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Biological activity and fate of trace quantities of intravenous chromium(III) in the rat

WALTER MERTZ, EDWARD E. ROGINSKI, AND RICHARD C. REBA
Divisions of Biochemistry and Nuclear Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C

MERTZ, WALTER, EDWARD E. ROGINSKI, AND RICHARD C. REBA. *Biological activity and fate of trace quantities of intravenous chromium (III) in the* rat. *Am.* J. Physiol. 209(3): 489–494. 1965.—Complexes of trivalent chromium were injected intravenously into rats raised on a low-chromium diet. Two hours after the injection of submicrogram amounts, impaired glucose removal rates were increased to near-normal values. The response of epididymal adipose tissue to insulin in vitro, as measured by the production of $C^{14}O_2$ from labeled glucose, was significantly enhanced in the chromium-injected animals. The amounts required for these effects were approximately 1/10,000 of doses producing acute toxicity. The whole-body disappearance of intravenously injected submicrogram amounts of chromium could be described by a multiexponential expression. Compartmental-type analysis revealed at least three regression rates. These rates were unaffected by previous dietary history with regard to chromium and were independent of absolute amounts injected. Intestinal absorption of chromium ranged around a few percent of the dose, regardless of amount applied, and was independent of dietary history.

chromium(III) metabolism glucose metabolism trace elements trace metals

COMPLEXES OF TRIVALENT CHROMIUM restore impaired glucose tolerance of chromium-deficient rats to normal 18hr after administration by stomach tube (20). Similar effects were observed in various in vitro systems in which the element increased glucose uptake, lipogenesis (13), and galactose entry rates (11), as well as swelling rates of liver mitochondria (4). Subsequently, the results of polarographic experiments suggested that chromium facilitated the tissue-insulin interaction by participating in the formation of a ternary complex (5).

Although these studies contributed to the knowledge of the mode of action of chromium at the cellular level they gave no information as to the quantitative aspects in the intact animal. For example, the problem of intestinal absorption of chromium compounds and its dependence on the chemical structure is poorly understood. While some complexes (chromic hydroxides) are believed not to be absorbed at all, others do get into the organism, as shown by observation of their biological effect and by the rise of tissue chromium levels on oral administration. It is not known what fraction of an oral dose is actually effective within the organism. Therefore, it was the purpose of the studies reported here to measure biological effects of intravenously injected chromium in two systems, to determine the doses required for these effects, and to compare the biologically effecuve dose levels with those producing acute symptoms of toxicity. The second part of the studies was designed to measure the absorption and excretion of doses found biologically effective and to detect any possible control mechanisms for these functions. To obtain such quantitative information appeared important in the light of experimental evidence accumulating from different sources (2, 8, 12, 16, 17) which implicates chromium as a dietary factor of considerable nutritional interest.

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EXPERIMENTAL PROCEDURES

Male Sprague-Dawley rats of the Walter Reed strain were maintained from weaning on a 30% Torula yeast ration low in chromium (19). The animals were kept in individual wire-mesh cages with free access to diet and water, and were treated according to the principles of laboratory animal care as promulgated by the National Society for Medical Research. All glassware used in these studies was washed by ultrasound and rinsed with dilute hydrochloric acid, triple-distilled water, and triple-distilled, deionized water. The latter was used to make up all solutions. Glucose tolerance tests, toxicity studies, and the injections of chromium 51 were performed on rats fasted overnight. Glucose tolerance tests were conducted as previously described (14), except that glucose was determined on 20-μl samples obtained at 15, 30, 45, and 60 min after the glucose injection. A modified case method was used (23) for the in vitro glucose oxidation assay. Rats were randomly selected to receive one of three dose levels of chromium by intravenous injection or to serve as controls. Two hours after the injection, the test

animals and a corresponding number of control rats were killed by decapitation and five pieces of epididymal fat tissue from each were incubated without and with four levels of insulin in a medium containing glucose 1-C^{14}. The details of the assay are described elsewhere (12). Chromalum solutions were prepared fresh daily as described (13); the hesa-urea chromium chloride was kindly synthesized for us by Dr. C. L. Rollinson, University of Maryland. A stock solution was prepared in water and used when 2–4 weeks old. The volume of all intravenous injections of chromium, made up in 0.9% NaCl solution, ranged around 0.2 ml/100 g body weight.

