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Toxic Effects of Trace Elements on the Reproduction of Mice and Rats

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Breeding mice and rats were exposed to low doses of six trace elements in drinking water in an environment controlled as to contaminating trace metals. Each group was carried through three generations. Compared to control mice given only doubly deionized water, selenate (3 ppm selenium) resulted in excess deaths before weaning, runts, and failures to breed. Lead (25 ppm) and cadmium (10 ppm) resulted in loss of the strain in two generations with many abnormalities. Molybdate (10 ppm molybdenum) was slightly toxic in this respect, and arsenic resulted only in elevated ratios of males to females. In rats, lead was very toxic, and titanium and nickel moderately toxic, resulting in many early deaths and runts. This method provides fairly rapid estimates of innate toxicities of trace elements in doses tolerable for growth and survival.

TERATOGENIC effects of certain trace elements have resulted when the element was injected into pregnant animals. Thus, cadmium sulfate has produced congenital abnormalities and resorbed embryos when doses of 2.0 mg/kg of body weight were injected into hamsters on the eighth day of pregnancy.[1] Orally administered selenium compounds are teratogenic, and livestock feeding on seleniferous grasses have had dead or deformed offspring.[2] Lead has been known to be toxic in this respect for many years, and injections into hamsters have caused specific abnormalities.[3] Injected arsenic was likewise teratogenic.[4]

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Innate toxic effects of 22 elements in 26 forms have been studied in this laboratory by giving small doses of soluble salts in drinking water to mice for their lifetimes (H. A. Schroeder, MD, unpublished data).[5-7] Likewise, 14 elements have been evaluated in rats.[8-10] In this way, effects on growth, survival, tumors, and longevity were ascertained. The experiments, however, were time-consuming, each lasting several years. A more sensitive and more rapid test of innate toxicity might become manifest by exposing breeding mice and rats to single elements in drinking water for several generations. Therefore, three elements with demonstrated innate toxicities, selenium, lead, and cadmium, and four elements without demonstrable long-term toxicity, arsenic, nickel, titanium, and molybdenum, were studied in mice or rats.

Methods

Mice of the Charles Rivers CD strain and rats of the Long-Evans BLU:(LE) strain were born in a laboratory especially designed and constructed to avoid environmental contamination from trace metals.[11] The diet used was composed of untreated seed rye flour (60%), dried skim milk (30%), corn oil (9%), and iodized sodium chloride (1%), with added iron and vitamins.[11] Drinking water from a forest spring was doubly deionized. To it were added single elements as soluble salts (ppm element): selenate, 3; arsenite, 5; lead, 25; molybdate, 10; cadmium, 10; nickel, 5; and titanate, 5.

The diet contained the following elements (ppm wet weight): zinc, 23.2; copper, 1.95; manganese, 12.25; cobalt, 0.18; molybdenum,

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0.45; selenium, 0.056; and chromium, 0.16; as well as the abnormal elements, arsenic, 0.06; lead, 0.20; cadmium, 0.10; nickel, 0.31; and titanium, 1.12.

Five pairs of mice or rats were randomly selected for each element from divided litters at the time of weaning, placed in separate cages and given the element in drinking water continuously. Mice were allowed to breed as often as they would up to 6 months of age and rats up to 9 months of age or longer. At weaning time, pairs were randomly selected from the first litter (F_1A generation), from the second litter (F_1B), and sometimes from the third (F_1C) and allowed to breed as they would to produce the F_2 generations. Surplus surviving animals were discarded at the time of weaning, after ascertaining the sex.

Pairs were likewise selected at random from the first F_2A litters and allowed to breed the F_2A generation, and sometimes from the second litter for the F_2B generation. Experiments were terminated when the strain was obviously dying out or when three generations had been weaned. Control animals received plain deionized water and were treated similarly.

Criteria used to estimate innate toxicity were the intervals between litters, the age at which the pair produced its first litter, the ratio of males to females, and the numbers of runts (animals with large heads and small bodies), deaths, stillborn offspring, failures to breed, and congenital abnormalities.

The doses of trace elements in drinking water were those which we have found, by lifetime experiments, not to affect the rates of growth of mice and rats as compared to controls, not to affect reasonably long-term survival or cause early mortality, and, except in the case of lead, not to affect longevity. In these doses, arsenic, nickel, molybdenum, and titanium showed no innate toxicity; cadmium induced arterial hypertension; lead shortened life span; and selenate produced malignant tumors (H. A. Schroeder, MD, unpublished data).[5,12]

Results

Effects of Selenate, Arsenite, Lead, Molybdate, and Cadmium in Mice.—Control mice bred normally for four generations, with average litters of 10 to 11 offspring (Table 1). There were no stillbirths, one maternal death, seven deaths of young before weaning, two runts, and no congenital abnormalities. There were no signs of deterioration as this strain became inbred. The average age of a pair at birth of the first litter was 64 days, and for F_2 and F_3 litters, 62 to 64 days. The average intervals between litters were 28 to 30 days, and the ratios of males to females were 0.94 to 1.03.

