Life-term Studies in Rats: Effects of Aluminum, Barium, Beryllium, and Tungsten[1]

HENRY A. SCHROEDER[2] AND MARIAN MITCHENER
Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire 03755, and Brattleboro Memorial Hospital, Brattleboro, Vermont 05301

ABSTRACT Recondite toxicities of small doses of aluminum, barium, beryllium, and tungsten were evaluated by feeding weanling rats each metal in drinking water for life. Three hundred and thirty-four rats of the Long-Evans strain were divided by sex. Their drinking water contained 5 ppm soluble salts of aluminum, barium, beryllium, or tungsten in a basal water containing zinc, copper, manganese, cobalt, molybdenum, and chromium. The diet fed was low in trace elements. These metals were virtually innocuous as measured by median life-span, longevity, incidence of tumors, serum cholesterol, glucose, and uric acid. There was slight enhancement of growth from tungsten and barium and slight depression from beryllium. There was also slight shortening of longevity from tungsten. At this dose level these metals had little detectable effects in rats. J. Nutr. *105:* 421–427, 1975.

INDEXING KEY WORDS trace elements · trace metals · life-term studies · toxicity

We have exposed weanling rats and mice to each of some 21 to 29 "abnormal" trace elements for life in order to ascertain recondite toxicity (1). The present report concerns aluminum, barium, beryllium, and tungsten given as soluble salts in drinking water to rats.

METHODS

The low metal diet, the drinking water, and the metal-free environmental conditions in the laboratory have been reported in detail (1–5). Random-bred pregnant rats of the Long-Evans (BLU:LE) strain were purchased,[3] and their offspring were born and weaned in our laboratory. Littermates were divided into groups; the sexes were separated and placed four to a cage at weaning time, where they remained all their lives until natural death. Groups of 52 of each sex were given 5 ppm aluminum as the potassium sulfate, barium as the acetate, or beryllium as the sulfate, and 35 to 37 animals were given tungsten as sodium tungstate in drinking water. The water also contained 5 ppm chromium (III) as the acetate, 50 ppm zinc as the acetate, 5 ppm copper as the acetate, 10 ppm manganese as the chloride, 1 ppm cobalt as the chloride, and 1 ppm molybdenum as sodium molybdate. The diet was made of seed rye flour (60%), dried skim milk (30%), corn oil (9%), iodized sodium chloride (1%), and assorted vitamins (2).[4] The rats were weighed at weekly intervals at first, and at monthly intervals for a year, and then at 3-month intervals. They were disturbed as little as possible, only for sampling of blood and for cleaning cages. At natural death, they were weighed, dissected, and gross pathological changes were described. Heart, lung, kidney, liver, spleen, and tumors were fixed in Bouin's solution, sectioned, stained with hematoxylin and eosin, and examined under light microscopy.

Serum glucose was measured by the glucose oxidase method, using premixed re-

Received for publication August 12, 1974.

[1] Supported by Public Health Service Research Grant ES-00699-15, National Institutes of Health, CIBA-GEIGY Corporation, Cooper Laboratories Inc., and Foremost-McKesson Foundation, Inc.

[2] Present address: 9 Belmont Avenue, Brattleboro, Vt. 05301.

[3] Blue Spruce Farms, Altamount, N.Y.

[4] To each kilogram of diet was added (in mg): ferrous sulfate, 100; calcium pantothenate, 10; niacin, 50; pyridoxine hydrochloride, 1.0; and vitamin A, 5,000 IU; vitamin D, 1,000 IU.

