiple Range Test to distinguish among groups (41).

RESULTS

Growth

By 18 days of age, when chicks were first weighed, there were significant weight differences between exposed and control chicks (Kruskal-Wallis $\chi^2 = 14.2$, $df = 2$, $p < 0.0008$), and these persisted throughout the experiment (Fig. 1, Table 1).

Begging

Control birds started pecking significantly earlier when exposed to food than either chromium or manganese birds, but the number of pecks in the first 15 s following initiation of pecking did not differ significantly. However, the control birds ate for a longer total period during each feeding session, and thus consumed more food.

Other Behavior

Behavioral tests were routinely performed on three exposure groups from 18–48 days of age; results are presented for the first or initial test at 18–20 days, and for the mean of the tests overall (Table 2). There were significant differences among groups for time to right themselves, thermoregulation behavior, and performance on a balance beam, incline plane, visual cliff apparatus, and actual cliff. Time to right themselves normally increases slightly with age as the birds become larger and heavier (compare Initial and Overall in Table 2); control birds took less time to right themselves than manganese birds initially, and than both experimental groups overall. Thus, the effect of chromium seemed to be slightly delayed. Similarly, on the balance beam, control birds performed better than either experimental group (Table 2).

On the incline plane, where the board was slowly raised, the manganese birds waited significantly longer before standing than did the other two groups (Table 2). Control birds moved farther along the board than did birds in the other groups. Chromium birds fell off faster than birds in the other groups.

In a thermoregulation test, the control birds generally located the shade before the other birds, and remained in the shade the longest. Although initially the chromium birds reached the shade as soon as the manganese birds did, they remained there for less time. Moreover, the performance of the chromium birds did not improve over time (Table 2). On both the actual and visual cliffs, the control birds gave significantly more calls overall than did the birds in the other two groups (Table 2).

DISCUSSION

Methodologic Considerations

There have been few studies examining the effects of chromium and manganese on vertebrates other than laboratory rodents, and fewer still with birds (2,30,44). Thus, the levels that might produce subtle sublethal effects were not clear. Even though toxicity for chromium exposure in marine ani-

TABLE 1

BEGGING BEHAVIOR OF HERRING GULLS AT 5–7 DAYS POSTINJECTION AS A FUNCTION OF TREATMENT

	Number of Chicks	Time to Initiate Pecking (Latency)	No. of Pecks per 15 s (Frequency)	Final Weight (g)*
Control	12	0.2 ± 0.09(A)	6.7 ± 0.8	821 ± 1(A)
Chromium	12	1.0 ± 0.3(B)	5.4 ± 1.1	528 ± 30(B)
Manganese	12	1.2 ± 0.3(B)	4.9 ± 0.8	546 ± 31(C)
ANOVA $p<$		0.007	0.34	0.0001

Number of pecks per 15 s were times after the chick first pecked. Letters indicate significant differences using Duncan's Multiple Range Test.

*Forty days of age.

