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SENSITIZATION AND TESTING OF GUINEA PIGS WITH POTASSIUM BICHROMATE

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Abstract. In this report, on the one hand, the efficacies of different methods of sensitization in inducing chromium allergy ($K_2Cr_2O_7$) in albino guinea pigs are compared, and on the other, the capacity of different test methods to detect whether the method of sensitization applied has induced chromium allergy. We found that the combined method, with both injection and painting, gave better sensitization than did injections alone.

On comparing ointment, intracutaneous and patch tests, it was found that the first two mentioned gave the most reliable results, based on both macroscopic and microscopic assessment.

A survey of the literature (Table I) shows that, in the attempts made to sensitize guinea pigs with potassium bichromate ($K_2Cr_2O_7$), widely varying methods have been used. In broad outline, a distinction can be made between painting methods and injection methods. As is evident from the table there are great differences among the authors with regard to the $K_2Cr_2O_7$ concentrations, the total amount of chromium administered, the vehicle, etc. Recently Polak & Turk (9) reported a combined method with both injection and painting with $K_2Cr_2O_7$ in different vehicles and administered at different times.

A number of test methods have also been given for determining whether the respective sensitization method causes chromium allergy. These are summarized in Table II. Here the concentrations of $K_2Cr_2O_7$, the vehicle etc. also varied considerably between different methods. Moreover, many questions arise; for example, it is not always possible to ascertain when the test was made in relation to onset of sensitization treatments.

Each author used his own sensitizing and testing method. Nowhere has it been possible to find any comparative investigation indicating the effectiveness of different sensitization methods in inducing chromium allergy. Nor did we find any investigation where one method had been applied for sensitization and then tested parallel with different test methods. Thus, both sensitization and test methods are intimately associated with one another, and it is not possible to state that a special sensitization method is the most effective on the basis of only one method of testing.

When planning our experiments, which were intended to demonstrate the passive transfer of chromium allergy to guinea pigs (11), it was found necessary first to compare different test methods on animals sensitized in various ways.

MATERIALS AND METHODS

Experimental animals: albino guinea pigs, average weight about 300 g when sensitization was begun.

Sensitization methods

I. According to *Hunziker* (2): In this method 1.0 ml of 0.05% $K_2Cr_2O_7$ (J. T. Baker Chemical C., Phillipsburg, New Jersey, USA) in Freund's complete adjuvant (Difco, Detroit 1, Michigan, USA) are injected subcutaneously, twice at an interval of one week (total 1 mg of $K_2Cr_2O_7$).

II. According to *Magnusson* (5): In short, this method consists in injecting intracutaneously 0.1 ml of 1% $K_2Cr_2O_7$ in Freund's adjuvant, daily, 2 days in succession (total 2 mg of $K_2Cr_2O_7$).

III. According to *Polak & Turk* (9) in the following manner: (*a*) Single injection intramuscularly of 0.1% $K_2Cr_2O_7$ in Freund's adjuvant divided into 0.2 ml amounts administered in five different places (total 1 mg of $K_2Cr_2O_7$). (*b*) 2 weeks later 0.1 ml of 0.025% $K_2Cr_2O_7$ in 0.15 *M* NaCl is injected intracutaneously. Repeated 4 times at intervals of 1 week. (*c*) At the same time painting is begun with 0.5% $K_2Cr_2O_7$ in Triton X100 (Alkyl phenoxy polyethoxy ethanol, Rohm and Haas, Philadelphia, USA.) once a week for 4 weeks.

Table I. *Review of literature on sensitization experiments with $K_2Cr_2O_7$ on guinea pigs*

	Painting methods (ref. 4, 6–8, 10, 12)	Injection methods (ref. 1, 2, 5, 6, 9)
$K_2Cr_2O_7$, conc. %	0.05–1.0	0.001–1.0
Total amount $K_2Cr_2O_7$ administered, mg	?,[a]	0.75–3
Vehicle	?,[a] H_2O, Na-lauryl-sulfate, Triton X 100, La8Et.	?[a], H_2O, Freund's adjuvant, NaCl
Pretreatment	Na-lauryl-sulfate, Ether	—
Volume	?,[a] 4 drops, 0.2 ml	0.1–1.0 ml
Area	?,[a] 8 cm^2, 36 cm^2	—
Method of injection	—	Intracutaneous, subcutaneous, intramuscular
Total number of injections and paintings respectively	3–10	1–10
Painting time	?,[a] 2 min	—
Interval between treatments, days	1–2	1–7
Weight of guinea-pigs, g	?,[a] 200–400	?,[a] 300–500

[a] Signifies that in the respective reference no data are given on this point.

