

From med. dep. F, Copenhagen County Hospital, Glostrup, Denmark.
Head: M. Schwartz, M.D.

CYTOLOGICAL EXAMINATION OF EXPERIMENTAL CONTACT ALLERGY USING THE SKIN WINDOW TECHNIQUE¹⁻²

By

PER WOLF-JÜRGENSEN

INTRODUCTION

Contact allergy may be defined as an inflammatory process in the skin, following sensitization with a specific sensitizing agent (3). Experimental contact allergy may be produced by a large number of compounds; 2-4 dinitrochlorobenzene (DNCB) is most frequently used because of its particularly strong sensitizing effect. Most normal persons can be sensitized if suitably exposed to this agent and usually a "flare up" reaction develops in 9 to 24 days. A superficial erythema, with sharply demarcated vesicles, appears at the site of application (2, 3). A positive reaction is easily recognized and may vary in strength from a few papules to a marked erythema with numerous vesicles. If the allergen is reapplied after sensitization a new, stronger reaction develops within 24 hours. All subjects do not react to the first application. (10).

Histological studies have shown that the reaction is predominantly limited to the dermis and superficial part of the corium. In the corium, vasodilatation and perivascular round cell infiltration have been described, and in the dermis infiltration by polymorphonuclear leucocytes and monocytes has also been reported. (2, 3, 11, 12).

¹ Presented at meeting of "Dansk Selskab for Allergiforskning" on Dec. 7th, 1962.

² Supported by a grant from "Kong Chr. X's Fond".

No reports indicate that contact allergy differs histologically from less specific states of irritation in the skin.

As there are no reports of cytological examination of contact allergy using the skin window technique, such examinations have been made on patients after the production of contact allergy using DNCB.

MATERIAL AND METHODS

a) Sensitization and production of contact allergy.

Patients who were expected to remain in hospital for at least four weeks were selected for the investigation. Most of those selected were suffering from coronary occlusion or fractures. Patients with dermatological or haematological diseases were excluded. A synthetic product, 2-4 dinitrochlorobenzene (DNCB), was used to induce the contact allergy, according to the method used by Schwartz (10). 0.1 ml of 1 % DNCB in acetone is applied to the volar surface of the forearm in a petroleum jelly ring (area about 3 sq.cm.). The acetone evaporates and DNCB is bound to the skin. The patients were seen 10 days later and subsequently with a few days interval. If no "flare up" reaction developed within 24 days DNCB was reapplied and if necessary applied again 4 days later. If the patients were not sensitized by this procedure the experiment was abandoned.

b) Previous studies of contact allergy have been based on isolated biopsies. Most examinations have been made using mice and guinea pigs (2, 11). Single biopsies, however, only give a static picture of the contact allergy which is of a dynamic nature. Using biopsies a single process cannot be studied.

In 1940 *Rebuck* developed a new method for the examination of skin changes, the so-called skin window method. This method which has been the basis of my experiments has not previously been used for the examination of contact allergy. Sterile coverslips were used as skin windows. Using a sterile knife, the superficial part of the dermis was scraped from a small area of the forearm. The lesion must not bleed. DNCB, (1 % in acetone), or in the control experiments, acetone, was applied at zero-time and the lesion was covered with a coverslip. The cover slips were changed hourly during the first 24 hours and at intervals of several hours during the second 24 hours.

The cover slips were dried in air and stained according to the May-Grünwald-Giemsa method and mounted in Canada balsam on a microscope slide. A few imprints of the allergy reaction itself were also made using this technique.