TABLE 1

Weights of rats given various trace metals

Age days	Control-5	Aluminum	Tungsten	Barium	Beryllium
No. rats initially	52	52	52	52	52
Males					
30	79 ± 1.39[1]	74 ±1.35	84.7±1.56	84.6±2.64	90.1±3.13[2]
60	211 ± 3.04	220.4±0.83	218.3±2.72	216.5±0.54	186.6±3.13[2]
90	298.2± 3.98	303.2±2.76	307.3±4.93	302.6±2.23	262.2±9.5[2]
120	350.3± 4.02	352 ±3.4	364.2±4.60	341.4±0.84	315.6±1.3[2]
150	373.9± 5.17	358.2±5.06	380.2±5.27	385.2±6.4	360.2±8.7
180	409.6± 5.17	420 ±5.32	433.4±7.1[3]	421.3±7.55	383.6±9[3]
360	484.5± 6.3	511.1±1.11[4]	529.1±8.2[5]	487.6±9.55	485.8±9.3
540	501.3±11.8	547.1±3.61[6]	539.3±9.1[6]	485.9±9.75	496.9±8
No. rats at 540 days of age	49	47	32	47	49
Females					
No. rats initially	52	52	35	52	52
30	75 ± 1.59	74.6±1	77.7±4.03	81.9±1.49	89.2±6.74[2]
60	166.5± 2.50	165.8±1.52	167.5±1.68	174.6±2.16	170 ±4.41
90	200.7± 4.10	208.4±3.6	208.5±3.52	221 ±2.73	202 ±4.32
120	226.9± 3.51	237.7±4.61	235.8±4.73	245.1±5.3[2]	231.6±4.15
150	241.9± 3.15	249 ±3.37	229.1±9.13	259.1±3.38[2]	244.4±5.54
180	250.3± 4.8	257.4±4.73	265.5±3.9	289.6±7[2]	254.6±4.86
360	277.9± 5.52	284.3±5.64	297 ±6.3[6]	282 ±8.15	282.1±1.63
540	290.8± 5.52	315.4±9.7	315.2±6.5	323.5±6.2[2]	305.1±5.54
No. rats at 540 days of age	50	50	30	48	48

[1] ± SEM. [2] Significance of difference in mean value from controls by Student's *t* test, $P < 0.001$. [3] $P < 0.01$. [4] $P < 0.05$. [5] $P < 0.005$. [6] $P < 0.025$.

agents.[5] Blood samples for fasting levels were obtained from 12 rats of each sex and each group deprived of food for 18 hours. Cholesterol and uric acid were measured by premixed reagents also.[5] Urine was tested semiquantitatively by sensitized paper for protein, pH, and glucose.[6]

Tissues were not analyzed for trace metals, as the methods available to us were unsatisfactory with the equipment at hand. At 20 months of age, an epidemic of pneumonia was controlled by penicillin added to the water for 2 weeks. The epidemic killed 139 males and 113 females, fairly equally divided among the groups. In the calculations on life-span, these animals were subtracted from the total of each group.

The controls numbered 52 of each sex and were littermates of the others. They were treated exactly the same way with the same basal water without any of the abnormal metals. They have been named control-5 in the text and tables, because they were the 5th control group that we have studied for life.

RESULTS

Growth rates. Compared with the controls, aluminum did not affect growth rates in females significantly, but males were heavier after a year of age (table 1). Likewise, tungsten did not affect weights until 180 days of age in males and 360 days of age in females, when they were enhanced. Barium had no significant effect on growth of males, but increased that of older females at four intervals. The effect of beryllium on the growth of males was unique, growth being somewhat depressed from 2 to 6 months of age. There was no such effect in females. The number of intervals at which the average weights differed from the controls was as follows: aluminum 2, tungsten 4, barium 4, beryllium 6, out of 16 in each group.

[5] Searle/BMI Instruments, Worcester Medical Supply Co., Worcester, Mass. 01609.
[6] Combistix, Ames Company, Elkhart, Ind.

TABLE 2

Average heart and body weights and ratio × 1,000 at autopsy in rats given additional metals in their drinking water

Metal	No. rats autopsied	Heart weight and range	Body weight and range	Ratio mean wt × 1,000
		mg	*g*	
Males				
Controls	26	1,241 (765–2,200)	421 (425–576)	2.95
Aluminum	25	1,409 (1,070–1,886)	415 (456–635)	3.40
Barium	30	1,420 (1,022–2,260)	370 (462–569)	3.84
Tungsten	25	1,280 (1,007–1,595)	448 (486–577)	2.86
Beryllium	33	1,342 (1,040–1,982)	385 (450–538)	3.49
Females				
Controls	24	937 (585–1,772)	239 (260–326)	3.92
Aluminum	19	1,062 (681–1,368)	234 (280–377)	4.54
Barium	33	962 (548–1,339)	287 (292–369)	3.35
Tungsten	20	1,137 (750–1,330)	392 (277–353)	2.90
Beryllium	17	965 (650–1,294)	259 (273–333)	3.73

Zinc, copper, manganese, cobalt, molybdenum, and chromium were given in water of all groups. Blanching of the incisor teeth was observed in no male rats; in three control females, one in the barium group and two in the beryllium group, this sign appeared.

Blanching of the incisor teeth in older rats varied from 2.1 to 27.3% in previous series (2). In the present series it did not occur in males (table 2), but was found in 6 out of 113 females examined (5.3%).