Chromium 51 chloride ($Cr^{51}Cl_3 \cdot 6H_2O$) in acidic solution, specific activity approximately 126 mc/mg chromium (Volk Radiochemical Co., Chicago, Ill.), was diluted to volume (see above) and administered as described in the tables. Stable chromium carrier solutions were prepared fresh immediately before injection. The animals were counted for radioactivity in a small animal counter (Packard Instrument Co.) with proper corrections applied for background and for natural decay of chromium 51. In the first experiment, four rats (140–160 g) on a laboratory chow were administered 0.1 ml/100 g of a chromium 51 solution (100 μc/μg, 100 μc/ml) intravenously. After allowance for complete intravascular mixing (10–15 min), the animals were counted, and all subsequent counts were expressed in percent of this initial activity. Whole-body retention was plotted against time in days on semilogarithmic coordinates. The curve was reduced to a minimum of three exponential components by simple graphic analysis. Specifically, the slowest component was obtained first by a least-squares best fit of the terminal linear portion of the plot. Additional components were obtained by serial subtraction in the usual manner and the slopes of subsequent terminal portions again obtained by the least-squares method. The compartmental constants and the half-times of disappearance were likewise available from these calculations. In a separate study, a chromium-deficient and a chromium-supplemented rat were similarly assayed for radioactivity remaining following the intravenous injection of 0.2 ml (10 μc Cr^{51}) containing 1 μg of chromium. On day 9 following this initial injection, the rat on the chromium-supplemented diet received 0.5 μg of nonlabeled chromium; the chromium-deficient rat was injected with 100 μg on day 27. Another group was followed for 20 days after intravenous injection of 0.15 and 1.1 μg of Cr^{51}, 100 g; one of these rats was loaded with 1,000 μg of nonlabeled chromium 3 days prior to the Cr^{51} injrction.

TABLE 1. *Effect of chromium(III) on glucose tolerance of rats on a low-chromium diet*

No. of Rats	Supplement,* μg Cr/100 g	Glucose Removal Rates† (% Excess Glucose/min)	
		Before	After supplement
6	None	1.8±.2	2.5±.3‡
6	0.1	1.9±.3	3.2±.6‡
6	0.25	1.7±.3	3.5±.5§
6	0.5	1.8±.3	3.3±.3§

Twenty-four rats with previously established low glucose removal rates (before) were divided into four groups for application of four treatments (supplement). Glucose tolerance tests were repeated 2 hr following intravenous injection of chromium. Thus *each* animal served as its own control. * Intravenous injection (0.2 ml/100 g) as neutralized chromalum 2 hr before glucose tolerance test. † Mean ± SE. $Difference between "before" and "after" not significant: $P > 0.05$. § Difference between "before" and "after" significant: $P < 0.01$.

No. of	Supplement,*	mμmoles/100 mg Tissue†			
				1,000	2,000
22	None	0±4	3±4	24±6	47±11
a	0.01	(−6)	4±4	28±7	53±10
10	0.05	6±3	32±8‡	57±14§	115±24‖
10	0.1	0 r 3	12±8	29±6	66±14

§

The insulin used in these studies (lot no. 765666) was obtained from Eli Lilly & Co., Indianapolis, Ind. through the courtesy of Dr. O. Behrens.

RESULTS

The results of the intravenous glucose tolerance tests are shown in Table 1. The average glucose removal rate of 1.8%/min, measured in 24 rats on the Torula yeast diet, is approximately one half of rates found in animals raised on chromium-sufficient rations. These low rates were significantly increased in the subsequent test when from 0.25 to 0.5 μg of chromium was injected intravenously. The apparent increase in the control animals not receiving chromium is nor statistically significant ($P > 0.05$); it may reflect normal fluctuations. A comparison of the effect of 0.25 with that of 0.5 μg suggests that a plateau of activity is reached with the higher dose. The rates of 3.5%/min produced by these doses are close to those found in rats on natural or synthetic, chromium-mfficient rations (15). This finding is in accordance with results of previous in vivo and in vitro studies in which chromium supplementation restored impaired functions to close to normal but not beyond. Furthermoat, the results in Table 1 indicate that the effect of chromium is obtained within 2 hr or less and that the 18-hr period required for observation of activity on stomach tubing is not directly related to the mechanism of action. A comparison of the required intravenous

doses (0.25–0.5 μg) with those effective on stomach tubing (20–50 μg) indicates that approximately 1/100 of the oral dose is sufficient to elicit an effect when introduced directly into the blood stream.

