

DISAPPEARANCE OF CHROMIUM (III) TRICHLORIDE INJECTED INTRACUTANEOUSLY

N. Bang Pedersen and Y. Naversten

*From the Departments of Dermatology and of Radiation Physics, University of Lund, Lund,
Sweden*

Abstract. The disappearance of 0.1 ml 1 mM $^{51}\text{CrCl}_3$ injected intracutaneously was studied in two chromium hypersensitive and two non-sensitive men. Chromium disappeared at a decreasing rate. Half of the injected chromium had disappeared in 5-12 days, while after 3 months 39-44% remained at the injection site. Chromium in blood was demonstrated only in plasma. The main part of the chromium not bound at the injection site was excreted in the urine.

Delayed reactions to chromium in allergic human subjects may be elicited both by epicutaneous and intracutaneous testing (7). In studies concerning the strength of a test reaction in relation to the quantity of hapten used, it may be more convenient to employ intracutaneous testing in order to avoid the problem of transepidermal absorption and the adherence of hapten to the patch (2). In investigations of contact allergy to chromium, hexavalent chromium has usually been used as a hapten, but trivalent chromium also elicits positive reactions when injected intradermally into persons allergic to potassium dichromate (8). The purpose of the present study was to trace the disappearance of intracutaneously injected chromium trichloride in chromium-allergic and non-allergic subjects.

MATERIALS AND METHODS

The investigation was carried out on 5 men. Subject E, aged 59, and subject F, aged 62, had earlier had chromium dermatitis and reacted positively to tests with potassium dichromate. Control subject G, aged 60, had never had any skin disease, while the two other control subjects, H and I, aged 39 and 38, had several months previously had a moderate irritant dermatitis of their hands.

The test-solutions from $^{51}\text{CrCl}_3$ (Radiochemical Centre,

Amersham, Bucks, UK) were mixed with an appropriate volume of a solution of inactive chromium trichloride in order to obtain concentrations of 1 mM and 0.1 mM, and an activity of 250-500 $\mu\text{Ci/ml}$. The concentrations of test-solutions were controlled by an atomic absorption spectrophotometer (Perkin-Elmer 303).

The *intracutaneous tests* were performed with plastic syringes (Johnson & Johnson Ltd., Slough, Bucks, UK), graded in 0.01 ml. To minimize adhesion of chromium trichloride to the surface of the syringe, the solution was kept in the syringe only a short while. During the test and subsequent measurements, the test subject was reclining with his arm supine. 0.1 ml of the test solution was injected intracutaneously on the volar side of the forearm. The 1 mM-solution was used in both chromium-hypersensitive persons and in two control subjects. The solution of 0.1 mM chromium trichloride was used in the third control person.

The *measurements* of the activity (1) at the test site were done with a NaI (Tl) scintillation detector (Φ 7.5 cm $\times$ 5 cm) shielded by a lead collimator having a cylindrical opening 24 mm in diameter and 80 mm long. As soon as possible after injection, i.e. 10-30 seconds, the collimator was placed with barely perceptible pressure against the skin around the test site. The activity during the first hour was recorded continuously for 96- or 50-second periods by means of a multichannel multi-scaling analyser technique (Inter technique SA 40 B). The disappearance curve was extrapolated to time 0 to obtain the starting value. All the individually counted values were corrected to represent the correct count-rate in successive time intervals of 100 seconds. The same time histogram representative for the activity-disappearance was thus obtained in all the 5 subjects. After the first hour, the pulses were registered for periods of 100 seconds with a single-channel analyser (Nuclear Enterprises), at first several times daily, and later, when the disappearance-rate decreased, at longer intervals. The 100-second measurements were performed four times on each occasion. The activity at the test site was observed for 3-4 months in the 4 subjects where testing with 1 mM chromium trichloride had been performed. In the fifth subject tested with 0.1 mM chromium trichloride, the activity was observed for a period of 4 weeks.