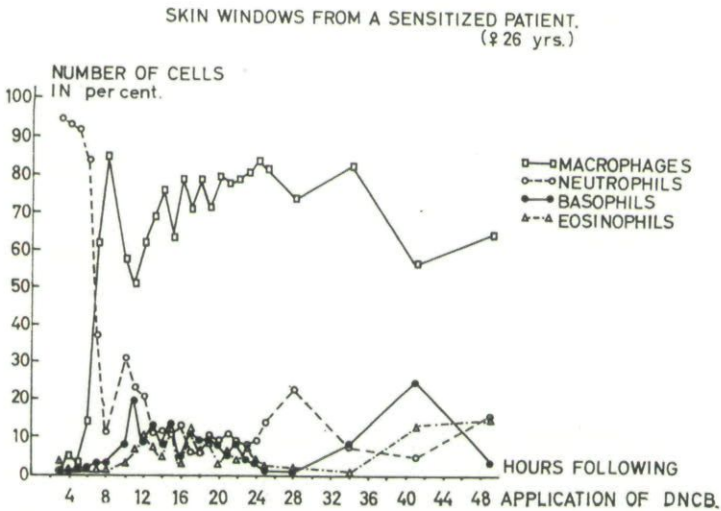
The leucocytes in the blood were also examined during the period of sensitization. When white blood cell counts were made, the basophils were stained metachromatically with toluidine blue (6), and eosinophils were stained with eosin in acetone (13). Other leucocytes were counted in the same counting chamber as the basophils.

RESULTS

A total of 48 patients was followed after application of DNCB, of these 16 were sensitized, but some patients were discharged from hospital before the "flare up" reaction could be expected to be seen. A few refused to participate further in the investigation.

9 sensitized patients were examined using the skin window technique, and application of DNCB besides acetone as a control on skin windows on the other arm. 2 non-sensitized patients were examined using the same technique. A series of single imprints of the "flare up" reactions was also made. 700 cytological preparations were made, viewed under the microscope and counted. When possible 500 cells were counted to give an accurate count of the less frequently occurring cells.

Four cell types were observed; polymorphonuclear leucocytes, macrophages, eosinophils and basophils. A differential



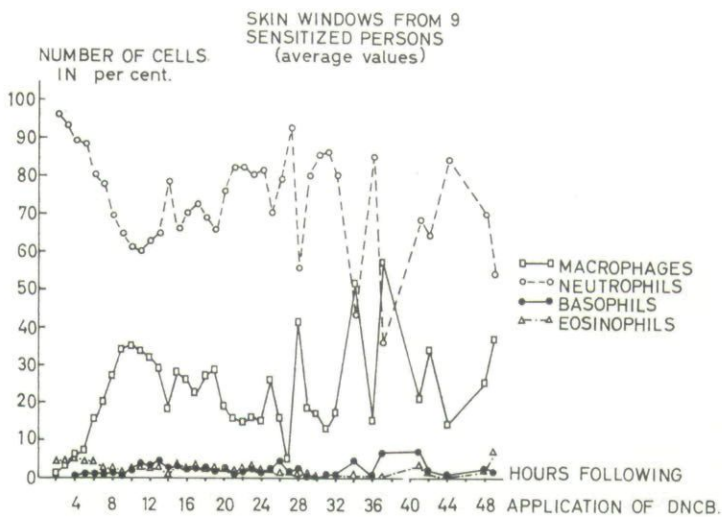
Curve 1.

The composition of the cellular exudate in skin windows from one sensitized patient during 48 hours. DNCB is applied at 0. The exudate contains many eosinophils and basophils.

count was made for each preparation and plotted graphically. The graphs represent the progress of contact allergy.

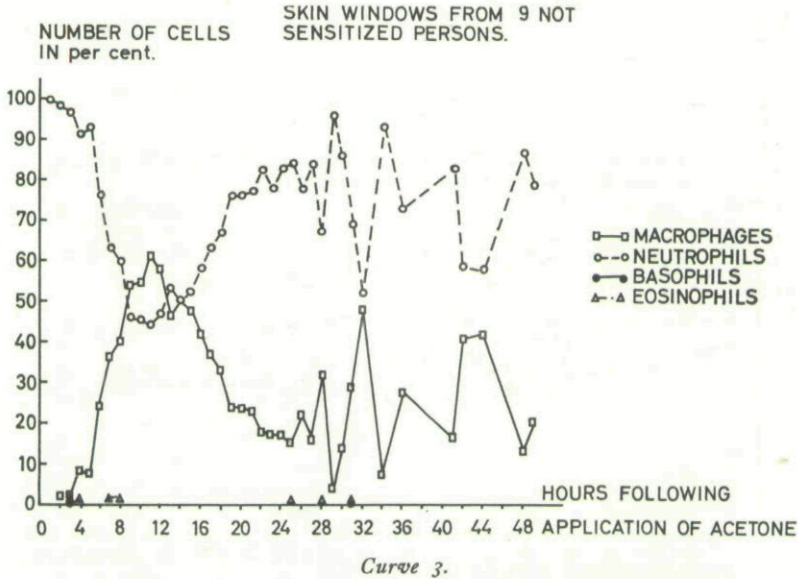
After 1 to 2 hours most of the migrating cells were polymorphonuclear leucocytes, after 2 to 3 hours eosinophils and macrophages appeared, and after 4 to 7 hours basophils appeared. The relative number of polymorphonuclear leucocytes fell gradually and the number of macrophages rose correspondingly. Later the number of polymorphonuclear leucocytes usually increased again. After 2 to 4 hours usually 4 to 5 % (in some preparations 10–12 %) of the cells were eosinophils, and after 7 to 8 hours there were about 3 % eosinophils. The eosinophil count continued to decrease during the first thirty hours of the experiment and then increased slightly.