In table 2 are shown the heart and body

TABLE 3

Life-spans of rats given various metals

Metal	No. Rats[1]	50% dead	75% dead	90% dead	Last survivor	Longevity[2]
				days		
Males						
Control-5	33 (52)	907	1,039	1,142	1,178	1,126±18.2
Barium	40 (52)	757	907	1,005	1,173	1,094±33
Aluminum	39 (52)	891	971	1,079	1,123	1,064±19.7
Tungsten	24 (37)	825	900	962	995	983± 7.3[3]
Beryllium	32 (52)	865	966	1,038	1,187	1,101±28.3
Females						
Control-5	41 (52)	841	978	1,142	1,239	1,139±29.6
Barium	46 (52)	785	999	1,075	1,152	1,106±25.6
Aluminum	38 (52)	857	993	1,104	1,186	1,150±17.3
Tungsten	25 (35)	900	943	995	1,088	1,063±22.8
Beryllium	44 (52)	892	1,025	1,167	1,464	1,250±30

[1] The numbers surviving epidemic are given; the number in parenthesis in the original number. [2] Mean of last surviving 10% or five animals. [3] Significance of difference from controls by Student's t, $P < 0.005$.

TABLE 4

Urinary abnormalities in rats given various metals

Metal	Group	Age, days	Protein +	Glucose +	pH units
Control-5	Male	162	11.5	3	7
	Female	162	14	4	6–8
Aluminum	Male	172	12.5	1.5	6–8
	Female	172	12.5	3	6–7
Barium	Male	173	22.5[1]	4	6–8
	Female	173	14.5	5	6–8
Beryllium	Male	117	13	8	6–9
	Female	117	11.5	14[2]	6–9
Tungsten	Male	162	13.5	4	6–7
	Female	162	14.5	10	6–8

The figure for protein represents the total number of pluses found by testing 12 rats in a group (out of a possible 48), with a response of ± counted as half a plus. The figure for glucose was derived in the same way. pH shows the range found.
[1] Differs from controls by Chi-square analysis, $P < 0.05$.
[2] $P < 0.025$.

weight ratios of the various groups. The smallest hearts were found in the controls and tungsten groups. Differences however were not significant.

Survival. Life-spans of these rats are given in table 3. In males, the longevity of rats given tungsten was significantly depressed. In females, barium and tungsten also showed slight increases in mortality at two intervals. Beryllium was noted for its lack of toxicity. There were no marked effects exhibited by any of the four metals.

Urinary findings. In table 4 are shown results of testing the urine with sensitized paper. Glycosuria occurred in females given beryllium. Proteinuria occurred in males given barium to a greater extent than in the controls ($P < 0.05$).

Serum glucose and cholesterol. Fasting serum glucose levels are shown in table 5. Levels declined with age in males, as occurs commonly in those given chromium (6). Less of a decline occurred in males given aluminum and barium, whereas they became elevated with age in the beryllium and tungsten groups. In females, age-linked trends were confined to the group given barium. Rats of both sexes given tungsten behaved erratically in this respect. Fasting serum cholesterol levels are shown in table 6. No trends with age are evident.

Fasting uric acid levels. Levels are shown in table 7. There were no marked changes or differences.

Tumors. Table 8 shows the number of rats in the various groups having gross tumors at autopsy, as well as the number of tumors considered malignant, because

TABLE 5

Fasting serum glucose in rats given various metals (± *SEM*)

Metal	Age, days	Males		Females	
		mg/100 ml	*P*[1]	mg/100 ml	*P*
Control-5	90	114±3.3	—	95±5.4	—
	414	101±5.5	—	90±2.1	—
	537	95±4.7	—	89±3.7	—
	769	66±6.06	—	110±4.18	—
Aluminum	97	116±2.1	—	98±2.4	—
	425	107±3.2	—	73±5.3	<0.01
	770	97±7.26	—	111±7.77	<0.005
Barium	98	122±3.1	—	77±6.8	<0.05
	532	53±9.13	<0.005	90±2.96	—
	773	96±5.0	<0.01	112±3.97	—
Beryllium	475	73±5.0	<0.001	83±4.7	—
	719	115±4.42	<0.001	107±2.93	<0.01
Tungsten	116	149±1.7	<0.001	132±3.1	<0.001
	181	74±4.2	—	90±4.8	—
	511	153±4.39	—	154±7.82	—
	525	97±2.7	—	45±5.0	<0.001
	783	140±5.44	<0.001	130±7.64	<0.001
	824	111±5.92	<0.001	90±2.57	—

[1] Significance of difference from controls at comparable age by Student's *t*. There were 12 animals in each group, at each age and of each sex.

