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ABSORPTION OF INORGANIC, TRIVALENT AND HEXAVALENT CHROMIUM FOLLOWING ORAL AND INTRAJEJUNAL DOSES IN RATS

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The intestinal absorption of trivalent and hexavalent chromium (Cr) given orally (experiment I) or infused in the intestine (experiment II) was investigated in rats. The nonabsorbable form of chromium ($^{51}Cr_2O_3$) and water-soluble and more absorbable $Na_2{}^{51}CrO_4$ (the hexavalent form of Cr) were compared. Total retention of chromium given orally ranged around 15 percent of the dose, regardless of the chromium compounds applied. The absorption rate of chromic oxide, which is considered a nonabsorbable compound, was 14.4 as a percentage of chromium intake. This result indicates that some loss of chromium has to be taken into account in metabolic trials made by the indicator method. In isolated rat intestine, from the injected Cr 2.5% of chromic oxide and 43.2% of sodium chromate were absorbed during an hour (experiment II). The absorbed chromium was transferred to the liver where the liver tissue retained 10.9% of chromic oxide and 51.1% of sodium chromate. Radioactivity of v. cava caudalis following intestinal injection of Na_2CrO_4 was thirtyfold greater than after Cr_2O_3 dosing. This phenomenon can be explained by the lower blood clearance of chromate. Different absorption rate of chromate depending on the route of administration could be due to the fact that the hexavalent form given orally was reduced to Cr^{3+} in the acidic environment of the stomach. When Na_2CrO_4 was infused directly in the intestine of rats, such reduction could not occur. This means that the acidic gastric juice might play a role in inhibiting the intestinal absorption of Na_2CrO_4 when this compound is given orally.

Key words: Chromium, absorption, excretion, retention, rats

The use of chromium as a reference marker in absorption tests or balance studies is based on observations suggesting that this substance is absorbed poorly (Underwood, 1977). Nevertheless, it seems likely that some chromium is absorbed, since it is found in the tissues and urine of animals. The necessity of investigating chromium absorption is emphasised by the reported effects of trace quantities of chromium on cellular metabolism and enzyme activity.

The problem of intestinal absorption of chromium compounds and its dependence on the chemical structure are poorly understood. Some compounds

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(Cr_2O_3) are believed not to be absorbed at all, while others get into the different organs, as shown by observation of their biological effect and by the rise of tissue chromium levels after an oral administration. The purpose of our studies reported here was to measure the absorption, excretion and retention of two different chromium compounds dosed orally. The second part of the studies was designed to measure the absorption and distribution of intrajejunally injected chromium compounds. The final aim of these experiments was to compare the intestinal absorption of trivalent and hexavalent chromium given orally or infused into the intestine.

Materials and methods

Two experiments (I and II) were conducted with rats to investigate the absorption of Cr. The experimental design was arranged according to the following table:

	Experiment I		Experiment II	
	Group 1	Group 2	Group 1	Group 2
Number of rats	6	6	7	7
Administered compound	$^{51}Cr_2O_3 + Cr_2O_3$	$Na_2{}^{51}CrO_4 + Na_2CrO_4$	$^{51}Cr_2O_3 + Cr_2O_3$	$Na_2{}^{51}CrO_4 + Na_2CrO_4$
Administration route	oral	oral	intrajejunal	intrajejunal

In experiment I, 6 male albino rats (WISTAR, SPF, HUMAN, Gödöllő, Hungary) per group, averaging 120 g, were fed pelleted rat feed (CRLT/N, Charles River Hungary Ltd.). The animals were kept in individual wire-mesh metabolism cages with free access to water. The rats were divided into two groups according to the treatments. One group was given a nonabsorbable form of chromium (Cr_2O_3) and the other group was treated with the water-soluble and more absorbable sodium chromate (hexavalent form of Cr). Chromic oxide was chosen from the possible trivalent compounds because this substance is often used in metabolic experiments as a marker. The rats were fed 10 g of the diet daily. After receiving the diet during the 3-day experimental period, daily doses of 1500 kBq of ^{51}Cr and 15 μmol of Cr as Cr_2O_3 were administered to each rat orally in the first group and 1500 kBq of ^{51}Cr and 15 μmol of Cr as Na_2CrO_4 in the second group. The applied amount of chromium was the same as generally used in metabolic trials made by the indicator method. Faeces and urine were quantitatively collected for 3 days. The rats were treated according to the principles of laboratory animal care as promulgated by the Hungarian Committee on Animal Care. Faeces and urine were analysed for ^{51}Cr by a scintillation counter equipped with ∅ 40 × 2.5 mm NaI(TI) crystal.