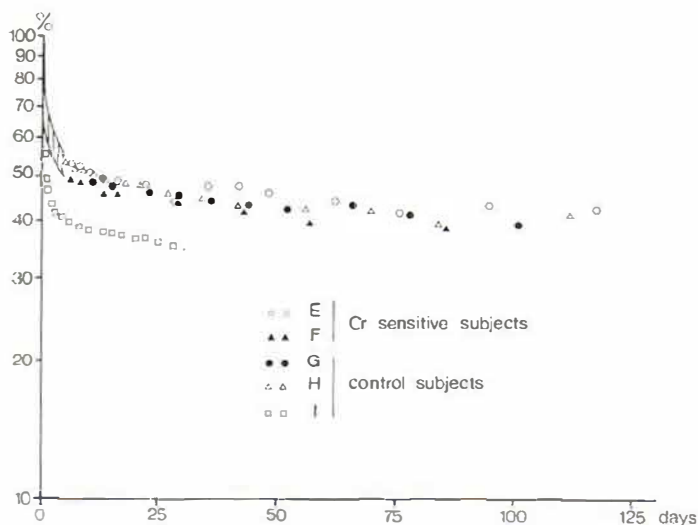


Fig. 1. Skin depot retention after intracutaneous injection of chromium as percentage of the starting value. 1 mM CrCl_3 was given to subjects E, F, G and H. 0.1 mM CrCl_3 was given to subject I. One standard deviation on each observation is less than indicated by the borders of the symbols.

The background pulse-rate was counted over the corresponding site on the other forearm.

Blood samples from the subjects tested with 1 mM chromium trichloride were taken twice the first day and then every 1–5 days during 2–3 weeks after the injection. The blood was centrifuged and separated in a portion of blood cells and in a portion of plasma (1). The activity of the blood fractions was measured in a well-type NaI (Tl) scintillation detector (Nuclear Enterprises).

Urine from the subjects tested with 1 mM chromium trichloride was collected in the form of 24-hour specimens for 2 weeks, and from control subject H, urine was collected for a further 30 days. The activity of the urine was measured in a plastic container standing on a cylindrical NaI (Tl) scintillation detector (ϕ 7.5 cm $\times$ 5 cm).

Standards. A standard source used for adjustment of

the single-channel analyser consisted of a test patch containing 0.1 ml of the test solution. The test patch was sealed between 2 adhesive tapes. During the adjustment, the standard source was placed in a jig at a fixed distance from the detector. Furthermore, a standard source used as reference for the urine activity was made from a container of the same type as used for urine sampling. 0.1 ml of the test solution was thoroughly diluted in 900 ml water. To prevent adhesion of the radionuclide, a surplus of chrome-carrier was added. To prevent precipitation and subsequent sedimentation of the chromium trichloride, the pH of the solution was adjusted to 3 by hydrochloric acid.

Two ml of the above-mentioned solution was used as a reference for measurements of radioactivity in blood fractions.

In order to estimate the errors in administering a volume of 0.1 ml with a plastic syringe, 0.1 ml of a 1 mM test solution was drawn up in each of 25 syringes. The content of each syringe was emptied on a test patch and the activity of each of the patches was measured. This test gave a reproducibility of S.D. = 2.3%.

RESULTS

In one of the chromium-hypersensitive subjects a positive test reaction was observed after 1 day. A redness about 5–7 mm in diameter, and an infiltrate, remained at the test site for 3 months. In the other chromium-hypersensitive person the test was positive from the 2nd to 5th day after injection. The intracutaneous test was negative in the 3 control individuals.