TABLE 6

Fasting serum cholesterol in rats given various metals (±*SEM*)

Metal	Age, days	Males		Females	
		mg/100 ml	P[1]	mg/100 ml	P[1]
Control-5	90	84 ±1.9	—	74 ±4.0	—
	115	114 ±5.0	<0.005[2]	110 ±2.7	<0.001[2]
	204	89 ±4.57	—	86 ±4.52	—
	516	84 ±2.40	—	78 ±3.86	—
	769	86.7±2.55	—	77.3±4.25	—
Aluminum	97	73 ±3.5	<0.025	68 ±2.8	—
	425	62 ±3.4	<0.001	82 ±4.3	—
	770	85 ±9.17	—	92.4±6.03	—
Barium	98	77 ±2.5	—	78 ±4.1	—
	532	90 ±4.6	—	99 ±4.3	<0.001
	773	91 ±1.74	—	110 ±4.87	<0.001
Beryllium	475	90 ±5.0	—	105 ±5.3	<0.005
	719	80.2±5.17	—	96 ±4.09	<0.025
Tungsten	117	94 ±3.2	<0.01	86 ±3.7	<0.025
	180	96 ±4.1	—	106 ±6.0	<0.05
	525	73 ±2.6	<0.001	87 ±4.5	—

[1] Significance of difference from controls at comparable age by Student's *t*. There were 12 animals in each group, at each age and of each sex. [2] Differs from controls at younger age.

they were multiple in the same animal. Male rats given aluminum had more gross tumors than their controls.

Effect of epidemic pneumonia on the various groups. The number of rats dying at 20 months of age from an epidemic of pneumonia can be obtained from table 3. The mortality in males was 34.9% and in females was 22.2%. In no single group was the mortality significantly excessive. The order of frequency of mortality was: males, aluminum < barium < beryllium < tungsten; females, barium < beryllium; controls, aluminum < tungsten. The higher mortality in the tungsten-fed animals was not significantly different from that of any one of the other groups nor from all of them combined in both sexes.

DISCUSSION

The four metals studied in rats are the 14th to 17th elements so evaluated. In progress are life-term studies on mercury, titanium, and boron, and one on molybdenum has been completed. When finished these studies will provide knowledge on the innate toxicity of 21 metals and metalloids in terms of life-span and longevity (1).

The present study is important more for its negative than its positive findings. Aluminum showed a slightly elevated incidence of gross tumors in male rats but not in females. Tungsten appeared to shorten life-span somewhat in male rats. Proteinuria was slightly increased in male rats given barium ($P < 0.05$). There was some increase in glycosuria in females fed beryllium. Effects on serum lipid and uric acid levels were not observed. There was some suppression of growth in males by

TABLE 7

Serum uric acid in rats given various metals

Metal	Age	Males	Females
	days	*mg/100 g*	
Control-5	204	3.0, 2.8	1.5, 1.8
	516	3.5	2.8
	769	2.0	3.2
Aluminum	425	2.8	3.0
	770	4.0, 2.8	4.0, 2.4
Barium	532	3.0	2.8
	773	3.3	3.5
Beryllium	475	3.2	3.5
	719	4.8	3.5
Tungsten	181	2.0, 2.3	1.5, 2.3
	511	3.0, 4.0	3.8, 3.5
	783	4.0	3.0
	921	2.8	2.6

Data are based on pooled sera. Two values represent repeated analyses of a single pool.

TABLE 8

Gross tumors in rats given various trace elements

Metal	No. autopsied	Rats with tumors		P[1]	Malignant tumors	
		No.	%		No.	%
Males						
Control-5	26	4	15	—	2	50
Aluminum	25	13	52	<0.005	6	46
Barium	30	8	26	NS	6	75
Tungsten	25	4	16	NS	2	50
Beryllium	33	9	27	NS	4	44
Females						
Control-5	24	17	70[2]	—	8	47
Aluminum	19	14	73	NS	6	42
Barium	33	15	45	NS	9	60
Tungsten	20	13	65	NS	5	38
Beryllium	17	14	82	NS	8	57

[1] Significance of difference from control group by Chi-square analysis. [2] Differs from male control group, $P < 0.0005$.

beryllium. None of these effects was consistent.

These metals have not been noted for their toxicity to mammals, except for inhaled beryllium and injected barium (9, 10). Aluminum is the seventh most abundant element on the earth's crust (7). It is ubiquitous in human tissues, especially in lungs where it accumulates with age (8). Orally it has a low order of toxicity. As the leading nonferrous metal, it has some 4,000 terminal uses. It is poorly absorbed in the gastrointestinal tract, although the reference man contains about 61 mg, most of it being in the lungs (8). Large amounts fed to experimental animals may bind organic phosphate (9). For many years preparations containing aluminum have been used in large doses in the therapy of gastric hyperacidity.