In the cases of mice given lead, the strain died out rapidly, so that by the second generation there were insufficient numbers to continue (Table 2). Only eight litters, two of them stillborn, appeared in the F_1 generation and only two in the F_2. There were nine deaths of young mice and 69 runts out of 72 live offspring (95.6%) in the F_1 generation, of which four grew enough to breed at 70 to 75 days of age.

Selenium was toxic according to these criteria. The strain did not begin to die out until the F_3 generation, which produced only three litters with 23 animals, of which 16 were runts. Seven pairs failed to breed. In all, there were 23 young deaths, one stillborn litter, 93 runts out of 389 live offspring (23.9%) and one maternal death. The male-female ratios ranged from 1.27 to 1.65 in the various subgroups.

Cadmium was also toxic to breeding mice, with the strain not lasting beyond the second (F_2B) generation. A congenital abnormality, sharp angulation of the distal third of the tail, appeared in five litters, comprising 4 of 255 live offspring (16.1%) of the F_1 and F_2A generations. There were also 34 runts (13.3%) in the offspring living beyond weaning, 87 deaths before weaning (30.5%), two maternal deaths, and two dead litters. Three of five pairs failed to breed in the F_2B generation, and the experiment was discontinued.

Molybdenum in the dose used was moderately toxic, in that there were 15 early deaths of 238 offspring in the F_1 generation (6.3%), seven early deaths of 242 offspring in the F_2 generation, and five dead litters with one maternal death in the F_2 generation. The F_3A generation of four pairs died out, as two failed to breed and there were only two litters, one of 11 runts and one of two males. The F_3B generation had one failure to breed, one dead litter and mother, 67 offspring of which 13 died, and a litter with only one male. The F_3C generation had 21 deaths of 43 offspring, three dead litters, and three maternal deaths. The experiment was discontinued.

Table 1.—Summary of Breeding Experiments in Mice Exposed to Trace Elements

	Control	Selenium	Arsenic	Lead	Molybdenum	Cadmi
F_1 generation						
No. of litters	19	16	19	8	21	14
Pair age at first litter, days	64	67	70	70.1	64.4	80.0
Interval between litters, days	30	34	28	48	30	34.6
Average litter size	11.0	12.3	8.2	12.0	11.3	13.1
M-F ratio	0.94	1.50	0.93	1.05	1.05	1.0
F_2 generation						
No. of litters	23	17	25	2	26	11
Pair age at first litter, days	64	54	69	73	66	69
Interval between litters, days	28	33	32	...	43	56
Average litter size	10.3	10.0	9.6	11.5	10.3	9.2
M-F ratio	1.03	1.44	1.30	0.92	1.32	0.7
F_3 generation				Discontinued		Discontin
No. of litters	22	3	7		14	
Pair age at first litter, days	62	54	63		79	
Interval between litters, days	28	...	22		25	
Average litter size	10.5	7.6	8.1		8.8	
M-F ratio	1.00	1.30	1.71		0.90	

Table 2.—Deaths and Abnormalities of Mice Bred While Exposed to Trace Elements

	Control	Selenium	Arsenic	Lead	Molybdenum	Cadmium
F_1 generation						
Maternal deaths	0	0	0	0	0	0
Dead litters	0	1	0	2	0	0
Young deaths	0	13*	1	9†	15‡	39†
Failures to breed	0	2	0	0	0	0
Runts	0	36*	0	69†	0	25†
No. of mice	209	197	147	72	238	184
F_2 generation						
Maternal deaths	1	1	0	0	1	2
Dead litters	0	0	0	0	5	2
Young deaths	6	10	7	0	7	48†
Failures to breed	0	2	1	1	1	3
Runts	2	41†	0	0	2	9*
No. of mice	248	169	290	23	242	101
F_3 generation				Discontinued		Discontinue
Maternal deaths	0	0	0		4§	
Dead litters	0	0	0		4§	
Young deaths	1	4	0		34†	
Failures to breed	0	3	0		3	
Runts	0	16†	0		11†	
No. of mice	230	23	57		123	
Total No. of mice	887	389	494	95	603	285

* Differs from controls by χ^2 analysis; $P < 0.0005$.
† $P < 0.0001$.
‡ $P < 0.001$.
§ $P < 0.05$.

The mice fed arsenic survived well through the third generation. There were no runts in 494 progeny, eight young deaths, one failure to breed, and no maternal deaths. The ratio of males to females increased from 0.93 to 1.71 (Table 1). The only other abnormality noticed was a reduction in size of the litters, with the mean size in each successive generation being 8.2, 9. and 8.1 mice. In fact, eight of 51 litte (16.0%) had 3 to 5 mice, compared to fo of 61 in the molybdenum group (7.4%), o of 64 in the controls (1.6%), 3 of 36 in t selenium group (8.3%), none of eight in t lead group, and none of 25 in the cadmiu group. The number of small litters in mi

Table 3.—Summary of Breeding Experiments in Rats Exposed to Nickel, Titanium, and Lead

	No. of Litters	Pair Age at First Litter, Days	Interval Between Litters, Days	Average Litter Size	M-F Ratio
F_1 generation					
Control	10	89	41	11.4	1.14
Nickel	11	101	42	11.0	1.20
Titanium	11	87	49	9.4	1.43
Lead	19	139	43	9.1	0.68
F_2 generation					
Control	10	87	40	11.3	1.10
Nickel	15	98	38	10.5	1.18
Titanium	16	87	39	10.9	0.99
Lead	32	92	38	10.3	1.15
F_3 generation					
Control	11	86	41	11.0	1.06
Nickel	10	92	30	8.1	0.44
Titanium	2	88	---	8.0	0.60
Lead	6	93	30	8.0	0.91

fed arsenic differed from that in the controls ($P < 0.05$).

