

Oral Bioavailability of Chromium from a Specific Site

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Analysis of soil from a specific site in New Jersey indicated a low level of sodium and chromium present as a calcium compound. Chromium was then administered orally to young, mature male rats at a level of 240 $\mu\text{g}/\text{kg}$ for 14 days as chromium-contaminated soil, as CaCrO_4 , and as an equimolar mixture of the soil and calcium salts for 14 days. The rats were sacrificed 24 hr after the last dosing, and tissues were taken immediately for chromium analysis. Blood, muscle, and liver contained the highest levels of chromium in these animals, although kidney contained the highest concentration per gram of tissue. The total amount of chromium in the tissues was less than 2% of the administered chromium. In a study of the excretion of chromium, the animals were dosed orally for 8 days (with CaCrO_4 or contaminated soil, each at the level of 240 $\mu\text{mole Cr}/\text{kg}$), and the chromium in feces and urine was determined on days 1, 2, 7, and 8. After cessation of dosing for 27 days, the same rats were dosed for 2 days at the same level, and chromium in urine and feces was determined for the 2 days. The animals administered the chromium in soil had higher levels of chromium in both urine and feces on all days compared to the group fed the CaCrO_4 . The total recovery of chromium in any of the 2-day periods was less than 50% of the chromium administered during that period.

Introduction

Chromium has recently been identified in several sites in New Jersey in high concentrations as a result of several decades of chromium work and subsequent dumping of waste materials. Lagoons containing chromium and the appearance of chromium on walls of several buildings near the sites of chromium deposits have also been identified. The proximity of these dumping sites to schools and playgrounds has caused reasonable concern about the oral bioavailability of the chromium in the soil following possible ingestion by children. Very little is known about the absorption of chromium from the gut, particularly in children, so this is a legitimate concern. It is known that trivalent chromium is a dietary requirement in trace amounts (1), but hexavalent chromium has been shown by epidemiological studies to cause respiratory cancers (2,3). This study was thus

originated because of concerns of the New Jersey Department of Environmental Protection about the oral absorption from soil from specific chromium-contaminated sites in Jersey City. Analyses of soil samples from the specific sites using the scanning electron microscope indicated that the hexavalent chromate was present largely as the calcium salt, a common salt of chromium found in soil. Therefore, our studies used samples of soil, calcium chromate, and a mixture of the two for the bioavailability determinations. The resultant bioavailabilities of the three sources of chromium were then compared to determine whether the soil matrix had an effect on chromate uptake by the mammalian system. The objectives of the study were thus the determination of chromium from the specific site in Jersey City and the comparison of the bioavailability of the chromium from the soil and from the calcium chromate salt.

A previous report of part of this work (4) reported on the tissue distribution of the chromium after up to 14 days of oral administration of sodium and calcium chromates as well as contaminated soil, along with excretion data from soil and calcium chromate following 2 days of treatment. This report includes some data on tissue distribution as well as the data for excretion of chromium on days 1, 2, 7, and 8 of treatment and the excretion data 27 days after cessation of treatment and renewal of treatment for 2 days.

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Methods

Chromium was determined using either the Baird inductively coupled plasma (ICP) method (in which the chromium is detected by atomic emission and the atomization is carried out by argon plasma), or by atomic absorption, using a graphite furnace to heat the chromium to the gaseous state. The ICP method is more sensitive and was used for concentrations in the parts per billion range. Neither instrument differentiates between chromium in the different oxidation states, however. Samples were prepared for analysis by a modification of the acid procedure, as previously described (4). Briefly, 0.1 g or more of the chromium-containing sample (tissue or original soil) was weighed into a 20-mL test tube and 1 mL of concentrated HNO_3 was added slowly, followed by 1 mL of 30% H_2O_2 . After the bubbling ceased, the solution was warmed in a water bath to 60°C and kept at that temperature until the solution cleared (16 hr or more). The solutions were then made up to 25 mL volume each with double distilled water, and aliquots were taken for chromium analysis, with filtering when necessary. Chromium solutions obtained from Sigma Chemical Company were used to standardize each instrument.

