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Effects of Chronic High-Level Manganese Exposure on Male Behavior in the Japanese Quail (*Coturnix coturnix japonica*)¹

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ABSTRACT Male Japanese quail were chronically exposed to 5000 ppm manganese (Mn) as particulate manganese oxide (Mn₂O₃) in their diet from hatching to 75 days of age. No decrements in growth or in other indices of general toxicity were noted. There were significant (P<.05) age-related increases in general locomotor activity in the control group, although no significant (.05<P<.10) increases were seen in the Mn-treated group. Both control and Mn-treated groups had significant (P<.05) age-related increases in aggressive behavior with an overall significant (P<.05) treatment-related depression. Serum testosterone concentration was only slightly depressed (.05<P<.01) in the 75 day-old, Mn-treated quail. Both the control and Mn-treated quail had higher liver Mn concentrations than previously reported in rodents. Both control and Mn-exposed quail accumulated 5 to 10 times more Mn in their livers than similarly treated rodents. This study indicated that the Japanese quail was less sensitive to particulate Mn₂O₃ exposure than rodents treated comparably.

(Key words: Japanese quail, aggressive behavior, locomotor activity, manganese, testosterone)

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INTRODUCTION

Manganese (Mn) is an essential trace mineral. Deficiencies result in poor growth and reproductive development in mammals (Boyer *et al.*, 1942; Kemmerer *et al.*, 1931) and depressed bone and tendon growth in birds (Caskey *et al.*, 1939; Schaible and Bandimer, 1942). It is among the least toxic trace minerals in mammals and the chronic median lethal dose (LD₅₀) (30 day) in young rats has been shown to be in excess of 20 µg Mn/kg body weight (BW)/day for MnCl₂ (Deskin, 1979) and 70 µg Mn/kg BW/day for Mn₂O₃ (Rehnberg *et al.*, 1982). Manganese as MnSO₄ causes reduced growth of turkeys at a dietary level of 4800 ppm (Vohra and Kratzer, 1968).

Chronic ingestion of particulate Mn, as Mn₂O₃, at high dietary levels alters reproductive development in rats and mice (Laskey *et al.*, 1982; Gray and Laskey, 1980; Gray *et al.*, 1978). These alterations were not overt in that fertility was not significantly impaired. In rats, there were subtle changes in the developmental pattern in the secretion of follicle-stimulating hormone (FSH) and testosterone (T), but in male mice treated through puberty, Mn treatment resulted in smaller testes and sex accessory gland weights.

The young rat has been shown to be particularly sensitive to Mn accumulation. Exposure to Mn from birth resulted in high Mn concentrations in the pituitary and hypothalamus by 20 to 25 days postpartum (Rehnberg *et al.*, 1980). It is likely that accumulation in the young rat is the result of the high pinocytotic activity of the neonatal gastrointestinal tract and the absence of biliary excretory route prior to 17 days postpartum (Miller *et al.*, 1975). In birds, it has been reported that similar pinocytotic activity, and therefore the possibility of particulate ingestion, occurs in the bursa of Fabri-

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cius, cecum, and Peyer's patches (Bockman and Cooper, 1973).

Development of the male reproductive system in the Japanese quail can be characterized by increasing 1) testicular weight, 2) serum T concentrations, 3) cloacal gland (proctodeal protuberance) area, and 4) number of mating attempts when placed with a female. The onset of these parameters has been shown to occur at 22, 28, 32, and 34 days of age, respectively, reaching their maximum at 49 to 57, 44, 50, and 52 days of age, respectively (Mather and Wilson, 1964; Adkins, 1975; Ottinger and Brinkley, 1978, 1979; Hutchison, 1978). In the rat, the ontogeny of testicular growth and increases in serum T concentration are similar. However, maximal levels are reached 10 to 29 days later (Odell and Swerdloff, 1976).

This study was designed to determine whether chronic dietary Mn exposure at high dietary levels would retard reproductive development in the Japanese quail as it does in rodents.

MATERIALS AND METHODS

Quail were obtained on the day of hatching, sexed by the method of Siopes and Wilson (1973) and Homma *et al.* (1966), and the selected males were caged in electrically heated brooders for 21 days. All evaluations were made on male birds, which were kept under constant light throughout the study. Birds were allowed *ad libitum* access to food (North Carolina Agricultural Research Service, standard quail ration) and water throughout the study. Particulate Mn_3O_4 was added to the basal diet to achieve a supplemental concentration of 5000 ppm Mn. Control birds received the basal diet, containing 56 ppm Mn (as $MnSO_4$), while the treated birds received the Mn supplemented diets from hatching through 75 to 80 days of age. The Mn_3O_4 particle diameter was determined to be 1.02 μ with 78% of the particulate less than 1.8 μ in diameter (Rehnberg *et al.*, 1982; Laskey *et al.*, 1982).

