[CANCER RESEARCH 29, 892–895, April 1969]

Ref 0454

Life Term Studies on the Effect of Trace Elements on Spontaneous Tumors in Mice and Rats[1]

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SUMMARY

Rats numbering 830 provided with a diet low in many trace elements and raised in an environment relatively free from contamination were given small amounts (5 ppm) of arsenite, germanate, stannous, chromic, cadmium, or lead ions in drinking water for their lifetimes. Mice numbering 540 taking the same diet were given 5 ppm of zirconium, antimony or niobium, or 10 ppm fluorine (as fluoride), for life. Sections of five tissues and gross tumors were made. Compared to controls, significant differences in the incidences of spontaneous tumors and malignant tumors did not appear. Tumors other than in lung, liver, adrenal, and mammary gland were significantly fewer in the germanium-fed rats. None of the trace metals was carcinogenic or tumorigenic in the doses given.

INTRODUCTION

In the process of exposing small mammals during their life times to low doses of various trace elements in drinking water (9, 12), we observed some suppression of spontaneous tumors in mice given arsenite and germanate, with no demonstrable effect of stannous or vanadyl ions (4). The present report concerns rats given the same diet and the same doses of arsenite, germanate or stannous ions, as well as trivalent chromium, cadmium or lead. In addition, mice were exposed for their lifetimes to zirconium, niobate, antimony, and fluoride ions. The results were essentially negative in that suppression or enhancement of tumors by any of these elements did not occur.

MATERIALS AND METHODS

The conditions of the experiment and the relatively metal-free environment have been reported in previous publications (12, 13). White Swiss mice of the Charles River Strain (CD-1) numbering 540 were born from random-bred pregnant females and, at the time of weaning, were divided according to sex into groups of 54, six to a cage. To their doubly deionized drinking water, which contained the essential trace elements chromium, manganese, cobalt, copper, zinc, and molybdenum as soluble complexes (4, 12), were added 5 μg/ml zirconium as the sulfate, 5 μg/ml niobium as sodium niobate, 5 μg/ml antimony as the potassium tartrate, or 10 μg/ml fluorine as sodium fluoride (11). An equal number of animals served as controls. The diet of seed rye flour (60%), dried skim milk (30%), corn oil (9%) and sodium chloride (1%) contained (μg/gm wet weight) zirconium (2.66) and niobium (1.62); antimony and fluorine were not detected.

Random-bred rats of the Long Evans strain numbering 830 were born in our laboratory from pregnant females purchased from the supplier (Rockland Farms, New York, N. Y., for those given cadmium, chromium, and lead; Blue Spruce Farms, Altamont, N. Y., for those given arsenic, germanium, and tin). From the time of weaning, groups of 50 or more of each sex, four to a cage, were given one of the following trace metals at 5 ppm in drinking water: sodium arsenite, sodium germanate, stannous chloride (10), cadmium acetate, chromium (III) acetate, and lead acetate (9). The water contained the same essential trace metals as were given to mice. The same diet contained (μg/gm wet material): arsenic, 0.46; germanium, 0.32; tin, 0.28; cadmium, 0.02; chromium, 0.10; lead, 0.19.

Two series of experiments on rats were made, each taking four years. In the first, on chromium, cadmium, and lead, data on which have been reported (9), chromium acetate was given only to one group, the controls and others being considered marginally deficient in this element. In the second, on arsenic, germanium, and tin, 1 ppm chromium as the acetate was added to the water, for this trace metal was found to be a growth factor for rats and mice, promoting longevity (9) and inhibiting spontaneous aortic atherosclerosis in older rats (6).

Dead animals were weighed and dissected, gross lesions were recorded, and tissues were fixed in Bouin's solution. Sections were stained with hematoxylin and eosin and examined under light microscopy. Only visible tumors were sectioned (4). Necropsies were done on 489 mice and 686 rats, some of which showed postmortem autolysis to the extent that tissues were not fixed. Sections were made on 370 mice and 534 rats. Numerical data were treated by Chi-square analysis and by Student's *t* test. Criteria for grading microscopic lesions as *pretumorous*, *benign* tumors, and *malignant* tumors were the same as used in our previous report (4).

[1]Supported by USPHS Grant HE 05076, U. S. Army Grant Contract 2595, the American Cancer Society, and the CIBA Pharmaceutical Company.

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Received September 20, 1968; accepted December 2, 1968.

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RESULTS

Mice

The numbers, types, and major locations of tumors found are shown in Table 1. No significant differences from the control group appeared.

