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ALLERGIC CONTACT DERMATITIS OF THE MOUSE EAR*

Halvor Möller

Departments of Dermatology and Experimental Research, University of Lund, Malmö General Hospital, Malmö, Sweden

Abstract. Allergic contact dermatitis to picryl chloride on the mouse ear was registered by measuring the increasing wet weight during the inflammatory reaction. This quantitative technique permitted the use of small experimental groups of regular laboratory mice.

Full sensitization to picryl chloride is achieved as early as 3 days after a single painting with the hapten. The allergic reaction peaks at 24 h after challenge. Sensitization and challenge are not inhibited, whether by antihistamine, histamine liberator, or antiserotonin. The hypersensitivity state lasts at least 4 months.

Key words: Contact dermatitis; Delayed allergy; Sensitization; Mouse; Picryl chloride

The mouse has been far too neglected by dermatologists as an experimental animal in studies on allergic contact dermatitis, but it has been widely used by immunologists for studies on delayed-type hypersensitivity as well as pathogenically related immediate-type allergy. The contact dermatitis proper, being less eczematous than in the guinea pig and in man, has been insufficiently studied. The present work provides data on time prerequisites for optimal sensitization and challenge to picryl chloride in the mouse as well as the influence of an antihistamine, a histamine liberator, and an antiserotonin.

MATERIAL AND METHODS

Animals. Female CBA albino mice were obtained from Anticimex AB, Stockholm, Sweden. Their weight was about 30 g, their age 2–3 months when starting the experiments.

Drugs. Picryl chloride was purchased from BDH, Poole, U.K.; before delivery 20% water is added, thus reducing the figures given below to the same degree. Clemastine (Tavegyl®) and methysergide (Sansert®) were obtained from Sandoz AB, Täby, Sweden. According to the manufacturer the mouse LD^{50} by intravenous administration is 43 mg/kg for clemastine, and 180 mg/kg for methysergide. The dose of clemastine used, 1.5 mg/kg i.p., is about 50 times that recommended for human intramuscular administration. The methysergide dose of 6.0 mg/kg i.p. was chosen on the basis of LD^{50} of the two preparations; also, higher doses were not tolerated by the animals. Polymyxin B sulphate was purchased from Pfizer, Brussels, Belgium. It was given in dose of 10 mg/kg i.p. on 3 consecutive days, which is of the same order as that which liberates 53% of the histamine in the mouse ear, and 38% of the serotonin (16).

Sensitization was performed by a single painting onto a 3–4 cm^2 area of the shaved abdomen with picryl chloride 7% in 0.1 ml 99.5% ethanol. The fluid was allowed to evaporate, after which the animal was not bandaged or otherwise restrained.

Challenge was performed by a single painting on both sides of the ear with picryl chloride 1% in 0.05 ml olive oil.

Evaluation. The animal was killed by a blow on the head and the challenged ear excised. The wet weight of the ear tissue was calculated by comparing the weight before and after one hour of heating in a 110°C oven. A similar method has been used successfully in our laboratory to assay phototoxic dermatitis in the mouse tail (6). As controls we used ears from animals 'sensitized' with ethanol only, or from animals sensitized with picryl chloride but 'challenged' with olive oil only. Thus, the inflammatory reaction of the mouse ear may be expressed as the wet weight increase (%) over controls. Mean values from 5–10 animals were used for statistical evaluation which was performed with Student's *t*-test.

RESULTS

Time course of allergic reaction. Twenty mice divided into four groups of 5 animals were sensitized with picryl chloride and challenged one week later. The animals were killed 12 h, 24 h, or 48 h, after challenge. Controls 'challenged' with oil were killed after 24 h. The wet weight increase was: after 12 h, 15%; 24 h, 25%; and 48 h, 14%. The absolute values are given in Table I. The statistical difference between reactions in controls and specifically challenged animals, as well as between 24 h responses on the one hand and 12 h and 48 h responses on the other, was highly significant.

