Zirconium, Niobium, Antimony, Vanadium and Lead in Rats: Life term studies[1]

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ABSTRACT To evaluate innate effects of the trace elements zirconium, niobium, antimony and vanadium, and to reevaluate those of lead, 603 rats of the Long-Evans strain were fed a diet containing relatively small amounts of these elements in an environment reasonably free of trace contaminants. Groups of 100 or more divided as to sex were given 5 ppm (as metal) either zirconium, niobate, antimonite, or vanadyl ions, and 25 ppm lead (males only) in drinking water from the time of weaning until natural death, and compared with an equal number of controls. Chromium 1 ppm was in the water. These doses were tolerable for growth which was enhanced in the male niobium group. Innate toxicity in terms of life span and longevity occurred in the antimony groups. In rats given antimony, nonfasting serum glucose levels were lower than fasting, an unusual finding. Increased incidences of glycosuria occurred in the zirconium, niobium and lead groups. Serum cholesterol was abnormal in the antimony and vanadium groups. Antimony and lead accumulated in soft tissues, the former with age. No element was tumorigenic. Lead-fed males lost weight from 24 to 30 months of age and their coats were poor, the only signs of toxicity. A previous series of chromium-deficient rats given the same dose of lead showed early mortality, shortened life span and decreased longevity. Chromium may be antagonistic to lead toxicity.

Innate biological effects of small doses of trace elements in drinking water, given to mice and rats from the time of weaning until death are being studied in an environment relatively free of contaminating metals. The purposes of these time-consuming experiments are to ascertain whether an orally given element — usually a metal — has favorable or adverse effects on growth and survival or is inert biologically, and whether or not a common human chronic disease is reproduced in these animals. We have reported effects of cadmium, lead, chromium and its deficiency (1–5), arsenic, germanium and tin (6) in rats. Under study are selenium, tellurium, nickel and molybdenum. The present report concerns zirconium, niobium, antimony and vanadium. The previous study on lead was made in chromium-deficient rats, a fact of which we were not aware at that time (2); therefore it was repeated in males supplemented with chromium. All of these trace elements are found in human tissues, and have been given to mice (7, 8).

METHODS

The diet of seed rye flour (60%), dry skim milk (30%), corn oil (9%) and iodized sodium chloride (1%) with added vitamins and ferrous sulfate, the basal drinking water containing soluble salts of zinc (50 ppm), manganese (10 ppm), copper (5 ppm), trivalent chromium (1 ppm), cobalt (1 ppm) and molybdenum (1 ppm), and the environmental conditions of the laboratory have been described previously (8). To the water was added one of the following as trace metal concentration: zirconium sulfate 5 ppm, sodium niobate 5 ppm, potassium antimony tartrate 5 ppm, vanadyl sulfate 5 ppm or lead nitrate 25 ppm.[3] The diet contained 2.66 μg/g zir-

Received for publication June 20, 1969.

[1] Supported by Public Health Service Research Grant no. HE-05076 from the National Heart Institute, Contract DA 2595 from the U.S. Army, Cooper Laboratories, Inc., and the CIBA Pharmaceutical Products, Inc.

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[3] Previous reports (1, 2) have stated that the dose of lead was 5 ppm. The dose was incorrect. A typographical error in our laboratory instructions resulted in 5 times the desired dose being used, both in the experiments begun in 1960 and in those here reported which were begun in 1965. When the error was discovered, the larger amount was continued.

conium, 1.62 μg/g niobium, 3.2 μg/g vanadium and 0.2 μg/g lead. Antimony was not detected. All values are in terms of dry weight.

Random-bred pregnant female rats of the Long-Evans strain were purchased [4] and their offspring born and weaned in our laboratory. Groups of 50 or more of each sex, 4 in a cage, were given the basal water and one of the metals. Lead was given only to males. Controls received the basal water. The total number of rats used was 603.

Animals were weighed at weekly intervals from weaning time to six weeks of age, then at monthly intervals. They were disturbed for measurements of blood pressure which required anesthesia, for sampling of blood which required warming, and for cleaning their cages at weighing time. Animals dying a natural death were weighed and dissected; grossly visible tumors, other lesions, and heart, lung, kidney, liver and spleen were described and fixed in Bouin's solution. Portions of the same tissues were frozen in polyethylene bottles and later ashed and analyzed for the elements given. A low-temperature asher [5] was used for antimony; for other elements tissues were ashed at 450° in muffle furnaces. Analytical methods and their sensitivities have been reported in the study on mice (7); for lead, both colorimetric methods (2) and atomic absorption spectrophotometry were used. Extraction of antimony in 2% ammonium pyrrolidine dithiocarbamate and analysis by atomic absorption spectrophotometry improved sensitivity considerably above that previously found (7). The sensitivity of the method for vanadium was suspect; therefore, analyses for this element were not done. Methods for serum constituents, urinalysis and blood pressure have been reported in other similar studies (6). Fasting serum glucose levels were measured in animals deprived of food for 18 hours; nonfasting levels came from animals allowed free access to food.