Analyses of several samples from the site in Jersey City indicated that the chromium content varied between 0.2 and 3.6%. Samples were obtained with the cooperation of the New Jersey Department of Environmental Protection and they had been sized when received by our group. Soil samples with the highest chromium concentration (Pacific Avenue Fines [PAC]) were used for the *in vivo* experiments to allow for the greatest possible oral ingestion from such samples. Analysis for the oxidation states of chromium using the carbazide method (5) indicated that 30 to 35% of the chromium was hexavalent, but samples were not homogeneous, and because the oxidation state of chromium changes with storage under various conditions, this analysis was not considered to be accurate but was considered to be an indication of the maximum hexavalent concentration. Analysis for some elements other than chromium was carried out with preliminary experiments using a Hitachi Scanning Electron Microscope S-570 equipped with a PGT System 4 Microanalyzer. The high calcium content of the soil along with a low sodium content suggested that the chromium was present as a calcium salt.

The Pacific Avenue sample analysis was in agreement with the chromium analysis but analysis for other elements was limited to 0.1% accuracy. No manganese was found in the soils tested by this procedure.

Healthy, male Sprague-Dawley rats of 80 to 120 g weight (Taconic Farms, Boyertown, PA) were used as the experimental animals. The rats were acclimated to the vivarium for several days before experiments and were kept with a light/dark cycle of 12 hr each through all studies. Calcium and sodium chromates were purchased from Aldrich Chemical Company (Milwaukee,

WI) and were the purest available. All chromates and soil were administered *per os* at the doses indicated for each experiment, and the rats were sacrificed 24 hr after the last treatment.

Animals treated with chromium or control solutions were weighed and dosed daily between 9 and 10 A.M. and sacrificed by either anesthesia and exsanguination through the abdominal aorta. All organs for analysis were immediately removed and weighed; total organs were used except for the liver. A small piece (approximately 100 mg) of the liver was taken for analysis. The following organs were taken for analysis after dosing, except as otherwise noted: liver, lung, spleen, kidney, muscle, brain, and testes. An aliquot of blood from the abdominal aorta was also analyzed for chromium.

Experimental Design and Results

Initial pilot studies with oral administration of 0, 20, 40, and 100 $\mu\text{mole/kg}$ of hexavalent chromium (as $\text{Na}_2\text{CrO}_4 \cdot 4\text{H}_2\text{O}$, dissolved in distilled water) to four groups of rats for 7 days indicated that, of the organs studied, liver, blood, and kidney contained the highest amount of chromium. With the concentration expressed as micrograms per gram organ, however, the kidney contained the highest concentration of chromium (4). In the animals dosed with 100 $\mu\text{mole/kg}$, the total amount of chromium in the tissues represented only 1.7% of the amount administered in the previous 24 hr. Animals given doses less than 100 $\mu\text{mole/kg}$ showed less than 0.1% recover of chromium in tissues.

Doses of 120 $\mu\text{mole Cr/kg}$ were administered orally to four groups of rats for 7 days, using four sources of chromium: a) Na_2CrO_4 , b) CaCrO_4 , c) PAC-soil, and d) a mixture of soil and calcium chromate containing 60 $\mu\text{mole Cr/kg}$ each. Subsequent analysis of the tissues showed that the kidney contained a higher concentration of chromium (ng/g tissue) than any other tissue studied (4). On a total organ basis, the liver contained the greatest amount of total chromium. In these studies, the absorption from the soluble sodium salt was generally higher in all tissues studied than from either the calcium salt, soil, or a mixture of calcium salt and soil (4). However, the recovery of chromium in the tissues studied accounted for less than 2% of the total administered dose. If the calculations were made on the basis of the chromium administered in the last dose prior to sacrifice, the percentage was approximately 4%.