Test methods

The guinea pigs were tested 3 weeks after the last sensitization injection or painting.

I. *Patch test* (5) 0.5%, 0.1% $K_2Cr_2O_7$ in distilled water, and only distilled water were applied to the clipped and shaved flanks of the guinea pigs with occlusion for 24 hours. Reading after removal of test patch and 24 hours later.

II. *Intracutaneous test.* We decided to use the concentrations employed by Gross, Katz & Samitz (1): 0.024%, 0.012% and 0.006% $K_2Cr_2O_7$ in distilled water, and only distilled water, 0.1 ml being injected as superficially as possible into the skin, which had previously been shaved to facilitate readings, which were taken after 24 and 48 hours.

III. *Ointment test.* This method has been developed by us, and we have tested by routine with 5.0%, 2.5%, 1.25% and 0.62% $K_2Cr_2O_7$ in petrolatum and only petrolatum. (Vaselinum, Pharm. Nord.); 0.25 ml of the respective test ointment was applied to a clipped area 20 mm in diameter. Occlusion was not used. The animals were fastened for 24 hours, after which the first reading was taken. The second reading was taken 48 hours after the test ointment was applied.

IV. *Painting methods.* These have been described by Wikström (12) among other authors, but our preliminary experiments showed that they gave throughout weaker test reactions and are, moreover, time-consuming and difficult to uniform.

V. *"Nipple-test".* This method is considered to be especially effective (3) in revealing allergy, since spongiosis can be clearly produced in guinea pigs. However, after making some experiments we discontinued the method as guinea pigs have only 2 usable mamillae, which is not sufficient when it is desired to compare different concentrations and to include controls.

Macro- and microscopical assessment

Skin reactions in patch and ointments tests were assessed with the naked eye according to the following scale:

No change	0
Spotty erythema	1
Slight erythema	2
Erythema	3

In the intracutaneous tests redness was measured in mm^2.

The biopsies were made centrally at the test sites with a pair of scissors. They were fixed in formalin, embedded in paraffin, cut at right angles to the surface of the skin, and stained with hematoxylin-eosin.

Table II. *Review of literature on test methods with $K_2Cr_2O_7$ on guinea pigs*

	Painting (ref. 2, 4, 7, 8, 10, 12)	Patch test (ref. 5)	"Nipple" (ref. 2)	Injection (ref. 1, 2, 6)
$K_2Cr_2O_7$, conc. %	0.05–1.0	0.1–0.5	0.5	0.006–0.03
Vehicle	H_2O, Na-lauryl-sulfate La8et, Triton X 100	H_2O	Triton X 100	H_2O NaCl
Volume	?,[a] 1 drop, 0.2 ml	?[a]	?[a]	0.1 ml
Area, cm^2	?,[a]	0.8	?[a]	—
Method of injection	—	—	—	Intracutaneous
Total number of paintings, injections, etc.	1–3	1	1	1
Exposure time before reading, days	?,[a] 0.5–3	1–3	?[a]	2
Interval between sensitization and testing, days	?,[a] 13–28	14	20–44	?,[a] 20–33
Weight of guinea-pigs, g	?,[a] 200–400	—	?[a]	?,[a] 300–500

[a] Signifies that in the respective reference no data are given on this point.