The basophils appeared after 4–7 hours, their number increased to about 4 % (in some preparations 15 %) at the 13 hour stage and fell slowly until a small secondary increase



Curve 2.

The composition of the cellular exudate in skin windows from 9 sensitized patients (average values) after application of DNCB. The preparations contain 2–5 per cent eosinophils and basophils.



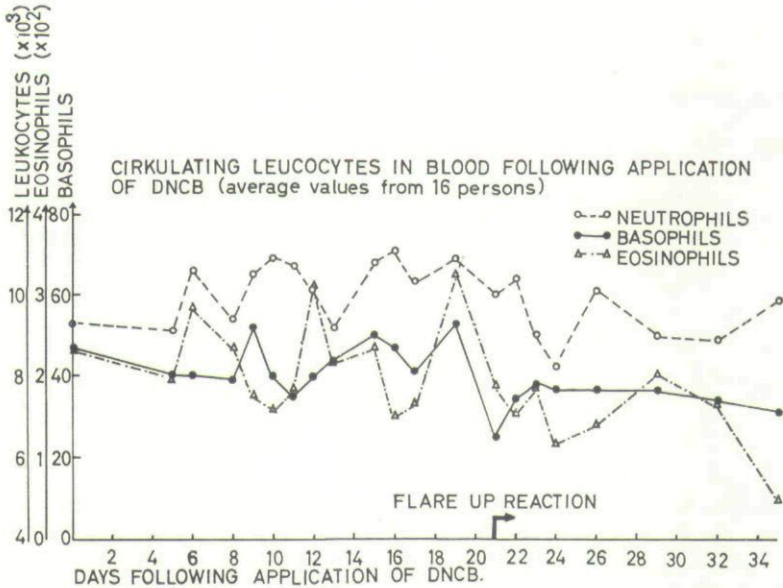
The composition of the cellular exudate in control skin windows from the same 9 patients (average values) after application of acetone. Only 0-0.5 per cent eosinophils and basophils are found.

occurred after 30 hours. After the 10 hour stage more basophils than eosinophils were found.

In the acetone control experiments, and in the DNCB experiments in unsensitized patients only 0 to 0.5 % eosinophils and basophils were found in the preparations.

In the single imprints from "flare up" reactions a relatively constant number of eosinophils and basophils (2 to 5 %) were found during three days.

Eosinophil, basophil and total white cell counts made on blood in the sensitization period from 16 patients were normal. There seems to be a slight fall in the number of eosinophils and basophils near the time of sensitization. Only 4 of these patients were with certainty sensitized before discharge from hospital.



Average values of white cell counts in peripheral blood in 16 patients during the period of sensitization. All values are within the normal range. After the "flare up" reaction a moderate fall in the eosinophil and basophil counts occurs.

DISCUSSION

Riis concludes from his studies of the inflammatory exudate in skin windows that the cells originate from the blood (8). The macrophages are thought to be small and big lymphocytes and monocytes; eosinophils and basophils in the exudate do not differ morphologically from eosinophils and basophils in blood. The basophils in the exudate may be distinguished from connective tissue mast cells by their lobulated and polymorph nucleus, coarse granules and more regular cell shape.

