

EXPERIMENTAL TOXIC AND ALLERGIC CONTACT DERMATITIS

II. A HISTOPATHOLOGIC STUDY

J. PROCHAZKA FISHER, M.D., AND ROBERT A. COOKE, M.D., NEW YORK, N. Y.

WITH THE TECHNICAL ASSISTANCE OF SONYA STROYMAN AND GLORIA GORDON

INTRODUCTION

THE early investigators of the allergic contact type of dermatitis of the guinea pig¹⁻⁵ reported and emphasized that the histologic changes in allergic contact dermatitis of guinea pigs resemble those in contact dermatitis of human beings. Later investigators⁶⁻¹¹ not only abstained from likening the histology of allergic contact dermatitis of the guinea pig to that of human lesions, but some also stated that morphologic and histologic changes seen regularly in human skin cannot be induced readily, if at all in the skin of guinea pigs. Of special interest, therefore, are the recent publications of Jadassohn, Bujard, and Brun,^{12, 13} DeWeek and Brun,¹⁴⁻¹⁶ and Baer, Rosenthal, and Sims,¹⁷ who have revived the controversy. These investigators have demonstrated in the epidermis of the guinea pig mammilla and in the skin, after the epidermal cells have been exogenously induced to proliferation and acanthosis, histologic changes which can be compared to those observed in human epidermis at the sites of contact dermatitis.

The terms describing histologic changes seen in experimental contact dermatitis are derived from the terminology used for histopathologic description of several types of eczematous dermatitis of human skin before the specific etiology and pathogenesis of contact dermatitis were recognized and acknowledged as manifestations of induced sensitivity. An attempt has been made to fathom the concepts and interpretations in original texts by authors who suggested terms or used them for histopathologic diagnosis of eczematous dermatitis of human skin:

Alteration cavitaire (Leloir¹⁸): Perinuclear vacuolization of epidermal cells.

Acanthosis (Unna¹⁹): A thickening through hyperplasia of rete malpighii.

Spongioide umwandlung (Unna¹⁹), *Spongiose* (Besnier and Doyon²⁰): Spongiosis through intercellular edema of the epidermis which, when marked, progresses to vesiculation.

*Vesicle de Willan*²¹ (Brocq²²): A circumscribed globoid space within the epidermis, filled with serous fluid usually containing a number of lymphocytes. The compression of epidermal cells surrounding the vesicle suggests its rapid development through localized intercellular pressure.

Vesicle primordiale sous-cornee (Civatte²³): A primary vesicle located in the uppermost portion of the epidermis, beneath the keratinized surface, arising in place of disintegrating epidermal cells.

From the Institute of Allergy, Roosevelt Hospital.

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Recently Miescher²⁴⁻²⁶ studied chronologically the development of histologic changes occurring in human skin at sites of contact dermatitis and primary toxic dermatitis. He added a concept which alludes to a physiopathologic process in the development of contact dermatitis (*Spongiose lymphocytaire ascendante precoce en foyer*). He demonstrated, in biopsies obtained from early lesions of contact dermatitis induced in sensitive patients by means of patch tests, evidence of focal spongiosis associated with a precocious ascent of lymphocytes through the dermis and the dilated intercellular spaces of the epidermis. Similar histologic findings are reported by Charpy, Stahl, and Castelain.²⁷ These authors, however, are of the opinion that the changes seen in the epidermis are only epithelial manifestations of an exudative allergic process of the papillary portion of the dermis (Manifestation epithéliale de allergie exudative du derme papillaire).

In the present study is contrasted the development of the histopathology of allergic dermatitis and of primary toxic dermatitis of the guinea pig in an effort to establish a difference, if any exists, between the physiopathologic processes of the two types of inflammatory reactions. The allergic dermatitis is represented by contact dermatitis of guinea pigs made extremely sensitive to a simple chemical (1-chloro-2,4-dinitrobenzene, referred to as DNCB). The primary dermatitis is represented by toxic dermatitis induced in normal animals by the same, but a more concentrated, chemical. It should be noted at this time that in guinea pigs sensitized to contact with DNCB and other simple chemicals, by any procedure (epicutaneous contact or parenteral injections with or without adjuvant), the delayed and the immediate types of sensitivity are always* induced simultaneously.²⁹⁻³¹

MATERIALS AND METHODS

The techniques used (1) to determine the toxicity of DNCB in olive oil to skin of normal guinea pigs, (2) to sensitize guinea pigs, (3) to test the contact type of sensitivity, and (4) to obtain skin biopsies are described in detail in the first part of this study.³² As stated there, only one lesion was induced in each guinea pig at one time, and only one biopsy specimen was removed. For a detailed examination of the histologic changes at the site of allergic dermatitis, some highly sensitive guinea pigs were used two to three times. The resting period between biopsies was at least six weeks. For a detailed examination of the histologic changes at the sites of primary toxic dermatitis, normal guinea pigs with no prior contact with the chemical were used.