Fig. 1 shows the chromium disappearance from the skin depot. The values measured during the 1–4 month periods are presented as percentages of the starting value. One standard deviation of

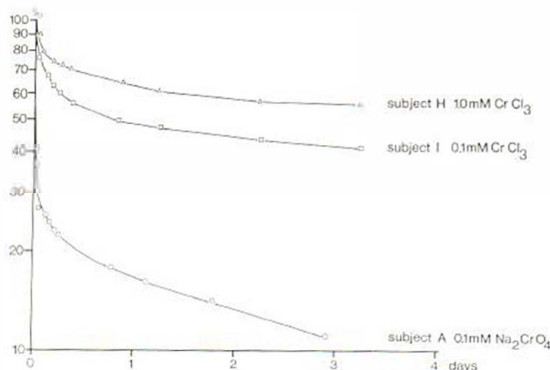


Fig. 2. Early depot retention after intracutaneous injection of chromium in 3 control subjects, i.e. 1 mM CrCl_3 (subject H) and 0.1 mM CrCl_3 (subject I) in the present study, and 0.1 mM Na_2CrO_4 (subject A) in an earlier study (1).

the mean value of the four measurements made on each occasion was always between 0.2–0.8 % of the initial value. The disappearance rate decreases with time, and after 2 weeks seems to be rather constant. Individual regression analysis was performed for that part of the disappearance course covering the 4 subjects tested with 1 mM chromium trichloride. As four measurements were made on each occasion, a test of linearity was also carried out, but it was not possible to adequately match the disappearance courses to a monoexponential function. For estimating the disappearance rate, all measurements for the subjects from 2 weeks onward were pooled and analysed by the best fit of the least squares deviation and the half-life time was found to be 380 days.

The disappearance of chromium from the test site of the subject given 0.1 mM chromium trichloride was initially more rapid than for the other cases, as is seen in Figs. 1 and 2. Half of the injected chromium had disappeared during the first 24 hours compared with 5–10 days in the other cases.

Fig. 3 shows the plasma concentration of chromium in relation to time. Maximum activity was found in samples No. 2 taken 8–11 hours after injection. The blood corpuscles were not labelled at all or were labelled with insignificant activity.

Fig. 4 gives the accumulated amount of chromium excreted in urine during 2 weeks in the 4 persons tested with 1 mM CrCl_3 . The excretion was on the level of 600–900 ng on the first day, and decreased to a level of 30–40 ng per day after 10 days. In control H the urine was collected for as long as 43 days. Throughout this period, chromium was still detectable in the urine, as the activity was more than 50 % above background

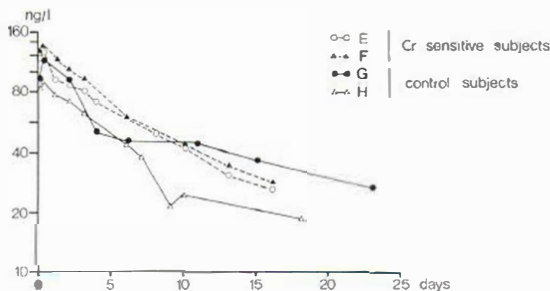


Fig. 3. The chromium concentration (ng/l) in blood plasma after intracutaneous injection of 1 mM CrCl_3 .

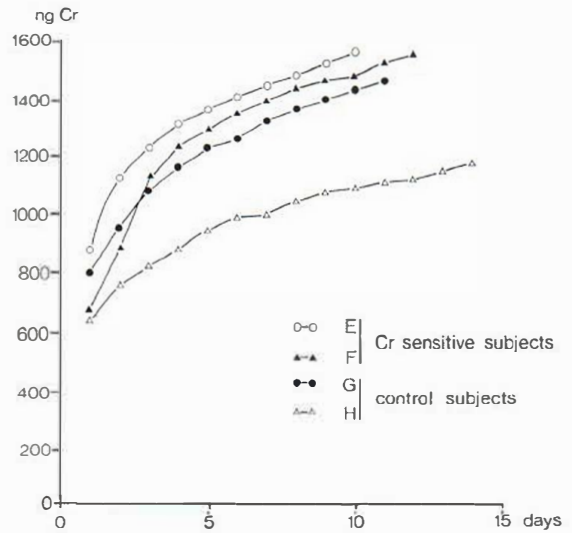


Fig. 4. The accumulated chromium excretion in urine after intracutaneous injection of 1 mM CrCl_3 .

level. During the first day 12–17 % of the amount injected was excreted in the urine, and during the first 10 days 23–30 %.

