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EFFECTS OF DIETARY VANADIUM IN MALLARD DUCKS

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Adult mallard ducks fed 0, 1, 10, or 100 ppm vanadyl sulfate in the diet were sacrificed after 12 wk on treatment; tissues were analyzed for vanadium. No birds died during the study and body weights did not change. Vanadium accumulated to higher concentrations in the bone and liver than in other tissues. Concentrations in bones of hens were five times those in bones of drakes, suggesting an interaction between vanadium and calcium mobilization in laying hens. Vanadium concentrations in most tissues were significantly correlated and increased with treatment level. Lipid metabolism was altered in laying hens fed 100 ppm vanadium. Very little vanadium accumulated in the eggs of laying hens.

INTRODUCTION

Vanadium is an essential element in certain animals (Schwarz and Milne, 1971; Hopkins and Mohr, 1974); in sufficient quantities, however, it may induce toxic effects (Hatchcock et al., 1964; Hafez and Kratzer, 1976a) and interfere with normal metabolic processes (Bernheim and Bernheim, 1939; Curran, 1954). Vanadium is a natural constituent in petroleum, reaching concentrations of 500 ppm or higher (Committee on Biologic Effects of Atmospheric Pollutants, 1974). Waterfowl could therefore be exposed to vanadium introduced into the environment as a result of oil spills or discharges. We found no information concerning the extent of such exposure nor its possible effects.

We conducted a chronic feeding study to determine the extent of vanadium accumulation in eggs, blood, brain, fat, liver, kidney, and bone (femur) of mallard ducks (*Anas platyrhynchos*) and to examine the effects of vanadium on lipid metabolism.

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METHODS

Twenty pairs (one male, one female) of first-year adult mallards obtained at the Patuxent Wildlife Research Center were randomly assigned to 90 X 90 X 60 cm vinyl wire cages supplied with running water. After a 2-wk equilibration period, the ducks were fed *ad libitum* with commercial duck breeder mash coated with either 1, 10, or 100 ppm (wet weight) vanadyl sulfate dissolved in propylene glycol. Propylene glycol alone was mixed into the control diet. Three feed samples from each treatment group were taken at intervals throughout the study and stored frozen for vanadium analysis. Five pairs of birds were randomly assigned to each of three treatment groups, to be fed 1, 10, or 100 ppm vanadium in the diet; five pairs served as controls. Food intake was estimated on a daily basis.

Ducks were weighed and bled at 3-wk intervals during the study. After 12 wk, all birds were killed and tissues were saved for vanadium analysis. All eggs laid during the treatment period were collected; three from each group were randomly selected for vanadium analysis.

Vanadium analyses were conducted at the Environmental Trace Substances Research Center, Columbia, Missouri. The frozen tissue samples were freeze-dried and homogenized by thorough grinding; blood samples were dried under a heat lamp. A portion of each sample was analyzed by the neutron activation method using the ^{51}V reaction. The lower limit of quantification averaged 0.1 ppb on a wet-weight basis.

Triglycerides in blood plasma were enzymatically hydrolyzed to glycerol and free fatty acids; the cholesterol esters were hydrolyzed to cholesterol and free fatty acids. Cholesterol and triglyceride concentrations in plasma were determined by spectrophotometric methods, using Calbiochem kits (Bucolo and David, 1973; Richmond, 1973).

RESULTS

Mean levels of vanadium in feed samples were 1.87, 2.84, 10.36, and 110 ppm (wet weight) for the control, 1, 10, and 100 ppm groups, respectively. No birds died and all appeared healthy throughout the study. The ducks consumed an average of 121 g of food daily and maintained body weights averaging 1170 g. Food consumption and body weights did not differ significantly ($p > 0.05$, analysis of variance) among treatment groups throughout the study.

Vanadium concentrations in the blood of mallards sampled at 3-wk intervals are shown in Table 1. Residues in blood were very low; there was no difference ($p > 0.05$) in blood vanadium levels between the controls and the groups fed 1 and 10 ppm. However, at all sampling intervals blood vanadium levels were about 100-fold higher in the 100 ppm group than in the controls. Residues did not increase significantly ($p > 0.05$) with time.

Average residue accumulations in the various tissues are shown in Table 2. In all but one case, the mean values represent levels in both males

TABLE 1. Accumulation of Vanadium in Blood of Mallard Ducks

	Vanadium (ppb, wet weight)[a]			
Treatment	3 wk	6 wk	9 wk	12 wk
Control	0.8 ± 0.3	0.4 ± 0.2	0.7 ± 0.3	0.2 ± 0.1
1	1 ± 1	0.5 ± 0.1	1 ± 0.2	0.6 ± 0.2
10	3 ± 1	2 ± 1	3 ± 0.4	2 ± 0.4
100	79 ± 9	56 ± 8	106 ± 19	89 ± 15

[a]Mean ± SE; n = 10 per treatment.

and females; differences between sexes fed the same diet were not detected except in bone of ducks fed 100 ppm vanadium. Females accumulated at least 5 times more vanadium in the femurs than did males, and one laying hen in the 100 ppm group accumulated 11 times as much. Residues in all tissues were very low, although they increased according to treatment level. Higher levels were reached in femur and liver than in other tissues, but they seldom exceeded 1 ppm. Vanadium accumulated to lower levels in the brain than in any other tissue.