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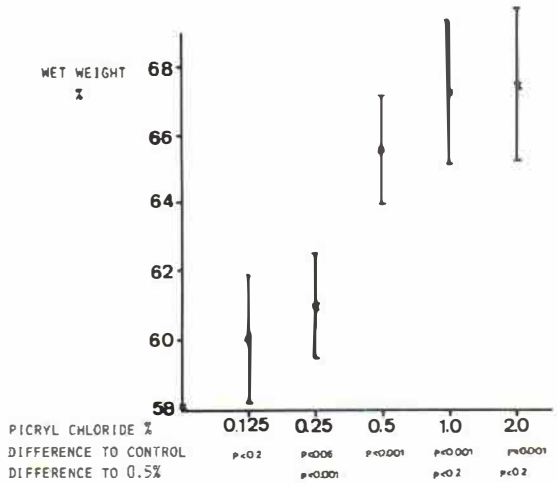

Fig. 1. Epicutaneous ear testing in mice sensitized to picryl chloride: reaction at 24 h. Mean values and S.D.

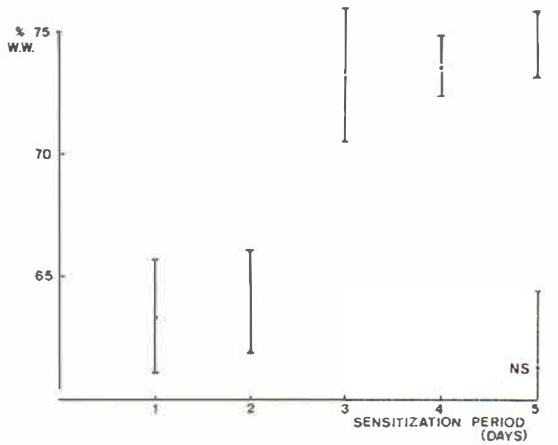

Fig. 2. Contact dermatitis in mice challenged 1–5 days after sensitization to picryl chloride. NS = non-sensitized controls. Mean values and S.D.

Challenge dose and vehicle. Thirty mice divided into six groups of 5 animals were sensitized to picryl chloride and challenged one week later with doses of 0.125–2.0% picryl chloride in olive oil (controls: oil only). The animals were sacrificed 24 h after challenge. As shown in Fig. 1 there was a weak edematous reaction to 0.25% picryl chloride ($p<0.05$) and strong reactions to 0.5, 1.0 and 2.0% ($p<0.001$). There was no statistical difference between the responses to the three highest challenge doses.

The role of the challenge vehicle was examined by painting 10 non-sensitized mice with olive oil on the right ear, and 10 other mice with 95% ethanol on the right ear. The animals were sacrificed after 24 h and both ears assayed for wet weight. It was found (Table II) that oil application increased ($p<0.05$) the wet weight of the ear tissue by 2.8% while ethanol had no effect.

Sensitization time. Five groups of 5 animals each were sensitized to picryl chloride and challenged 1–5 days later. As control I used a non-sensitized group which was challenged with picryl chloride after 5 days. All mice were sacrificed 24 h after challenge. As can be seen in Fig. 2 there was no allergic reaction in animals challenged 1 or 2 days after sensitization, but a clear reaction in those challenged after 3–5 days ($p<0.001$). There was no statistical difference between responses obtained in animals challenged 3, 4 and 5 days after sensitization.

Duration of allergy. Five groups of 5 animals each were sensitized to picryl chloride and challenged 1–16 weeks later. As controls I used five groups of non-sensitized animals but challenged with picryl chloride at the same time as the earlier described groups. All mice were sacrificed 24 h after challenge. As shown in Table III an allergic response could be elicited after all intervals tested.

Influence of an antihistamine on sensitization and challenge. Mice sensitized to picryl chloride

Table I. *Time course of challenge reaction in mouse ear after sensitization with picryl chloride*

	Controls	12 h	24 h	48 h
n	5	5	5	5
Mean w.wt. %	57.5	66.4	71.8	65.8
S.D. ±	1.6	1.4	2.3	2.4
p (vs 24 h)	<0.001	<0.001		<0.001

Table II. *The role of the challenge vehicle*

Animals were painted with olive oil or ethanol on right ear. All ears were assayed for wet weight 24 h later

No. of animals ...	10		10	
	Left	Right	Left	Right
Ear treatment	–	Oil	–	Ethanol
Mean w.wt %	56.6	58.2	56.2	55.6
S.D. ±	1.5	1.7	1.4	1.1
Statist. difference	$p<0.05$		–	