During the 4 years of these experiments, an epidemic of virulent pneumonia struck the rat colony, killing a sizable number of animals before it was controlled by oral penicillin. Fortunately, enough rats in each group survived to continue the experiment. Numbers of rats dying during the 3 weeks of the epidemic were as follows: Males, control 19, zirconium 5, niobium 12, antimony 9, lead 22, vanadium 17; females, control 12, zirconium 4, niobium 6, antimony 3, vanadium 17. These animals were removed from the series and survival curves corrected from that time, using the smaller numbers.

RESULTS

Growth rates. None of the four metals affected growth or mature weights of females consistently (table 1). The oldest females in all but the vanadium group were significantly heavier than their controls. Males fed zirconium were heavier than the controls at three intervals and lighter at two. Males fed niobium were heavier at six intervals. Antimony, vanadium and lead had negligible effects on growth and mature weight. Therefore, niobium appeared to enhance the growth of male rats; the opposite effect was observed in mice of both sexes (7).

Survival and longevity. In table 2 are given mean and median ages and 75% and 90% life spans of the various groups. There were no significant differences from controls in the zirconium, niobium, lead and vanadium groups. Antimony, however, was innately toxic, males surviving 106 days and females 107 days less than the controls at median life spans, and 70 and 165 days less when 90% were dead. Survival curves are shown in figures 1 through 4. This toxicity of antimony was not seen in mice (7).

Innate toxicity was also demonstrated when longevities, defined as the mean age of the last surviving 10%, were calculated. In the antimony groups these ages were significantly reduced compared to control ages. The oldest antimony-fed male lived 202 days and the oldest female 152 days less than their controls. Males of the niobium group also had decreased longevity, and the last survivor was 171 days younger than his control. The opposite effect was seen in male mice (7).

Blood and urinary findings. Fasting serum glucose levels are given in table 3.

[4] BLU: (LE) strain, Blue Spruce Farms, Inc., Altamont, New York.
[5] Tracerlab 500-A, Richmond, Calif.

Weights of rats

Age	Control	Zirconi
days	*g*	*g*
Males		
30	72.1 ± 4.2 [1]	88.5 ±
60	189.5 ± 6.0	204.0 ±
90	270.0 ± 8.9	285.7 ±
120	312 ± 9.3	313.7 ±
150	341.5 ± 8.9	377.5 ±
180	364.7 ± 8.7	392.0 ±
360	443.8 ± 14.9	405.2 ±
540	507.4 ± 16.4	469.0 ±
Females		
30	64.7 ± 2.1	82.1 ±
60	154.2 ± 6.0	159.3 ±
90	197.1 ± 5.4	204.4 ±
120	225.2 ± 5.3	232.0 ±
150	238.8 ± 4.0	250.0 ±
180	250.5 ± 4.9	263.7 ±
360	262.6 ± 5.9	267.0 ±
540	262.4 ± 9.8	299.2 ±

[1] Mean ± SEM. Differences fr
notes 2, 3, 4 and 5.
[2] $P < 0.005$.
[3] $P < 0.025$.
[4] $P < 0.01$.
[5] $P < 0.05$.

Metal	No. rats	M
Control ♂	52	
♀	54	
Zirconium ♂	56	
♀	58	
Niobium ♂	52	
♀	56	
Antimony ♂	51	
♀	59	
Lead ♂	52	
Vanadium ♂	52	
♀	61	

[1] Mean ± SEM of last 10
[2] Differs from controls, P
[3] Differs from controls, P

Females fed zirconium showed somewhat elevate to their controls. The lc was in the lead group. N levels were higher than f groups but the antimony, lower. In males, differer cant in all but the vanadi female zirconium and the two glucose levels cantly different. This un sponse of serum glucose mony-fed rats of both se duplicated in groups of ra

