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PASSIVE TRANSFER OF CHROMIUM ALLERGY IN GUINEA-PIGS

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Abstract. Passive transfer has been carried out by cutaneous parabiosis and by arteriovenous cross transfusion. The donors were guinea-pigs sensitized with potassium bichromate ($K_2Cr_2O_7$) and dinitrochlorobenzene (DNCB). A positive (successful) transfer was based on the microscopic changes. Both $K_2Cr_2O_7$ and DNCB reactions could be transferred but not in every experiment despite satisfactory blood exchange. The macroscopic and microscopic reactions did not always correspond, which was observed in both donors and recipients.

In a previous work (9) we have reported on the results of different methods for sensitizing and testing guinea-pigs with potassium bichromate ($K_2Cr_2O_7$). It was found that the most pronounced test reactions were obtained after sensitization according to the combined method (5) with both injection and painting. These reactions were elicited with $K_2Cr_2O_7$ either in water, as a patch test, or in oinment, as an "open patch test", as well as by intracutaneous injection. The ointment tests and the intracutaneous tests were the most reliable test methods.

Despite the greatly increased interest in chromium allergy in recent years, which is evident, for example, in the large number of reports (see 9) that deal with sensitization and test methods for guinea-pigs, there is only one investigator [Wikström (11)], who has tried to show that these sensitization procedures actually cause the production of antibodies of the type which, for example, occur in the dinitrochlorobenzene sensitization (DNCB) of guinea-pigs. Wikström performed cutaneous parabiosis and obtained successful transfer in 5 out of 25 experiments.

In the present paper the results of attempts to demonstrate antibodies through passive transfer are described. The experiments have been carried out as a comparison between guinea-pigs sensitized with $K_2Cr_2O_7$ and DNCB respectively. This passive transfer was effected by means of cutaneous parabiosis and through continuous blood exchange.

Table I. *Parabiosis experiments with $K_2Cr_2O_7$-sensitized donors*

Patch test. Score and assessment, see text

Exp. no.	Communication, cm	Donors						Recipients						Evaluation
		Macroscopic			Microscopic			Macroscopic			Microscopic			
		0.5%	0.1%	aq. dest.	0.5%	0.1%	aq. dest.	0.5% 1.5[a]	0.1% 0.5[a]	aq. dest. 0.2[a]	0.5% 3.5[a]	0.1% 2.4[a]	aq. dest. 2.1[a]	
1	3	3	2	0	7	7	2	0	0	0	1	1	2	Neg.
2	5	2	1	1	9	8	6	2	1	0	4	2	1	Pos.
3	5	2	1	0	6	2	1	2	1	0	5	1	1	Pos.
4	5	2	2	0	6	4	4	2	1	0	7	4	3	Pos.
5	5	2	2	0	7	6	2	2	1	1	7	5	4	Pos.
6	6	2	2	0	6	7	1	2	1	0	9	6	2	Pos.
7	5	2	1	1	5	3	3	2	2	0	5	5	3	Pos.

[a] Mean score for control animals at 48 h (9).

Table II. *Parabiosis experiments with DNCB-sensitized donors*

Score, see text

Exp. no.	Communication, cm	Donors				Recipients			
		Macroscopic		Microscopic		Macroscopic		Microscopic	
		1.0%	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5%
1	3	3	3	Pos.	Pos.	0	0	Neg.	Neg.
2	3	3	1	Pos.	Pos.	0	0	Neg.	Neg.
3	5	3	2	Pos.	Pos.	0	0	Neg.	Neg.
4	5	2	2	Pos.	Pos.	2	0	Neg.	Neg.
5	5	2	2	Pos.	Pos.	2	0	Neg.	Neg.
6	2	0	0	Pos.	Pos.	0	0	Neg.	Neg.
7	3	3	3	Pos.	Pos.	0	0	Neg.	Neg.
8	3.5	3	3	Pos.	Pos.	0	0	Neg.	Neg.
9	5	3	2	Pos.	Pos.	3	1	Pos.	Pos.
10	3	3	2	Pos.	Pos.	0	0	Pos.	Pos.
11	5	3	3	Pos.	Pos.	2	0	Pos.	Pos.
12	6	2	1	Pos.	Pos.	1	1	Pos.	Pos.
13	3	3	3	Pos.	Pos.	3	0	Pos.	Pos.
14	5	3	3	Pos.	Pos.	1	2	Pos.	Pos.
15	4.5	1	0	Pos.	Pos.	1	2	Pos.	Pos.