Biopsy specimens from sites of allergic dermatitis were obtained three, six, nine, twelve, twenty-four, and forty-eight hours and three, four, and five days after epicutaneous challenge with nontoxic (0.1 per cent, 0.01 per cent) concentrations of DNCB in olive oil. Six to ten guinea pigs were biopsied at each interval. Biopsies for investigation of histologic changes at the sites of primary toxic dermatitis were made in the same sequence, using normal guinea pigs and toxic (2 per cent, 3 per cent, and 5 per cent) concentrations of DNCB in olive oil. In addition, biopsies obtained from lesions of six, seven, and nine days' duration, induced by 5 per cent DNCB in olive oil, were examined. At each interval four to five animals were biopsied from 2 per cent and 3 per cent DNCB lesions and six to ten from 5 per cent DNCB lesions.

Plain olive oil proved slightly irritating to the skin of 5 per cent of normal animals.³² Furthermore, it has been reported that a daily application of plain olive oil to guinea pig skin for ten consecutive days induces an average twofold acanthosis of the epidermis.^{33, 34} Therefore, a number of biopsies obtained from normal and sensitive animals from sites

*Substantiated by our unpublished experiments using passive cutaneous anaphylaxis²⁸ for demonstration of serum antibodies.

tested with plain olive oil were histologically examined. One application of plain olive oil did not induce any significant changes in the epidermis or dermis, even if an irritation had been observed at the time the biopsy was made or before. Occasional epinuclear vacuolization of superficial epidermal cells was seen. However, such epinuclear vacuolizations were also found in the epidermis of skin areas shorn of hair with an electric hair clipper without application of olive oil.

Preparation of Histologic Sections.—Part of each skin biopsy specimen used for measurement of histamine content³² was examined histologically. These specimens were fixed in formalin. Additional biopsies, made to gather supplementary histologic information, were fixed in Bouin's fixative.

The fixed specimens were mounted in paraffin and secured in the microtome in such position as to yield cross sections of the hair follicles (the hair shafts pointing at the microtome blade at an angle of about 30 degrees). Twelve serial sections were made at two levels of each specimen, leaving one-half of each. After microscopic examination of these sections, the remaining half of some were cut for more thorough examination. Some were remounted and the paraffin blocks were fixed in the microtome in such position as to yield longitudinal sections of the hair follicles.

Routine Harris' hematoxylin and eosin stain was used.³⁵ Six serial sections of each biopsy specimen were stained with Lillie's azure A-eosin B method³⁵ to reveal tissue mast cells and blood basophils and eosinophils. None of these cells were found in excess.

The microphotographs of the histologic changes were made in color on Anscochrome film and then were converted into black and white negatives for printing and reproduction.

RESULTS

Histologic Changes at the Site of Allergic Contact Dermatitis.—Three hours after a nontoxic concentration (0.1 per cent and 0.01 per cent) of DNCB in olive oil has been applied to the shorn skin of highly sensitized animals, the epidermis is not affected. The main changes are in the dermis. The capillaries of the upper and middle portions of the dermis are dilated, and a slight interstitial edema is present. The infiltrating cells, exclusively mononuclears, are clinging to the vascular endothelium, many leaving the lumen (Fig. 3). A number of these cells are perivascular, and a small amount are diffuse between the collagen fibrils (Fig. 2). Some of the infiltrating cells are in a linear arrangement, giving an impression of trails ascending toward the epidermis (Figs. 1 and 2). Here a few cells which have reached the basement membrane of the epidermis or are in close proximity are escorted or surrounded by a vacuole. A formation of vacuoles adjacent to the mononuclear cells was not observed deeper in the dermis.