In another experiment using the same three sources of chromate (calcium salt, soil, and a mixture of the calcium salt and soil) all orally administered as corn oil suspensions, with a much higher dose (240 $\mu\text{mole Cr/kg}$) and longer time of administration (14 days of treatment), the total tissue levels of chromium from any of the groups mimicked the distribution of the previous experiments with the exception that the blood levels were higher than those of the kidney (on a chromium/gram tissue basis) (Fig. 1). The recovery in the tissues again represented a small percentage (<1.5%) of the total

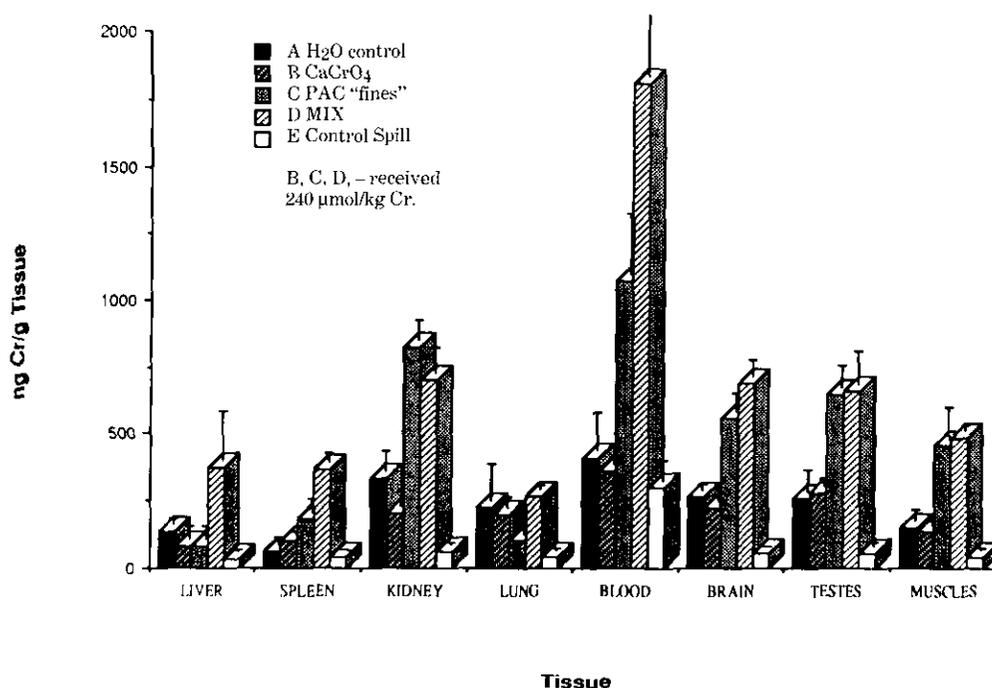


FIGURE 1. Tissue levels of chromium. Rats were administered either calcium chromate, chromate-contaminated soil, or a mixture of the two, orally, as designated in the figure, for 14 days at a level of 240 $\mu\text{mol Cr/kg}$ body weight. They were sacrificed on day 15 by ether anesthesia and bleeding from the abdominal aorta. Tissues were analyzed for chromium using the ICP method. Chromium levels are per gram tissue.

chromium administered. The recovery was also very low even if the percentage was based on the amount given in the last dose before sacrifice (4). This small percentage of recovery from both a hexavalent (CaCrO_4) and trivalent source (soil was calculated to contain about 70% of the chromium as the trivalent form) suggested an unusually low absorption from oral administration, even from the hexavalent compound, with a very rapid excretion of the chromium (within 24 hr of absorption), or a redistribution of absorbed chromium followed by rapid excretion. Another possibility is that untested tissues contained high amounts of chromium.

