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Time Course of Contact Hypersensitivity to DNFB and Histologic Findings in Mice

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This experiment pursued the time course of contact hypersensitivity to 2,4-dinitro-1-fluorobenzene (DNFB) and histologic changes of the cutaneous reaction in mice.

The contact hypersensitivity reached a maximum 4 days after sensitization (96.9±6.7% vs. 22.7±1.3% in control) and persisted for 3 weeks. The cutaneous hypersensitivity reaction showed peak reactivity at 24 hr after challenge (96.2±4.7% vs. 11.5±1.7% in control), and presisted up to 96 hr (13.2±2.1%). Prime histologic changes observed in this experiment were the exocytosis of lymphoid cells and epidermal thickening which appeared at 20 hr after challenge. Edema, vasodilatation and increased mast cells were observed within the dermis at 4-8 hr. However, edema and vasodilatation disappeared gradually, but numbers of mast cell increased up to 96 hr. The dermal infiltrates were maximum at the 28-72 hr after challenge.

Key Words: *Contact hypersensitivity.*

INTRODUCTION

The experimental allergic contact dermatitis has traditionally been studied in the guined pig. The mouse has many practical advantages for experimental delayed-type hypersensitivity as well as pathologically related immediated-type hypersensitivity, ascribed to the rich occurence of mast cells (Gershon et al.,1975) and has been used as an in vivo model for experimental allergic contact dermatitis by many investigators (Asherson & Ptak, 1968; Phanuphak et al., 1974).

This study was designed to investigte the time course and intensity of contact hypersensitivity (CHS) to 2.4-dinitrol-1-fluorobenzene (DNFB) and histologic findings.

MATERIALS AND METHODS

Animal:

Female albino mice (hybrid BALB/c), about 8 weeks old weighing 15-20 gram were used.

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Antigen:

DNFB (Sigma Chemical Co., St. Louis, Missouri, U.S.A.) was dissolved in vehicle of acetone-olive oil (v/v=4/1) to make 0.5% and 0.2% solution for sensitization and challenge, respectevely.

Sensitization and challenge:

All mice except the control group were sensitized 25 ul of 0.5% DNFB solution on the clipped abdomen once a day for two consecutive days. Mice for experimental and control groups were challenged with 20ul of 0.2% DNFB solution on the dorsum of right ear, and 20 ul of vehicle were applied on the left ear as a control.

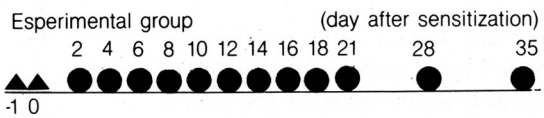
Measurment of the reaction and histologic preparation:

A constant area of the ear was measured with Mitsutoyo engineer's micrometer. The increment rate of ear swelling was expressed as follow.

$$\% \text{ of increment} = \frac{\text{thickness of the right ear-thickness of the left ear}}{\text{thickness of the left ear}} \times 100$$

Biopsy specimens were taken from the right ear (challenge site with DNFB) by 3 mm punch, immediately after measurement of ear thickness. The specimens were fixed in 10% buffered formalin solution, and 3-4 um sections were stained with hematoxylin-eosin and Wright-Giemsa.

Experiment I. Time course of the contact hypersen-h sitivity.

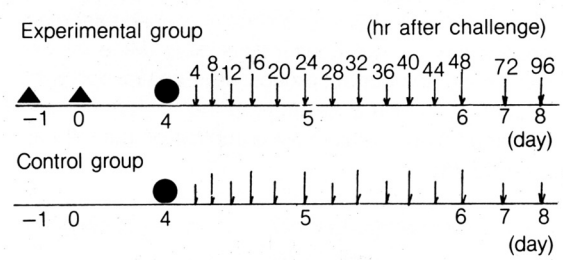


▲ Sensitized with 25 ul of 0.5% DNFB in acetone-olive oil (v/v=4/1) solution on the lower abdomen once a day for two consecutive days.

● Challenged with single application of 20 ul of 0.2% DNFB in acetone-olive oil (v/v=4/1) solution on the dorsum of right ear, and with 20 ul of vehicle(acetone-olive oil, v/v=4/1) on the dorsum of left ear, in each group of 10 animals and in each group of day.
Control group: Ten unprimed animals were challenged with DNFB on the right ear and vehicle on the left ear same as in the experimental group.
Twenty-four hr after challenge, the right and left ear thickness were measured, and the increment rate of ear swelling was calculated as follow:

$$\% \text{ of increment} = \frac{\text{thickness of the right ear-thickness of the left ear}}{\text{thickness of the left ear}} \times 100$$

Experiment II. Time course of ear thickness changes of the reaction after challenge.