Basophils are thought to contain histamine and heparin and may be of importance for the organisation of oedema fluid in connective tissue ground substance. They probably do not contain serotonin. Eosinophils were previously thought to contain histamine, but new investigations suggest that eosinophils contain anti-histamines which block histamine receptors

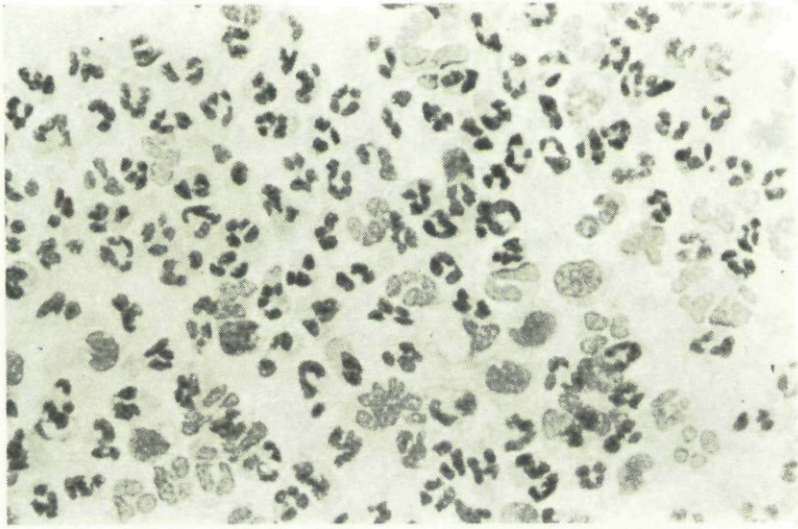


Fig. 1.

Nineteen hours of inflammation in normal control. Only neutrophils and macrophages are seen. ($\times 560$).

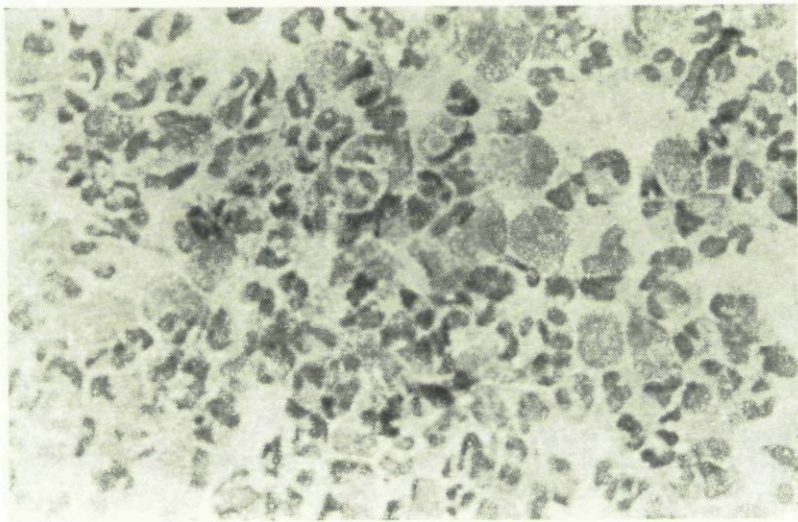
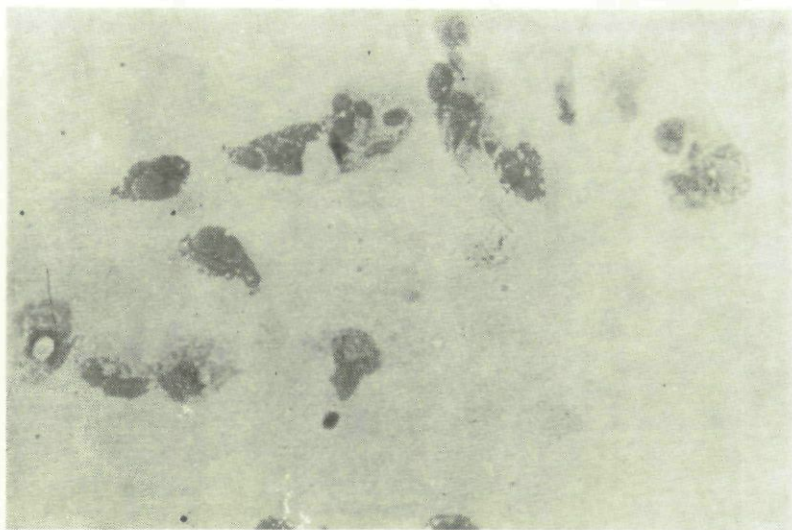


Fig. 2.