EXPERIMENTAL TECHNIQUE

Experimental animals. Albino guinea-pigs of both sexes and of various breeds, weighing between 350 and 750 g. They were fed on pellets, hay and oats.

Parabiosis. The animals were connected for about 10 cm via the skin in the flanks (7). Tests were made simultaneously on both donor and recipient after 5–7 days of parabiosis; reading and biopsy 48 hours later. The vascular communication between the animals was assessed by separation in the part stitched together and measuring the length of the bleeding surface.

Continuous blood exchange. This is an arteriovenous cross transfusion originally elaborated on rats (4). The experimental technique has been subsequently applied to guinea-pigs by Schröpl & Rippman (6), and gives about 10 times better blood exchange than cutaneous parabiosis. We have further modified the method. The donor's carotid artery is connected by a silicon-treated polyethylene tube (Intramedic Polyethylene Tubing, Clay Adams, New York, USA) (0.23″ is inserted into the vessels and 0.32″ used as connecting joint) with the jugular vein in the recipient and vice versa (Fig. 1). The operation was performed

Table III. *Arteriovenous cross transfusion with $K_2Cr_2O_7$-sensitized donors*

Ointment tests and intracutaneous tests. Score and assessment, see text

Exp. no.	Transfusion, h	Donors											
		Macroscopic						Microscopic					
		Ointment test score			Intracutaneous test, mm²			Ointment test score			Intracutaneous test score		
		2.5%	1.25%	petrol.	0.024%	0.012%	aq. dest.	2.5%	1.25%	petrol.	0.024%	0.012%	aq. dest.
1	24	4	2	1	156	90	4	4	3	1	6	6	2
2	48	4	2	0	100	100	0	5	2	2	6	6	4
3	24	4	2	0	48	36	25	8	1	2	5	5	2
4	24	2	1	0	56	64	0	7	5	4	7	4	3
5	24	2	2	0	120	49	0	2	3	2	5	5	2
6	48	2	1	0	80	56	0	6	4	4	6	3	3
7	24	4	3	2	156	130	42	6	4	3	7	3	3
8	48	3	3	0	—[b]	—[b]	—[b]	6	3	1	—[b]	—[b]	—[b]
9[a]	48	2	1	0	0	0	0	5	3	1	4	4	4
10	24	2	2	0	—[b]	—[b]	—[b]	6	6	3	—[b]	—[b]	—[b]

[a] Donor ill, died one hour after reading. [b] Not carried out. [c] Mean score for control animals at 48 h (9).

