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MEASUREMENT AND DIFFERENTIATION OF THE CELLULAR INFILTRATE IN EXPERIMENTAL TOXIC CONTACT DERMATITIS

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Abstract. Toxic (irritant) contact dermatitis was elicited by epicutaneous application of dinitrochlorobenzene (DNCB) and croton oil in unsensitized guinea pigs. The course of the skin reaction was studied with the naked eye, low-power microscopy, and a method based on counting of infiltrating cells in the upper corium. A weakly toxic dose of DNCB (100 $\mu g/cm^2$) gave marked erythema and moderate swelling, more pronounced 6 h after application of the DNCB than at 24 h or 48 h. A significant increase in mononuclear cells in particular but also in neutrophil and eosinophil granulocytes was noted in the upper corium. A more strongly toxic dose (500 $\mu g/cm^2$) gave a similar visible reaction, this too most marked at 6 h, but here there was an increase in neutrophil granulocytes in particular. Over and above predominant mononuclear-cell infiltration, croton oil (10 $\mu g/cm^2$) caused a slight increase in basophil cells. The reaction thus resembled a contact allergic reaction. In the light of earlier findings in allergic contact reactions the results suggest that weak stimuli, toxic or allergenic, elicit a non-specific skin response. With stronger stimulation the histological picture is modified and the cellular response acquires a pattern characteristic of toxic or allergic contact dermatitis.

Key words: Toxic contact dermatitis; Irritant contact dermatitis; Microscopic assessment; Cellular infiltrate; Mononuclears; Polymorphonuclears

Investigations in large groups of patients have shown that toxic (irritant) contact eczema is commoner than allergic contact eczema in many categories of workers (1, 4, 20, 21). Nevertheless, little interest has been directed towards the cellular reaction in toxic eczema compared with contact allergy. This may be because the skin is readily accessible for studies on the cell-mediated immune response which contact allergy represents. For the individual patient with eczema it may be important to know whether the contact eczema is allergic or toxic, with regard to prognosis.

It may be difficult or impossible to distinguish the two types of reaction with the naked eye. Histological criteria for a toxic reaction have been searched for in skin biopsy preparations from humans (2) and laboratory animals, notably guinea pig (6, 11, 12, 13, 16, 23). Epidermal necrosis and separation from the dermis accompanied by cellular infiltration in the dermis are said to be characteristic. According to some researchers (2, 6, 11, 23) the dermal infiltrate consists of both mononuclear and polymorphonuclear cells, whereas others (12, 13, 16) maintain that the polymorphonuclears predominate. Polymorphonuclear cells also characterize the cytological appearance of the blister fluid (18). Certain authors, however, maintain that it is impossible to distinguish between an allergic and a toxic reaction by means of the histological findings (10, 17, 19). It has been reported that isotope labelling of round cells and epithelial cells differes in allergic vis-à-vis irritant patch test reactions (8, 14). The visible skin

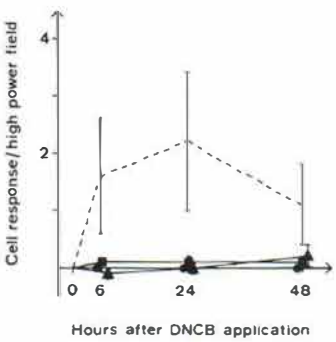


Fig. 1. Quantitation of mononuclear-cell and polymorphonuclear-cell infiltration in the upper corium in 10 unsensitized guinea pigs after epicutaneous application of 20 μg of DNCB/cm^2 (means and standard error of the mean). Cell response is given as the number of cells per high-power field in tested skin minus the corresponding number of cells in normal skin. All animals were tested 6, 24 and 48 h before the specimens were taken. ---, Mononuclear cells; ●, neutrophil granulocytes; ▲, eosinophil granulocytes; ■, basophil granulocytes.