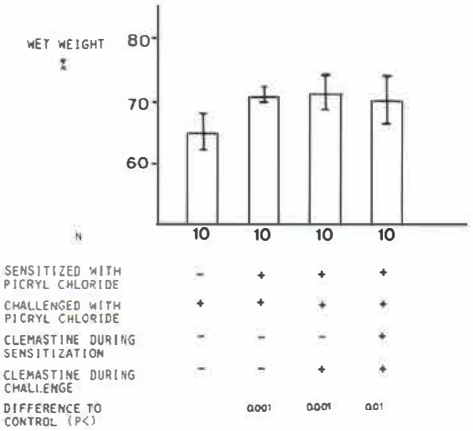

Fig. 3. Allergic contact dermatitis to picryl chloride in the mouse ear induced in animals treated and not treated with clemastine during sensitization and challenge. Mean values and S.D.

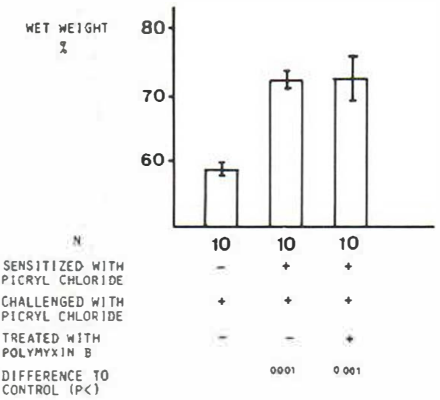

Fig. 4. Allergic contact dermatitis to picryl chloride in the mouse ear induced in animals treated and not treated with polymyxin B before and during challenge. Mean values and S.D.

were treated once daily from sensitization with clemastine 1.5 mg/kg intraperitoneally. Simultaneous with challenge one week later they were given the same dose which was repeated after 12 h; the animals were sacrificed after a further 12 h. One group of animals were treated during the 24 h of challenge only. As controls I used one group sensitized and challenged with picryl chloride but not treated with clemastine, and one group not sensitized but challenged with picryl chloride. The results are presented in Fig. 3.

It can be seen that the challenge induced an allergic contact dermatitis in all three groups sensitized and challenged with picryl chloride. The response was not inhibited in animals treated with clemastine during development of the ear edema, nor in those also treated during the sensitization period.

Table III. *Duration of allergy*

Five groups of 5 mice each sensitized to picryl chloride as well as 5 non-sensitized control groups were challenged after 1–16 weeks

Challenge time (weeks)	Controls (w.wt % ±S.D.)	Sensitized (w. wt % ±S.D.)	Increase (%)
1	64.0±1.1	73.8±1.8	15.3
2	61.2±2.1	71.7±2.5	17.2
4	62.6±4.3	72.3±2.1	15.5
8	59.0±2.1	66.5±2.4	12.7
16	55.8±1.0	67.4±4.6	20.8

Influence of a histamine liberator. Mice sensitized with picryl chloride were treated on 3 consecutive days with polymyxin B, 10 mg/kg i.p. On the last day they were challenged with picryl chloride and sacrificed 24 h later, this being 8 days from sensitization. As controls I used one group sensitized and challenged with picryl chloride but not treated with polymyxin B, and one non-sensitized group challenged with picryl chloride but not treated with polymyxin B. As shown in Fig. 4 the challenge reaction in sensitized animals was similar, whether they had been treated with polymyxin B or not.

Influence of an antiserotonin on sensitization and challenge. Mice sensitized to picryl chloride were treated once daily from sensitization with methysergide 6 mg/kg intraperitoneally. Simultaneous with challenge 4 days later they were given the same dose which was repeated after 12 h; the animals were sacrificed after a further 12 h. One group of animals were treated during the 24 h of challenge only. As controls I used on group sensitized and challenged with picryl chloride but not treated with methysergide, and one group not sensitized but challenged with picryl chloride. The results are presented in Fig. 5.

It can be seen that the challenge induced an allergic contact dermatitis in all three groups sensitized and challenged with picryl chloride. The response was not inhibited in animals treated with methysergide during development of the ear edema, nor in those also treated during the sensitization period.