DISCUSSION

Chromium disappeared from the injection site at the same rate in both hypersensitive and control subjects tested with the same amount of hapten. This is in agreement with the results of an earlier study (1). In some studies (11, 19, 23) antigens have been reported to disappear from the test site of sensitized guinea pigs at a rate different from that in the control animals.

The disappearance-rate decreases with time. The main disappearance, i.e. during the first days following injection, is probably by way of the blood and lymph. This assumption is supported by rapid labelling of plasma 2–2.5 hours after injection. The further disappearance at a slower rate may reflect transport of chromium bound to protein via the lymph, as trivalent chromium is easily bound to proteins (5, 13, 16, 22). There are great regional differences of transport-rate of intracutaneously injected albumin (6), but recently Cavill & Jacobs (4) found a 21-hour half-life of albumin in the skin of the forearm.

The slower disappearance may also be caused by the development of various chromium compounds. At the pH of the body trivalent chromium will

slowly start building olates and polymeric complexes with such substances as organic acids, amino acids, peptides, proteins etc. (20). These compounds are firmly bound to collagen *in vitro* and thus possibly influence the rate of disappearance. Furthermore, trivalent chromium may form a precipitate (14) of chromium hydroxide. Chromium hydroxide is practically insoluble, as the solubility-constant at room temperature is 10^{-30} (3). Olates of chromium or other chromium compounds are firmly bound to collagen *in vitro* (15). The turnover of collagen in animals is very low (12). The estimated half-life time of 380 days may reflect a turnover of collagen or a disappearance of macromolecules.

Statistical analysis of counting values showed a wider spread of data from time to time than could be expected from the accuracy of the counting statistics (Fig. 1). This indicates a slight irreproducibility of the counting system from time to time, and therefore no attempt was made to divide the disappearance curve into monoexponential components.

The present investigation may be compared with an earlier one (1). In the present study, 5.2 μg chromium as trichloride was injected intracutaneously in 4 of the subjects, and in the earlier study 0.5 μg chromium as sodium chromate was used. There was thus a difference of a factor of 10 in the two studies, but the quantities of the two chromium compounds were chosen to elicit test reactions of the same strength.

A comparison of the transport-rates shows that sodium chromate disappears much more rapidly. Half of the injected sodium chromate disappeared within 10–15 minutes, and after 2 days, when the delayed reaction was well developed, only 15% was left at the injection site.

Ten minutes after injection of 1 mM chromium trichloride, only 6–7% had disappeared, after 2 days 58–60% was found in the skin, and half of the injected chromium trichloride had not disappeared until 5–12 days after injection. In control subject I, 0.1 ml of a solution of 0.1 mM chromium trichloride was injected intracutaneously. The amount of chromium was thus the same as in the earlier study done with sodium chromate, but one-tenth of the amount otherwise used in the present investigation. Half of the injected 0.1 mM chromium trichloride disappeared in 1 day. The disappearance curve is inter-

mediate to that of 1 mM chromium trichloride and that of 0.1 mM sodium chromate (Fig. 2).

The different disappearance rates of the same amount of chromium given as chromium trichloride and sodium chromate show that the chemical properties of the chromium compound are of importance for the disappearance rate. The disappearance rate of chromium trichloride depends on the concentration, as the initial slope of the disappearance curve of 0.1 mM chromium trichloride is steeper than that of 1 mM chromium trichloride (Figs. 1 and 2). This indicates a threshold in the mechanism of transport. If the disappearance rate had depended on the binding capacity at the injection site, the opposite results would have been found.

van Kooten & van Neer (18) studied the disappearance of sodium bichromate and chromium trichloride injected intracutaneously in guinea pigs. They found that sodium bichromate at first disappeared rapidly from the skin of non-sensitized guinea pigs, and later at a slower rate, compared with the disappearance of bichromate from the skin of sensitized animals. The disappearance of chromium trichloride from the skin of normal guinea pigs had an intermediate course.