▲ Sensitized same as in the experiment I.

● Challenged same as in the experiment I, each group of 5 animals.

↓↓ The ears were measured and biopied.

Fig. 1. Experimental designs

Study deisng

Experiment I: Time course of contact hypersensitivity.

A total of one hundred and twenty mice were sensitized on Day-1 and Day 0. Each experimental group composed of ten mice was challenged at Day 2, 4, 6, 8, 10, 12, 14, 16, 18, 21, 28 and 35 respectively. Ten umprimed mice challenged with DNFB were taken as a control group. The thickness of both ears were measured immediately after being killed by wringing the neck 24 hr after challenge.

Experiment II: Time course of ear thickness and histologic changes of the reaction after challenge.

One hundred experimental mice were sensiti :ed on Day-1 and Day 0 same as the experiment I and challenged on Day 4. At the same day (Day 4), the other one hundred unprimed mice of control group were challenged. The thicknesses of both ears were measured at 4 hr intervals form 4 hr to 48 hr, and at 72 hr and at 96 hr after challenge, and biopsy specimens were taken from the right ear (Fig. 1)

RESULTS

Experiment I: Time course of the contact hypersensitivity (Table 1).

The increment rate of ear thickness was 10.7±2.3% in the Day 2 group (challenged at 2 days after sensitization) and 96.9±6.7% in the Day 4 group which was the maximum reactivity among all the experimental groups. Thereafter, the CHS reaction decreased gradually up to 35 days after sensitization (15.4±1.6%).

Experiment II: Time course of ear thckness and histologic changes of the reaction after challenge (Table 2).

The challenge was performed on Day 4 in all the experimental groups, which represented the maximum reactivity at 4 days after sensitization in experiment I. The initial increment of the ear thickness was observed at 4 hr after challenge in both primed and unprimed animals (experimental group; 71.2±3.6%, control group; 72.4±2.8%), and then, the thickness gradually decreased. At 16 hr, the secondary increment appeared (67.±3.8%) and reached in maximum reaction at 20-28 hr (over 90%) but the control group did not show the second peak, and thereafter a slow decrement was shown in the experimental group and reached lowest ebb at 96 hr (13.2±2.1%).

The earliest changes observed at 4-8 hr after challenge were marked edema, vasodilation and in-

Table 1. Time course of the contact hypersensitivity

Group* (day)	2	4	6	8	10	12	14
Increment (%)**	10.7 ± 2.3	96.9 ± 6.7	82.8 ± 5.5	68.3 ± 6.5	49.2 ± 5.3	35.4 ± 3.2	28.8 ± 2.9
Group (day)	16	18	21	28	35	control	
Increment (%)	24.5 ± 3.5	22.7 ± 1.3	24.2 ± 1.9	16.3 ± 2.7	15.4 ± 1.6	10.4 ± 1.1***	

*After sensitization on Day-1 and Day 0, 10 animals were randomly chosen for challenge on each day.
**Increment of the thickness of right ear (challenged with DNFB) against to the left ear (challenged with vehicle only).
***Each value represents the mean ± S.D.

Table 2. Time course of the reaction after challenge

Group * (hour)	4	8	12	16	20	24	28
Increment (%)**							
experiment group	71.2 ± 3.6	36.5 ± 4.9	39.5 ± 4.1	67.6 ± 3.8	90.1 ± 5.2	96.2 ± 4.7	92.7 ± 5.2
control group	72.4 ± 2.8	33.3 ± 2.9	30.1 ± 1.8	22.8 ± 2.1	13.2 ± 2.5	11.5 ± 1.7	10.8 ± 2.1
Group (hour)	32	36	40	44	48	72	96
Increment (%)							
experiment group	85.0 ± 6.4	73.8 ± 4.4	69.5 ± 2.9	56.6 ± 4.7	43.4 ± 4.2	22.3 ± 3.8	13.2 ± 2.1***
control group	11.4 ± 3.1	12.5 ± 2.7	10.3 ± 1.2	8.4 ± 1.1	8.8 ± 1.5	5.3 ± 0.7	2.7 ± 0.8

*After sensitization on Day -1, Day O and challenge on Day 4, 5 animals were ranoomly chosen for measurement of reaction on each time.
**Increment of the thickness of right ear (challenged with DNFB) against to the left ear (challenged with vehicle only); experiment group (group of primed animal with DMFB), control group (group of unprimed animal).
***Each value represents the mean ± S.D.