In the following experiment, reported in Table 2, the result of intravenous chromium injections on the response of epididymal fat tissue to insulin was measured. Previous studies had shown that dietary supplementation and also direct in vitro addition of chromium enhanced the effect of small doses of insulin on various parameters o glucose metabolism. Therefore, if the observed effect o intravenous chromium on glucose tolerance in vivo was to be considered physiological, an increase of the tissue response to insulin in vitro would also have to be demonstrated. This was indeed the case. In the control animals, assayed concurrently with the injected rats, the response of fat tissue to four doses of insulin was small and the slope of the dose-response curve quite flat. Injection with 0.01 μg of chromium/100 g did not produce an increase, but five times this amount led to a significantly greater response to the hormone, particularly when 100 μU of insulin were given. Here, as in the in vivo experiments described above, a further increase in the amount of chromium did not result in greater activity; indeed, the high level was less effective than the 0.05-μg dose. The amounts of chromium required in this in vitro system were less than those found effective in the intravenous glucose tolerance test in vivo. This may be related to a greater sensitivity of adipose tissue to chromium than that of the other tissues participating in glucose removal in the intact rat, or the hexa-urea comples used for the in vitro studies may possess a higher biological activity than the hexa-aquo complex.

A similar, though less pronounced, difference in activity between forms of chromium was observed when toxic amounts were injected. The hexa-urea compound was more toxic than the hexa-aquo preparation. However, with both materials acute toxic symptoms appeared only with milligram amounts, thus establishing a ratio of therapeutic-to-acute toxic doses of approximately 1:10,000 (Table 3). Lethal doses usually caused convulsions and death within a few minutes following the injection.

Finally, some aspects of chromium metabolism were studied with the use of chromium 51 hesa-aquo chloride ($Cr^{51}Cl_3 \cdot 6H_2O$). It is unlikely that this is the form normally entering the blood stream under physiological conditions but, as is shown below, it appears to be handled in the same way as chromium absorbed through the intestines. In the first experiment total body retention after intravenous injection of 0.1 μg/100 g was assayed in four rats for 72 days (Fig. 1). At this time, 13.5% of the injected dose remained. In this interval there appear to be at least three major factors determining rates of elimination, each probably representing multiple physiochemical processes mathematically integrated to define the over-all chromium concentration regression. Whole-body retention of chromium as a function of time could be represented by the general polynomial,

TABLE 3. *Acute toxicity of intravenous chromium(III) in rats*

No. of Rats	Dose, mg Cr/100 g	No. of Dead	% Dead
	A: chromalum		
4	1.0	0	0
6	1.5	2	33
4	2.0	3	75
	B chromium(III) hexa-urea chloride		
6	0.5	0	0
6	0.75	1	17
6	1.0	3	50
6	1.25	6	100

$C_t = A_1e^{-k_1t} + A_2e^{-k_2t} + \cdots A_ne^{-k_nt}$, where C_t is the concentration at any time, t; A_1, A_i, and A, are the respective fractions of the total amount of chromium injected which appear to be regressing at different rates, k_1, k_2, and k_n. At least three distinct regression terms could be demonstrated and are defined by the equation $C_t = .44e^{-1.4t} + .33e^{-.12t} + .25e^{-.009t}$, where C_t is the percent of the dose remaining at any time t (days) after intravenous injection.