Of the 63 benign epithelial tumors, 47 were adenomata of lung and 11 of liver. Carcinomata were found in 10 lungs, one liver and four mammary glands. There were only four nonepithelial benign tumors: one myoma, one fibroma, and two mammary fibroadenomata. Of the 14 malignant tumors of this type, there were five leukemias, three lymphomata, two fibrosarcomata, two reticulosarcomata, one seminoma, and one osteosarcoma. No element enhanced or suppressed any type of tumor significantly.

The life spans of these mice are indicated in Table 2. Males fed fluorine survived one or two months longer than did the controls, whereas those fed zirconium survived a month or more less. At two intervals, female mice had shorter life spans when given zirconium, niobium, and antimony than their controls. These differences were not reflected in the incidences of tumors.

Weights of mice at three intervals are given in Table 3. Older fluorine-fed females weighed somewhat more than did their controls, and older niobium-fed females weighed less. None of the elements appeared to affect the weights of males significantly.

Table 1

	Control	Zirconium	Niobium	Antimony	Fluorine
No. of mice	71	72	79	76	72
Type of tumor					
Epithelial					
Benign	16	10	11	10	16
Malignant	4	4	0	4	3
Nonepithelial					
Benign	0	0	2	2	1
Malignant	4	1	5	2	2
Pretumorous, liver	1	0	2	0	2
Total lesions	25	15	20	18	24
Location of tumor					
Lung	15 (3)	9 (3)	11	10 (2)	12 (2)
Liver	4	3 (1)	0	1	4
Mammary gland	1 (1)		2	3 (2)	2 (1)
Other	4 (4)	3 (1)	5 (5)	4 (2)	4 (2)
Total tumors	24 (8)	15 (5)	18 (5)	18 (6)	22 (5)
% with tumors	33.8	20.8	22.8	23.7	30.5

Types of tumors in mice fed zirconium, niobium, antimony, or fluorine for life. Numbers in parentheses indicate number malignant.

Table 2

Element given	Male 50% dead	Male 75% dead	Male 90% dead	Female 50% dead	Female 75% dead	Female 90% dead
Control	570	637	692	625	745	770
Zirconium	543	599	645	558	660	800
Niobium	563	603	787	560	644	752
Antimony	582	626	651	576	659	742
Fluorine	599	682	752	630	707	789

Life spans (days) of mice given trace elements.

Table 3

	60 days (gm ± S.E.)	180 days (gm ± S.E.)	540 days (gm ± S.E.)
Males			
Control	38.8 ± 0.33	51.5 ± 1.38	57.0 ± 1.95
Zirconium	39.4 ± 1.01	51.4 ± 1.16	51.3 ± 2.23
Niobium	38.1 ± 0.38	51.1 ± 0.67	51.8 ± 1.71
Antimony	38.3 ± 0.48	51.6 ± 1.01	51.6 ± 2.51
Fluorine	38.6 ± 1.16	53.6 ± 1.05	54.8 ± 1.36
Females			
Control	28.5 ± 0.62	44.8 ± 0.72	55.5 ± 1.60
Zirconium	31.5 ± 1.13	44.0 ± 0.91	52.0 ± 1.15
Niobium	29.5 ± 0.29	42.0 ± 1.26	45.2 ± 1.17[a]
Antimony	29.0 ± 0.43	43.6 ± 1.12	53.1 ± 1.97
Fluorine	29.4 ± 0.62	48.8 ± 0.90[a]	59.6 ± 2.24[b]

Weights of mice given various trace elements at selected ages.
[a]Differs from controls, $P < 0.005$.
[b]Differs from controls, $P < 0.01$.

Fatty degeneration of the liver was found in all groups, but it was more prevalent in the niobium-fed mice than in the others. Of 58–99 livers examined in each group, this lesion occurred in 22.2% of the controls, 15.9% of the antimony group, 22.4% of the fluorine group, 25.5% of the zirconium group, and 43.7% of the niobium group ($P < 0.005$).

Of the elements fed to mice, fluorine was not detected in soft tissues although it was undoubtedly present in bone. Much zirconium was found in the tissues of controls and zirconium-fed animals, 10–64 μg/gm wet weight in heart, lung, kidney, liver, and spleen and in two or four tumors, without significant differences between groups. Niobium accumulated in heart and spleen to levels of 11–16 μg/gm in those fed the element, with 0.2–1.7 μg/gm in those not so fed. Antimony was not found in the controls, but levels of 6–14 μg/gm were found in those fed the element, being especially elevated in kidney, lung, and spleen. These data have been reported (11).