To determine whether the major portion of the orally administered chromium was rapidly excreted, urine and feces were collected and analyzed for chromium following dosing of rats with 240 $\mu\text{mol Cr/kg}$ suspended in corn oil. The rats (three/group) were housed in metabolic cages and dosed daily at this level with chromium as the calcium salt and as the chromium-contaminated soil. Control rats received corn oil. Dosing was carried out for 8 days, once daily, between 9 and 10 A.M. Urine and feces were collected at 6, 12, and 24 hr after dosing. In the first part of the experiment, urine and feces were collected on days 1 and 2 and on days 7 and 8. The experiment was continued after day 8 by cessation of chromium administered from 27 days and then redosing the animals for 2 days (designated days 1B and 2B) during which time the urine and feces were again collected at the same time periods, for 2 days. In all these experiments the metabolic cages were plastic and con-

tained nalgene partitions which separated the urine and feces immediately. Both urine and feces were prepared for analysis of total chromium as previously described, and aliquots were analyzed using the ICP method. The excretion results showing the total chromium found in each time period for the several groups and the comparison of the total chromium excreted for each group are shown in Figures 2 through 9.

The data in these figures indicated that chromium is not excreted to an appreciable extent in the urine from either of the treated groups, but significant amounts are excreted in some feces. The chromium content in urine from CaCrO_4 rats on days 1 and 2 was < 0.5% of the total administered dose. The urinary excretion in the rats dosed with the soil was somewhat higher, the average amount being 1.80% of the 2-day dose. The chromium content of the feces of rats that were dosed with soil on days 1 and 2 was much greater than that in feces of rats fed the calcium salt, the recover percentages being 19 and 1.80%, respectively. The urinary excretion values can be used as a measure of absorption of chromium from the gastrointestinal tract, and thus the values seem to substantiate the conclusions based on tissue levels, that little chromium is absorbed from the oral route. However, the low fecal values indicated that the total administered chromium was not excreted rapidly.

The excretion patterns in urine and feces on days 7 and 8 as well as on days 1 and 2 in the second part of the experiment also indicated that more of the chromium from soil is excreted in both feces and urine than

CHROMIUM EXCRETION IN URINE DAYS 1 AND 2

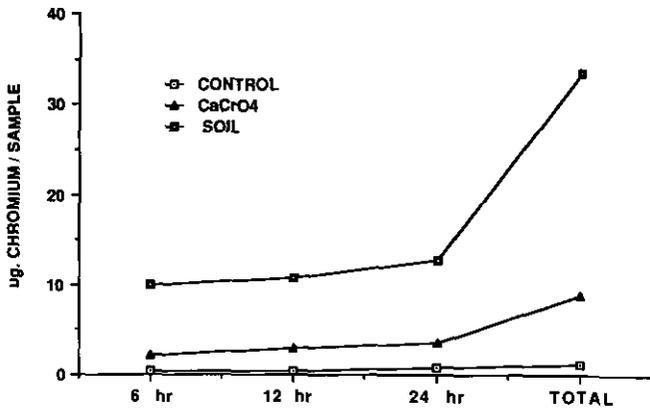


FIGURE 2. Chromium excretion in urine on days 1 and 2. Rats were treated orally with either control vehicle (corn oil), calcium chromate, or chromium-contaminated soil at a level of 240 $\mu\text{mole Cr/kg}$ daily between 9 and 10 A.M., during these 2 days. Urine and feces were collected at 6, 12, and 24 hr after dosing and prepared for chromium analysis by oxidation as described in the text. Chromium was determined on aliquots of the samples using the ICP method. Chromium levels shown are for each time period as well as for the total 24-hr period.

the chromium from the CaCrO_4 -treated rats. For urine collected on days 7 and 8 of the study, the percentage of the recovery of chromium for soil-treated animals was 1.12% of the dose and that for CaCrO_4 -treated animals was 0.21%. Fecal excretion was 40.6% and 12.35% for the soil and CaCrO_4 groups, respectively. Data for CaCrO_4 -treated rats in the resumed studies (days 1B and 2B) show that the urinary excretion of chromium averaged 0.44% of the dose for soil-treated rats and averaged 0.1% for the CaCrO_4 -treated animals. Fecal excretion was much higher for both groups: 42.92% for the soil group and 2.92% for the CaCrO_4 -treated group.

