THE LEUCOCYTE MIGRATION TEST IN CHROMIUM HYPERSENSITIVITY*
HENNING THULIN, M.D. AND HUGH ZACHARIAE, M.D.

ABSTRACT

Nine patients with patch tests positive for potassium bichromate were investigated by the leucocyte migration test. Specific inhibition of migration of leucocytes from patients with allergy to chromium was obtained with 3 different antigens: complexes of hexavalent chromium and bovine albumin, trivalent chromium and bovine albumin and hexavalent chromium and human dermal proteins.

The highest degree of inhibition was seen with hexavalent chromium-bovine albumin. This indicates that the specificity of the carrier protein apparently is of lesser importance than earlier assumed. It is of interest to notice that both hexavalent and trivalent chromium may be used in the antigen complexes, probably due to reduction of hexavalent to trivalent chromium prior to binding.

No specific inhibition of migration was found in patients with contact allergy to compounds other than chromium.

Antigen-induced inhibition of migration of macrophages (1, 2) or leucocytes (3, 4) has proved useful in clinical research and has been proposed as an in vitro parameter of cellular (delayed type) hypersensitivity (1, 2, 3). Contact allergy belongs to the group of cellular hypersensitivity (5, 6), and preliminary experiments have indicated that the leucocyte migration test (LMT) may be used for detection of contact sensitivity to certain drugs (7, 8). However, this method has not so far been used to demonstrate contact hypersensitivity to simple chemicals.

The present study, using the LMT in the modification of Bendixen and Søborg (9, 10, 11), is an attempt to demonstrate specific inhibition of leucocytic migration from patients with allergy to chromium. The antigens used were extracts from human skin treated with hexavalent chromium and bovine albumin treated with both hexavalent and trivalent chromium.

MATERIALS AND METHODS

Inhibition of leucocytic migration by chromium, bound to bovine albumin and skin proteins, was studied in 9 patients with a history of allergy to chromium and a positive patch test to potassium bichromate (0.5 per cent in petrolatum) as well as in 9 controls. The controls were patients attending our clinic for venereal diseases. Inhibition of migration of leucocytes, using the same antigens, was also investigated in 8 patients with allergic contact dermatitis without positive patch tests to chromium, but with positive patch tests to other compounds.

Skin-chromium antigen was prepared by binding chromium to skin proteins. This was carried out by the methods used by Anderson (12). Human skin, obtained from surgical operations, was soaked in a 1 per cent solution of potassium bichromate, stored at 4°C for a period of 12 hours, and then suspended in a vessel with running tap water. Washing was carried out for 12 hours to make sure that only chromium bound to dermal proteins remained. The tissue was homogenized in leuco­cyte culture medium (Difco TC medium 199 code 5477) using a motor driven glass homogenizer. The homogenate was stored at 4°C over night and then centrifuged at 1000 g for 30 minutes. The protein concentration of the supernatant was determined by Lowry’s method (13) and adjusted to 2.5 mg protein per ml. The supernatant was used as antigen in two different concentrations, 5 µl or 10 µl per culture chamber (protein concentrations of 0.125 mg/ml or 0.25 mg/ml).

An antigen consisting of hexavalent chromium bound to albumin was prepared in the following manner. Two grams of bovine albumin were dissolved in 6 ml sterile water and 4 ml of 1 per cent potassium bichromate was added. The solution was then stored at 4°C for 3 days and afterwards dialyzed against sterile water for 3 days. The concentration of albumin after dialysis was 10 000 mg%, and no free chromium could be demonstrated. Twenty µl of the solution, corresponding to 2000 mg% albumin, were used as antigen in the culture chambers.

Another antigen was made from bovine albumin treated with trivalent chromium. Five hundred mg of bovine albumin were dissolved in a solution of 10 ml containing 50 mg chromium sulphate (trivalent chromium). After 3 days of incubation at 4°C the solution was dialyzed against sterile water and the amount of albumin was approximately 2500 mg%. Fifteen µl of the solution was used as antigen per culture chamber. The concentration of albumin in the culture chambers was approximately 375 mg%.

The solutions of antigens were adjusted to pH 7.3 before use.

The presence of chromium in skin homogenates as well as in albumin solutions, after dialysis, was demonstrated by gas chromatography (14).

Heparinized blood was obtained from patients as well as from controls and allowed to sediment spontaneously for 1 hour at 37°C. The white blood cells were removed and washed 3 times in Hanks balanced salt solution. The cell suspensions then were transferred to capillary tubes and the leucocytes allowed to migrate for 24 hours in the culture chambers (3). Identical sets of culture chambers were set up for controls and patients, two

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control cultures within each set without antigen, filled with TC medium 199, and duplicate cultures for each antigen. After 20 hours the migration areas of the leucocytes around the opening of the capillary tubes were measured in a projection microscope. The average migration area for two identical cultures, containing antigen (Mx) and TC medium alone (Mo) from the same blood sample determines the migration index (Mi):

\[ \text{Mi} = \frac{\text{Mx}}{\text{Mo}} \]

A value of less than 1.0 indicates inhibition of migration due to the added antigen.