Rats

The number of rats with benign tumors, *pretumorous* lesions, and malignant tumors are given in Tables 4 and 5. Unlike mice (4), arsenic-fed rats had no significantly decreased incidence of tumors but did show a rather high proportion of pretumorous lesions in the livers (20.9%), whereas those fed germanium and tin had a low proportion (5.2%) of this type of lesion. The difference was significant ($P < 0.001$).

There was a surprisingly large number of adrenal tumors in the group fed chromium (17.9%) as well as of tumors other than hepatic or mammary types (Table 5). Rats in this group had more tumors of all kinds than did those given lead ($P < 0.025$), possibly due to their increased longevities (9). The first tumor appeared at 29 months of age, compared to 14 months in the control group and 23 months in the lead group.

The predilection of rats to develop nonepithelial tumors is evident in all groups. There were 5 myosarcomata, 11 fibrosarcomata, and 5 reticular cell sarcomata of the 29 malignant nonepithelial tumors, the remainder arising from bone (two),

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Table 4

	Control	Arsenic	Germanium	Tin
No. of animals	82	91	98	94
Type of tumor				
Epithelial				
Benign	2	8	2	3
Malignant	2	1	1	1
Nonepithelial				
Benign	20	14	21	20
Malignant	7	2	1	4
Pretumorous, liver	10	19[a]	5	5
Total lesions	41	43	30	34
Location of tumor				
Lung	0	1 (1)	0	0
Liver	1	5	1	0
Mammary gland	4	7	7 (1)	12
Adrenal	9 (1)	4	13	7
Other	17 (8)	8 (2)	4 (1)[b]	10 (5)
Total tumors	31 (9)	25 (3)	25 (2)	29 (5)
% with tumors	37.8	27.4	25.5	30.8

Types of tumors in rats fed arsenic, germanium, and tin for life. Numbers in parentheses indicate number malignant.
[a]Differs from germanium and tin groups, $P < 0.0001$.
[b]Differs from controls, $P < 0.005$.

Table 5

	Control	Cadmium	Chromium	Lead
No. of animals	34	47	56	32
Type of tumor				
Epithelial				
Benign	1	3	2	1
Malignant	0	2	0	1
Nonepithelial				
Benign	7	12	19	4
Malignant	2	5	7	1
Pretumorous, liver	8	8	8	5
Total lesions	18	30	36	12
Location of tumor				
Lung	0	1	0	1 (1)
Liver	1	2	1	0
Mammary gland	2	7 (1)	7 (1)	3
Adrenal	2	5 (1)	10 (1)	2
Other	5 (2)	7 (5)	10 (5)	1 (1)
Total tumors	10 (2)	22 (7)	28 (7)	7 (2)[a]
% with tumors	29.4	46.8	50.0	21.9

Types of tumors in rats fed cadmium, chromium, and lead for life. Numbers in parentheses indicate number malignant.
[a]Differs from chromium group, $P < 0.025$.

salivary gland (one), adrenal medulla (two), and lymphoid tissue (three). There were only eight carcinomata, two from lung, one each from breast, pancreas, intestine, stomach, and adrenal cortex, and an undifferented type. No metal appeared to affect any particular type of malignant tumor. Of the 100 benign nonepithelial tumors, 42 were in the mammary glands (often reaching enormous size and sometimes weighing more than the remainder of the carcass) and 37 were in the adrenal medulla. Of the 21 benign epithelial tumors, 10 were in liver, four in adrenal cortex, three in ovary or prostate, and the rest in other locations.

The growth rates of those rats given cadmium, lead, and chromium have been reported (13). Chromium was a growth stimulant, both at 1 ppm and 5 ppm in water, and animals taking it were significantly larger at a year and at 21 months of age. Weights of rats given arsenic, tin and germanium at three selected ages are shown in Table 6. There were no significant differences at any period, except for the group fed germanium at 18 months of age.

The survival rates of these rats are indicated in Table 7. Although life spans of animals fed cadmium and lead were shortened compared to their controls, the rats given cadmium had more tumors (Table 5). Shortening of life span also occurred in germanium-fed rats. Ten to 12 animals of each group lived more than 1000 days, and one arsenic-fed rat lived for 52.5 months without a tumor appearing.

DISCUSSION

The intakes of the trace elements given in water to mice can be roughly calculated, at 7.0 ml of water/100 gm body weight/day, as 35 μg in the cases of zirconium, niobium, and antimony and 70 μg in the case of fluorine. The additional amount in food, at 6.0 gm/100 gm weight/day, would supply 16 μg zirconium and 9.7 μg niobium. Therefore based on these data, it is doubtful that any of these four trace elements can be considered carcinogenic when taken orally. Human equivalent doses would amount to 24.5 mg (49.0 mg in the case of fluorine) or more daily from the water alone and are much higher than the normal or usual intakes of these elements.