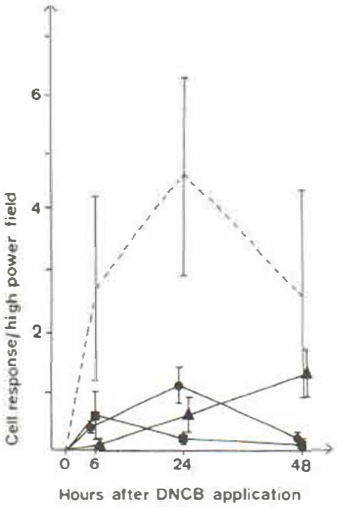


Fig. 2. DNCB 100 μg/cm². The animals were the same as those in Fig. 1. ---, Mononuclears; ●, neutrophils; ▲, eosinophils; ■, basophils.

reaction is often characteristic for each individual toxic substance (3), and the histological picture apparently shows some variations, depending on the substance concerned (11). The concentration of substance required to induce a toxic reaction varies between individuals (3).

A method for absolute and differential counting of the cellular infiltrate in the upper corium in allergic contact eczema in guinea pigs has been described previously (9). This was employed in the present investigation to analyse the cellular infiltrate in toxic reactions to various doses of DNCB in unsensitized animals. Croton oil was used for comparison.

MATERIAL AND METHODS

Animals

19 female albino guinea pigs (Dunkin–Hartley strain) weighing ca. 300 g were used. They were kept at 22–25°C in separate cages, and were fed on standard pellets (Astra-Ewos AB, Sweden).

Testing

Both flanks were shaved at least 2 h before testing. In 10 unsensitized guinea pigs 20, 100 and 500 μg of dimtrochlorobenzene (DNCB) dissolved in acetone was applied. These three single test doses were applied to different areas of 1 cm² on the flanks 48, 24, and 6 h before reading the tests. In 9 guinea pigs 10 μg of croton oil dissolved in acetone was applied to different areas of 1 cm² on the flanks 72, 48, 24, and 6 h before reading. The tests were examined with the naked eye, the animals were killed, and a biopsy specimen 4 mm in diameter was taken from each test area and from intact skin.

Naked-eye assessment

The reactions were graded as follows: 0=no change, or uncertain reaction, + = redness, often slightly irregular in distribution, ++ = redness with slightly palpable induration, +++ = redness and obvious swelling.

Histological technique

The specimens were fixed in 10% neutral, phosphate-buffered formalin and embedded in glycol methacrylate and polyethylene glycol (JB-4 Plastic Embedding Kit®, Polysciences, USA). 3-μm thick sections were cut and stained with May-Grünwald-Giemsa stain.

Classification and counting of cells in the corium

Several widely-separated sections were taken from each specimen. Counts were made of mononuclear and polymorphonuclear cells in 20 fields in the upper corium (1 000×, oil-immersion). The result is given as number of cells per high-power field. The number of cells in the test area was reduced by the number of cells in normal skin in the same animal. The difference is the number of new cells in the test area after application of test substance, and is called the *mononuclear response* or *polymorphonuclear response* in the corium. When differential counts of polymorphonuclear cells were made, the terms neutrophil, eosinophil, and basophil response are used. The various polymorphonuclear groups and mast cells were identified

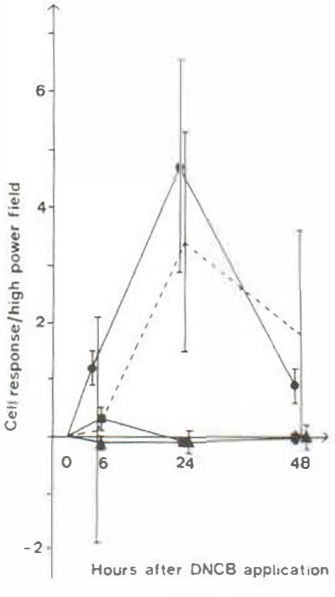


Fig. 3. DNCB 500 μg/cm². The animals were the same as those in Fig. 1. ---, Mononuclears; ●, neutrophils; ▲, eosinophils; ■, basophils.