Table III. *Results of patch tests on guinea pigs chromium-sensitized according to Magnusson (5), and on the control animals*

$K_2Cr_2O_7$, conc. % ...		0.5	0.1	Aq. dest.
Control animals (No. 15)	24 h, macr.,[a] mean ± S.E.	0.5 ± 0.2	1.0 ± 0.3	0
	24 h, micr.,[b] mean ± S.E.	2.5 ± 0.1	2.9 ± 0.2	2.4 ± 0.2
	24 h, micr., ratio	1.0	1.2	1.0
	48 h, macr., mean ± S.E.	1.5 ± 0.4	0.5 ± 0.2	0.2 ± 0.1
	48 h, macr., ratio	7.5	2.5	1.0
	48 h, micr., mean ± S.E.	3.5 ± 0.2	2.4 ± 0.2	2.1 ± 0.2
	48 h, micr., ratio	1.7	1.1	1.0
Chromium-sensitized (No. 10)	24 h, macr., mean ± S.E.	1.7 ± 0.4	1.3 ± 0.3	0.1 ± 0.1
	24 h, macr., ratio	17.0	13.0	1.0
	24 h, micr., mean ± S.E.	4.9 ± 0.4	4.6 ± 0.5	2.5 ± 0.3
	24 h, micr., ratio	2.0	1.8	1.0
	48 h, macr., mean ± S.E.	2.2 ± 0.3	1.4 ± 0.3	0.1 ± 0.1
	48 h, macr., ratio	22.0	14.0	1.0
	48 h, micr., mean ± S.E.	6.4 ± 0.5	4.4 ± 0.5	2.9 ± 0.2
	48 h, micr., ratio	2.2	1.5	1.0

[a] macr. = macroscopic assessment.
[b] micr. = microscopic assessment.

The changes were assessed after microscopic examination of about nine sections taken from different levels of the specimen. Changes of the following types were noted and graded:

Acanthosis	1–3
Edema in the epidermis	1–3
Cell infiltration	1–3

RESULTS

For each test series 10–15 animals were used. The tables give the mean values ± the standard error of the mean (S.E.) for each series. The reaction of the individual experimental animals are not given, as we do not regard them of essential importance in this connection. In order to show to what extent the mean values differ from those of the controls (distilled water and petrolatum respectively), ratios between these means have been calculated, and these are given in the tables.

Patch test. In Table III the results are presented of tests performed on 15 control animals and 10 sensitized animals according to Magnusson (5). As a rule stronger reactions were obtained with 0.5 % than with 0.1 % $K_2Cr_2O_7$ both macroscopically and microscopically, with the exception of control animals after 24 hours. There were throughout practically no macroscopic reactions with distilled water, but clear reactions microscopically. In the control animals the difference between 0.1 % $K_2Cr_2O_7$ and distilled water was minimal microscopically.

In order to facilitate the assessment of the results obtained, ratios between the different mean values were calculated. These are shown in Table IV for chromium-sensitized compared with control animals, and for readings after 48 hours compared with those after 24 hours. No unambiguous results were obtained when readings after 48 and after 24 hours were compared. In control animals stronger macroscopic reactions were obtained with 0.5 % after 48 hours; with 0.1 % concentration the opposite result was obtained. In the chromium-sensitized animals the reaction was stronger

Table IV. *Ratios (see text) based on the numerical values in Table III*

		Ratios		
$K_2Cr_2O_7$, conc. % ...		0.5	0.1	Aq. dest.
Chromium-sensitized/ control animals	24 h, macr.[a]	3.2	1.3	1.0
	24 h, micr.[b]	1.9	1.6	1.0
	48 h, macr.	1.5	3.0	0.5
	48 h, micr.	1.8	1.8	1.4
Normal animals	48/24 h, macr.	2.8	0.5	1.0
Normal animals	48/24 h, micr.	1.4	0.8	0.9
Chromium-sensitized	48/24 h, macr.	1.3	1.1	1.0
Chromium-sensitized	48/24 h, micr.	1.3	1.0	1.2

[a] macr. = macroscopic assessment.
[b] micr. = microscopic assessment.