The amount of antigen available for a certain period of time after the intracutaneous injection may be of importance in causing a delayed reaction. It is necessary to inject more chromium trichloride than hexavalent chromium in order to elicit a positive test in hypersensitive humans (9). The difference in immunological capacity is not due to the faster disappearance of chromium trichloride. On the contrary, more trivalent chromium than hexavalent chromium is retained in the skin (Fig. 2). This indicates that less of the retained test substance, chromium trichloride, is present in the form of a hapten as compared with sodium chromate.

Positive test reactions elicited by sodium chromate in the earlier study (1) healed within the first week after the intracutaneous test. One of the positive test reactions in this study healed in 4 days, while the test reaction in the other subject (F) was positive, with signs of redness, infiltration and periodic itching during the entire 3 months.

Fregert (10) has reported two cases of repeated "flare-up" reaction at test sites up to 3 and 4 years after intracutaneous testing with chromium

trichloride. Chemical analysis of the excised test sites revealed remanent chromium. Thus, the presumed precipitates of chromium trichloride are not totally insoluble and may still be immunologically active.

The labelling of the blood and the disappearance of chromium from the blood was the same in hypersensitive and non-sensitive subjects. There was, as a rule, no labelling of blood corpuscles. Sometimes activity slightly above background level was found, but it was too low to be converted to significant nanogram values. The distribution of chromium between plasma and blood corpuscles is the same as if chromium trichloride had been injected intravenously (13). Maximum labelling of the plasma was seen on the first day (Fig. 3). The activity of samples No. 2, taken 8–11 hours after injection, exceeded that of samples No. 1 taken 2–2.5 hours after the injection. This may be due to an initially faster transport from the blood to urine and extravascular compartments than the transport from the injection site to the blood.

van Tongeren & Majoor (22) found the half-life of ^{131}I -albumin in blood after intravenous injection to be 21–24 days, while the half-life of ^{51}Cr given as chromium trichloride or as chrome-albumin was, in both cases, 6–9 days, probably because chromium is bound to, or shifts to, transferrin (5, 16, 22). Jarnum & Lassen (17) found a half-life of 8.7 days for ^{131}I -transferrin. The disappearance rate of plasma activity in our study indicates a binding of chromium to transferrin rather than to albumin.

The rate of chromium excretion in urine decreased with time. During the first day 12–17% of the amount injected was excreted, which is the same as 35–44% of the chromium fraction that had disappeared from the skin depot. The proportion excreted in urine in the present study is somewhat higher than the proportion excreted after injection of CrCl_3 directly in the bloodstream (21, 22). According to van Tongeren & Majoor (22), a higher excretion rate may indicate that some of the chromium has been bound to other compounds than plasma proteins.

ACKNOWLEDGEMENTS

The investigation was supported by grants from the Faculty of Medicine, University of Lund, the Alfred Österlund Foundation and Riksförbundet mot Allergi.