Deodorant sticks containing zirconium have produced granulomas of the skin of the axilla (3); similar lesions were produced in the lungs of rabbits by exposure to zirconium in a mist (5). In animals, niobium has been found to inhibit hepatic succinic dehydrogenase (2). To our knowledge, life-term experiments with these elements have not been done previously although carcinogenic effects in man have not been suspected.

The daily intake of drinking water by rats of this strain, measured for two years, was 6.8 ± 0.5 ml/100 gm body weight for males and 7.5 ± 0.3 ml/100 gm for females. As the trace metals were given in doses of 5 μg/ml, males consumed 34 μg and females 37.5 μg/100 gm/day of each metal, or 12.4 and 13.7 mg per year in water respectively. The yearly amount in food, at an estimated daily ingestion of 6 gm/100 gm body weight, varied according to the element from 110 μg in the case of arsenic to 700 μg of germanium. All of these trace metals accumulated to some extent in rat tissues (9, 10). Levels of arsenic up to 300 ppm in blood and tissues were found in older rats (10). None of these metals appeared to be tumorigenic or carcinogenic, nor were the incidences of specific tumors affected significantly, except perhaps in the germanium-fed group.

The approximate daily human intake of the trace metals studied has been measured and calculated as follows (mg): arsenic, 0.9; germanium, 1.5; tin, 4.0; cadmium, 0.2; chromium, 0.2; and lead, 0.3; there were wide variations in arsenic, tin, cadmium, and lead. The intake by these rats, however, usually far exceeded even the largest human intakes on a comparable weight basis, amounting to approximately 25 mg daily for a 70-kg man. Only in the cases of tin, where 40 mg/day for

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Table 6

	60 days (gm ± S.E.)	180 days (gm ± S.E.)	540 days (gm ± S.E.)
Males			
Control	190 ± 6.0	365 ± 8.7	507 ± 16.4
Arsenic	185 ± 8.8	380 ± 7.2	479 ± 11.0
Germanium	193 ± 11.2	363 ± 8.6	455 ± 8.7[a]
Tin	181 ± 8.2	355 ± 11.3	463 ± 10.5[b]
Females			
Control	154 ± 6.0	251 ± 4.9	267 ± 9.8
Arsenic	148 ± 7.4	242 ± 5.0	274 ± 5.4
Germanium	132 ± 5.4	243 ± 4.5	301 ± 9.2[c]
Tin	149 ± 6.7	241 ± 3.8	283 ± 5.2

Weight of rats fed arsenic, germanium, and tin at selected ages.
[a] $P < 0.01$.
[b] $P < 0.025$.
[c] $P \leq 0.005$.

Table 7

Metal given	Male 50% dead	Male 75% dead	Male 90% dead	Female 50% dead	Female 75% dead	Female 90% dead
Control	872	979	1052	895	1050	1167
Arsenic	825	917	1093	912	1064	1175
Germanium	738	902	1018	833	932	1044
Tin	876	930	1022	830	998	1096
Control	987	1016	1083	945	1036	1156
Cadmium	822	996	1134	805	902	1016
Chromium	922	1099	1143	950	1099	1189
Lead	729	903	1069	727	925	1069

Life spans (days) of rats given various trace metals.

an adult man could be calculated in extreme cases (8), and of arsenic, where the maximal allowable limits in food could provide 4.8 mg/day (7), were the rat and human intakes of the same relative order of magnitude.

Therefore, from our present data and that previously reported (4), the oral ingestion of any one of the following trace elements cannot be considered carcinogenic in the doses given: arsenite, stannous tin, germanium, vanadyl vanadium, fluoride, zirconium, niobate, antimony, cadmium, chromium (III), and lead. Two of these elements, germanium and arsenite, appeared to suppress certain tumors in mice, whereas none exhibited such an effect in rats.

Byron *et al.* (1), continuously fed rats large amounts of arsenate and arsenite (15.63–250 ppm). At high dosage levels, enlargement of the common bile duct was frequently found, a result of fibrosis and inflamatory changes. No effects on the incidence of tumors, benign or malignant, at the various levels of dosage were observed. It is apparent that arsenite is not carcinogenic to rats and mice when fed for their life times.

ACKNOWLEDGMENTS

We are indebted to Professor Kurt Benirschke for the sections and to Marian Mitchener for care of the animals.

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