Table 3. Time dependent patterns of histologic changes

Group (hr)	Epidermal				Dermal changes											
	Epidermal cell layer		Exocytosis		Microabscess		Edema		Infiltrates		MNC:PMNL		Mastcell		vessel	
	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C
4	1-2	1-2	−	−	−	−	+ + +	+ + +	0	0	≫	≫	+	+	+	+
8	1-2	1-2	−	−	−	−	+ +	+ +	0	0	≫	≫	+	+	+	+
12	1-2	1-2	−	−	−	−	+ +	+ +	0	0	>	>	+ +	+ +	+	+
16	1-2	1-2	−	−	−	−	+ +	+ +	+	+	≧	≧	+ +	+ +	+	+
20	2	1-2	+	+	−	−	+ + +	+ +	+	+	≥	≃	+ +	+ +	+	+
24	2-3	1-2	+	+	+	−	+ + +	+	+	+	≧	≃	+ +	+ +	+	±
28	2-3	1-2	+ +	+	+	−	+ + +	+	+ +	+	≃	≃	+ +	+ +	+	±
32	2-3	1-2	+ +	−	+	−	+ + +	—	+ + +	0	≃	≃	+ + +	+	+	±
36	2-3	1-2	+ +	−	+ +	−	+ +	—	+ + +	0	≃	≧	+ + +	+	+	−
40	3	1-2	+ +	−	+ +	−	+ +	—	+ + +	0	≃	>	+ + +	+	+	−
44	3	1-2	+ +	−	+ +	−	+ +	—	+ + +	0	≥	>	+ + +	+	+	−
48	3	1-2	+ +	−	+	−	+ +	—	+ + +	0	≥	≫	+ + +	+	+	−
72	4-6	1-2	+	−	—	−	+ +	—	+ +	0	>	≫	+ + +	+	—	−
96	4-8	1-2	+	−	—	−	+	—	+	0	>	≫	+ + +	+	—	−

MNC: mononuclear cell PMNL: polymorphonuclear cell E: experimental group C: control group
Exocytosis & Microabscess: absent (−), a little evidence (+), prominent (+ +)
Edema: nearly normal (−), slight (+), moderate (+ +), marked (+ + +)
Infiltrates: nearly absent (0), slight (+), moderate (+ +), marked (+ + +)
Mast cells: a few (+), moderate (+ +), many (+ + +)
Vessel: nearly normal (−), suspicious dilatation (±), definite dilatation (+)
Proportion of infiltrates (MNC:PMNL): almost composed of MNC (≫), mostly composed of MNC (>), nearly equal proportion but predominently composed of MNC ( ), nearly equal proportion ( )

creased mast cells within the dermis. At 20 hr, the exocytosis of lymphoid cells, microabscess and proliferation of epidermal cells were observed, and then, the changes became intense with time. The cellular infiltrates in the dermis was gradually increased and reached to maximum at 28-72 hr, and particularly, the mast cell which posseses single round nucleus, round to oval or polygonal cell contour and basophilic granules within the abundant cytoplasm persisted up to 96 hr (Table 3, and Fig, 2-9).

DISCUSSION

Contact hyperseneitivity to DNFB in mice is a form of cell-mediated immunity which can be assessed by challenging the sensitized animals on the ears and measuring increased ear swelling with an engineer's micrometer.

The maximum contact hypersensitivity was observed as early as 4 days after sensitization, and these data consistant to some other previous studies (Phanuphak et al., 1974; Claman & Miller, 1980; Moller, 1981). De Sousa and Parrott (1969) observed the proliferation of pyroninophilic blast cells in the thymus-dependent area of draining lymph nodes at 3 days after sentization with picryl chloride and Zembala et al (1975) established the transfer of CHS by lymphoid cells 4 days after sensitization.

We noted that CHS to DNFB in mice declined with time after an apex. However, the declining of CHS has been caused by the mediation of suppressor T-cell or a specific antibody. Polak and Rinck (1977) suggested suppressor T-cell mediated decrement of CHS which was proved by an enhance CHS when suppressor T-cell was eliminated by cyclophosphamide. Sy et al. (1979) suggested that the anti-receptor antibody appears in the serum can suppress the passive transfer of sensitivity to DNFB when co-transferred to unprimed recipients together with T_{DH} from sensitized donors.