CHROMIUM EXCRETION IN URINE DAYS 7 AND 8

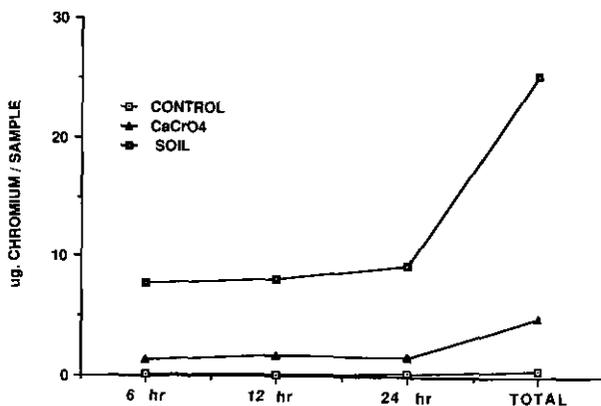


FIGURE 4. Chromium excretion in urine on days 7 and 8. Rats were treated orally with control vehicle or with calcium chromate or chromium-contaminated soil at a level of 240 $\mu\text{mole Cr/kg}$ daily, as described in the text, for a period of 8 days. Urine and feces were collected 6, 12, and 24 hr after dosing on days 7 and 8. Urines were prepared for analysis by oxidation as described in the text. Chromium was determined on aliquots using the ICP method. Chromium levels are as described for Figure 2.

CHROMIUM EXCRETION IN FECES DAYS 1 AND 2

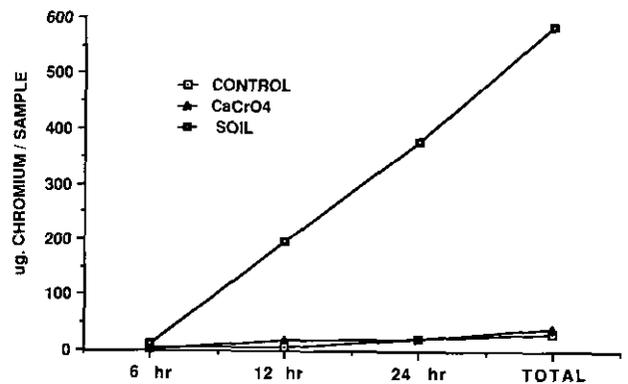


FIGURE 3. Chromium excretion in feces on days 1 and 2. Rats were treated orally with control vehicle or with calcium chromate or chromium-contaminated soil as described for Figure 2. Feces were prepared for analysis by oxidation as described in the text, and the chromium was determined using the ICP method. Chromium levels are shown as described for Figure 2.

The rats had a low feces output in the 12 hr after the oral dose, and the 12- to 24-hr period feces contained the major percentage (about 90% of the fecal output) of the chromium for the rats in both of these groups.

In two-thirds of the rats in the second part of the study, there were no feces in the first 6 hr. The urinary patterns were more level in that the chromium output at each time period was higher from the soil-treated animals. These studies also show that the excretion is not so rapid as to clear the chromium from the system within 24 hr, so the low tissue recovery percentages are not accounted for by rapid excretion.