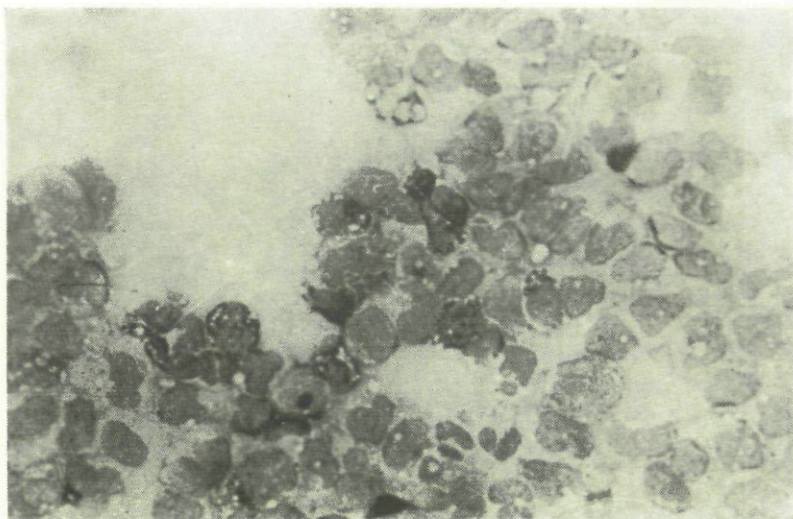
Numerous eosinophils and neutrophils at the 8 hour stage of contact allergy. ($\times 560$).

Fig. 3-6.

The exudate in contact allergy at different intervals in the same patient.

*Fig. 3.*

4 hour preparation. Only a few cells are seen, eosinophils and neutrophils. ($\times 560$).

*Fig. 4.*

12 hours preparation. Basophils, macrophages, neutrophils and a few eosinophils. ($\times 560$).

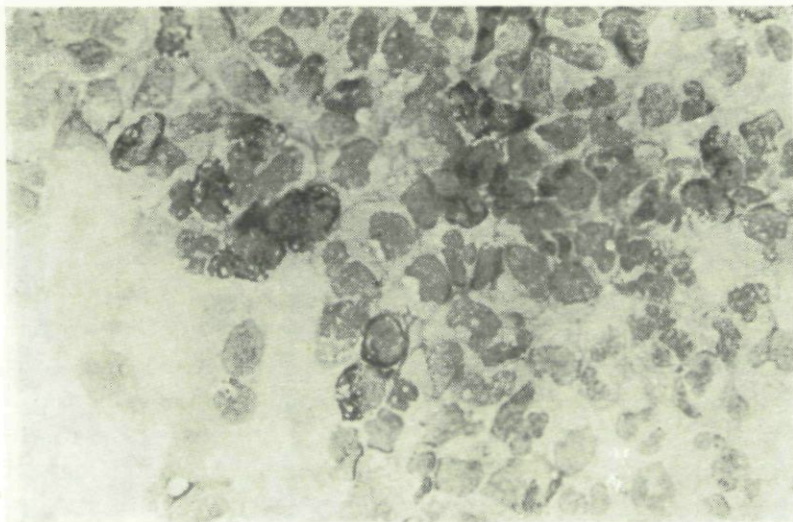


Fig. 5.

14 hour preparation, basophils and macrophages dominate. ($\times 560$).

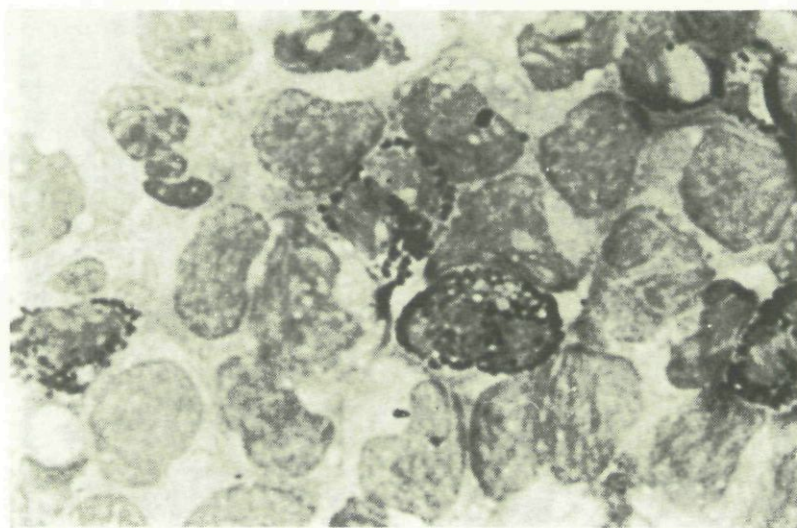


Fig. 6.