In experiment II, 7 male albino rats of the same stock, averaging 250 g in weight, were used in each group. Absorption of chromic oxide (Cr III) and sodium chromate (Cr VI) was studied in isolated intestine of rat using radiochromium marker. The rats were fasted for 24 h prior to surgery to ensure an empty intestinal lumen. They were anaesthetised with an intraperitoneal injection of sodium pentobarbital. An abdominal incision was made and the small intestine was exposed.

Absorption was measured over a 15-cm segment of jejunum. The two ligaments were placed on the same part of the jejunum. The intestinal solution was infused into the proximal end of the gut sac. The composition of perfusate was different. In the first group 15 μmol Cr and 1500 kBq ^{51}Cr as Cr_2O_3 and in the second group as Na_2CrO_4 were given. The injected Cr concentration corresponded to the doses applied in experiment I.

Following a single intestinal tracer dose of chromium the laparotomy incision was sutured. After 60 min, blood samples were taken from the v. portae, v. hepatica and v. cava caudalis, then the rats were killed with sodium pentobarbital. The gut sac was removed and washed. The liver was also removed. Infusion fluid from the gut sac, liver and blood samples were analysed for ^{51}Cr by the same method as described earlier.

Results

Experiment I

For both trivalent chromium and chromate, the faeces was the predominant route of excretion (Table 1). However, rats that had been given chromate excreted smaller amount of chromium through the faeces. Faecal Cr excretion was lower in Group 2 (36.89 μmol) than in the rats of Group 1 (39.15 μmol). Thus, calculated Cr excretion was also lower, 80.66% vs. 85.58% of the dose. Total urinary Cr excretion of the first and second group was 0.29 and 0.99 μmol, respectively. Rats that had been given chromate (Group 2) had significantly higher urinary Cr as a percentage of the oral dose. No significant difference was found in the retention of chromium. From the total Cr excretion it was calculated that in case of Cr_2O_3 13.78% and in rats fed chromate (Group 2) 17.16% of the Cr dose was retained.

Experiment II

The absorption of intrajejunally administered chromic oxide was much lower than that of chromate (Table 2). Because of the lower absorption smaller amount of chromium could be detected in the v. portae and in the liver. Chromium concentrations of the v. portae and liver tissue were 2.87 nmol/ml and 40.2 nmol, respectively.

Table 1

Excretion rate of orally administered chromium compounds (experiment I)

		Group 1	Group 2
Number of rats		6	6
Administered compound		$^{51}Cr_2O_3 + Cr_2O_3$	$Na_2{}^{51}CrO_4 + Na_2CrO_4$
Cr intake	µmol/day	0.138 + 15.109	0.138 + 15.109
Total Cr intake	µmol	45.742	45.742
Activity	kBq/day	1500	1500
Total activity	MBq	4.5	4.5
Cr content of faeces	µmol	39.15 ± 1.09	36.89 ± 2.27
	%	85.58 ± 2.38	80.66 ± 4.96
Cr content of urine	µmol	0.29 ± 0.11	0.99* ± 0.28
	%	0.64 ± 0.23	2.17* ± 0.60
Total Cr excretion	µmol	39.44 ± 1.05	37.89 ± 2.07
	%	86.22 ± 2.30	82.83 ± 4.53
Retention	µmol	6.30 ± 1.05	7.85 ± 2.07
	%	13.78 ± 2.30	17.16 ± 4.50