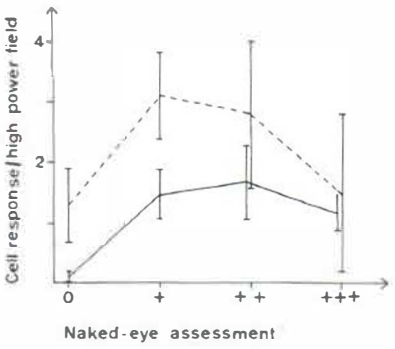


Fig. 4. Mononuclear-cell and polymorphonuclear-cell infiltration in 10 unsensitized guinea pigs after epicutaneous application of DNCB, compared with naked-eye assessment (means and standard error of the mean). ---, Mononuclears; —, polymorphonuclears.

by the appearance of the nucleus and the cytoplasmic granules. For details of classification and counting of cells, see (9).

Statistical method

Student's *t*-test was used.

RESULTS

DNCB

20 μg of DNCB/cm² gave slight erythema after 6 h in 8 of 10 animals, but no reaction could be seen by the naked eye at 24 or 48 h. Microscopy of all specimens showed that this dose caused no obvious changes in the epidermis over and above small patches of slight spongiosis. In normal-looking corium, cell counting disclosed a tendency to slight increase in the mononuclear-cell count, but this was not statistically significant (Fig. 1). The polymorphonuclear-cell count was the same as in normal skin.

100 μg of DNCB/cm² gave marked erythema and induration that were more intense at 6 h than at 24 or 48 h. The tests were positive at 24 and 48 h, but the erythema was less marked. Microscopy showed epidermal acanthosis and spongiosis from 24 h and later (Fig. 7), and at 24 h intra-epidermal vacuoles were present and the epidermis had partly separated from the corium. At 48 h the most superficial part of the epidermis was necrotic, took on a strikingly deep colour with May-Grünwald-Giemsa stain, and was sharply delimited from the rest of the epidermis (Fig. 8). An increase in the number of cells was seen in the upper corium from 24 h and on,

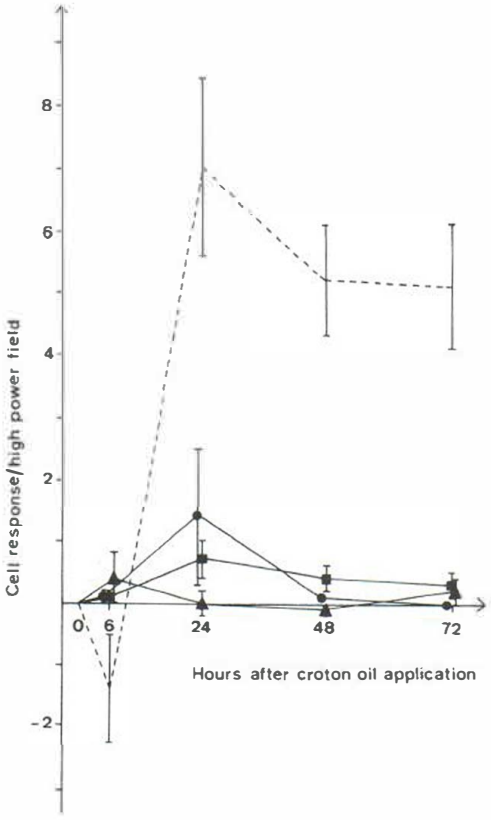
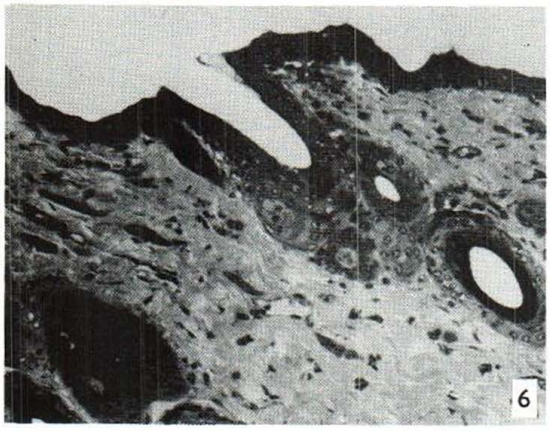
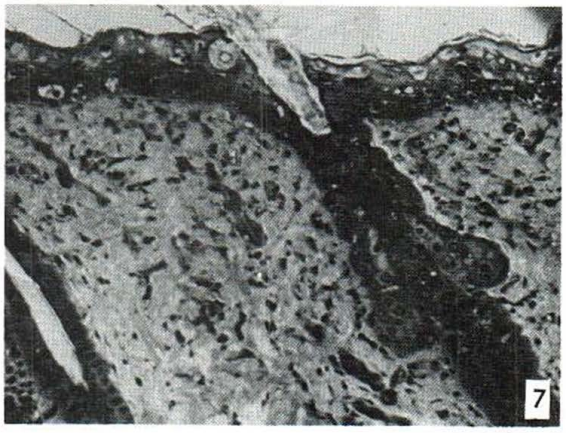