19 hour preparation ($\times 1400$). Many basophils. No degranulation is observed but some of the granules of the basophils are ballooned.

(1). *Ham* (4) states about these cells:—"They constitute a subject for research rather than for discussion".

Local tissue eosinophilia was first reported by *Klini* (1932) (5), who injected histamine into allergic patients. *Riis* concludes that local tissue eosinophilia is often associated with eosinophilia in the blood, but not always. *Rorsman* (9) states that local tissue basophilia, at the site of injection of protein, has been observed in rabbit and guinea pigs. Local tissues basophilia in man has been reported by *Rebuck* (1960) who found an increased amount of basophils in the inflammatory exudate from the skin of patients with ulcerative colitis using diphtheria toxoid as inflammatory stimulus (7). *Rorsman* has found basopenia in blood in urticaria and concludes that the basophils degranulate. (9) The skin window technique is well suited for studying the granulation of leucocytes and no sign of degranulation of the basophils was observed.

Riis has examined the cellular exudate in skin windows in a number of normal subjects and patients but usually only found a few eosinophils and rarely found basophils. It may be concluded that in normal persons eosinophils and basophils are less numerous in the exudate than in blood. The present investigation therefore indicate that the cellular exudate in contact allergy differs from that in a nonspecific inflammatory process, especially because the proportion of basophils is 4 to 8 times that found in blood.

SUMMARY

An increased number of eosinophils and especially basophils were found in the exudate in experimental contact allergy, using 2-4 dinitrochlorobenzene as sensitizing agent and the skin window technique. Seven hundred cytological preparations were made. Biopsies have previously shown round cell infiltration in the dermis and perivascular and intercellular oedema. The cytology of the exudate in experimental contact allergy seems to differ from the cytology in less specific states of inflammation where only few basophils and eosinophils are

found. It is too early to assess the possible value of direct imprints in distinguishing between contact allergy and skin processes of a less specific kind.

REFERENCES

1. *Archer, R. K. Feldberg*: Antihistamin activity of horse eosinophils. *Brit. J. Pharmacol* 18, 101-108, 1962.
2. *Crowle, Alfred J.*: Delayed hypersensitivity in health and disease, p.p. 63-66, N.Y. 1962.
3. *Grolnick, M.*: Contact allergy of the skin. *Ann. New York Acad. Sc. Allergy* 50, 718-742, 1949.
4. *Ham, Arthur W.*: Histology, p. 148. Montreal 1953.
5. *Klini, B. S., Cohen, M. B. & Rudolph, J. A.*: Histologic changes in allergic and non allergic wheals. *J. Allergy* 3, 531, 1932.
6. *Moore, J. E. & James, G. W.*: *Proc. Soc. Exper. Biol. & Med.* 82, 601, 1953.
7. *Priest, R. J., Rebuck & Havey*: A new qualitative defect of leucocyte function in ulcerative colitis. *Gastroenterology* 38, no. 5, May 1960.
8. *Riis, Powl*: The cytology of inflammatory exudate. Thesis. Copenhagen 1959.
9. *Rorsman, Hans*: Studies on basophil leucocytes with special reference to urticaria and anaphylaxis, p.p. 4-10. Lund 1962.
10. *Schwartz, M.*: Eczematous sensitization in various age groups. *J. Allergy* 24, 143-148, 1953.
11. *Waksman, B. H.*: Pathological study of delayed hypersensitive reactions. Cellular aspects of immunity, p.p. 286-294. London 1960.
12. *Waldbott, Georg L.*: Contact dermatitis, p.p. 8-2. N.Y. 1953.
13. *Uhrbrand, H.*: *Ugeskr. f. læger* 114, 385, 1952.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.