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Chronic Toxicity Studies

II. Hexavalent and Trivalent Chromium Administered in Drinking Water to Rats

ROBERT D. MacKENZIE, Ph.D.; RICHARD U. BYERRUM, Ph.D.; CLARENCE F. DECKER, Ph.D.; CARL A. HOPPERT, Ph.D., and ROBERT F. LANGHAM, Ph.D., East Lansing, Mich.

Acute toxicity of chromium by topical exposure or inhalation resulting in ulcer-like lesions has been recognized as an industrial hazard for many years.[1] Prolonged exposure to chromate mists has been suggested as contributing to the high incidence of lung cancer in certain industries.[2,3] Acute poisoning by ingestion is well documented.[4,5] Animal studies have also been made to determine symptoms of acute toxicity.[6] However, few investigations have been made on chronic toxicity of ingested chromium. The longest previous study involved periods of two to three months in which 134 parts per million (ppm) or more was shown to produce toxic symptoms in rats.[7]

Extensive industrial use of chromium and disposal of chromium wastes have resulted in contamination of many water supplies in the United States. In one survey, the chromium content of 24 municipal water supplies ranged from 0.001-0.04 ppm,[8] and well waters have been reported to contain up to 25 ppm of chromium.[9] At present the maximum allowable chromate concentration in potable water supplies is 0.05 ppm expressed as chromium.[10] This is assumed to be well below the lowest harmful level and is supported by the observation that a family used water containing 1 ppm of chromium for three years with no apparent ill effects.[9]

Since so little is known about the hazards of prolonged ingestion of small amounts of chromium, water containing up to 25 ppm was given to rats for a period of one year.

Methods and Materials

In the first of two experiments, Sprague-Dawley albino rats, 34 days old, were arranged into six groups. The control group (I) was given distilled water, and Groups II to VI were given water containing 0.45, 2.2, 4.5, 7.7, and 11 ppm of chromium (as K_2CrO_4) respectively. Except for the control group, which contained 10 rats of each sex, each group consisted of 8 males and 8 females. The rats were then grown and examined for pathological changes in both blood and tissues as described in the preceding paper.[11]

In the second experiment, rats were divided into three groups, each composed of 9 females and 12 males. Group VII, the control group, received distilled water. Group VIII was given water containing 25 ppm of chromium as potassium chromate, and Group IX was given 25 ppm chromium in the form of chromic chloride. All other experimental conditions were the same as outlined previously,[11] except that no animals were killed at six months.

Livers, kidneys, and femurs from rats killed at six months were analyzed for chromium. In addition to these, spleen was analyzed in animals killed at 12 months. A weighed sample of each tissue was wet-ashed on a hot plate in 250 ml. Phillips beakers covered with watch glasses using concentrated sulfuric acid to char and then concentrated nitric acid to oxidize the organic matter. Saltzman's micromethod[12] was used for chromium analysis.

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Departments of Chemistry and Pathology, Michigan State University.

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Present address: Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill. (Dr. C. F. Decker); Biochemistry Department, Wm. S. Merrell Company, Cincinnati (Dr. R. D. MacKenzie).

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TABLE 1.—*Chromium Concentration in Tissues of Rats Ingesting K_2CrO_4 in Drinking Water for One Year*

Group No.	Ppm Cr in Drinking Water as CrO_4	Liver μg. Cr/Gm. Wet Tissue		Kidney μg Cr/Gm. Wet Tissue		Bone μg Cr/Gm. Wet Tissue		Spleen μg Cr. Gm. Wet Tissue	
		Male	Female	Male	Female	Male	Female	Male	Female
I	0				0.25±.02		0.72±.08		
II	0.45	0.02±.002 *	0.08±.007	0.14±.007	0.39±.04	0.58±.04	0.76±.04	0.95	0.91±.11
III	2.2	0.08±.017	0.17±.03	0.29±.02	0.48±.07	1.27±.06	1.48±.04	0.68±.18	1.14±.1
IV	4.5	0.15±.04	0.47±.06	0.45±.17	1.09±.13	2.14±.25	2.44±.25	3.41±.44	4.48±.71
V	7.7	0.70±.04	0.55±.06	3.30±.03	2.39±.09	3.43±.83	5.10±.35	5.24±.20	4.73±.8
VI	11.2	1.22±.06	1.62±.14	4.40±.36	3.98±.32	3.84±.49	6.06±.58	9.91±.83	11.1 ±.86

* ± values are standard errors.