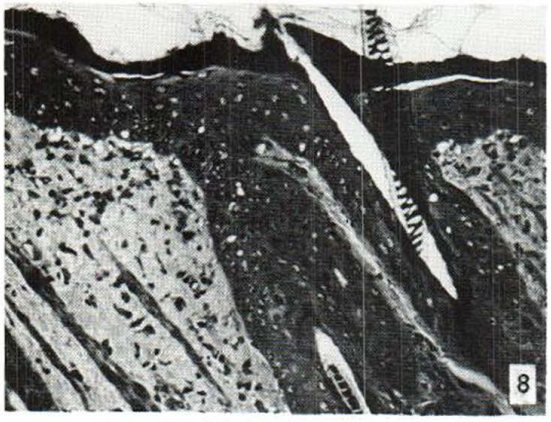
Fig. 5. Quantitation of mononuclear-cell and polymorphonuclear-cell infiltration in 9 guinea pigs after epicutaneous application of 10 μg croton oil/cm² (means and standard error of the mean). All animals were tested 6, 24, 48 and 72 h before biopsy. ---, Mononuclears; ●, neutrophils; ▲, eosinophils; ■, basophils.

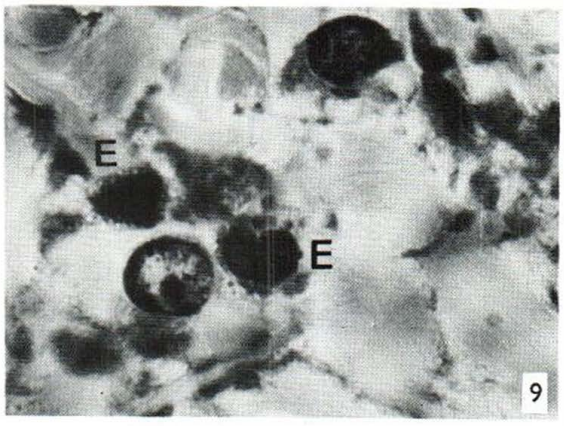
but no changes were found in the lower corium. No perivascular accumulations of cells were seen. An increase in the mononuclear-cell count in the upper corium was noted at 24 h compared with the untested skin ($p<0.05$) (Fig. 2). The increase was no longer significant at 48 h. The number of neutrophil granulocytes was raised at 24 h ($p<0.01$), but at 48 h was below the 24-h value ($p<0.01$). The count of eosinophil granulocytes was raised at both 24 h ($p<0.05$) and 48 h ($p<0.01$) (Fig. 9). No significant change in the basophil granulocyte count was noted at 6, 24 or 48 h compared with normal skin.