The percent excretion of the administered chromium in days 7 and 8 is significantly higher than that for the first 2 days of administration and slightly higher than

CHROMIUM EXCRETION IN FECES DAYS 7 AND 8

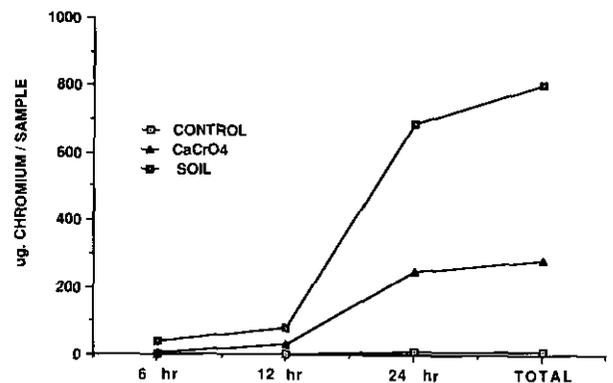


FIGURE 5. Chromium excretion in feces on days 7 and 8. Rats were treated orally with control vehicle or with calcium chromate or chromium-contaminated soil as described for Figure 2. Feces were collected 6, 12, and 24 hr after dosing on days 7 and 8. Feces were prepared for analysis by oxidation as described in the text. Chromium was determined on aliquots using the ICP method. Chromium levels shown are as described for Figure 2.

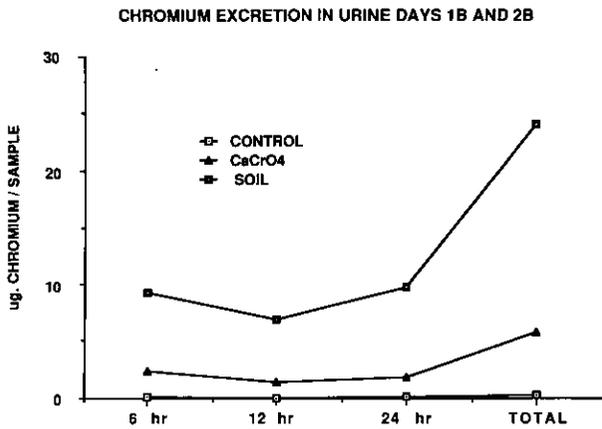


FIGURE 6. Chromium excretion in urine on days 1B and 2B. Rats received no doses of chromium for 27 days after 8 days of treatment and were treated again for 2 days after the 27-day interval. Doses of calcium chromate and soil were as in Figure 2 for the 2 days. Urine and feces were collected at 6, 12, and 24 hr after dosing, and samples were prepared for analysis by oxidation as described in the text. Chromium was determined on aliquots using the ICP method. Chromium levels are as described for Figure 2.

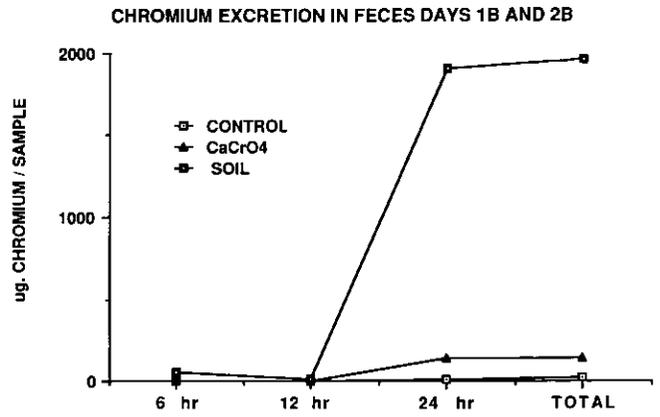


FIGURE 7. Chromium excretion in feces on days 1B and 2B. Feces of the rats treated as described for Figure 6 were analyzed for chromium using the ICP method. Chromium levels are as described for Figure 2.

on the 2 days after readministration after cessation of chromium treatment. The increased levels in days 7 and 8 suggest excretion of some residual chromium, and it appears that the biological half-life of chromium is at least several days. It also suggests that this storage of chromium negates the use of urinary or fecal chromium detection as a quantitative indication of recent oral exposure. The biological half-life of chromium in humans has been estimated to be 30 days (6), but there have been no reported half-life values for rats.

