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PATCH TESTING AND ABSORPTION OF CHROMIUM

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Abstract. The fate of chromium in patch tests with $Na_2{}^{51}CrO_4$ in 2 chromium allergic and 2 non-allergic subjects was studied. 20.8 μg chromium was applied on the patch test. Neither disappearance measurement nor examination of blood cells and plasma revealed any absorption. At removal of the patch 7.8–10.3% of the applied chromium remained at the site of application. One month after the test there were 0.22 and 0.13 μg, respectively, at the application sites in the non-allergic subjects, compared with only 0.02 μg in one of the allergic subjects, and none in the other.

The percutaneous absorption of chromium in guinea-pigs has been studied in vivo (2, 9, 10, 11) and in vitro (12). The penetration of chromium through excised human skin has also been investigated (8, 12). The experiments in the guinea-pig indicate a penetration of epicutaneously applied chromium through the skin, as have in vitro studies in man.

Percutaneous absorption of chromium has also been investigated in vivo in man by Mali et al. (5). They claim that chromate ions penetrate equally well as water under patch test circumstances and that the absorbed chromium quickly disappears into the body. However, the experiments reported by these authors and the conclusions made are contradictory and do not lend support to their statement. Further investigation on chromium absorption in man is therefore desirable.

The purpose of the present study was to analyse the percutaneous absorption from conventional patch tests in chromium allergic and non-allergic subjects.

MATERIALS AND METHODS

The investigation was carried out on 4 men between October 1969 and March 1970. Two of the men, aged 28 and 42, had earlier had a chromium contact dermatitis. The other 2 persons, aged 37 and 44, had never had any skin disease.

For *testing* an unbuffered 20 mM solution of Na_2CrO_4 containing ^{51}Cr (AB Atomenergi, Studsvik, Sweden) with and activity of 6–12.5 mCi/ml was used. The chromium concentration was controlled by a spectrophotometric method with diphenylcarbazid as reagent (4).

Using a micropipette 20 μl of the solution was dropped on a test patch (Al-test unit — IMECO (Astra Agency Co.) AB, Stockholm, Sweden, and Astra-Hewlett, Watford, England). The amount of chromium was thus 20.8 μg and the activity between 125–250 μCi. The patch was applied for 48 hours on the volar side of the forearm by a 5×8 cm piece of adhesive tape (Leukoplast—Beiersdorf, Hamburg, DBR).

A *standard source* identical with that used in the test consisted of Leukoplast, test patch and chromate solution, but to prevent any loss of chromate it was sealed with a piece of Leukoflex—(Beiersdorf, Hamburg). For standard measurements the standard was applied to the corresponding site of the other forearm.

All the *measurements* of the gamma radiation were made with a NaI(Tl) scintillation detector with a coarse collimator and with a single channel spectrometer (Nuclear Enterprises) for 100 sec. The radiation measured was between 270 and 370 keV. Before the measurements were started the detector was placed 5 cm above the standard source and the amplifier was adjusted to standard amplification.

The *test subject* was recumbent with his arm supine. The detector was placed at a distance of 5 cm from the test area. To minimize the effect of variation of the position of the arm, distance to the detector, fluctuations in the analyser etc., the measurement was repeated 25 times. After every measurement the detector was removed and the person was allowed to move his arm, before the arm and the detector were replaced in the same position. Measurements of the activity over the test site were made immediately after application of the test patch, immediately before and after removal of the test, and again after 1 month.

After the first series of measurements of the test site at 48 hours the patch was removed. The test area was covered with a piece of Leukoplast and Leukoflex to avoid contamination of the detector during the following series of measurements. No stripping of the test area

Table I. *Chromium at the test site in 2 control subjects (A and B) and in 2 chromium hypersensitive subjects (C and D). The applied quantity is given as 100.00%. The figures are the means of 25 measurements and the standard errors of the means*

Subject	Immediately after application of test	2 days after application of test *before* removal of patch	2 days after application of test *after* removal of patch
A	100.00±0.17	99.72±0.20	7.88±0.02
B	100.00±0.15	100.05±0.21	7.82±0.02
C	100.00±0.18	99.99±0.27	8.48±0.02
D	100.00±0.11	99.79±0.16	10.29±0.03

occurred, because the adhesive surfaces of the plasters faced each other. After these measurements the test area was left unprotected.

Background radiation was measured for 1 000 sec over the forearm after removal of the standard preparation.

The spectral response of the scintillation spectrometer system was slightly count rate dependant. The amplifier was adjusted with the help of a high activity source giving a count rate corresponding to that found at the standard measurement. A subsequent measurement of a low activity source with a count rate corresponding to that found at the measurement of the test site after one month then gave a count rate 4% lower than the true value.

Blood samples were obtained in heparinized tubes before and 1 and 2 days after application of the test patch. The samples were centrifugated for separation of plasma and blood cells (1) and examined for radioactivity in a well type scintillation detector (Nuclear Enterprises).

All values were corrected for physical decay and background radiation and possible variations at different count rates.