500 μg DNCB/cm² also gave a stronger visible reaction at 6 h than at 24 and 48 h, with erythema and induration. Low-power microscopy showed marked epidermal separation from the corium at 24 h, but no acanthosis or spongiosis. Massive









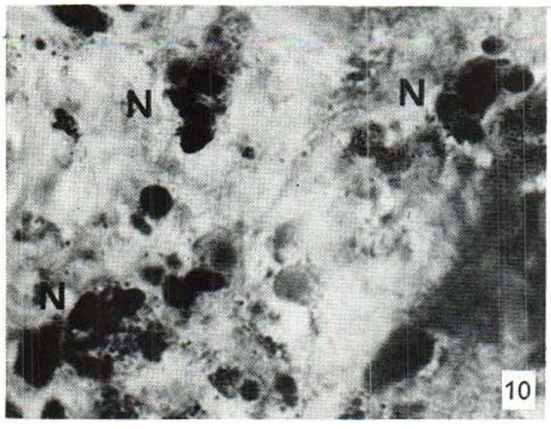


Fig. 6. Normal guinea pig skin. ×160.
Fig. 7. 24-h test reaction, 100 μg DNCB/cm². Epidermal acanthosis and spongiosis, particularly in the upper layers. Slight cellular infiltration in the upper corium. ×160.
Fig. 8. 48-h test reaction, 100 μg DNCB/cm². Upper epithelial layer homogeneously dark-stained, necrotic. Structures in the basal layers starting to regenerate (cf. 24 h). ×160.
Fig. 9. 48-h test reaction, 100 μg DNCB/cm². Eosinophil granulocytes (E) and mononuclear cells in the upper corium. ×1600.
Fig. 10. 24-h test reaction, 500 μg DNCB/cm². Neutrophil granulocytes (N) with multilobulated nuclei in the upper corium. ×1600.

granulocyte infiltration was seen in parts of the upper corium and between the corium and the detached epidermis. Extensive necrosis was seen in the upper parts of the epidermis at 48 h, and new, regenerating epidermis could be discerned under the detached areas. Owing to injured neutrophil granulocytes and often abscess-like accumulations at 24 h, counting of isolated cells in the corium was sometimes difficult by the method used here, and counting was therefore limited to abscess-free areas in the upper corium. Even so, the count of neutrophil granulocytes was distinctly raised at both 24 h ($p<0.05$) and 48 h ($p<0.01$) (Figs. 3 and 10). If continuous counting including the abscess-like re-

gions had been done, the figures would have been still higher. At 24 h the mononuclear-cell count was insignificantly increased, by up to 3 cells per field. Neither eosinophil nor basophil granulocytes were increased, compared with normal skin. The mean number of mast cells was less than 1 cell per field in both normal and tested skin.

Fig. 4 illustrates the relationship between the visible reaction and the increase in cell count in all DNCB-tested animals. The comparison is made irrespective of dose, or interval between application of DNCB and reading of the test.

Croton oil

This substance gave weak erythema within the test area at 6 h. The visible reaction increased and was strongest at 24 h, after which it subsided slightly. Further fading was noted at 72 h, and slight scaling was seen in most animals. At 6 h there were no microscopical changes in the epidermis or corium. At 24 h, epidermal acanthosis and spongiosis were present; these were further accentuated at 48 h, and persisted at 72 h. Like DNCB, croton oil caused cellular infiltration, notably in the upper corium. One animal showed granulocyte infiltration in the walls of the hair follicles. Cell counting in the upper corium (Fig. 5) revealed an increase in mononuclear cells 24 h ($p<0.01$), 48 h ($p<0.001$), and 72 h ($p<0.001$) after application of croton oil. An insignificant increase in neutrophil granulocytes (by 1 cell per field) was found at 24 h. The basophil granulocyte count was increased at 24 h ($p<0.05$), but not at 48 or 72 h. The mean number of mast cells was less than 1 cell per field in both normal and tested skin.