The percentage of chromium accounted for in tissues and excreta in these experiments is not above 50% of the administered dose even for the soil-treated animals, which have the highest recovery of the administered material. Langard's group (7) and others have found that

the lungs, liver, and blood contain the major amounts of absorbed chromium. Tissues that remain to be studied, e.g., skin, bone, intestinal, and gastrointestinal tract lining, may contain high levels of chromium and have not yet been studied by our group. Values for these tissues are not included in the literature. We have failed to find chromium in bile of chromium-treated rats with cannulated bile ducts (C. M. Witmer, unpublished results), but these animals received low doses of chromium (100 μ mole/kg) and the experiments must be repeated. Some of the older literature includes negative determinations for chromium in other tissues, but these determinations were carried out prior to the use of sensitive detection methods and therefore these results may have to be reinterpreted.

An important question to be answered that was raised previously by our group is the location of the chromium that was not found in the tissues examined and was not excreted in the 24 hr after dosing. The red blood cells

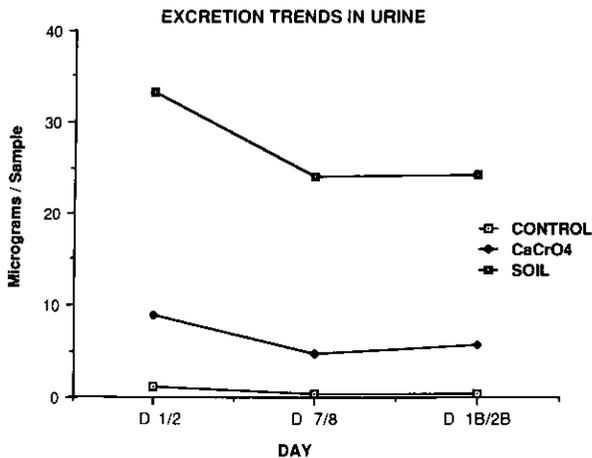


FIGURE 8. Excretion trends in urine. Total urinary chromium levels are shown for the rats as determined on days 1 and 2, 7, and 8, and 1B and 2B.

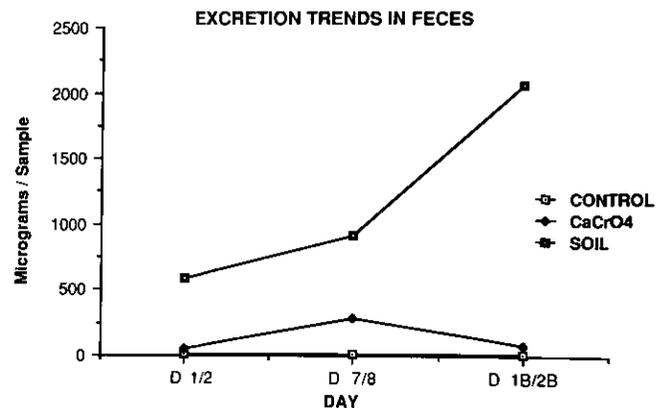


FIGURE 9. Excretion trends in feces. Total fecal chromium levels are shown for the rats as determined on days 1 and 2, 7 and 8, and 1B and 2B.

are known to store chromium bound to hemoglobin, but this amount was included in our tissue studies. The apparent low absorbance of the hexavalent chromium was not unexpected as the hexavalent form has been shown to be reduced in both saliva and gastric juice (8), and the resultant trivalent material would be poorly absorbed through the intestinal wall. However, trivalent compounds can be absorbed slowly. The mammalian system appears to limit absorbance of the orally administered material to a low amount; the reduction in the blood would also prevent distribution to many tissues. It seems that absorbance following the oral route of exposure needs to be explored further to ascertain the limits of absorbance and the biological half-life under different conditions, such as intermittent exposure and with chromium from several salts which differ in their solubilities. Such studies of mechanism of chromium uptake and transport are necessary for the carrying out of assessment of risk of oral exposure to chromium in soil or from any other source. The question of other components of soil that may change the oxidation state of the chromium as well as the binding of the chromium to the soil compound the problem and indicate that we cannot easily solve the problem of oral absorption without investigating each specific site.

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