TABLE 2

COMPARISON OF BEHAVIOR (MEAN ± SE) OF HERRING GULL CHICKS AS A FUNCTION OF EXPOSURE

	Control	Manganese	Chromium	ANOVA (*p*)
Time to right (s)				
Initial	2.7 ± 0.9(A)	5.1 ± 1.9(B)	2.8 ± 0.2(A)	0.001
Overall	4.0 ± 0.7(A)	5.3 ± 1.2(B)	5.2 ± 1.2(B)	0.0002
Time on balance beam before falling (s)				
Initial	23.2 ± 2.3(A)	16.3 ± 3.6(B)	17.1 ± 1.6(B)	0.09
Overall	23.6 ± 1.0(A)	18.1 ± 1.7(B)	18.7 ± 1.7(B)	0.002
Incline plane				
Angle to stand or move				
Initial	48.3 ± 2.2(A)	53.8 ± 2.1(B)	48.0 ± 2.1(A)	0.05
Overall	49.0 ± 0.9(A)	51.8 ± 1.7(B)	48.4 ± 1.2(A)	0.04
Angle to fall off				
Initial	54.6 ± 1.3(A)	55.2 ± 1.3(A)	49.5 ± 1.1(B)	0.009
Overall	51.0 ± 1.0(A)	53.1 ± 1.0(B)	49.3 ± 1.1(A)	0.03
Distance moved (cm)				
Initial	18.2 ± 4.3(A)	13.1 ± 4.2(B)	11.0 ± 2.1(B)	0.06
Overall	20.6 ± 5.3(A)	14.6 ± 4.0(B)	10.0 ± 2.3(B)	0.06
Thermoregulation	10.7 ± 4.4	6.6 ± 3.4	7.4 ± 4.1	NS
Time to reach cover (s)				
Initial	0.4 ± 0.4	1.2 ± 0.7	0.4 ± 0.4	NS
Overall	0.7 ± 0.3	0.5 ± 0.2	0.3 ± 0.2	NS
Time in shade (s)				
Initial	22.8 ± 4.8(A)	16.0 ± 4.3(B)	6.3 ± 3.2(C)	0.08
Overall	19.8 ± 2.9(A)	16.9 ± 3.1(B)	8.6 ± 2.1(C)	0.06
Time to reach shade (s)				
Initial	14.5 ± 2.5(A)	31.3 ± 3.5(B)	28.7 ± 10.8(B)	0.01
Overall	16.9 ± 2.8(A)	18.1 ± 2.7(A)	26.0 ± 4.6(B)	0.05
Scores				
Initial	1.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.5	NS
Overall	1.0 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	NS
Actual cliff				
Calls at edge				
Initial	27.9 ± 7.2(A)	19.6 ± 11.4(B)	11.0 ± 1.0(C)	0.01
Overall	18.3 ± 2.9(A)	13.9 ± 2.7(B)	6.8 ± 2.4(C)	0.01
Peers over edge				
Initial	4.8 ± 1.2	5.6 ± 2.7	2.4 ± 0.2	NS
Overall	3.2 ± 0.4	3.1 ± 0.6	2.4 ± 0.3	NS
Visual cliff				
Calls at edge				
Initial	18.9 ± 5.0	12.8 ± 5.2	13.0 ± 4.6	NS
Overall	20.8 ± 0.1(A)	13.2 ± 2.4(B)	12.1 ± 2.5(B)	0.01
Peers over edge				
Initial	3.7 ± 0.5(A)	4.5 ± 0.9(A)	1.7 ± 0.4(B)	0.02
Overall	4.5 ± 0.4	4.4 ± 0.4	3.7 ± 0.4	NS
Score				
Initial	3.8 ± 0.5	3.2 ± 0.3	3.0 ± 0.4	NS
Overall	3.4 ± 0.5	4.1 ± 0.3	3.5 ± 0.5	NS

Initial refers to performed tests at 18–20 days of age; overall equals mean of all tests performed (18–48 days of age). NS, Not significant. Letters indicate significant differences using Duncan's Multiple Range Test.

mals is similar to lead [and manganese is half as toxic (30)], we used lower levels than we normally use with lead exposure, partly to reduce the dangers of high mortality in a preliminary study. This experiment represents the first in a series of dose–response tests that are required before the actions of these metals can be understood. Determining the dose–response relationship for chromium and manganese requires a series of experiments with different doses. However, we thought it necessary to observe behavioral changes on this initial exposure experiments to maximize the use of these wild animals. Future experiments will use lower doses.

Behavioral observations are time-consuming, making it difficult to have large sample sizes for any group. Moreover, we used the same assistant for observations on any given test to reduce interobserver variability.

The major methodologic problem with this study is that the behavioral tests were performed from 18–48 days of age (from 16–46 days postinjection). We initially did not weight or test the birds because we wanted to avoid added stress. We used this protocol because, without previous research on sublethal effects of chromium and manganese, we were unsure of the appropriate dose. However, future studies should examine the immediate effects of exposure to chromium and manganese.

Exposure in Nature

Exposure in humans, particularly for occupational exposure, and other animals is often via inhalation (2,3,20,45), yet we exposed the gulls via injection. Wild birds are not normally exposed to the high or prolonged levels that might occur in occupational exposure. In nature, herring gulls would encounter chromium and manganese regularly via ingestion from their food or inhalation from fumes. Both chromium and manganese bioaccumulate through the food chain in marine organisms (30), and herring gulls obtain their food mainly from clams, crabs, and fish from nearby bays and estuaries, as well as from garbage.

Feathers can be used as an indicator of exposure in wild birds (10), and feather values average 8.8 ± 1.2 ppm (SD) for chromium ($N = 17$ studies, range = 0 − 17.9 ppm) and 11.8 ± 20.1 ppm (SD) for manganese ($N = 19$ studies, range = 0 − 119.9 ppm). Values for wild fledgling herring gulls from Captree averaged 2.8 ± 0.6 ppm for chromium and 3.7 ± 0.3 ppm for manganese the same year we examined chicks in the laboratory. The feathers of the laboratory gulls averaged 1.2 ± 0.3 for chromium and 0.9 ± 0.2 for manganese. Thus, exposure in the laboratory was not excessive.