TABLE 1

Weights of rats given zirconium, niobium, antimony, vanadium and lead

Age	Control	Zirconium	Niobium	Antimony	Vanadium	Lead
days	*g*	*g*	*g*	*g*	*g*	*g*
Males						
30	72.1 ± 4.2 [1]	88.5 ± 2.3 [2]	91.9 ± 1.4 [2]	89.3 ± 1.5 [2]	76.4 ± 3.9	55.7 ± 3.1 [2]
60	189.5 ± 6.0	204.0 ± 4.7	193.0 ± 3.7	207.7 ± 4.3 [3]	184.2 ± 6.3	171.2 ± 3.3 [4]
90	270.0 ± 8.9	285.7 ± 6.2	309.8 ± 4.3 [2]	257.7 ± 3.8	253.6 ± 9.5	265.8 ± 4.5
120	312 ± 9.3	313.7 ± 8.7	362.6 ± 4.2 [2]	312.0 ± 7.2	306.7 ± 8.7	314.0 ± 6.8
150	341.5 ± 8.9	377.5 ± 6.3 [2]	385.3 ± 5.9 [2]	334.4 ± 9.4	331.4 ± 2.7	353.1 ± 8.1
180	364.7 ± 8.7	392.0 ± 6.5 [4]	392.8 ± 5.5 [4]	350.0 ± 8.7	370.0 ± 9.3	369.7 ± 5.2
360	443.8 ± 14.9	405.2 ± 8.4 [5]	473.1 ± 6.9 [5]	453.0 ± 9.0	427.7 ± 11.7	443.7 ± 8.1
540	507.4 ± 16.4	469.0 ± 8.1 [3]	497.1 ± 11.3	475.0 ± 10.2	472.8 ± 17.3	461.5 ± 12.0 [3]
Females						
30	64.7 ± 2.1	82.1 ± 2.1 [2]	89.7 ± 0.9 [2]	82.9 ± 1.6 [2]	63.2 ± 2.7	
60	154.2 ± 6.0	159.3 ± 1.9	162.8 ± 3.3	163.5 ± 2.4	152.1 ± 3.7	
90	197.1 ± 5.4	204.4 ± 1.9	216.4 ± 3.3	199.8 ± 2.6	193.4 ± 5.9	
120	225.2 ± 5.3	232.0 ± 2.7	239.7 ± 3.3	217.6 ± 4.4	223.0 ± 6.1	
150	238.8 ± 4.0	250.0 ± 2.5 [4]	247.0 ± 3.9	238.2 ± 2.9	235.7 ± 2.7	
180	250.5 ± 4.9	263.7 ± 4.3	262.4 ± 3.1	246.2 ± 3.2	246.0 ± 9.3	
360	262.6 ± 5.9	267.0 ± 4.2	271.5 ± 7.0	283.1 ± 3.9 [2]	274.1 ± 11.7	
540	262.4 ± 9.8	299.2 ± 5.3 [2]	282.8 ± 4.3 [5]	295.2 ± 4.0 [2]	282.9 ± 10.0	

[1] Mean ± SEM. Differences from controls have been analyzed by Student's *t* test, giving the *P* values of footnotes 2, 3, 4 and 5.
[2] $P < 0.005$.
[3] $P < 0.025$.
[4] $P < 0.01$.
[5] $P < 0.05$.

TABLE 2

Survival and longevity of rats, days

Metal	No. rats	Mean age	50% dead	75% dead	90% dead	Last	Longevity [1]
Control ♂	52	819	872	974	1057	1232	1160 ± 27.8
♀	54	910	912	1050	1157	1347	1304 ± 36.0
Zirconium ♂	56	870	881	1019	1077	1189	1127 ± 23.0
♀	58	935	947	1099	1187	1291	1247 ± 17.4
Niobium ♂	52	853	892	959	1035	1061	1045 ± 4.1 [2]
♀	56	994	998	1087	1207	1342	1247 ± 21.3
Antimony ♂	51	746	766	955	987	1030	999 ± 7.8 [2]
♀	59	797	805 [3]	900	992	1195	1092 ± 30.0 [2]
Lead ♂	52	877	883	931	951	1262	1071 ± 66.0
Vanadium ♂	52	813	860	918	1091	1218	1147 ± 35.5
♀	61	922	961	1051	1170	1313	1269 ± 34.5

[1] Mean ± SEM of last 10% of animals surviving.
[2] Differs from controls, $P < 0.001$, by Student's *t* test.
[3] Differs from controls, $P < 0.025$, by chi-square analysis.

Females fed zirconium and vanadium showed somewhat elevated levels compared to their controls. The lowest male value was in the lead group. Nonfasting glucose levels were higher than fasting ones in all groups but the antimony, where they were lower. In males, differences were significant in all but the vanadium group. In the female zirconium and antimony groups the two glucose levels were not significantly different. This unusual lack of response of serum glucose to food in antimony-fed rats of both sexes has not been duplicated in groups of rats fed seven other elements, and occurred only in males given selenate and females given tellurite.

Glycosuria [6] was found in 23% of 90 controls, 43% of 23 in the antimony group, 52% of 56 in the zirconium group, 63% of 16 in the lead group, 71% of 24 in the niobium group and 12% of 17 in the vanadium group. By chi-square analysis the differences from the controls were significant in the zirconium ($P < 0.01$), the lead ($P < 0.005$) and the niobium groups (*P*

[6] Glucose in the urine was measured by Combistix (Ames Co., Elkhart, Ind.) on nonfasting mature rats about 18 months of age.