Residue levels in the different tissues were significantly correlated ($p < 0.01$); blood and kidney, blood and liver, and blood and femur showed the highest correlations (Table 3). Brain and fat and femur and fat were not significantly correlated ($p > 0.05$).

Very little vanadium accumulated in eggs of laying hens (Table 4). Only two hens in the control, 1, and 100 ppm groups and three hens in the 10 ppm group laid eggs. These numbers were not sufficient to test for differences in egg laying among groups. Egg laying was also sporadic. Some hens did not start laying until the last week of the study, whereas others laid eggs near the onset but stopped entirely after a few days. However, residue levels in eggs from hens fed 100 ppm vanadium were very similar, regardless of when the eggs were laid.

The effects of vanadium on lipid metabolism were studied by

TABLE 2. Residues in Mallard Ducks Fed Dietary Vanadium

	Residue (ppb, wet weight)[a]			
Tissue	Control	1	10	100
Brain	0.5 ± 0.1	0.8 ± 0.2	0.5 ± 0.2	9± 1
Fat	2 ± 1	3 ± 2	11 ± 7	20 ± 11
Kidney	1 ± 0.3	3 ± 0.7	18 ± 2	273 ± 49
Liver	19 ± 5	17 ± 4	61 ± 20	657 ± 113
Femur	100 ± 22	92 ± 15	251 ± 80	274 ± 47 (♂) 3327 ± 2208 (♀)

[a]Mean ± SE; n = 5 pairs per treatment.

TABLE 3. Correlations between Vanadium Residues in Various Tissue Combinations[a]

Tissue	Blood	Brain	Fat	Kidney	Liver	Femur
Blood[b]	1	.6086[c]	.4819[c]	.9486[c]	.9165[c]	.8831[c]
Brain		1	.2239	.6305[c]	.5997[c]	.4335[c]
Fat			1	.4879[c]	.4407[c]	.2132
Kidney				1	.8323[c]	.6858[c]
Liver					1	.7607[c]

[a]Values are product-moment correlation coefficients following log transformation of combined values for all treatment groups (n = 40 per tissue).
[b]Blood residues from the 12-wk sampling period were used in the regression analyses.
[c]$p < 0.01$, linear regression analysis.

measuring plasma cholesterol and triglyceride concentrations at 3, 6, 9, and 12 wk of treatment. In drakes there were immediate elevations in plasma cholesterol that were proportional to the vanadium accumulation in the tissues (Table 5). These increases were also evident at 9 and 12 wk. Maximal cholesterol increases were 30–50% above the respective control values. By contrast, there was only a transient increase in plasma triglycerides at 3 wk that was 57% above the control value for that interval.

Alterations in lipid metabolism were most pronounced in females since they were affected by reproductive activity as well as vanadium. Laying controls and laying hens fed 1 or 10 ppm vanadium illustrate the normal depletion of plasma cholesterol associated with egg production (Table 6). However, the laying hens fed 100 ppm vanadium failed to exhibit plasma cholesterol depletion during any of the sampling intervals, as did laying hens after 12 wk on 10 ppm vanadium.

In general, the triglycerides of laying hens were about twofold higher

TABLE 4. Residues in Eggs of Mallard Hens Fed Dietary Vanadium

	Residue (ppb, wet weight)	
Treatment	Mean[a]	Range
Control	<0.2	0
1	<0.2	0
10	< 0.2	0
100	63	59–68

[a]n = 3 eggs per treatment.

TABLE 5. Lipids in Blood of Mallard Drakes Fed Vanadium

Treatment	Lipid (mg/dl) 3 wk	6 wk	9 wk	12 wk
		Cholesterol		
Control	128 ± 16[a]	144 ± 7	141 ± 14	149 ± 10
1	142 ± 6	146 ± 23	153 ± 28	157 ± 27
10	167 ± 21	122 ± 6	169 ± 18	161 ± 21
100	184 ± 25	141 ± 14	221 ± 30	195 ± 31
		Triglycerides		
Control	116 ± 5	151 ± 27	96 ± 15	130 ± 9
1	146 ± 38	138 ± 29	99 ± 11	102 ± 13
10	127 ± 10	162 ± 14	122 ± 10	134 ± 10
100	182 ± 19	120 ± 9	120 ± 19	101 ± 4

[a]Mean ± SE; $n = 5$ per treatment.

than those of nonlaying hens, irrespective of the vanadium concentrations in the feed (Table 7). Thus the triglyceride/cholesterol ratio was fivefold higher in laying than in nonlaying hens, except in laying hens fed 100 ppm vanadium. This did not result from any effect on triglycerides, but from an almost complete inhibition of cholesterol depletion in laying hens fed the high concentration of vanadium.