*Significant difference at $P < 0.05$

Table 2

Chromium absorption from the jejunum (experiment II)

		Group 1	Group 2
Number of rats		7	7
Administered compound		$^{51}Cr_2O_3 + Cr_2O_3$	$Na_2{}^{51}CrO_4 + Na_2CrO_4$
Activity	kBq	1500	1500
Intrajejunally injected Cr	µmol	15	15
Cr retention in the jejunum	µmol	14.63 ± 0.04	8.52 ± 0.50
Absorbed Cr	µmol	0.37 ± 0.04	6.48 ± 0.48
Cr content of liver	nmol	40.2 ± 6.9	3309.4 ± 772.6
Cr content of v. portae	nmol/ml	2.87 ± 0.60	48.18 ± 6.13
Cr content of v. hepatica	nmol/ml	2.57 ± 0.65	24.01 ± 3.28
Cr content of v. cava caudalis	nmol/ml	1.37 ± 0.16	40.29 ± 6.81

The chromium concentration of the v. hepatica dropped to 2.57 nmol/ml. The liver retained 10.9% of chromium from the blood. The v. cava caudalis contained less chromium (1.37 nmol/ml) than the v. hepatica. The difference detected between these two veins was 47%. Intrajejunal administration of Na_2CrO_4 resulted in a significant absorption as indicated by the increased radioactivity of the v. portae. This rate was high because 6.48 µmol chromium absorbed from the hexavalent form of chromium (more soluble than chromic oxide). An average of 43% of the infused chromium was absorbed. The large increase in Cr absorption after Na_2CrO_4 injection resulted in increased chromium content of the liver (3309 nmol of Cr). The chromium level of the v. hepatica was 10-fold higher in

the group with hexavalent chromium infusion than in the group given chromic oxide. After Na_2CrO_4 infusion, 1.7 times higher chromium content was measured in the v. cava caudalis than in the v. hepatica.

Discussion

In the first experiment the excretion rate and retention of trivalent and hexavalent chromium compounds were compared using ^{51}Cr isotope.

When either Cr_2O_3 or Na_2CrO_4 was administered orally to rats, most of the radioactivity could be recovered in the faeces. Absorption of orally given chromate was greater than that of the trivalent compound. Urinary Cr excretion was higher but could not follow the absorption rate, therefore retention was also higher. Chromium in the more soluble form absorbed better, and 81% of the chromium intake was excreted via the faeces. Total retention of chromium ranged around 15 percent of the dose, regardless of chromium compounds applied. The oral dose of chromate administered to rats resulted in an increased intestinal absorption of chromium, but this increment was much lower than expected. However, it was a great surprise that the absorption rate of chromic oxide, regarded as a nonabsorbable compound, amounted to 14.4 percent of the chromium intake.

Our results do not confirm previous observations (Whitby and Lang, 1960; Hansky and Connell, 1962; Gabriel et al., 1963) which indicated that the absorption of trivalent chromium is so small that it can be used as a convenient nonabsorbable marker for studies of intestinal absorption and excretion. Visek et al. (1953) reported intestinal absorption of less than 0.5% of an oral dose in rats. The proportion of an oral dose of chromic chloride absorbed by the rat appears to be independent of the amount given and of the nutritional status of the animals (Hopkins and Schwarz, 1964; Mertz et al., 1965). Donaldson and Barreras (1966) found that 0.1–1.2% of an oral dose appeared in the urine. A higher retention of 2–3% was calculated from results of total body counting of rats receiving $CrCl_3$ by stomach tube (Mertz et al., 1965). The absorption of chromate is higher, with 2.1% of a dose appearing in the urine of man (Donaldson and Barreras, 1966) and 3–6% in rats (MacKenzie et al., 1959). It can be assumed, however, that urinary chromium output underestimates the amount of chromium actually absorbed (Mertz, 1969).