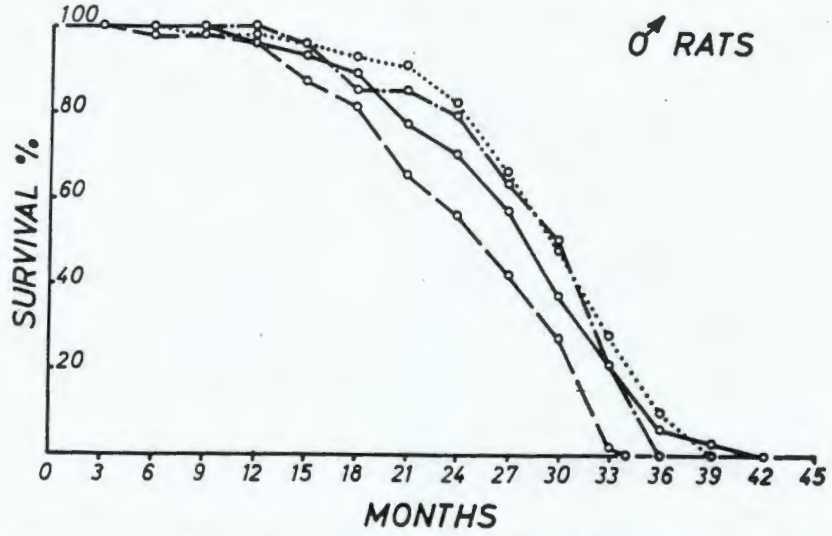

Fig. 1 Survival curves of male rats. Solid line, controls; dotted line, zirconium-fed; dot-dashed line, niobium-fed; dashed line, antimony-fed. The lessened survival and longevity of the group fed antimony is apparent, although there was only one significant difference by chi-square analysis at the 33-month interval ($P < 0.0005$). There were 52 to 56 rats in each group.

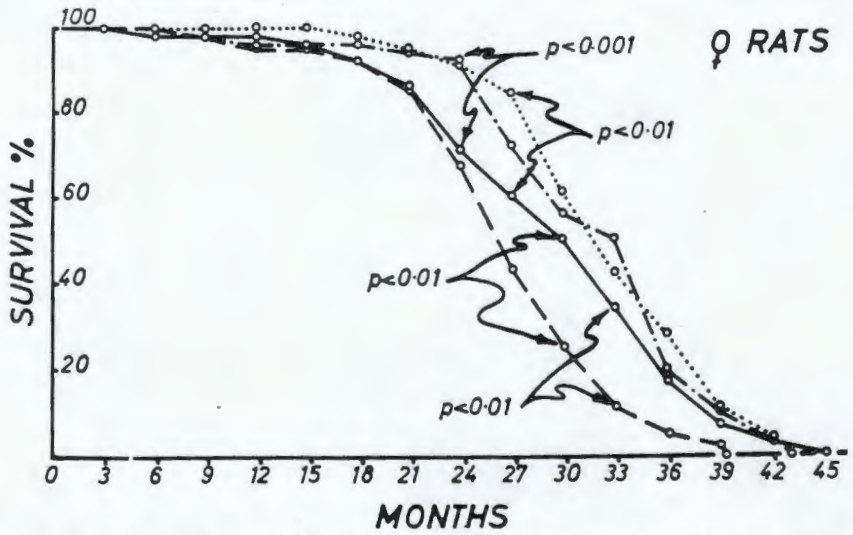

Fig. 2 Survival curves of female rats. Solid line, controls; dotted line, zirconium-fed; dot-dashed line, niobium-fed; dashed line, antimony-fed. The decreased survival of the group fed antimony is obvious; it was significant by chi-square analysis at 30 and 33 months of age. The zirconium group had a significantly lower mortality at 24 and 27 months of age, as did the niobium group at 24 months of age. There were 56 to 64 rats in each group.

< 0.0001). No significant differences in proteinuria were found between the several groups, although niobium-fed animals of both sexes and vanadium-fed females showed no samples with 3- or 4-plus protein, whereas 17 to 31% of the others had these amounts. There was half as much protein in the urine of niobium-fed females as in the controls.