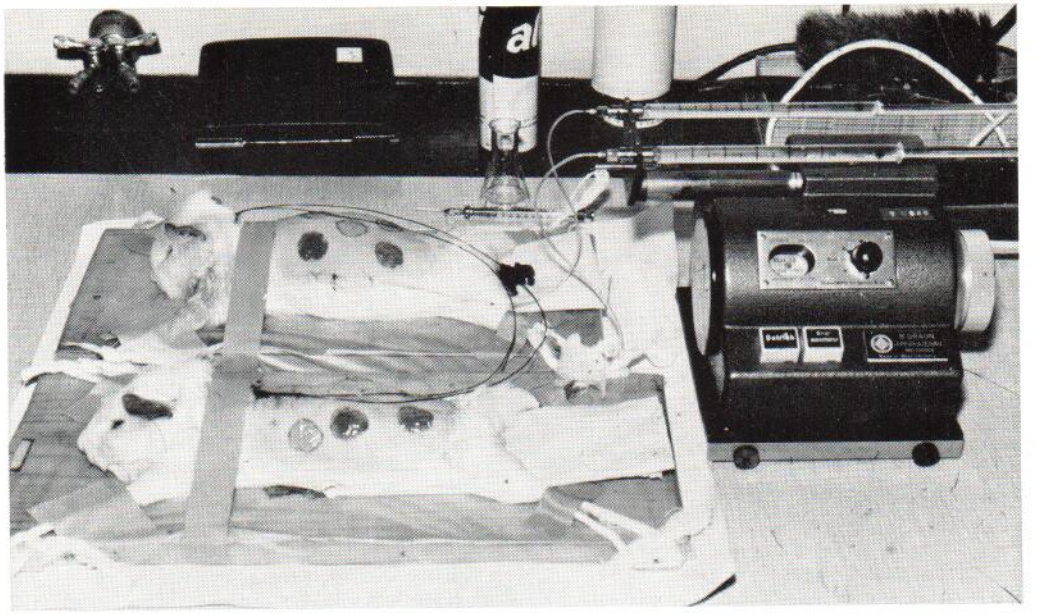

Fig. 1. This is an arteriovenous cross transfusion in progress. The animals are fastened to wooden boards, the polyethylene tubes from the vessels are drawn out through the dorsal skin and connected. Herapin is continously administered subcutaneously by means of an infusion pump.

under nembutal (Nembutal®, Abbot) anesthesia (0.05 mg per 100 g body weight, intraperitoneally). The animals were fastened, after dissection of the carotid arteries and the jugular veins, on special operation boards with the back upward. The polyethylene tubes from the vessels were threaded under the skin and out through the dorsal skin. After stabilizing the circulation the polyethylene tubes were connected as described above. During the whole experimental period heparin (Heparin, Vitrum, Sweden) was continuously administered subcutaneously by an infusion pump (Braun, Melsungen, West Germany), 150–200 IU/hour depending on the weight of the animal. Tests were made simultaneously on both donor and recipient as soon as the animals were connected and the cross

Recipients														
Macroscopic						Microscopic						Evaluation microscopic		
Ointment test score			Intracutaneous test, mm²			Ointment test score			Intracutaneous test score					
2.5%	1.25%	petrol.	0.024%	0.012%	aq. dest.	2.5% 2.7[c]	1.25% 2.6[c]	petrol. 2.0[c]	0.024% 2.3[c]	0.012% 2.1[c]	aq. dest. 2.2[c]	Ointment	Intracutaneous
2	2	0	56	25	30	3	3	2	7	6	3	Neg.	Pos.
2	1	0	25	25	0	2	2	1	5	5	1	Neg.	Pos.
4	2	0	120	100	48	2	2	1	5	5	2	Neg.	Pos.
2	1	0	56	80	42	1	1	1	3	3	2	Neg.	Neg.
2	2	1	36	20	0	3	4	2	4	2	2	Pos.	Pos.
2	1	0	30	30	0	3	3	2	6	5	4	Neg.	Pos.?
3	2	0	100	42	0	3	3	1	4	3	3	Pos.	Neg.
2	1	0	—[b]	—[b]	—[b]	7	4	1	—[b]	—[b]	—[b]	Pos.	—
3	2	1	100	56	49	5	3	2	4	4	3	Pos.	Neg.?
0	0	0	—[b]	—[b]	—[b]	3	2	2	—[b]	—[b]	—[b]	Neg.	—