It can be concluded that the first experiment measuring chromium excretion and retention in rats fed two different inorganic chromium compounds (1) failed to detect that trivalent chromium is poorly absorbed and (2) no such increment was observed after an oral dose of the more soluble sodium chromate.

In the second experiment the intestinal absorption of trivalent and hexavalent Cr in the rat jejunum was examined by the use of a gut sac procedure.

In this manner four components of the absorptive pathway could be quantified: (1) Cr uptake from the intestinal lumen; (2) Cr transport into the blood stream (Cr concentration of v. portae); (3) Cr retention in the liver; (4) Cr retention in the tissues and excretion rate (Cr concentration of v. cava caudalis).

The mechanism responsible for the intestinal absorption of chromium is not well understood. It is unclear whether Cr is absorbed passively or with the aid of carrier proteins located in the intestinal mucosa. Mertz et al. (1965) reported that the absorption of trivalent Cr does not appear to be a saturable process, which suggests that it is absorbed by passive diffusion. Mertz and Roginski (1971) reported contrary evidence. They found that the percentage of trivalent Cr absorbed by everted gut sacs decreased as the Cr concentration increased in the incubation medium. This observed saturation effect suggests that carrier proteins are involved in Cr absorption. However, in the experiment of Dowling et al. (1989) it was concluded that inorganic, trivalent Cr is absorbed by the nonmediated process of passive diffusion in the small intestine of rats fed a Cr-adequate diet.

According to the results of this study, 2.5% of chromic oxide and 43.2% of sodium chromate were absorbed during an hour. The absorbed chromium was transferred to the liver where the liver tissue retained 10.9% of chromic oxide and 51.1% of sodium chromate. The chromium absorption result agrees well with that of MacKenzie et al. (1959) and Donaldson and Barreras (1966) who found that Na_2CrO_4 absorption was greater than that of $CrCl_3$ and that blood radioactivity following intestinal infusion of Na_2CrO_4 was three- to fivefold greater than that observed after $CrCl_3$ administration. In our study, the radioactivity of the v. cava caudalis following intestinal injection of Na_2CrO_4 was thirtyfold greater than the value after Cr_2O_3 dosing. These results could be explained by the lower blood clearance of chromate. The reasons can be the following: (*i*) The affinity of tissues for the trivalent form is greater than for the hexavalent. (*ii*) All chemical forms, except chromate, clear the blood quite rapidly. (*iii*) The accumulation of chromium from chromate may represent the chromium bound to red cells.

The experimental data revealed great differences in the absorption rate depending on the administration route of chromate. In rats given chromate orally nearly 20% of the dose could have entered the body from the intestine. In contrast to oral administration, more than 40% of the infused radioactivity was absorbed when Na_2CrO_4 was placed directly in the intestine. This observation is in agreement with previous experiments (Hansky and Connell, 1962; Donaldson and Barreras, 1966) where one fourth to one half of the administered radioactivity appeared to be absorbed when Na_2CrO_4 was placed directly in the intestine of humans or rats. This can be explained by the fact that the hexavalent form is easily reduced to Cr^{3+} in the acidic environment of the stomach. Thus, ingested chromium, regardless of its original form, is most likely to exist as Cr^{3+} by the time it reaches the small intestine. This means that the acidic gastric juice might play a role in inhibiting intestinal absorption of Na_2CrO_4 when this compound is

given orally. This possibility was supported by the observations that patients with gastric achlorhydria absorbed significant amount of orally administered Na_2CrO_4 and treatment of Na_2CrO_4 with acidic gastric juice inhibited absorption of this compound when it was placed directly in the intestine.

Conclusions

The experimental results indicate that trivalent chromium in the form of Cr_2O_3 can absorb from the intestine. This observation does challenge the validity of using chromic oxide as a means of estimating digesta flow rate and digestibility of nutrients in metabolic trials.

Due to the fact that the hexavalent form is easily reduced to Cr^{3+} in the acidic environment of the stomach big differences can be detected in the absorption rate of chromate after oral administration and intrajejunal infusion.

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