Fasting serum cholesterol levels, previously published (5), are shown in table for comparison. In both sexes significant

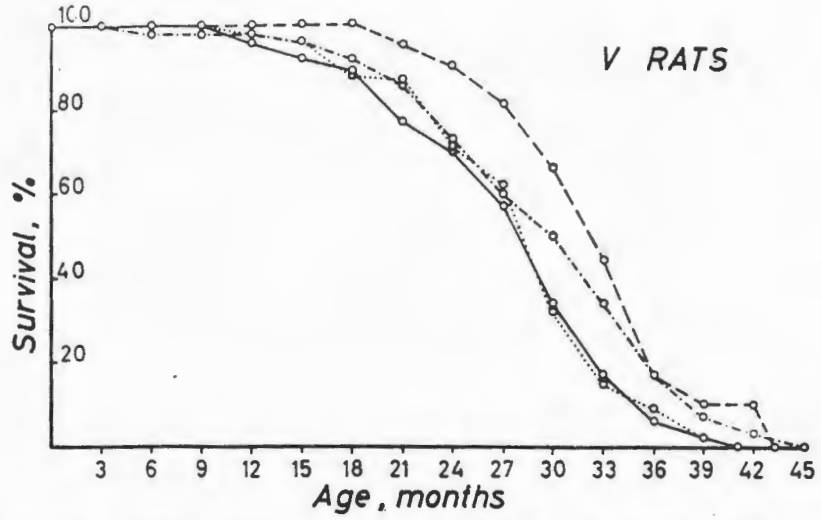

Fig. 3 Survival curves of vanadium-fed rats. Solid line, male controls; dotted line, males fed vanadium; dash-dotted line, female controls; dashed line, females fed vanadium. By chi-square analysis, the curve of the vanadium-fed females differed significantly from that of their controls at 24 months ($P < 0.01$) and at 27 months ($P < 0.05$), mortality being less. There were 52 to 61 rats in each group.

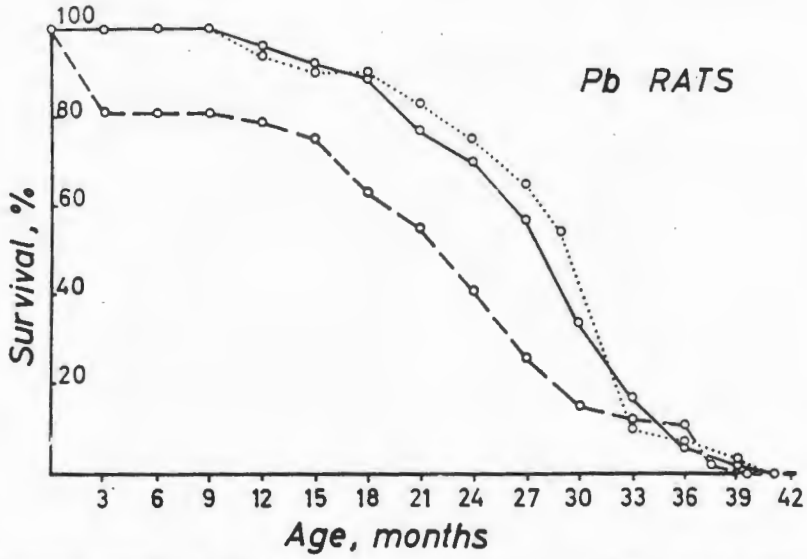

Fig. 4 Survival curves of lead-fed male rats. Solid line, controls; dotted line, present series of rats fed 25 ppm lead, with 1 ppm chromium in water of both groups. The hatched line is the curve of the previous series of lead-fed rats (2) without chromium in water, and includes the nearly 20% early mortality. This curve was significantly lower ($P < 0.05$ to < 0.0005) than that of their controls (not shown) from 18 to 30 months of age when the early mortality was excluded (2). There were 52 to 62 rats in each group.

differences from the controls occurred in the vanadium and antimony groups and in the male zirconium and female niobium groups, values being higher in males and lower in females. The high level in females was probably the result of insufficient chromium, for we have shown that 5 ppm is required for normal levels of serum cho-

TABLE 3

Serum glucose and cholesterol levels in rats fed zirconium, niobium, antimony, lead and vanadium [1]

Metal	Age	Glucose		Cholesterol
		Fasting	Nonfasting	
	days	*mg/100 ml*		*mg/100 ml*
Males				
Controls	718	106.5±3.6 [2]	134.4±5.1	77.5±2.1
Zirconium	921	106.1±9.9	133.3±4.7	89.7±5.6 [3]
Niobium	889	112.3±5.7	137.5±9.9	75.7±2.7
Antimony	852	114.9±6.8	94.5±6.2 [4]	97.6±4.9 [4]
Lead	737	81.8±3.6 [3]	147.3±5.7	86.6±6.5
Vanadium	697	107.8±8.9	121.3±5.4	91.6±5.1 [4]
Females				
Controls	698	79.6±8.2	114.2±5.4	116.0±6.0
Zirconium	921	111.4±5.6 [4]	120.5±3.3	100.7±9.0
Niobium	907	94.3±5.3	107.5±8.0	78.6±4.8 [4]
Antimony	859	86.2±5.0	82.5±7.0 [3]	97.0±5.6 [3]
Vanadium	711	96.2±2.5 [5]	116.1±5.9	67.9±9.2 [4]