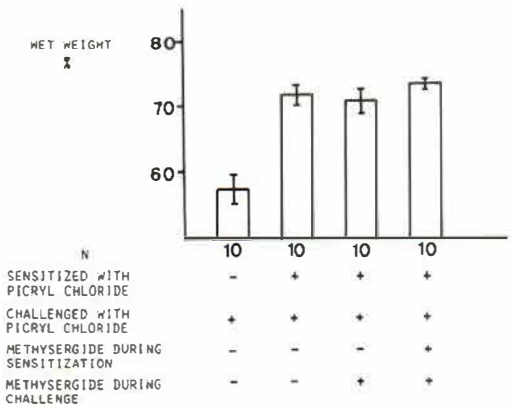

Fig. 5. Allergic contact dermatitis to picryl chloride in the mouse ear induced in animals treated and not treated with methysergide during sensitization and challenge. Mean values and S.D.

Actually, the inflammatory response was slightly increased in the group treated with methysergide during sensitization and challenge when compared with that treated during challenge only ($p<0.02$) or with the non-treated group ($p<0.01$).

DISCUSSION

Experimental allergic contact dermatitis has traditionally been studied in the guinea pig. The choice of experimental animal has been based on the similarity of macroscopic and microscopic challenge reactions to those in man, and the relative ease with which sensitization has been induced. Crowle & Crowle (2) should be credited for introducing the regular laboratory mouse as an in vivo model for allergic contact dermatitis. They used flank skin for sensitization and challenge and the latter reaction was recorded on the basis of diameter, thickness and necrosis. The method was elaborated by Asherson & Ptak (1) who found the challenge reaction to be more easily elicited on the ear. This has been ascribed to the rich occurrence of mast cells containing histamine and serotonin in auricular dermis (4, 16). For the same reason, allergic contact dermatitis is more easily provoked in the mouse's paw than in the flank skin.

The registration procedure was improved by Asherson & Ptak (1). The allergic contact dermatitis of the mouse ear is in essence a dermal reaction, not eczematous. Therefore, with the mice under light ether anaesthesia, they could measure the increment of edematous thickness with an engineer's micrometer. The method has become very popular among immunologists for studying the delayed-type allergy, using picryl chloride, oxazolone and dinitrofluorobenzene as principal sensitizers.

In the present study the ease with which laboratory mice can be sensitized with picryl chloride was confirmed. The allergic contact dermatitis was recorded by assaying the increase in wet weight of ear tissue over that of controls. In this way, standard deviations within animal groups were small, the groups could be limited in number to 5–10 mice, and graded inter-group comparisons were easily carried out. These conditions would probably be modified by using weaker sensitizers.

For several reasons it is recommended to use proper controls through all experiments. First, the wet weight of ear tissue from non-treated mice may vary from one batch to another (~58–64%), and second, it seems to decrease with age (Table III). Third, controls using identical vehicles are necessary, since application of olive oil alone may induce a slight wet weight increase (Table II).

One disadvantage with the method is, of course, that the inflammatory reaction could be assayed only once.

Earlier studies have suggested (1, 9, 13) that the challenge reaction is maximal at 24–48 h, with a regression to normal at 72 h, but the peak has never been defined. With the present method it was possible to demonstrate a maximal challenge reaction at 24 h, clearly stronger than that at 12 h and 48 h. Since it is known from studies in man that the maximal challenge reaction can vary from one antigen to another, it would be wise in future experimental studies in the mouse to define the peak challenge reaction for the particular antigen.

When testing different challenge doses in picryl chloride sensitized mice, 0.25% gave a weak reaction, and 0.5, 1.0 and 2.0% strong reactions without significant differences between the latter. Thus, a dose–response curve was indicated but could not be statistically confirmed.

All mice were sensitized within the optimal time range, 2–4 months of age (10). According to Crowle & Crowle (2) mice became sensitized 2 weeks after a single exposure to dinitrochlorobenzene, the sensitivity being maximal after 3 weeks, and disappearing after 4 weeks. With two exposures, contact allergy lasted at least 3 months. With picryl chloride the challenge reaction was found evident 7 days

after a single painting but not after 14 days (1). With two daily paintings of dinitrofluorobenzene or oxazolone a positive challenge reaction was observed as early as after 4 days (9).

In the present study with picryl chloride the shortest sensitization period was looked for and found to be 3 days (Fig. 2). This remarkably short induction period agrees well with other evidence of contact sensitivity obtained in the mouse: the proliferation of pyroninophilic blast cells in the thymus-dependent area of draining lymph glands at 3 days (13); the increased DNA synthesis there at 4 days (7); and the transfer of contact sensitivity by lymphoglandular cells already 4 days after sensitization (18). There was no evidence in the present experiments of an increased sensitivity in mice challenged later than 3 days.