Table V. *Results of ointment tests on guinea pigs chromium-sensitized according to Magnusson (5), and on the control animals, Microscopic assessment*

$K_2Cr_2O_7$, conc. % ...		5.0	2.5	1.25	0.62	Petrolatum
Control animals	24 h, mean ± S.E.	2.5 ± 0.4	2.6 ± 0.3	1.8 ± 0.2	1.7 ± 0.3	1.9 ± 0.3
(no. 10)	24 h, ratio	1.3	1.4	0.9	0.9	1.0
	48 h, mean ± S.E.	2.8 ± 0.3	2.7 ± 0.5	2.6 ± 0.3	2.3 ± 0.3	2.0 ± 0.4
	48 h, ratio	1.4	1.4	1.3	1.2	1.0
Chromium-sensitized	24 h, mean ± S.E.	3.1 ± 0.5	2.8 ± 0.3	2.4 ± 0.3	1.9 ± 0.2	1.8 ± 0.2
(no. 10)	24 h, ratio	1.7	1.6	1.3	1.1	1.0
	48 h, mean ± S.E.	5.1 ± 0.4	4.2 ± 0.3	3.5 ± 0.3	3.0 ± 0.4	2.3 ± 0.2
	48 h, ratio	2.2	1.8	1.5	1.3	1.0
Ratios						
Chromium-sensitized/	24 h	1.2	1.1	1.3	1.1	1.0
control animals	48 h	1.8	1.6	1.4	1.3	1.2
Control animals	48/24 h	1.1	1.0	1.4	1.4	1.1
Chromium-sensitized	48/24 h	1.6	1.5	1.5	1.6	1.3

after 48 hours with 0.5%; with 0.1% there was no difference. Agreement between the macro- and microscopic findings was satisfactory here.

Ointment test. Table V shows the results of tests made on 10 normal animals and on 10 animals sensitized according to Magnusson (5). Macroscopically the reactions were only slight and difficult to assess, and consequently only the microscopic reactions are reported.

In general the mean values increase with increasing concentrations of chromium. However, the values for 0.62% $K_2Cr_2O_7$ do not differ for certain from those for tests with petrolatum alone.

When comparing chromium-sensitized animals with control animals, higher ratios were obtained after 48 hours than after 24 hours.

Intracutaneuos test. In Table VI the results are given of tests made on 10 control animals and 10 animals sensitized according to Polak & Turk (1). As a rule the intensity of the test reaction decreases both macro- and microscopically with decreasing $K_2Cr_2O_7$ concentration. The greatest differences (ratios) were obtained macroscopically, on comparing chromium-sensitized animals with control animals.

Comparative chromium testing (patch tests, intracutaneous tests and ointment tests). Tables VII and VIII show the results of tests made on 10 animals sensitized according to Hunziker (2) and another 10 animals sensitized according to Polak and Turk (9). It was not possible to make macroscopic comparisons between the intracutaneous

Table VI. *Results of intracutaneous tests on guinea pigs chromium-sensitized according to Polak and Turk (9), and on the control animals*

$K_2Cr_2O_7$, conc. % ...		0.024	0.012	0.006	Aq. dest.
Control animals	48 h, macr.,[a] mean ± S.E. (mm^2)	18.5 ± 4.1	3.6 ± 3.0	11.3 ± 3.9	10.9 ± 3.9
(no. 10)	48 h, macr., ratio	1.7	0.3	1.0	1.0
	48 h, micr.,[b] mean ± S.E. (score)	2.3 ± 0.2	2.1 ± 0.2	1.7 ± 0.2	2.2 ± 0.2
	48 h, micr., ratio	1.0	1.0	0.8	1.0
Chromium-sensitized	48 h, macr., mean ± S.E. (mm^2)	104.9 ± 12.8	76.6 ± 10.3	43.3 ± 6.8	42.8 ± 12.5
(no. 10)	48 h, macr., ratio	2.5	1.8	1.0	1.0
	48 h, micr., mean ± S.E. (score)	6.9 ± 0.4	6.4 ± 0.4	5.6 ± 0.3	3.1 ± 0.4
	48 h, micr., ratio	2.2	2.1	1.8	1.0
Ratios					
Chromium-sensitized/	48 h, macr.	5.7	21.3	3.8	3.9
control animals	48 h, micr.	3.0	3.0	3.3	1.4

[a] macr. = macroscopic assessment.
[b] micr. = microscopic assessment.