Food consumption (24 hr) and body weight measurements were made weekly for individual birds. Because quail tended to waste food, food was offered in a container with a concave or funnel-shaped top, keeping food wastage to unmeasurable amounts. Starting at Week 3, males were housed singly. General locomotor activity and male aggressive behavior, which is dependent upon normal T secretion and is exempli-

fied by aggressive mating behavior, as described by Wade (1982), were determined weekly from Week 3 through Week 10. The individual locomotor activity was conducted using two Varimax Selective Activity Meters with a PC-2 printer (Columbus Instruments, Columbus, OH). Measurements were made for 30 min on individual birds, a control and a treated, between 0900 and 1200 hr in rooms isolated from the general housing facility. The same pairs of birds were monitored weekly throughout the study. Weekly aggressive behavior was also evaluated between the same pairs throughout the study during a 5-min test period. Birds were placed in a wire-bottomed plastic cage 25 by 45 cm, and an aggressive act was considered to be a combination of a neck grab and a mounting attempt.

When the males were 75 to 80 days old, food was removed and 16 hr later they were bled *via* cardiac puncture and killed. Serum was prepared from each blood sample and frozen at -40 C prior to T determination. Liver and testes were removed and weighed. Liver samples were frozen at -40 C for later analysis of Mn content.

Prior to Mn determination, liver samples were thawed, a portion removed, weighed, and solubilized using tetramethylammonium hydroxide (TMAH). Manganese assays were done by nonflame atomic absorption spectrophotometry using the methods defined previously (Rehnberg *et al.*, 1981). Standardization was accomplished by using aqueous standards prepared from a 1000 ppm Mn stock solution (Certified ACS reagent, Fisher Scientific Company). The solubilized tissue samples were diluted to be within the linear portion of the concentration curve, and the aqueous standard technique was verified by the method of standard additions. The adequacy of the analytical methods for Mn determination was confirmed by assaying NBS bovine liver containing 10.3 ± 1.0 ppm Mn. Serum T was assayed by the radioimmunoassay (RIA) procedure of Furuyama *et al.* (1970), as modified by Chen *et al.* (1971), using a T test set (Catalog No. TS-333, Wein Laboratories, Inc., P.O. Box 227, Succasunna, NJ 07876). All samples were assayed together, and to estimate interassay variation, a serum pool was included in four dilutions. Aliquots of serum were extracted once with methylene chloride and the dried extract reconstituted in assay buffer. The antiserum cross reacted with 5- α -dihydrotestosterone (55.5%). The minimum

detectable T in the assay was .15 ng/ml. The interassay coefficient of variation using the serum pool and serum T standards (Catalog No. R-9036 and 9009, 140 ± 30 and 635 ± 110 ng/dl, respectively; Wein Laboratories, Inc., P.O. Box 227, Succasunna, NJ 07876) was 4.6%.

Data were analyzed using the computer-based statistical analysis systems (Helwig and Council, 1979) containing analysis of variance. Significant differences between group means were evaluated using Duncan's (1975) multiple comparison procedure.

RESULTS

Because there were no statistical treatment differences ($P > .05$), the combined data on growth and on food and Mn consumption are presented in Figure 1. It appeared that the Mn-treated quail developed the male plumage earlier than the birds on the standard ration with Mn concentrations of 56 ppm. This would indicate, if verified, that the currently used diet may contain a marginal amount of Mn. Food consumption ranged from 115 g/kg BW/day in the adult to 195 g/kg BW/day in the rapidly growing quail (20 days old). Based on this information, Mn consumption in the treated birds

ranged from 575 to 977 mg Mn/kg BW/day, while in the control group consumption ranged from 6 to 10 mg Mn/kg BW/day.

General locomotor activity is presented in Table 1, and beginning with the activity in Week 7, the control group had activity significantly higher than its Week 3 activity. The Mn-treated group had only a slight increase in activity through Week 7 with no further increase through Week 10.

Aggressive behavior, measured from Week 3 through Week 10 of age, is presented in Table 2. When compared to Week 3, control animals show significant increases in this behavior by Week 6, while Mn-treated male birds did not show a similar increase until Week 7. Aggressive behavior in Mn-treated birds remained 25 to 50% lower than control birds throughout the study.

A summary of the data collected at the end of this study is presented in Table 3. Although Mn treatment depressed ($.05 < P < .10$) serum T concentration, statistical significance was not achieved when compared to controls of the same age. Dietary Mn treatment resulted in liver Mn concentrations of twice those found in control animals.

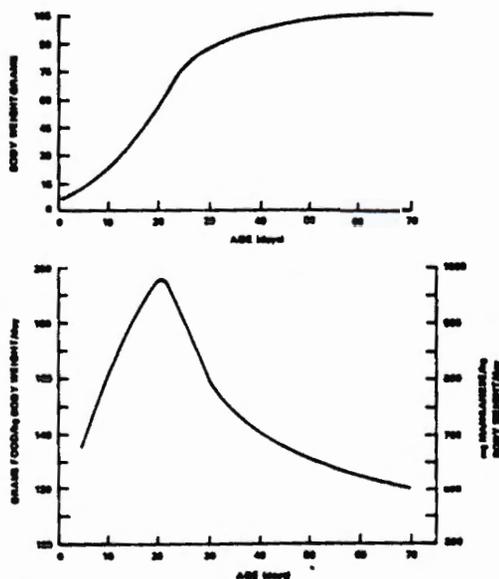


FIG. 1. Growth and food consumption in control and manganese (Mn)-treated groups combined and Mn intake with manganese-oxide-supplemented diet.