[1] Differences between fasting and nonfasting levels of glucose were significant in all groups of males ($P < 0.05$ to $P < 0.001$) but the vanadium, and in the control ($P < 0.001$), niobium ($P < 0.05$) and vanadium groups ($P < 0.005$) of females. Note decline in nonfasting level in antimony group in both sexes. Data on cholesterol previously published (5). Twelve animals in each group.
[2] Mean ± SEM. Differences from comparable controls have been analyzed by Student's *t* test, giving the *P* values of footnotes 3, 4 and 5.
[3] $P < 0.01$.
[4] $P < 0.005$.
[5] $P < 0.025$.

TABLE 4

Mean heart and body weights of rats and gross tumors

Metal	No. rats autopsied	Weight at death	Heart weight	Ratio ×1000 HW/BW	Tumors	
					No.	%
		g	*mg*			
Control ♂	50	334	1498	4.49	10	20.0
♀	39	234	949	4.06	14	35.9
Zirconium ♂	46	324	1280	3.95	7	15.2
♀	53	244	1019	4.18	20	37.7
Niobium ♂	46	346	1315	3.80	7	15.2
♀	52	234	992	4.24	16	30.8
Antimony ♂	50	340	1215	3.57	6	12.0
♀	47	243	982	4.04	18	38.3
Lead ♂	43	290	1204	4.15	7	16.3
Vanadium ♂	31	320	1223	3.83	13	42.0
♀	39	232	988	4.26	15	38.4
Normal,[1] killed age 315 days ♂	5	517	1311	2.54		
♀	5	300	904	3.01		

[1] Data from Blue Spruce Farms, Inc., Altamont, N. Y.

lesterol in females, whereas 1 ppm appears to be enough for males (5).

Elevated systolic blood pressure was not found in 10 animals of each sex and each group.

Heart weights. Mean heart and body weight at death and their ratios are shown in table 4. There were no obvious differences in body weights among the various groups. The hearts of males fed zirconium, niobium, antimony and vanadium weighed 14.6%, 12.2%, 18.9% and 18.3% less, respectively, than those of the controls, whereas the hearts of females weighed 3.5 to 7.4% more.

Tumors. None of these 5 metals was tumorigenic (table 4), as evidenced by visible tumors at necropsy. There were more than twice as many tumors in females as in males ($P < 0.0001$). No metal signifi-

cantly suppressed the incidence of tumors.

Accumulation of elements in tissues. Evidence for accumulation of zirconium in rat tissues was poor (table 5). The same phenomena were observed in mice (7). Deposition of antimony in tissues was clear (table 6) as it was in mice (7), none being detected in control samples and in the diet. Furthermore, antimony accumulated with age in rats. From ages 279 to 1070 days, or about 9 to 35 months, pooled samples showed a tendency to increase in concentration, with a correlation coefficient (r) of 0.525 ($P < 0.05$). The mean concentration of antimony in five tissues of all rats analyzed was 13.1 μg/gram. No obvious accumulation of niobium occurred in rats fed this element (table 7), with the possible exception of spleen, which was not controlled. Niobium accumulated in spleens of mice (7). There was a definite, but surprisingly small, accumulation of lead in soft tissues (table 8), considering the dose used.

Blanching of the incisor teeth, which has occurred in a number of older rats,

TABLE 5

Zirconium in rat tissues, wet weight [1]

Organ, sex	Controls [2]			Fed zirconium [3]		Difference
	No. rats	Mean	No. N.D.	No. rats	Mean	
		μg/g			μg/g	μg/g
Males						
Kidney	42	10.5	4	10	9.7	−0.8
Liver	35	10.5	3	9	11.2	+0.7
Heart	39	7.8	26 [4]	10	17.2	+9.4
Lung	35	6.4	3	3	7.4	+1.0
Spleen	33	3.1	3	10	35.1 [5]	+32.0
Tumors	3	2.7	1	—	—	—
Mean [7]	—	7.8	—	—	17.7	+9.9
Females						
Kidney	38	5.4	9 [5]	37	12.5	+7.1
Liver	38	3.4	0	35	6.7	+6.3
Heart	31	7.7	5 [6]	37	9.9	+2.2
Lung	38	9.6	0	37	10.5	+0.9
Spleen	31	22.3	4	35	19.9	−2.4
Tumors	3	4.5	1	—	—	—
Mean [7]	—	9.3	—	—	11.9	+2.6

[1] Tissues were pooled in groups of 2 to 16, usually 4 to 7.
[2] Control rats were 144 to 900 days old.
[3] Zirconium-fed rats were 427 to 1172 days old. All zirconium-fed rats had zirconium in their tissues. Limit of detection of the method was 0.01 to 0.017 μg/gram wet weight. N.D., not detected. Differences between controls and zirconium-fed rats have been treated by chi-square analysis, resulting in the P values of footnotes 4, 5 and 6.
[4] $P < 0.001$.
[5] $P < 0.005$.
[6] $P < 0.05$.
[7] Excluding tumors.