The time course of the reaction showed two peaks in experiment II. One is the initial reaction at 4 hr in both experimental and control group, and the other is the secondary reaction at 20-28 hr in experimental group. These suggest that the initial reaction may be an irritant reaction and the secondary reaction may be a allergic type of a delayed reaction.

The sequential biopsy specimens from the ear lobes showed three features in the epidermis and dermis. One is the increased number of mast cell. Dvorak (1976) demonatrated a high percentage of basophilic cells in skin infiltrates of CHS. These cell were, however, never found in delayed-type hypersensitivity reacion. Therefore, CHS is called cutaneous basophil hypersensitivity. It may occur in man and guinea pig and may be induced by sensitization with variety of antigens and elicited by skin testing with the specific antigens. Other interesting features are exocytosis of lymphoid cells and microabscess in the dermis.

In allergic contact dermatitis of the experimental group, exocytosis of lymphid cell started at 20 hr after challenge and became marked between 32 hr to 48 hr and declined gradually up to 96 hr. The microabscesses were observed from 24 hr to 48 hr which consisted of lymphocytes. The other change which tool place in the epidermis was that the increased number of epidermal cell layers in experimental group. Although the mechanism is uncertain, it may be supposed the cytokines from the infiltrates and some chemical substances during the inflammatory process play a role in the proliferation of epidermal cell.

The comparison of ear thickness to microscopic findings showed that the increment of ear thickness was well reflected by dermal edema, declined slowly in the experimental group, in contrast to the control group, which may account for the amount of dermal infiltrates.

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Explanation for Figures

Fig. 2. Microphotograph of normal mouse skin taken from the dorsum of ear lobe showing 1or 2 epidermal layer and some mononuclear cells including mast cells (*). (II & E stain, ×250)

Fig. 3. Microphotograph of experimental mouse skin at 4 hr after challenge with DNFB showing marked edema, vasodlatiation (*), and a few mast cells. (H & E stain, × 250)

Fig. 4. Microphotograph of experimental mouse skin at 48 hr after challenge with DNFB showing epidermal thickening, exocytosis (↑), microabscess (*) and marked infiltration of leucocytes. (H & E stain, ×250)

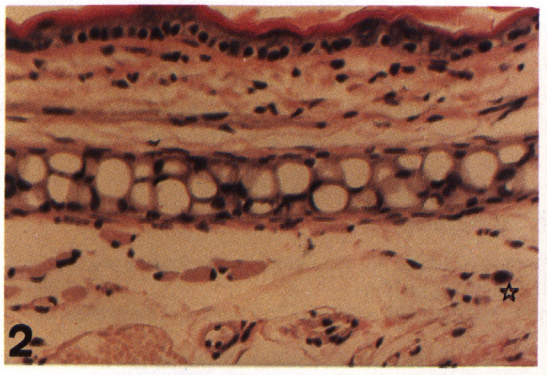
Fig. 5. Microphotograph of experimental mouse skin at 96 hr after challenge with DNFB showing exocytosis, moderate infiltration of leucocytes with mast cells, and considerable thickening of epidermis. (H & E stain, × 250)

Fig. 6. Microphotograph of control mouse skin at 4 hr after application with vehicle showing marked edema and vasodilatation. (H & E stain, ×250)

Fig. 7. Microphotograph of control mouse skin at 48 hr after application with vehicle showing nearly normal feature. (H & E stain, ×250)

Fig. 8. Microphotograph of normal mouse skin showing a few mast cells in the dermis. (Wright-Giemsa's stain, ×250)