Whether or not aluminum plays a necessary function in mammals has been the subject of some controversy (9). It is said to be involved in the succinic dehydrogenase–cytochrome system where it is activated in vitro. However, essentiality for any living thing remains to be proven.

Injected barium salts in animals are highly toxic, but most of them are insoluble. In experimental animals it causes prolonged contraction of all forms of muscle, probably by direct action on chromaffin cells, possibly by displacing calcium (9, 10). Very large amounts of insoluble barium sulfate are added to diesel fuel as a smoke suppressant. Inhalation of finely ground barium sulfate causes deposition in the lungs, demonstrable by X-ray, which produces few or no symptoms (10, 11).

Barium is present in the human body at about one-tenth of the concentration of strontium (8). As a member of group II A in the periodic table, barium is concentrated in bone (8). There is no evidence that barium has any essential role in the metabolism of living things.

Inhaled beryllium is highly toxic (11), and in man causes a chronic disease, berylosis, which is most difficult to treat, as beryllium is carcinogenic in human beings and in animals by inhalation (10). Osteosarcomas have been produced by intravenous injection of several beryllium salts (12). Beryllium sulfate is toxic when given by mouth (11). Beryllium rickets seen with larger doses than we use, was not observed in our animals, and beryllium ingested in foods has little toxicity (11).

Tungsten interferes with molybdenum metabolism by inhibiting xanthine oxidase. The rat, however, has an extremely low requirement for molybdenum and for tissue xanthine oxidase (9). Tungstate fed to rats at a 1,000 times the concentration of molybdenum has failed to cause reduced xanthine oxidation, although birds are susceptible to tungsten in this respect (9). Lambs have shown a growth response from supplementary molybdenum, although the mechanism has not been discovered (9).

Therefore, one expects that uric acid levels in rats would not be affected by tungsten. Under natural conditions molybdenum deficiency has never been reported in man or animals. Unlike beryllium, tungsten inhaled in the form of dust has little toxic effect (10). Large doses of sodium tungstate, 0.5 to 0.75 g, have been reported to be lethal in guinea pigs (10).

LITERATURE CITED

1. Schroeder, H. A. (1973) Recondite toxicity of trace elements. In: Essays in Toxicology, vol. 4, pp. 107–199, Academic Press, New York.
2. Schroeder, H. A., Vinton, W. H., Jr. & Balassa, J. J. (1963) Effect of chromium, cadmium and other trace metals on the growth and survival of mice. J. Nutr. *80*, 39–47.
3. Schroeder, H. A., Vinton, W. H., Jr. & Balassa, J. J. (1963) Effects of chromium, cadmium and lead on the growth and survival of rats. J. Nutr. *80*, 48–54.
4. Schroeder, H. A., Balassa, J. J. & Vinton, W. H., Jr. (1965) Chromium, cadmium and lead in rats: effects on life span, tumors and tissue levels. J. Nutr. *86*, 51–66.
5. Schroeder, H. A., Kanisawa, M., Frost, D. V. & Mitchener, M. (1968) Germanium, tin and arsenic in rats. Effects on growth, survival, pathological lesions and life span. J. Nutr. *96*, 37–45.
6. Schroeder, H. A., Mitchener, M. & Nason, A. P. (1971) Influence of various sugars, chromium and other trace metals on serum cholesterol and glucose of rats. J. Nutr. *101*, 247–258.
7. 1967 Mineral Facts and Problems (1970) U.S. Dept. of the Interior, Bureau of Mines, U.S. Government Printing Office, Washington, D.C.
8. Tipton, I. H. (1974) Gross and elementary composition of reference man. In: ICRP Report of Subcommittee II on Permissible Dose for Internal Radiation, chap. 2. Pergamon, Oxford (in press).
9. Underwood, E. J. (1971) Trace Elements in Human and Animal Nutrition, ed. 3, pp. 431–432, 425–427, Academic Press, New York.
10. Browning, E. (1969) Toxicity of Industrial Metals, ed. 2, chaps. 5 and 6, Butterworth & Co., New York.
11. Hamilton, A. & Hardy, H. L. (1974) Industrial Toxicology, ed. 3, sec. 2, chap. 5, Publishing Sciences Group, Inc., Acton, Mass.
12. Schepers, G. W. H., Durkan, T. M., Delehant, A. B. & Creedon, F. T. (1957) Biological action of inhaled Be sulfate. AMA Arch. Ind. Health *15*, 32.