The second part of this study is graphically displayed in Fig. 2, the semilogarithmic plot of the percent of whole-body retention for 41 days following the intravenous administration of 10 μc and 1 μg of chromium in defficient and supplemented rats. The handling of chdomium was similar in both. It is further apparent that the superimposed "burdens" administered on *days* 9 and 27 in no way affected for retention curves of the previously injected chromium. Further evidence for the independence bf fractional excretion rates from the absolute levels of the element in the organism is presented in Table 4. Doses of 0.15, 1.1, and 11 μg superimposed on a previous dose of 1,000 μg chromium 52 were excreted with an almost identical pattern.[1]

The findings that previous dietary history does not influence the excretion mechanism for chromium raised the question whether a control mechanism might perhaps exist at the level of intestinal absorption which would be able to reject the element, depending on the saturation of the animals. The results of experiments in which chromium 51 was given by stomach tube (Table 5) do nor support this possibility Regardless of the dose given, and regardless of previous dietary chromium supplementation, the fractional rates of loss of whole-body activity were not significantly different. The slight apparent difference observed when 1 μg was given, during *days 6–10*, does not mean much in view of the low counts (about 1% of initial activity) which were not much above the background, and taking into account the various degrees of coprophagy of the rats which may have influenced the data. It is difficult to judge from these figures what percentage of a given dose was actually absorbed, but a comparison of the remaining activity after 4–10 days with that following an intra-

[1] These results were obtained with freshly prepared, i.e., little-olated or nonolated chromium solutions. It is possible that the degree of alation may influence the turnover and metabolism of these compounds.

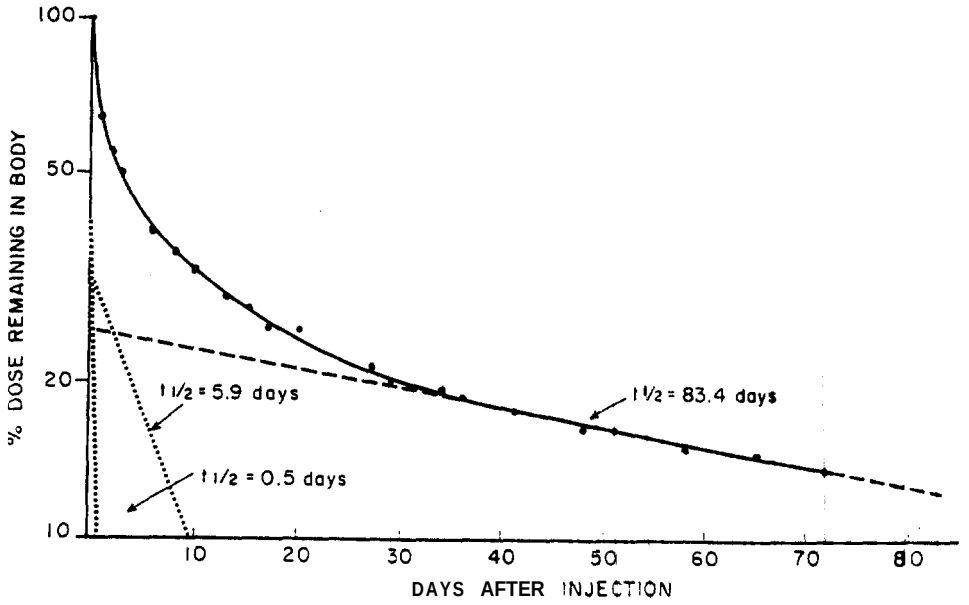

FIG. 1. Whole-body retention, as percent of initial count, of intravenously iniected chromium (0.1 μg/100 g, as $Cr^{51}Cl_3 \cdot 6H_2O$). Average of 4 rats.

venous injection would suggest that only approximately 2-3% of the oral dose did enter the organism from the intestines.

DISCUSSION

In the preceding part, biological effects of submicrogram amounts of chromium(III) on glucose metabolism were described for an in vivo and in vitro system, various aspects of the metabolism of such amounts were reported, and a comparison was made between biologically effective doses and those producing acute toxic symptoms. The data present evidence that passage of chromium through the gastrointestinal tract is not a requirement for biological activity and that the organism is well capable of transforming intravenously injected chromium into a (hypothetical) active form. That such a transformation may occur is suggested by the fact that two different compounds produce qualitatively similar effects. The quantitative difference between the activity of the hexa-urea and the hexa-aquo compounds which was detected not only in the physiological but also in the toxic range, may be related to the rate at which this transformation takes place. Both compounds become polynuclear complexes in aqueous solution, in which an unknown number of chromium ions are joined by OH bridges. The relation of the degree of olation to biological activity is being investigated.