RESULTS

The 2 chromium allergic subjects showed positive test reactions with erythema, infiltration and papules. One of these (D) also had vesicle formation. The 2 control subjects developed no inflammation at the test sites.

Table II. *Chromium (ng) in control subjects A and B and in chromium hypersensitive subjects C and D immediately after removal of the test patch after 48 hours, and after 1 month (for A 37 days, for B 31 days, for C 29 days, and for D 34 days). The figures are the means of 25 measurements and the standard errors of the means. The amount applied was 20.8 μg*

Subject	After removal of patch	After 2 month
A	1 639±4	222±1
B	1 626±4	129±0.6
C	1 764±4	17±0.3
D	2 141±5	0

In none of the control or allergic persons was there any measurable disappearance of chromium from the test site during the first 48 hours (Table I). With a probability of 95% the amounts possibly absorbed in the 4 subjects did not exceed 0.14, 0.08, 0.11 and 0.11 μg respectively.

After removal of the test patch only 8–10% of the applied chromium was still on or in the skin, corresponding to 1.6–2.1 μg (Tables I and II).

In control subject A the amount of chromium remaining at the test site after 37 days was 0.22 μg. In control subject B the amount persisting after 31 days was 0.13 μg. Less chromium was found at the test site in the allergic subjects. Thus, after 29 days only 0.02 μg was found in subject C, and in subject D no chromium could be detected after 24 days (Table II).

No radioactivity could be detected in blood cells or plasma from the 4 persons. Minimum detectable activity (95% confidence level) was 15 pCi ^{51}Cr/ml corresponding to 2.5 pg Cr/ml plasma or blood corpuscles.

DISCUSSION

The present study performed with quantities of chromium routinely used for epicutaneous testing did not show any percutaneous absorption of epicutaneously applied chromium during the 48 hour patch test (Table I). Our results obviously contradict the statement of Mali et al. (5): "Chromate ion may penetrate as well as water under patch test circumstances. The amount absorbed percutaneously will disappear very quickly into the body." In our study the conditions for determination of minimal absorption were, however, not optimal, since more than 90% of the chromium applied remained in the test patch and was included in the measurements, which increased the uncertainty of the determination.

Schwarz & Spier (9) demonstrated absorption of trivalent and hexavalent chromium in guinea-pig by examination of excreta and organs, and Czernielewski et al. (2) made similar investigations with hexavalent chromium. Wahlberg & Skog (10) demonstrated percutaneous absorption of sodium chromate in living guinea-pig by disappearance

measurements and organ analysis. When comparing results obtained in experimental animals with ours in man, it should be borne in mind that the techniques were very different. Furthermore, sodium chromate penetrates excised guinea-pig skin more readily than human skin (12).

Chromate may be reduced by the cellulose used in the patch test (6), a fact which may be of importance in the evaluation of our results, since reduced chromium may be less readily absorbed than chromate (11).

After removal of the test patch approximately the same proportion of the applied quantity of chromium was found at the test site in all 4 subjects, i.e. 7.8–10.3 % of the chromium applied. This is considerably less than was found by Ziegler et al. (13), who found 24–35 % left on the skin after removal of the patch, which was, however, of a different type than ours.

The results of the present study may be compared with those of an earlier one (1), where intracutaneous testing was performed with 0.52 μg of chromium as sodium chromate. Most of the injected chromium disappeared very quickly. After 48 hours, when the test reaction was pronounced, 14–18 %, i.e. less than 0.1 μg, was found at the test site.

In the present study 1.6–2.1 μg was found at the test site after removal of the test patch, but it is uncertain how much of it had passed through the skin barrier. Less than 0.08–0.14 μg of chromium, if any, had been absorbed to the inner body within 48 hours of application of the test, by which time the delayed reaction had developed. The amount of chromium persisting in the skin after 1 month (Table II), when the epidermis had been renewed (7) and any bound chromium consequently shed, gives an idea of the magnitude of trans-barrier absorption. However, the chromium found after 1 month may be located to some extent in hairs or in sweat glands, but probably not in the sebaceous glands, as the turn-over time of sebum in the glands is around 7 days (3).

Previous investigations have shown that chromium remaining in or on the skin after removal of the test patch disappears rather slowly (5, 13). In our study 0.22 and 0.13 μg persisted in the skin of the control subjects after 37 and 31 days, respectively, compared with only 0.02 μg in one of the allergic persons after 29 days, and none in the other allergic subject after 34 days. Thus, there was a pronounced difference between controls and allergic subjects in the present study. On the other hand, the same quantity of chromium was found at the test site of controls and allergic subjects 1 month after intracutaneous testing (1), indicating that chromium bound in the dermis seems to be dealt with in the same way in allergic and non-allergic subjects. The smaller amounts of chromium present at the test site 1 month after epicutaneous testing might, therefore, indicate that less chromium reached the dermis in the allergic subjects, possibly because more of the epithelium and applied chromium is shed by the inflammatory reaction.

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