Table IV. *Arteriovenous cross transfusion with DNCB-sensitized donors*

Score, see text

Exp. no.	Trans-fusion, h	Donors						Recipients					
		Macroscopic			Microscopic			Macroscopic			Microscopic		
		1.0%	0.5%	0.1%	1.0%	0.5%	0.1%	1.0%	0.5%	0.1%	1.0%	0.5%	0.1%
1	>4<24	2	2	1	Pos.	Pos.	Pos.		Dead		Neg.	Neg.	Neg.
2	17	2	2	2	Pos.	Pos.	Pos.	0	0	0	Neg.	Neg.	Neg.
3	>8<24	2	2	2	Neg.	Neg.	Neg.		Dead		Neg.	Neg.	Neg.
4	22	2	2	2	Pos.	Pos.	Pos.	1	0	0	Pos.	Pos.	Neg.
5	>7<24		Dead		Pos.	Pos.	Pos.	2	2	2	Pos.	Pos.	Pos.
6	20		Dead		Pos.	Pos.	Pos.	2	2	2	Pos.	Pos.	Pos.
7	24	2	2	2	Pos.	Pos.	Pos.	1	0	0	Pos.	Pos.	Pos.
8	26	2	2	2	Pos.	Pos.	Pos.	2	2	2	Pos.	Pos.	Pos.
9	24	2	2	2	Pos.	Pos.	Neg.	2	0	0	Pos.	Pos.	Neg.

circulation was functioning. Reading and biopsy after from 20 to 48 hours, i.e. the time the experiment lasted.

Sensitization. Regarding $K_2Cr_2O_7$ see previous report (9). DNCB sensitization (1.3-dinitro-4-chlorobenzene; Pro analysi, Merck. Mol. wt 202.56) was performed according to Chase (1) with the injection of 0.1 ml (= 0.0025 mg of DNCB) daily for 10 days, and thereafter 2 injections per week.

Testing. Testing with $K_2Cr_2O_7$ was carried out according to Skog & Wahlberg (9). For testing with DNCB a drop of the solutions mentioned below was used on an area of 1 cm^2.

At earliest 3 weeks after DNCB and 5 weeks after $K_2Cr_2O_7$ sensitization was started, and subsequently every week guinea-pigs with the most pronounced reactions were chosen as donors. On account of the restricted area for testing in the operated animals not all the test methods for the $K_2Cr_2O_7$ experiments could be used in parallel.

Test concentrations $K_2Cr_2O_7$.

Patch test. 0.5%, 0.1% in distilled water and water control.

Ointment test. 2.5%, 1.25% in petrolatum and petrolatum control.

Intracutaneous test. 0.024%, 0.012%, 0.006% in distilled water and water control.

Test concentrations DNCB. 1.0%, 0.5%, 0.1% in olive oil.

Macroscopic assessment. For the intracutaneous tests redness is given in mm^2, and for the other methods the reactions are graded according to the following scale: no reaction = 0, spotty erythema = 1, slight erythema = 2, erythema = 3, pronounced erythema and edema = 4.

Microscopic assessment. Biopsies were taken from all the test sites on both donors and recipients and the preparation of the histologic sections was done according to the technique earlier described (9). The following changes were assessed: in the $K_2Cr_2O_7$-reactions (9), the occurrence of acanthosis, edema and cell infiltration which were graded from 1 to 3 and in the DNCB-reactions especially the occurrence of spongiosis.

Successful transfer. A transfer was regarded as successful if the assessment of the chromium reactions was higher than the corresponding values for the control animals in accordance with the earlier investigation (9), and for DNCB the occurrence of spongiosis.

RESULTS

Parabiosis. Tables I and II show that in 6 of the 7 experiments $K_2Cr_2O_7$ reactions were transferred and in 7 of 15 experiments DNCB reactions. There was a discrepancy between macroscopic and the microscopic assessments in both donors and recipients, without a definite tendency.

Continuous blood exchange. The results of the $K_2Cr_2O_7$ experiments are presented in Table III, and show that 4 recipients in the ointment tests, and 4 (5) of the 10 in the intracutaneous tests developed microscopic reactions which were more pronounced than in the control animals (9). Nor did the macroscopic and microscopic findings correspond in these experiments either in the donors or the recipients. There was also discrepancy between the reactions elicited intracutaneously and epicutaneously. Corresponding experiments with DNCB-sensitized donors (Table IV) show that in 6 out of 9 experiments also the recipients had positive reactions.