TABLE 6

Antimony in rat tissues, dry weight, all ages [1]

Organ	Controls		Fed antimony		
	No. rats	Mean	No. rats	Mean	Range
		μg/g			μg/g
Kidney	9	N.D.[2]	58	10.14	4.6–34.4
Liver	9	N.D.	40	11.57	1.7–60.1
Heart	9	N.D.	66	12.10	4.0–28.0
Lung	9	N.D.	34	17.67	4.0–30.0
Spleen	9	N.D.	62	15.97	4.1–53.5

[1] Tissues were pooled in lots of 2 to 8.
[2] Not detected. Limit of detection of antimony by the method used was 0.9 μg/gram dry weight, or 0.25 μg/milliliter MIBK.

TABLE 7

Niobium in rats, wet weight [1]

Organ	Controls [2]			Fed niobium [3]		
	No. rats	No. N.D.[4]	Mean	No. rats	No. N.D.[4]	Mean
			μg/g			μg/g
Kidney	35	0	1.71	29	8	2.07
Liver	26	15	0.51	29	7	0.52
Heart	28	15	2.27	29	4	1.73
Lung	30	0	1.50	29	8	1.01
Spleen	—	—	—	29	4	7.23
Mean excluding spleen	119	30	1.53	116	27	1.33

[1] Tissues were pooled in groups of 2 to 15, usually 4 to 7.
[2] Controls were 300 to 911 days old.
[3] Niobium-fed rats were 276 to 780 days old.
[4] N.D., not detected. Limit of detection by the method was 0.1 μg/gram wet weight.

TABLE 8

Lead in rat tissues,[1] wet weight

Organ	Controls		Fed lead	
	No. rats	Mean	No. rats	Mean
		μg/g		μg/g
Kidney	21	0.50 [2]	40	2.65 [3]
Liver	22	0.64	30	2.06
Heart	12	0.82	25	1.28 [2]
Lung	26	1.24	33	1.04
Spleen	15	2.31 [2]	23	1.04 [2]
Mean	96	1.06	151	1.71

[1] Tissues were pooled in lots of 2 to 7.
[2] In control rats, lead was not detected in 2 kidneys and 2 spleens. In lead-fed rats, it was not detected in 5 hearts and 4 spleens. Limit of detection by the method was 0.05 μg/gram.
[3] Differs from controls by Student's t test, $P < 0.01$.

was found in the various groups with the following frequencies: zirconium 7, niobium 5, antimony 5, lead 9, vanadium 9 and controls 12. It was somewhat more prevalent in females than in males (9.7% and 6.0%, respectively).

DISCUSSION

In studies such as these we are attempting to evaluate some of the trace elements found in the human environment, human food and human tissues which have no known physiological roles and thus may be considered "abnormal," pending further investigation. We may obtain clues to biological activity, favorable or adverse, by examining growth, survival, longevity, tumorigenesis and certain serum constituents; microscopic sections may reveal pathological changes.

The present study and the previous one on mice (7) reveal no evidence that zirconium as fed has any biological activity, except possibly to affect body weight of older animals inconsistently. Nor was there good analytical evidence that zirconium was consistently absorbed by rats. A logical explanation was suggested by Blumenthal.[7] Zirconium sulfate in water solution olates to hydrous zirconia, $ZrO_2 \cdot H_2O$, in a long chain, just as do chromic salts; this complex is insoluble and probably nonabsorbable. Animals do absorb zirconium from food where it probably exists as a soluble chelate; it is probably broken down to insoluble and inert hydrous zirconia in tissues, in particles with diameters of 20 to 50 A.

Niobium as niobate was probably absorbed and excreted readily in the urine, as occurs in man (9). Its effect on body weight of male rats is unexplained. In terms of longevity, it appeared to be toxic also to males.

Antimony was more toxic to rats than to mice (7) in terms of survival and lon-

[7] Blumenthal, F. N., personal communication.

gevity. This element was tolerable for growth and its innate toxicity did not appear until later life, indicating that growth is not always a valid index of the adverse effects of an element. There appeared to be disturbances in glucose and cholesterol metabolism associated with its ingestion, but no signs of injury to the heart, as might be expected with larger doses (10).

No toxicity was demonstrated by tetravalent vanadium, according to any of the measurements made in this study. Growth, body weights and survival were almost identical to or slightly above the controls.