REFERENCES

1. Bang Pedersen, N., Bertilsson, G., Fregert, S., Lidén, K. & Rorsman, H.: Disappearance of chromium injected intracutaneously. *Int Arch Allergy* 36: 82, 1969.
2. Bang Pedersen, N., Fregert, S., Naversten, Y. & Rorsman, H.: Patch testing and absorption of chromium. *Acta Dermatovener (Stockholm)* 50: 431, 1970.
3. Bjerrum, J., Schwarzenbach, G. & Sillén, L. G.: Stability constants. Part II: Inorganic ligands. The Chemical Society, Burlington House, London, 1958.
4. Cavill, I. & Jacobs, A.: Skin clearance of iron in normal and iron deficient subjects. *Brit J Derm* 82: 152, 1970.
5. Cohen, Y., Wepierre, J. & Ponty, D.: Fixation du chrome-51 sur les fractions protéiques du sérum sanguin. *Radioaktive Isotope in Klinik und Forschung* 6: 273, 1965.
6. Ellis, J. P., Marks, R. & Perry, B. J.: Lymphatic function: The disappearance rate of ^{131}I albumin from the dermis. *Brit J Derm* 82: 593, 1970.
7. Epstein, S. E.: Contact dermatitis due to nickel and chromate. *Arch Derm (Chicago)* 73: 236, 1956.
8. Fregert, S. & Rorsman, H.: Allergy to trivalent chromium. *Arch Derm (Chicago)* 90: 4, 1964.
9. — Allergic reactions to trivalent chromium compounds. *Arch Derm (Chicago)* 93: 711, 1966.
10. Fregert, S.: Personal communication 1971.
11. Geczy, A. F. & Baumgarten, A.: Distribution of contact sensitizer, 1-fluoro-2, 4-dinitrobenzene, in the tissues of the guinea pig. *Immunology* 22: 381, 1972.
12. Gerber, G., Gerber, G. & Altman, K. I.: Studies on the metabolism of tissue proteins. *J Biol Chem* 9: 2653, 1960.
13. Gray, S. & Sierling, K.: The tagging of red cells and plasma proteins with radioactive chromium. *J Clin Invest* 29: 1604, 1950.
14. Grogan, Ch. H. & Oppenheimer, K.: Experimental studies in metal carcinogenesis. III. Behaviour of chromium compounds in the physiological pH range. *J Amer Chem Soc* 77: 152, 1955.
15. Gustavsson, K. H.: The Chemistry of Tanning Processes. Academic Press, New York, 1956.
16. Hopkins, L. L. & Schwarz, K.: Chromium (III) binding to serum proteins, specifically siderophillin. *Biochim Biophys Acta* 90: 484, 1964.
17. Jarnum, S. & Lassen, N. A.: Albumin and transferrin metabolism in infectious and toxic diseases. *Scand J Clin Lab Invest* 13: 357, 1961.
18. Kooten, W. J. van & Neer, F. C. J. van: Resorption of chromium compounds in guinea pigs after intradermal injection and iontophoresis. *Dermatologica* 132: 183, 1966.
19. Oort, J. & Turk, J. L.: The fate of ^{131}I -labelled antigens in the skin of normal guinea-pigs and those with delayed-type hypersensitivity. *Immunology* 6: 148, 1963.
20. Rollinson, C. L., Rosenbloom, E. & Lindsay, J.: Reactions of chromium (III) with biological substances. *Proc. 7th Internat. Congress of Nutrition, Hamburg, 1966*, p. 692.

21. Rootwelt, K.: Direct intravenous injection of ^{51}Cr chromic chloride compared with ^{125}I -polyvinylpyrrolidone and ^{125}I -albumin in the detection of gastrointestinal protein loss. *Scand J Clin Lab Invest* 18: 405, 1966.
22. Tongeren, J. H. M. van & Majoer, C. L. H.: Demonstration of protein-losing gastroenteropathy. The disappearance rate of ^{51}Cr from plasma and the binding of ^{51}Cr to different serum proteins. *Clin Chim Acta* 14: 31, 1966.
23. Witten, V. H. & March, C. H.: Studies of the mechanism of allergic eczematous contact dermatitis. II. Use of C^{14} labelled 2:4 dinitrochlorobenzene in guinea pigs. *J Invest Derm* 31: 97, 1958.

Received July 10, 1972

N. Bang Pedersen, M.D.
Department of Dermatology
University Hospital
S-221 85 Lund
Sweden