TABLE 1. Locomotor activity (mean counts/30 min \pm SEM) in the developing quail during manganese oxide exposure

Age ¹ (weeks)	Treatment group	
	Control	Manganese-treated
n	23	11
3	363 \pm 45 ^a	389 \pm 39 ^a
4	330 \pm 39 ^a	353 \pm 39 ^a
5	421 \pm 50 ^{ab}	401 \pm 40 ^a
6	424 \pm 52 ^{ab}	469 \pm 64 ^{ab}
7	593 \pm 52 ^b	469 \pm 75 ^{ab}
n	6	6
8	692 \pm 91 ^b	483 \pm 103 ^{ab}
9	671 \pm 88 ^b	526 \pm 72 ^{ab}
10	734 \pm 57 ^b	510 \pm 101 ^{ab}

^{a,b} Means with different superscript letters are significantly different ($P < .05$) by Duncan's multiple comparison test.

¹ Analysis of variance indicated significant ($P < .001$) week effects for Weeks 3 through 10 while no significant ($.05 < P < .10$) treatment effects were seen.

TABLE 2. Aggressive behavior (mean/5 min \pm SEM) in the developing quail during manganese oxide exposure

Age ¹ (weeks)	Treatment group	
	Control	Manganese-treated
n	23	6
3	.18 \pm .18 ^a	0 ^a
4	.18 \pm .18 ^a	0 ^a
5	1.73 \pm .52 ^{abc}	1.00 \pm .55 ^a
6	4.73 \pm 1.64 ^{bcd}	1.91 \pm .55 ^{abc}
7	5.82 \pm 1.75 ^{cd}	3.27 \pm .89 ^{bc}
n	6	6
8	9.16 \pm 1.50 ^d	5.50 \pm 2.56 ^{cd}
9	4.33 \pm .60 ^{bcd}	2.16 \pm 1.13 ^{abc}
10	6.00 \pm .68 ^d	3.80 \pm 1.07 ^{bcd}

^{a,b,c,d} Means with different superscript letters are significantly different ($P < .05$) by analysis of variance and Duncan's multiple comparison test.

¹ Analysis of variance indicated significant ($P < .001$) week effects and significant ($P < .05$) treatment effects.

DISCUSSION

Reproductive development in rodents is altered with chronic Mn (as Mn_3O_4) exposure (Gray *et al.*, 1978, 1980; Laskey *et al.*, 1982). These alterations included reduced weight of the testes, seminal vesicles, and preputial gland in mice, reduced postpubertal locomotor activity in rats and mice, dose-related changes in the postpubertal serum concentrations of FSH and T in rats, and a delay in reaching normal epididymal sperm content in rats. In the research reported here, the postpubertal loco-

motor activity in the Mn-treated birds failed to increase significantly over the prepubertal activity, which is consistent with the observations in mice and rats by Gray *et al.* (1978, 1980).

Aggressive and heterosexual behaviors in male Japanese quail are activated by T and its precursor delta-4-androstenedione in castrated birds (Wade, 1982). These behaviors were shown to be only minimally promoted by either estradiol or 5- α -dihydrotestosterone. Manganese treatment in this study reduced both the locomotor activity and the aggressive behavior by 16 and 40%, respectively. Additionally, serum T was depressed ($.05 < P < .10$) 24% in the Mn-treated group. Because the pattern of aggressive behavior (homosexual mating) measured in this study was indistinguishable from heterosexual mating behavior, it seems likely that aggressive behaviors and sexual behaviors could share a common mechanism(s) of action stimulated through conversion of T to estradiol by an aromatase in the central nervous system. We cannot state categorically that the 24% depression in serum T in the Mn-treated males was solely the cause of reduced aggressiveness or whether the decreased aggressive behavior was due to impaired reproductive development. Although dietary Mn concentrations were quite high (5000 ppm Mn as opposed to normal dietary concentrations of 50 to 90 ppm), no general toxic effects such as weight loss, tremors, etc. were noted.

Liver Mn concentrations were roughly doubled in the Mn-treated quail when compared to those receiving the standard ration. Livers from rats receiving a diet containing 3550 ppm Mn (as Mn_3O_4) for 60 to 100 days had only 1.2

TABLE 3. Summary of measurements (mean \pm SEM) on birds following chronic manganese exposure for 10 weeks.

	Experimental group	
	Control	Manganese-treated
n	14	6
Body weight, g	99 \pm 1.3	99 \pm 2.7
Testes weight, g	2.72 \pm .12	2.80 \pm .15
Liver weight, g	1.39 \pm .05	1.40 \pm .04
Liver Mn, g/g	5.09 \pm .34	12.30 \pm 1.03*
Serum T, ng/ml	7.00 \pm 1.02	5.30 \pm .78

*Significantly ($P < .05$) different from control by analysis of variance.