Fig. 9. Microphotograph of experimental mouse skin at 48 hr after challenge with DNFB showing many mast cells in the dermis. (Wright-Giemsa's stain, ×250)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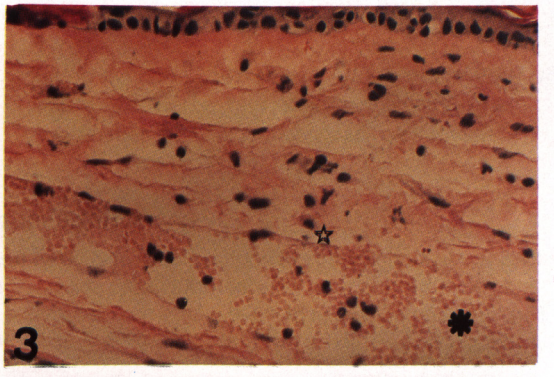
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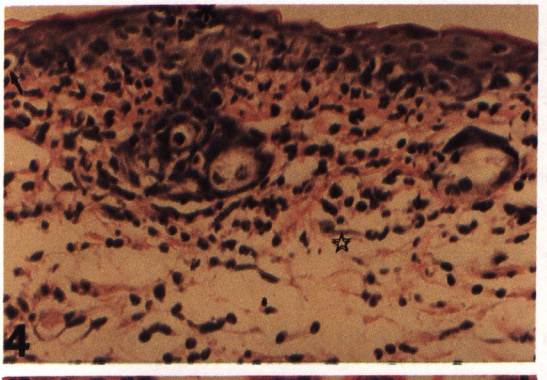
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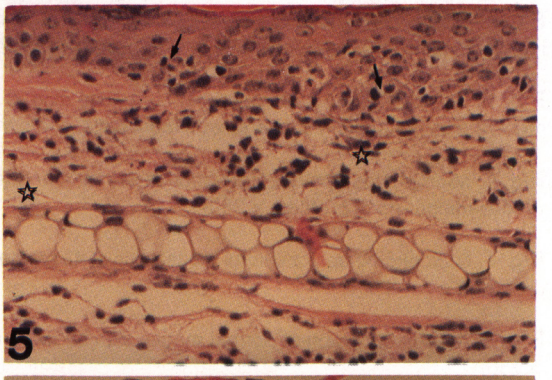
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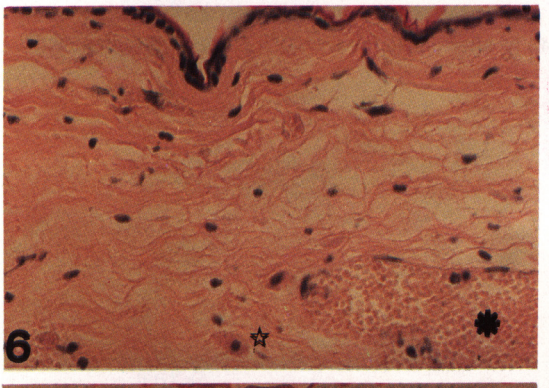
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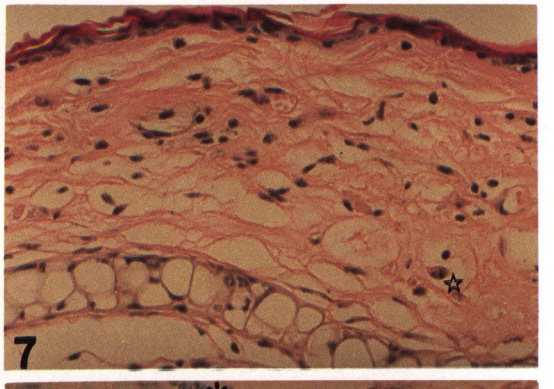
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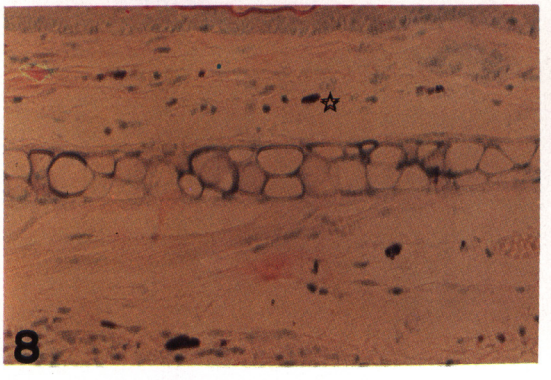
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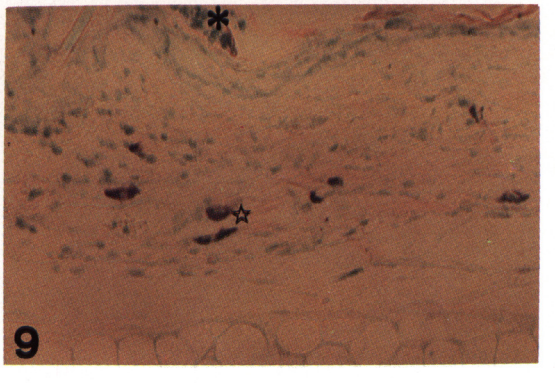
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