The therapeutic: toxic dose ratio of approximately 1 :10,000 classifies trivalent chromium among the safest elements, in accordance with previous studies (1-3, 6). On the other hand, it shows that very small amounts are biologically effective, and that contamination from various sources can seriously disturb biological assays involving chromium. The narrow range of doses effective when injected intravenously was not surprising, as similar results have been observed in other systems (11, 131.

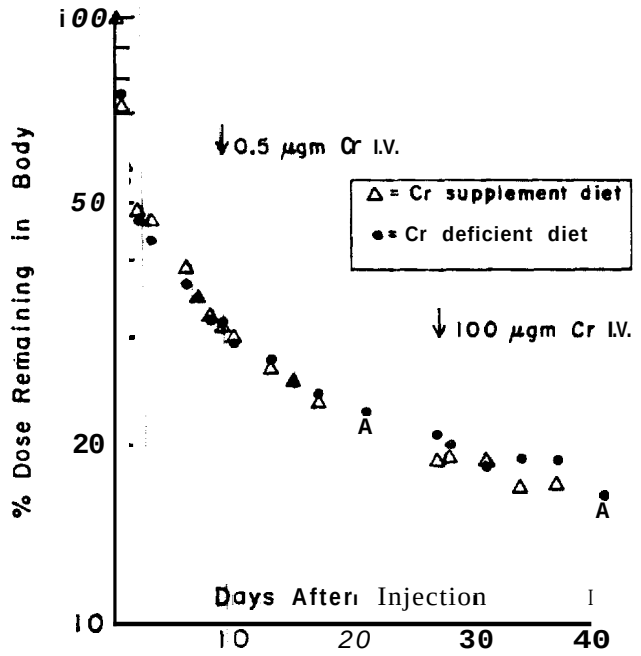

FIG. 2. Absence of effect of dietary history on whole-body retention of intravenously injected chromium (0.1 μg/100 g, as $Cr^{51}Cl_3 \cdot 6 H_2O$). A dose of 0.5 and 100 μg Cr^{51} was injected intravenously on *days 9* and *27*, respectively (mows).

Furthermore, such dose-response curves are known for a number of metals participating in enzymatic reactions (21, 22). They may be explained by an oversaturation of functional groups at or near the site of action of the metal. When chromium(III) is given by a more physiological route, for example as a constituent of the diet, this phenomenon has not been *observed.* Preliminary evidence in man emphasizes the importance of these considerations (10).

TABLE 4. *Independence of excretion rate of chromium(III) from dose (after intravenous injection of $Cr^{51}Cl_3 \cdot 6H_2O$)*

Days	Whole-Body Count as % of Initial Count		
	0.15 μg/100 g	15 μg/100 g	15 μg/100 g, 3 days after 1,000 μg Cr^{52}
0	100	100	100
1	49	47	50
2	42	40	41
3	37	36	37
6	30	30	30
7	29	28	
8	29	28	
10	25	25	
13	22	22	
16	20	20	
20	18	18	

TABLE 5. *Independence of intestinal absorption of chromium(III) from dose (administration by stomach tube as $Cr^{51}Cl_3 \cdot 6H_2O$)*

Days	Whole-Body Count as % of Initial Count					
	0.15 μg/100 g		1.0 μg/100 g		10.0 μg/100 g	
	Cr def. N = 2	Cr suppl. N = 2	Cr def.	Cr suppl.	Cr def. N = 2	Cr suppl. N = 2
0						
1						
2						
3						
4	·5					
7						
8						
10			.8	.1		

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Reba, F. J. Feldman, and W. Mertz, unpublished observations). The data presented here may explain why chromium does not accumulate in most tissues to the extent that some other metals do (17). Of the few percent of dietary chromium absorbed into the organism, more than half is excreted within the subsequent day. The rest will raise the tissue concentration only to that point where loss and intake become equal. On the other hand, a decrease of dietary intake will lead to a lowering of the chromium concentration in the body, until again loss and intake are equal. It is interesting to note in this connection that the average chromium concentration in the United States population declines from birth to old age to very low levels, suggesting an insufficient dietary intake (16). The relation of this observation to disturbances of carbohydrate metabolism in man is being investigated (10).

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