The duration of contact allergy in the mouse after a single exposure to the hapten has thus been claimed to be less than 4 weeks (2) or even less than 2 weeks (1). This would of course limit the usefulness of the technique for experimental work. In the present report, however, contact sensitivity was proved to last much longer: vigorous challenge reactions were elicited at least 16 weeks after sensitization (Table III). My results, at variance with the earlier investigations, are probably explained by the refined recording procedure.

It has been repeatedly shown that experimental contact allergy in the mouse and guinea pig is not a pure cell-mediated delayed-type hypersensitivity (2, 14, 17). The process definitely contains elements of immediate hypersensitivity, too. Thus, signs of a positive challenge reaction are discernible as early as after 4 h. Histologically, the cellular infiltrate in the mouse is dominated by polymorphonuclears (12, 13), while in the guinea pig substantial numbers of basophils appear (3). In the mouse, reaginic antibody is produced within a week of a single painting with picryl chloride (14).

These findings have renewed the old interest in the role of biogenic amines in cell-mediated skin reactions. Inderbitzin (5) demonstrated a marked increase of cutaneous histamine in the allergic contact dermatitis and tuberculin reaction of the guinea pig, but the reactions were not inhibited by an antihistamine. In the mouse, challenge reactions are more easily elicited in the skin areas where histamine- and serotonin-containing mast cells abound (4, 16). Crowle & Crowle (2) could not influence the contact sensitivity reaction in the mouse with an antihistamine but some inhibition was observed with an antiserotonin. Reserpin does not affect the total histamine content of the mouse, but it liberates a large amount of body serotonin (15). This drug which suppresses the capacity of mast cells to take up and/or store serotonin, was tested in mouse contact sensitivity (4); it partially inhibited the challenge reaction to oxazolone and dinitrofluorobenzene. However, reserpin also brings about the complete disappearance of cutaneous catechol amines (8); the importance of this effect was not ascertained. Recently, Roupe & Granerus (11) demonstrated an increased urinary excretion of histamine during provocation of contact dermatitis in the mouse without a concomitant change of histamine content in the challenged ear.

Against this background it seemed essential to examine the effect of a strong and long-acting antihistamine on allergic contact dermatitis in the mouse. Clemastine was tested both during the entire sensitization period to picryl chloride and/or during challenge, all without effect on the inflammatory reaction (Fig. 3). Nor did polymyxin B, an effective histamine liberator in the mouse (16), inhibit the development of contact dermatitis (Fig. 4). Nevertheless, it is interesting that polymyxin B induces a partial disruption and degranulation of cutaneous mast cells in the mouse (16), a similar event that has been observed during the contact sensitivity challenge reaction (12).

The role of serotonin in delayed-allergy reactions is poorly defined. Polymyxin B is not only a histamine but also a serotonin liberator (16) and therefore, since this drug did not influence the inflammatory response, serotonin too seems to be of little importance. In the original work by Crowle & Crowle (2) the effect of methysergide on the challenge reaction to dinitrochlorobenzene was inconclusive; that of another antiserotonin, however, inhibitory. By the present quantitative technique methysergide had no inhibitory effect on the challenge reaction to picryl chloride (Fig. 5). It should be pointed out that care was taken to avoid interference by any factors of humoral allergy by using a sensitization period of only 4 days. Surely, effect of reserpine on the delayed-allergy reaction described above must work by some other mechanism than by an influence on cutaneous serotonin.

It remains to be explained why methysergide treatment, when given during the sensitization period, resulted in a somewhat exaggerated chal-

lenge reaction. Should this be confirmed in another species, the role of serotonin will have to be considered from a new aspect.

CONCLUSION

Allergic contact dermatitis is easily achieved in the mouse, at least when using a strong sensitizer. A positive challenge reaction can be provoked after a few days and the hypersensitivity state persists for months. The new assay technique has improved quantification and this in vivo model should certainly be used more widely by experimental dermatologists, not least for reasons of economy.

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H. Möller, M.D.
Department of Dermatology
Malmö General Hospital
S-21401 Malmö
Sweden