DISCUSSION

The two transfer methods used had whole blood as the transfer medium and in that way differ from the purely cellular mode of transfer. The parabiosis technique has a long history; the arteriovenous

cross transfusion may be considered a technical development of this. Our modification of this method, originally described by Schröpl & Rippman (6), and adapted by them for guinea-pigs, means a considerable simplification. Thus we do not use special cages (4, 6), but only fasten the animals on wooden boards with comparatively short polyethylene tubes between the vessels and in addition they receive a continuous subcutaneous infusion of heparin instead of injection every 8th hour.

We found that the $K_2Cr_2O_7$ and DNCB reactions could be transferred by means of the two methods. As has been previously mentioned (9) the macroscopic and microscopic findings did not always correspond, which is probably due to different factors being recorded by the two methods of assessment. When reading with the naked eye, it is erythema, i.e. vascular dilatation that is taken into consideration, whereas microscopic assessment is based on the three parameters: acanthosis, edema in the epidermis and cell infiltration. The differences between the intracutaneous test and the ointment tests can be explained in the same way, but also owing to the allergen being administered differently. Despite technically successful conditions, in many experiments the recipients did not develop any reactions. The same conditions occurred also in Wikström's (11) parabiosis experiments. In order to successfully effect the passive transfer of contact hypersensitivity, it is apparently necessary for several events to happen simultaneously. The importance of the number of cells has been previously shown (8). Another factor may be the time for allergen stimulation and by that means the formation of antibodies. In our experiments the allergen was applied simultaneously to the donors and the recipients, but more systematic studies are required in order to shed light on this problem. Nor can the influence of humoral factors be excluded. An indication that this may be the case has appeared in the histologic investigation of the passive transfer of DNCB reactions by means of cells. Some investigators (2, 3, 10) have namely emphasized that these are mainly due to vascular reactions and less the result of epidermal changes and cellular infiltration. Our investigations also show, as mentioned above, a discrepancy between the microscopic and macroscopic findings in both donors and recipients, but not in any definite direction, consequently several experiments are necessary with varying technique and repeated biopsies to be able to draw further conclusions. Investigations are in progress in order to be able to answer some of these questions.

REFERENCES

1. Chase, M. W.: Inheritance in guinea-pigs of the susceptibility to skin sensitization with simple chemical compounds. J Exp Med *73:* 711, 1941.
2. Groth, O.: Studies on contact skin reactions and normal skin of passively sensitized guinea-pigs. Acta Soc Med Upsal *68:* 193, 1963.
3. Maibach, H. J. & Maguire, H. C.: Elicitation of delayed hypersensitivity in markedly panleukopenic guinea-pigs. J Invest Derm *43:* 123, 1963.
4. Müller-Ruchholtz, W., Dettweiber, W. & Peeiffer, E. F.: Kontinuerlicher Blutaustausch zwischen unnarkotisierten Ratten mittels doppelter arterio-venöser Kreutztransfusion. Z Ges Exp Med *135:* 368, 1962
5. Polak, L. & Turk, J. L.: Studies on the effect of systemic administration of sensitizers in guinea-pigs with contact sensitivity to inorganic metal compounds. Clin Exp Immun *3:* 245, 1968.
6. Schröpl, F. & Rippman, P.: Die passive Übertragung des DNCB-Kontaktekzems beim Meerschweinchen durch kontinuerliche Austauchtransfusion. Arch Klin Exp Derm *229:* 331, 1967.
7. Skog, E.: Parabiosis experiments. Acta Dermatovener (Stockholm) *35:* 264, 1955.
8. — Passive transfer of hypersensitivity to 2.4-dinotrochlorobenzene. Acta Dermatovener (Stockholm) *35:* 93, 1955.
9. Skog, E. & Wahlberg, J. E.: Sensitization and testing of guinea-pigs with potassium bichromate. Acta Dermatovener (Stockholm) *50:* 103, 1970.
10. de Weck, A. & Brun, R.: De l'eczéma expérimental. Acta Dermatovener (Stockholm) *36:* 360, 1956.
11. Wikström, K.: Epidermal treatment of guinea-pigs with potassium bichromate. Acta Dermatovener (Stockholm) *42:* Suppl. *49,* 1962.

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