Table VII. *Comparative $K_2Cr_2O_7$-testing on ten guinea pigs sensitized according to Hunziker (2)*

	Patch test			Intracutaneous test, mm²				Ointment test			
$K_2Cr_2O_7$, conc. % ...	0.5	0.1	Aq. dest.	0.024	0.012	0.006	Aq. dest.	2.5	1.25	0.62	Petro-latum
Macroscopic assessment (48 h)											
Mean ± S.E.	0.8 ± 0.3	0	0	28.9 ± 7.7	16.2 ± 5.6	10.5 ± 3.7	2.8 ± 1.7	2.0 ± 0.1	1.4 ± 0.2	1.1 ± 0.3	0.2 ± 0.1
Ratio	—	—	—	10.3	5.6	3.8	1.0	10.0	7.0	5.5	1.0
Microscopic assessment (48 h)											
Mean ± S.E.	2.2 ± 0.3	1.7 ± 0.3	1.4 ± 0.2	4.7 ± 0.4	3.6 ± 0.2	3.6 ± 0.2	2.6 ± 0.3	5.3 ± 0.4	5.2 ± 0.3	3.8 ± 0.4	1.5 ± 0.2
Ratio	1.6	1.2	1.0	1.8	1.4	1.4	1.0	3.5	3.5	2.5	1.0

tests and the other test methods because the reactions were recorded differently.

Macroscopic. Sensitizing according to Hunziker gave for both intracutaneous and ointment tests, ratios that were about 10 for the highest chromium concentrations. For the lowest concentration in the ointment test (0.62%) the ratio was as high as 5.5.

When sensitizing according to Polak and Turk the highest ratios were obtained with the intracutaneous tests.

Microscopic. When sensitizing according to Hunziker we obtained the highest ratios in the ointment tests, whereas sensitization according to Polak and Turk did not cause any differentiation between the various test methods.

DISCUSSION

All three methods of sensitization were found to cause chromium allergy revealed by all three test methods. Possibly somewhat better results were obtained with the sensitization method of Polak and Turk, which, however, is more time-consuming. Thus, apparently, the test reactions were stronger relative to an increasing number of injections given. The simultaneous epicutaneous application of the chromium salt may also be of importance. This has been pointed out by Magnusson (5) among other investigators. A disadvantage of Hunziker's method was that the animals did not gain weight and the mortality was high.

Comparative testing showed that the distinct differences (higher ratios) that were evident macroscopically between the chromium tests and the controls (distilled water and petrolatum respectively) did not correspond to equally large microscopical differences. On the other hand, in the patch tests with only distilled water, microscopic changes occurred which did not correspond with any skin changes observable with the naked eye. This is probably due to the fact that different parameters are recorded in macroscopic and microscopic assessments, which emphasizes the importance of using both methods of evaluation.

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Table VIII. *Comparative $K_2Cr_2O_7$-testing on ten guinea pigs sensitized according to Polak and Turk (9)*

	Patch-test			Intracutaneous test, mm²				Ointment test			
$K_2Cr_2O_7$, conc. % ...	0.5	0.1	Aq. dest.	0.024	0.012	0.006	Aq. dest.	2.5	1.25	0.62	Petro-latum
Macroscopic assessment (48 h)											
Mean ± S.E.	1.6 ± 0.2	0.4 ± 0.2	0	100.2 ± 12.2	66.1 ± 8.8	37.6 ± 10.8	4.0 ± 2.7	2.4 ± 0.2	1.6 ± 0.2	1.2 ± 0.3	0.2 ± 0.1
Ratio	—	—	—	25.1	16.5	9.4	1.0	12.0	8.0	6.0	1.0
Microscopic assessment (48 h)											
Mean ± S.E.	5.2 ± 0.3	3.9 ± 0.4	2.1 ± 0.3	6.2 ± 0.5	5.4 ± 0.5	4.6 ± 0.3	3.1 ± 0.4	6.4 ± 0.3	5.1 ± 0.4	3.7 ± 0.4	2.2 ± 0.2
Ratio	2.5	1.9	1.0	2.0	1.7	1.5	1.0	2.9	2.3	1.7	1.0

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