TABLE 6. Cholesterol in Blood of Mallard Hens Fed Vanadium

Treatment	Cholesterol (mg/dl) 3 wk	6 wk	9 wk	12 wk
		Layers		
Control	–	–	60(1)[a]	44(2)
1	43(1)	33(2)	53(2)	53(2)
10	22(1)	17(1)	32(1)	79(3)
100	85(1)	80(1)	119(1)	106(2)
		Nonlayers		
Control	111(5)	113(5)	130(4)	117(3)
1	110(4)	122(3)	141(3)	139(3)
10	84(4)	86(4)	106(4)	187(2)
100	100(4)	92(4)	136(4)	131(3)

[a]Means with sample numbers in parentheses; insufficient sample size for statistical consideration; sample number of 5 divided between layers and nonlayers.

TABLE 7. Triglycerides in Blood of Mallard Hens Fed Vanadium

Treatment	Triglycerides (mg/dl) 3 wk	6 wk	9 wk	12 wk
		Layers		
Control	–	–	274(1)[a]	529(2)
1	413(1)	308(2)	364(2)	499(2)
10	584(1)	271(1)	540(1)	522(3)
100	581(1)	192(1)	285(1)	620(2)
		Nonlayers		
Control	132(5)	138(5)	177(4)	278(3)
1	188(4)	111(3)	126(3)	262(3)
10	151(4)	228(4)	270(4)	577(2)
100	253(4)	152(4)	187(4)	217(3)

[a]Means with sample numbers in parentheses; insufficient sample size for statistical consideration; sample number of 5 divided between layers and nonlayers.

DISCUSSION

Young rats fed 92 and 194 ppm vanadium lost body weight and exhibited gross pathological symptoms, and 56% of those fed 368 ppm vanadium died (Daniel and Lillie, 1938). Dietary vanadium at 25 ppm, fed either as ammonium vanadate or vanadyl sulfate, significantly depressed growth and caused mortality in young chicks (Hatchcock et al., 1964). In another study, growth was depressed in young chicks fed 30 ppm vanadium, but levels up to 200 ppm caused high mortality (Romoser et al., 1961). Egg production was suppressed in laying hens fed 300 ppm vanadium, but no other symptoms of toxicity were detected (Hafez and Kratzer, 1976b). Levels of dietary vanadium up to 300 ppm failed to inhibit egg production in *Coturnix* quail (*Coturnix coturnix japonica*) and did not cause growth depression or mortality in 1-day-old male *Coturnix* chicks fed for 4 wk (Hafez and Kratzer, 1976b).

In our study, dietary vanadium did not cause significant weight loss or mortality in adult mallards. However, the effect of diet composition on the potential toxicity of vanadium could be a factor, as discussed by Hafez and Kratzer (1976a). They point out that low concentrations of vanadium fed in a nutritionally inadequate diet produce toxicity symptoms in chicks, whereas similar levels in adequate diets are not harmful.

Radioactive vanadium accumulated to higher levels in the bone (femur) and kidney of young chicks than in other tissues, and in the kidney of adult rats (Hatchcock et al., 1964). In our study with mallards, higher

levels were reached in the bone and liver than in other tissues. Hens fed 100 ppm accumulated vanadium in the bone to about five times the levels in drakes. Since some hens in this group were laying eggs and all hens were in reproductive condition, the data suggest an interaction between vanadium and calcium mobilization. The only hen in the 100 ppm group that laid a large number of eggs (30) had the highest level of vanadium in the bone (12,000 ppb). A similar interaction between lead and calcium mobilization in bone of laying mallards was demonstrated by Finley et al. (1976).

Several studies have shown contradictory effects of vanadium on lipid metabolism in birds and mammals. Responses were dependent on species (Mountain et al., 1956; Hafez and Kratzer, 1976b), age (Somerville and Davies, 1962; Curran and Burch, 1967), the composition of the diets (Yeh and Leveille, 1973; Hafez and Kratzer, 1976a), and whether biochemical tests were conducted *in vitro* or *in vivo* (Curran and Burch, 1967). Our results in mallard ducks fed vanadium also varied depending on sex and breeding condition. The alterations in lipid metabolism caused by vanadium were considered biologically significant because they were demonstrable in ducks that had absorbed and accumulated only minute tissue concentrations of the metal. Aquatic birds could be exposed through the food chain by ingesting invertebrates with high vanadium concentrations (Underwood, 1971), or during oil spills since crude oils also contain excessive amounts of vanadium (Committee on Biologic Effects of Atmospheric Pollutants, 1974). These sources could easily provide enough vanadium to result in the toxic biochemical changes demonstrated in this study. Comparable changes in lipid metabolism of chicks required higher levels of vanadium (Hafez and Kratzer, 1976c), emphasizing the sensitivity of the adult mallard ducks to vanadium.

One of the most striking manifestations of vanadium, failure of plasma cholesterol depletion in laying hens, should be reevaluated with a larger sample and the possible effects on embryonic development considered. Hafez and Kratzer (1976b) examined the egg yolk cholesterol content in chickens and were unable to detect differences, but no measurements were made of embryonic development, hatchability, or chick survival.

The results of this study will be useful in interpreting levels of vanadium that might be found in wild ducks. An increase in dietary vanadium was reflected by an increase in tissue residues, suggesting a relationship between body levels and environmental contamination. The bone and liver appear to be the best indicators of chronic vanadium ingestion.

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