The use of chromium-contaminated waste from the chromium processing industry resulted in dredge materials that provide the single largest input of chromium to the New York bight, with an additional large component derived from the Hudson River discharge (33,42). Although the chromium input from sludge decreased by nearly 50% from the early 1970's to the late 1980's in the New York Bight, inputs are still considerable. Currently, about a third each of the chromium input to the New York harbor area comes from storm water, wastewater, and tributaries (32). Manganese likewise is deposited in the New York Bight via river discharge, stormwater runoff, and spoil deposition.

That birds in the New York Bight are exposed to both chromium and manganese is evident by levels in their tissues (11,16,17). Moreover, young birds derive their entire burden from the local area because their body burden comes via the egg and from the foods their parents feed them (10). The contribution from the eggs (17) in the case of herring gulls also largely reflects local exposure, as many of the gulls remain over the winter and do not migrate farther south. The herring gulls that migrate south return to the Captree colony area in early March, several weeks before they lay eggs. Thus, the energy they use to produce eggs mainly comes from the New York Bight area.

Behavioral Deficits and Growth

The levels of exposure in this experiment did not result in immediate toxicity in that birds were not ill and did not show signs of being sick, they walked and moved well, and they ate when offered food. Any behavioral differences appeared 4–5 days after injection, not immediately, as would occur with an immediate toxic effect. However, there were behavioral deficits in begging behavior and growth rates. The differences in weight increased with age, rather than lessened. Thus, the manganese and chromium birds did not catch up with the control birds, even by 45 days of age, when they were beginning to fly. Furthermore, there were no significant weight differences between the chromium and manganese birds by fledging. Such growth deficits have not been noted in the literature for chromium and manganese exposure in birds, although lead causes similar deficits (12). The chromium and manganese exposed chicks in this study showed delayed pecking sufficient to suggest that, in nature, a nonimpaired chick would have the competitive advantage.

By 5 days postinjection there were significant differences in begging behavior; control chicks initiated pecking sooner than did the manganese or chromium birds. In herring gulls a parent returns to the nest with food, and begins to regurgitate when the chicks beg and peck at the bill. In the normal course of events, the chick that initiates feeding obtains the most food (7). If the same chick initiates feeding first all the time, it would normally gain weight faster than the other chicks, amplifying the behavioral differences and leading to even greater weight differences (24,27,34). In addition, being smaller, the chick is at a disadvantage in aggressive interactions (31). If the spiral continues, the disadvantaged chick starves.

There were behavioral deficits in many components of the behaviors examined, including righting, balance, thermoregulation, visual cliff, and actual cliff. The behaviors examined are those that relate directly to survival in the wild. Herring gull chicks can fall down small embankments, landing on their backs, and they must be able to quickly right themselves to avoid predation. Nearby neighbors as well as great black-backed gulls (*Larus marinus*) will kill and eat vulnerable chicks (6,7).

The coastal sand habitats where the gulls nest can be very hot, and during midday, with the sun radiating from the hot sand, young chicks normally seek shade under debris or vegetation. Being able to find shade quickly is critical to survival. This is particularly true for small, down-covered chicks that do not have the protection of dense feathers. Moreover, there is an increase in predation when chicks are under thermal stress (26).

Although most herring gulls nest on flat surfaces, some nest on debris or other objects, and in some places they nest on cliffs or ledges (25). Thus, it is critical for chicks to be able to perceive a cliff edge and avoid falling off.

It may be argued that some of the behavioral disadvantages we mention relate to competition among siblings for parental care (including food), and that in the normal course of events chicks would have equal exposure to contaminants. Although this is generally true, metal levels can vary among members of a brood because: a) females sequester the highest concentration of metals in the first egg laid (21); b) adoptions can occur, creating broods with young having different provisioning and exposure histories (19); and c) parents bring back a variety of foods, and the young can eat different diets either because they cannot compete for the largest food items or because they exhibit diet preferences (15).

It could be argued that the behavioral deficits observed were due to growth retardation and relative size. If this were the case, then the behavior of older, exposed (yet small) chicks should be similar to that of younger control chicks of equal size. This was not the case.

The behavioral deficits we observed in the laboratory relate directly to growth and survival of the chicks in the wild, and suggest that chromium- and manganese-impaired chicks would have lower survival if such exposure were achieved in the wild. Moreover, the deficits in weight would place the chicks at a disadvantage at fledging because they would have fewer reserves to tide them over during the time when they are learning to forage on their own. This is a particularly vulnerable time period of young seabirds, and accounts for a high percentage of first-year mortality (5).

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