Effects of Lead, Nickel, and Titanium on Breeding of Rats.—Lead was toxic to breeding rats, but to lesser degree than it was to mice (Table 3 and 4). Birth of the first litters was somewhat delayed. In the first generation there were 12 deaths before weaning, two maternal deaths, 40 runts, and a male-female ratio of 0.68, in 19 litters of 173 offspring. The five pairs of the F_2A generation produced 12 litters of 133 rats, of which 20 died and five were runts. In the F_2B generation there were 13 litters of 132 rats, with ten runts and seven deaths. Four of their five matings produced two F_3B litters of 22, with two deaths, one dead litter, and one failure to breed. This line was, therefore, discontinued. The F_2C generation had four of five successful matings, with seven litters of 46 rats, one dead litter, eight deaths, and 11 runts. Three litters had only three to four rats each. Two pairs of F_2C rats failed to reproduce, and this experiment was discontinued.

Nickel was less toxic than lead in respect to breeding, with 9.1% young deaths, one maternal death, and 80.6% runts in the first generation; 10.2% young deaths and 5.1% runts in the second; and 21.0% (17) young deaths and 6.2% (5) runts in the third. The size of the litters decreased somewhat with each generation, and, with two failures to breed, the number of rats was reduced. Few males were born in the third generation.

Titanium was nearly as toxic as nickel,

Table 4.—Deaths and Abnormalities in Rats Bred While Exposed to Nickel, Titanium, and Lead

	Control	Nickel	Titanium	Lead
F_1 generation				
Maternal deaths	0	1	0	2
Dead litters	0	0	0	2
Young deaths	0	11*	1	12†
Failures to breed	0	0	0	1
Runts	0	37‡	23‡	40‡
No. of rats	114	121	103	173
F_2 generation				
Maternal deaths	0	0	1	0
Dead litters	0	0	0	1
Young deaths	0	16‡	25‡	35‡
Failures to breed	0	2	4	3
Runts	1	8	14†	26§
No. of rats	113	157	174	311
F_3 generation				
Maternal deaths	0	0	0	0
Dead litters	0	0	0	1
Young deaths	1	17‡	0	2
Failures to breed	0	0	0	1
Runts	0	5†	6‡	4§
No. of rats	121	81	16	22
Total No. of rats	348	359	293	506

* Differs from controls by χ^2 analysis; $P < 0.005$.
† $P < 0.0001$.
‡ $P < 0.025$.
§ $P < 0.01$.

with a marked reduction in the numbers of animals surviving to the third generation. In the three generations there were 8.9% young deaths, 16.4% runts in the offspring living beyond weaning, and four failures to breed. Only two litters appeared in the third generation. The male-female ratio was progressively reduced. The controls continued to breed for four generations, with rare deaths and runts occurring.

Comment

These experiments provide a much more sensitive method for detecting toxicity of an element than feeding the element for life. Thus, selenate increased the incidence of spontaneous tumors and malignant tumors in mice and rats, when fed for a lifetime, without altering life span (H. A. Schroeder, MD, unpublished data). Lead shortened life span at this dose (25 ppm) without interfering with growth[5,8]; the effect on breeding mice and rats was unexpected. Cadmium feeding to rats resulted in arterial hypertension.[12] Injection of cadmium resulted in toxemia of pregnancy,[13] and, in males, testicular atrophy has been induced.[14] Toxic effects of cadmium can be antagonized by zinc or selenium.[1] Partial breeding out of the strain given molybdenum was unexpected, for no adverse metabolic effects have resulted from this dose fed to rats for life. The minor effect of arsenic was consiste with the absence of effects in rats and m fed this dose for life.[8,9] Nickel fed for l was not toxic to rats (H. A. Schroeder, M unpublished data) or to mice,[6] nor was tanium toxic to mice.[5]

Therefore, feeding of these trace elemen resulted in relative toxicities in the follo ing order: lead > cadmium > selenium nickel, arsenic, titanium, molybdenum. E posure of breeding mice and rats resulted the following relative toxicities: lead = ca mium > selenium > nickel > titaniu > molybdenum > arsenic.

Developing males in utero are believed to be more vulnerable to toxic substances tha are developing females. In these experi ments, however, the ratio of males to fe males born was increased in mice exposed t selenium and arsenic, compared to controls This ratio became reduced in mice exposed to cadmium and in rats exposed to titanium and nickel. The mechanisms of these alterations are unexplained.

From these experiments it is clear that certain trace elements fed to rats and mice in doses which do not interfere with growth or survival are intolerable for normal reproduction.

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