In these chromium-supplemented rats, lead was not toxic to males in terms of growth and survival. Older rats, however, appeared to have lost weight; their coats showed considerable loss of hair and they were sluggish in their movements. At 2 years of age, they weighed 421 ± 11.7 g (40 g less than at 1.5 years), whereas controls weighed 508 ± 13.1 g ($P < 0.005$); at 30 months of age their weight was 378 ± 32.0 g compared to the weight of the controls 444 ± 15.8 g ($P < 0.05$). At 3 years of age mean weights did not differ significantly. A similar difference in weight was observed in the former series of chromium-deficient lead-fed male rats (2) at 2 years of age ($P < 0.005$). There was a remarkable resistance to accumulation of lead in the soft tissue of these rats given 25 ppm in water; although bone was not analyzed, this tissue probably stored lead, as 91% of the lead in the human body is in bone. Whether or not the first series of lead-fed rats can be compared with the second done 5 years later is problematical; however, the diet and regimen were identical, the rats were of the same type, the environment had not altered and controls for both series had similar life spans and no early deaths. If the profound differences in mortality between the two series can be accepted, it appears that chromium protects against innate lead toxicity.

According to Browning (10), zirconium has a very low order of toxicity, whereas niobium is chronically toxic in doses more than 100 times those given here (11), inhibiting hepatic succinic dehydrogenase (12). In acute experiments vanadyl ions suppressed hepatic cholesterol synthesis (13); in our chronic ones depressant effects on circulating cholesterol were confined to female rats. Vanadium pentoxide in drinking water was highly toxic to rats at 49 μg/milliliter (14): the valence state of this metal may influence its toxicity. Effects of antimony potassium tartrate have been extensively studied (10); in rats oral doses up to 100 mg/kilogram body weight did not influence growth but consistently injured the heart (15). According to our experiences, doses calculated at 350 μg/kilogram were innately toxic; larger ones have shortened life spans (15).

To compare innate toxicities of these five elements with others up to half life spans, median ages at death of the six groups previously reported (2, 6) and the nine included here and to be reported later are shown in table 9. The amounts of chromium supplemented in the water are also indicated. In both sexes, the elements showing this early type of toxicity (early compared to the whole lives of the animals) were selenite, as reported (16); lead, reported in the first series (2); germanium (6); antimony and cadmium (2). Similar toxicities were observed in mice. No toxicity relative to the controls occurred with chromium, selenate, niobium, the second lead series (the present paper), zirconium, vanadium, tellurite, arsenite, or nickel. Tin was toxic to female rats and mice, but not to males. These data concern oral ingestion of the elements, and do not apply to elements absorbed into the lungs from polluted air or injected parenterally.

Median ages of male control rats in the three series differed by 106 and 18 days, and in the females by 33 and 34 days (table 9). The rats for the first series and the first lead, chromium and cadmium groups were obtained from a different supplier [8]; the remainder came from the present one.[9] Possibly males of the first strain were naturally long lived.

If any of the five trace elements in this study are essential for rats and mice, they are required at concentrations less than those present in our diet. There is no evidence, however, that antimony and lead are essential elements. Unfortunately, rel-

[8] Rockland Farms, New City, N. Y.
[9] See footnote 4.

TABLE 9

Median ages of rats fed various trace elements [1]

Element	Chromium in water	Male		Female	
		No. rats	Median age	No. rats	Median age
	ppm		*days*		*days*
Control I [2]	0	52	978	52	945
Selenate	5	49	964	55	1002
Chromium	5	54	922	54	950
Niobium	1	48	892	50	998 [3]
Lead II [2]	1	52	883	—	—
Zirconium	1	56	881	57	947
Tin	1	55	876	56	830 [3]
Control II [2]	1	53	872	80	912
Vanadium	1	52	860	61	961
Control III [2]	5	55	854	46	878
Tellurite	5	52	844 [3]	52	894 [3]
Arsenite	1	53	825 [3]	55	912 [3]
Cadmium	0	69	822 [3]	58	805
Nickel	5	52	822	51	928
Antimony	1	54	766	61	805
Germanium	1	55	738 [3]	52	833
Lead I [2]	0	62	729 [3]	60	727
Selenite	5	48	60 [3]	53	342 [3]
Total		971		953	

[1] All elements were given in water at 5 ppm, except for selenite and selenate at 3 ppm, tellurite at 2 ppm, and lead at 25 ppm.
[2] Meaning of Roman numerals: I refers to (2), II to the present paper, III to results yet to be published.
[3] Toxic to mice in terms of survival and longevity.

atively large amounts of zirconium, niobium and vanadium occurred naturally in the diet, the last two largely in corn oil; therefore, we have no evidence for or against essentiality of these three elements.

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