RESULTS

Figure 1 shows inhibition of migration of leucocytes from subjects with allergy to chromium compared with controls. Specific inhibition of migration of leucocytes from persons with allergy to chromium was obtained by using human skin as well as albumin, both treated with hexavalent chromium, as antigens. In all cases the migration indices from subjects with contact dermatitis to chromium were significantly lower than those obtained from controls (P < 0.02 when using 5 µl skin-chromium complex as antigen in the culture chambers and P < 0.05 when using 10 µl). Application of hexavalent chromium, bound to bovine albumin, resulted in highly significant lower migration indices among chromium allergic subjects compared with controls (P < 0.001). In the Figure the normal range of migration indices for controls is indicated as the mean value ± 2 × SD. All migration indices from patients allergic to chromium fall beyond the normal range for the controls. Table 1 shows a comparison between controls with and without allergy and controls with positive patch tests to other compounds than chromium. In this case hexavalent chromium, bound to bovine albumin, 20 µl per chamber, was used as antigen. No significant difference between migration indices in the two groups of controls could be demonstrated. In Table II migration indices from 5 patients with positive patch tests to chromium and 5 matched controls with contact allergy to other compounds than chromium are demonstrated. Trivalent chromium bound to bovine albumin was used as antigen. The difference between migration indices from patients allergic to chromium and controls allergic to other chemicals is significant (P < 0.01).

DISCUSSION

Our results show that delayed hypersensitivity to potassium dichromate may be demonstrated in vitro by LMT. The highest degree of specific inhibition of leucocytes was obtained by using chromium bound to bovine albumin. Both hexavalent chromium and trivalent chromium bound to albumin were found to be effective antigens in vitro.

![Figure 1](image-url)

**Figure 1.** Leucocyte migration indices in patients allergic to chromium (x) and normal human subjects (o). Chromium/skin extract (5 µl and 10 µl per chamber) and hexavalent chromium/albunin (20 µl per chamber) were used as antigens. For chromium/albunin the normal range for the controls is indicated as the mean value of the indices ±2 × SD.
LEUCOCYTE MIGRATION TEST

Table I Comparison of two control groups. One consisting of subjects without contact allergy, the other of patients with contact allergy to other compounds than chromium. Hexavalent chromium/albumin was used as antigen.

<table>
<thead>
<tr>
<th>Migration index</th>
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<tbody>
<tr>
<td>0.80</td>
<td>0.86 (lanolin)</td>
</tr>
<tr>
<td>0.95</td>
<td>0.84 (rubber)</td>
</tr>
<tr>
<td>0.85</td>
<td>1.01 (neomycin)</td>
</tr>
<tr>
<td>0.83</td>
<td>0.86 (p-phenylenediamin)</td>
</tr>
<tr>
<td>0.93</td>
<td>1.13 (nickel)</td>
</tr>
<tr>
<td>0.94</td>
<td>0.89 (Perubalsam)</td>
</tr>
<tr>
<td>1.00</td>
<td>1.09 (rubber)</td>
</tr>
<tr>
<td>0.84</td>
<td>0.76 (Perubalsam)</td>
</tr>
<tr>
<td>0.93</td>
<td>0.89 (rubber)</td>
</tr>
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</table>

Mean 90.55  Mean 93.00
SEM 0.0581  SEM 0.0458
p 0.1

Table II Leucocyte migration indices in patients with contact allergy to chromium and patients with contact allergy to other chemicals than chromium. These agents are indicated in brackets. Trivalent chromium/albumin was used as antigen.

<table>
<thead>
<tr>
<th>Migration index</th>
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<tbody>
<tr>
<td>0.78</td>
<td>0.94 (p-phenylenediamin)</td>
</tr>
<tr>
<td>0.87</td>
<td>1.33 (nickel)</td>
</tr>
<tr>
<td>0.68</td>
<td>1.08 (Perubalsam)</td>
</tr>
<tr>
<td>0.93</td>
<td>1.26 (rubber)</td>
</tr>
<tr>
<td>0.99</td>
<td>1.13 (Perubalsam)</td>
</tr>
</tbody>
</table>

Mean 0.85  Mean 1.14
SEM 0.05  SEM 0.06
p 0.01

albumin resulted in usable antigens. Skin/chromium extracts were used to achieve the specific protein-chromium complex. The importance of the carrier protein to the specific inhibition of the macrophage migration previously has been demonstrated (15, 16). In our case however, dermal proteins other than the chromium complex may have resulted in some non-specific inhibition. The marked inhibition found in the LMT, when chromium bound to albumin was used as antigen,
seems to indicate that the specificity of the carrier protein is of smaller significance than earlier assumed (1, 15). This is in accordance with the results of Cohen (17, 18), who found that patients suffering from contact dermatitis due to chromium showed positive intradermal reaction to chromium chloride and to complexes of chromium chloride and human serum albumin or heparin. Both trivalent and hexavalent chromium complexes resulted in usable antigens. Probably most hexavalent chromium undergoes reduction to trivalent chromium prior to binding to protein (19). However, in our case, the two experimental conditions are not comparable. Different amounts of albumin have been used in the two conditions. Also the toxicity of possible remaining amounts of non-protein bound chromium is far greater for hexavalent than for trivalent chromium. Our data show that contact allergy to other compounds than chromium does not seem to influence the test.

The results of our study suggest that LMT may find its place in dermatology. The advantages of an in vitro test are obvious. Risks of sensitization due to the procedure do not exist, and testing can be carried out at all clinical stages.

REFERENCES