Results

The chromium content of kidney, liver, spleen, and femur are given in Table 1. At concentrations 0-5 ppm chromate ion, relatively little chromium was found in any of the tissues. However, when the animals received between 5 and 10 ppm in drinking water, an appreciable increase in concentration in all the tissues examined occurred. The spleen contained significantly greater quantities of chromium than the other tissues. However, no significant differences in weight, food, and water consumption, or blood analyses between any of the experimental groups and the control group were observed. In the microscopic study of tissue sections, no significant changes were found that could be associated with ingestion of chromium.

Tissue chromium concentrations shown in Table 2 indicate that the group receiving chromate had an average tissue chromium content about nine times greater than that found in the group receiving trivalent chromium.

Even at these higher intakes of chromium no significant differences in weight gains and food consumptions were found among the groups. Neither gross changes in appearance nor pathological changes in blood or other tissues were observed.

Water intake, however, was decreased in animals receiving hexavalent chromium since females drank 77% and the males 84% as much as the controls.

Mortality due to respiratory infection occurred in both experiments, but there was no evidence that chromium had any influence on the prevalence of the respiratory infection.

Comment

Visek and co-workers[13] showed that hexavalent chromium, when injected intravenously, was excreted more rapidly than trivalent chromium. The fact that tissues from rats given hexavalent chromium contained nine times the amount in tissues of rats given trivalent indicates that hexavalent chromium is absorbed to a much greater extent. Consequently, hexavalent chromium must tentatively be considered potentially more hazardous than trivalent chromium when ingested. Reduction of hexavalent chromium to the trivalent form would appreciably decrease the potential hazard resulting from disposal of chromate wastes, as judged from study of 25 ppm chromium.

TABLE 2.—*Chromium Concentration in Tissues of Rats Given 25Ppm of K_2CrO_4 or $CrCl_3$ in Drinking Water for one Year*

Group No.	Ppm Cr Condition	Kidney μg Cr/Gm. Wet Tissue		Liver μg Cr/Gm. Wet Tissue		Bone μg Cr/Gm. Wet Tissue		Spleen μg Cr/Gm. Wet Tissue	
		Male	Female	Male	Female	Male	Female	Male	Female
VII	0-Control	0.28±.04 *	0.35± .05	0.05±.004	0.08±.012	0.77±.07	0.78± .09	0.90± .08	0.91± .078
VIII	25-Cr †	10.47±.97	13.79±1.4	3.2 ±.31	8.12± 74	4.07±.29	8.71±1.36	23.53±1.7	52.85±3.9
IX	25-Cr ‡	1.71±.29	2.07± .31	0.35±.045	0.53±.017	1.03±.2	1.23± .073	3.22± .45	3.88± .17

* ± values are standard errors.
† Administered as K_2CrO_4.
‡ Administered as $CrCl_3$.

No toxic symptoms were observed in any of the groups fed low concentrations of chromium over a period of one year, although quite high concentrations were found in the tissues. Apparently, tissues can accumulate considerable quantities of chromium before pathological changes result. Since both sexes ingested equal amounts of water, the chromium intake, in relation to body weight, was obviously greater in the females. This probably accounts for the higher tissue chromium concentrations in the females. The establishment of a higher allowable concentration than the present 0.05 ppm of chromium in water for human consumption will have to be postponed until further work on the metabolism of chromium has been done.

Summary

Five groups of rats were given water containing between 0.45 and 11 ppm of chromium as chromate ion for a year. No differences were observed between these groups and the controls as to water intake, food consumption, or weight gain; nor did an analysis of blood at monthly intervals or examination of tissues at six months or a year show any significant differences between any of the groups given chromium and the control group.

Kidney, liver, and femur were analyzed for chromium at the end of six months, and these plus spleen were analyzed at the end of one year. There was an abrupt rise in tissue chromium concentration when rats ingested water having concentrations above 5 ppm of chromate ion.

Two groups of rats were given water containing 25 ppm of chromium as hexavalent and trivalent chromium, respectively, for one year. No toxic symptoms were observed in either group. However, tissue concentrations of chromium were approximately nine times higher in the group given hexavalent chromium. This suggests that chromate ion is absorbed to a much greater extent than trivalent chromium at this level.

Wm. S. Merrell Company, Cincinnati (Dr. MacKenzie).

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