DISCUSSION

In this investigation DNCB was applied to the skin of unsensitized guinea pigs, three different concentrations being used. The aim was to study the course and pattern of the reaction, especially as regards the cellular infiltration occurring in toxic contact dermatitis. 20 $\mu g/cm^2$ was chosen, as in the strain of guinea pigs used this is regarded as a subtoxic dose. It was also the dose previously used for eliciting reactions in already-sensitized animals (9). 100 $\mu g/cm^2$ was chosen to bring about weak reactions and 500 $\mu g/cm^2$ for strong reactions.

The weak erythema that was obtained initially after 6 h in most animals tested with 20 $\mu g/cm^2$ had disappeared at 24 and 48 h. Low-power microscopy showed no convincing changes, however, and the erythema must therefore be interpreted as an early, transient vascular response. The visible response to testing with the two stronger concentrations of DNCB showed a similar course, with a more intense initial reaction.

With all three concentrations of DNCB the intensity of the visible reactions was thus greatest initially, in contrast to allergic reactions; and in toxic dermatitis caused by DNCB the visible skin reaction did not parallel the increase in cell count in the upper corium as occurs in contact allergy to the same substance (9). Other events, chiefly vascular reactions, are probably responsible for the visible skin reaction, and observations on contact allergy in animals treated with cytostatic drugs corroborate this (15, 22).

A small dose of DNCB, 20 $\mu g/cm^2$, caused a slight but insignificant increase in mononuclear-cell count in the upper corium in these unsensitized animals, whereas the polymorphonuclears showed no tendency to increase. The same dose, however, gave allergic eczema with marked increase in both mononuclears and polymorphonuclears in sensitized animals (9). This proves that the size of the dose per unit area of an agent that is both toxic and allergenic determines the nature of the reaction.

The difference between the cellular responses to the two toxic doses of DNCB, 100 and 500 $\mu g/cm^2$, is interesting. 100 $\mu g/cm^2$ gives an increase in mononuclear, neutrophil, and eosinophil granulocytes. The 500 μg dose causes cellular infiltration entirely dominated by neutrophils. A weakly toxic DNCB reaction, here elicited by 100 $\mu g/cm^2$, with only slight epidermal changes, some mononuclear response, and a slight polymorphonuclear response, could be confused with a contact allergic reaction. In an earlier study on cellular infiltration in the upper corium in allergic contact reactions in DNCB-sensitized guinea pigs (9) there was a highly significant polymorphonuclear response that was dominated during the observation period (72 h) by basophil granulocytes. According to the findings of this study and earlier observations (9), the cellular infiltrate in the upper corium of guinea pigs in toxic DNCB reactions differs from the infiltrate in allergic reactions in that the mononuclear-cell response in toxic reactions is weaker and the polymorphonuclear response consists of neutrophils or eosinophils instead of basophils. It remains to be

seen whether the same applies to other agents and other species.

10 μg of croton oil/cm^2 gave reactions of medium intensity. On microscopy only slight epidermal changes were found. A clearly significant mononuclear response and a slight basophil response occurred in the upper corium. Croton oil was chosen because it is said to elicit a reaction that resembles in many ways that seen in contact allergy (7). The present findings of marked increase in mononuclear cells and slight increase in basophil cells bear striking similarities to a contact allergic reaction, even with regard to the cellular infiltration.

The difference between an allergic and a toxic contact reaction appears to be least when the substance is applied in weakly toxic concentration. The skin may constitute a specific immune organ (5), and both allergic and toxic types of reaction could conceivably form part of the skin's defence mechanism against exogenic factors. It is possible that weak stimuli, allergenic or toxic, elicit certain non-specific responses in the skin, and that the stronger the allergic or toxic stimulus is, the greater is the modification of the response.

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