In the six-hour lesions the epidermal cells of some specimens show slight proliferating activity. The infiltration of the middle dermis has increased, and an intensive extravasation of the mononuclear cells is in progress. In the uppermost dermis the number of infiltrating cells has also increased; however, ascending mononuclear trails between the superficial capillaries and the epidermis, as well as the formation of vacuoles, are readily discernible. Singly or in groups, escorted by vacuoles, some mononuclears have entered the epidermis (Fig. 4).

In the nine- and twelve-hour lesions the trails of mononuclear cells can no longer be identified, since the diffuse cellular infiltration now extends into

the uppermost dermis. The epidermis has become slightly acanthotic (that is, from the original thickness of two to four cells to about three to six epidermal cells) and contains a number of regions invaded by groups of mononuclear

Fig. 1.

Fig. 2.

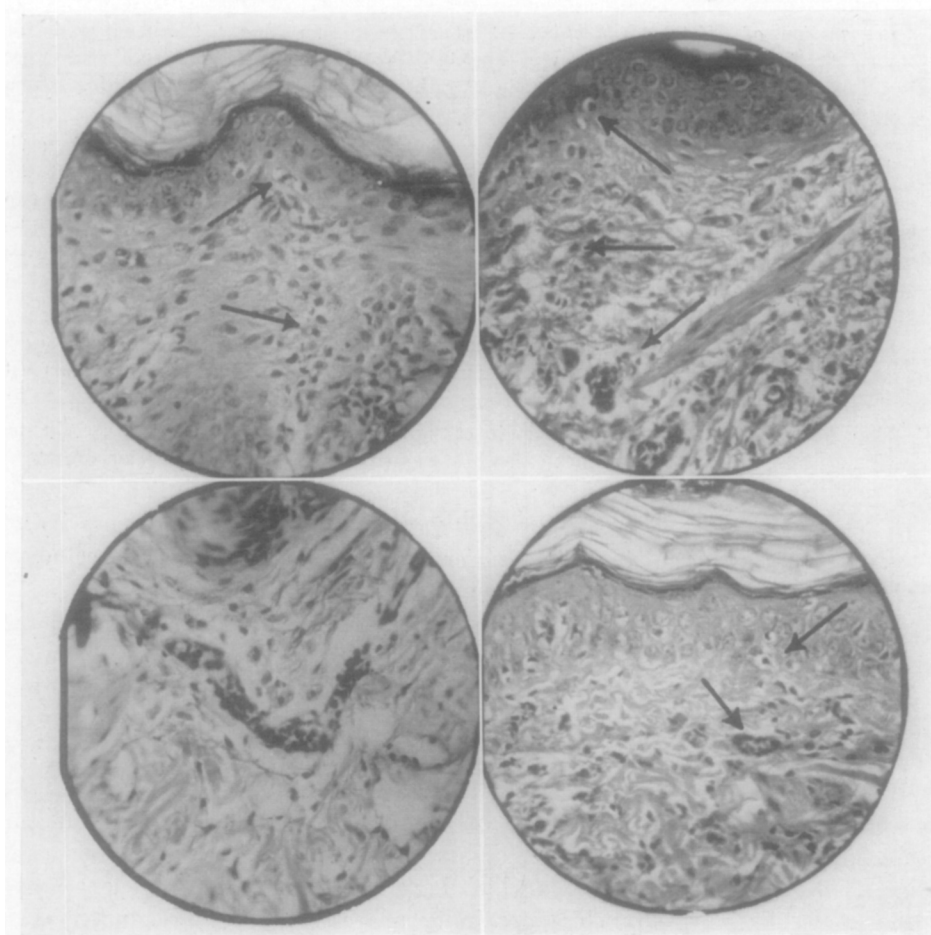


Fig. 3.

Fig. 4.

Fig. 1.—Three-hour lesion; 0.01 per cent DNCB. A trail of mononuclear cells. Note formation of vacuoles adjacent to the cells which are in proximity to the epidermis. (Magnification, $\times 400$.)

Fig. 2.—Three-hour lesion; 0.1 per cent DNCB. Extravasation of mononuclear cells. Note on the left a linear arrangement of infiltrating cells ascending toward epidermis, some escorted by a vacuole. (Magnification, $\times 400$.)

Fig. 3.—Three-hour lesion; 0.01 per cent DNCB. A capillary in the upper dermis with mononuclears crowding the vessel and leaving the lumen. (Magnification, $\times 400$.)

Fig. 4.—Six-hour lesion; 0.1 per cent DNCB. Infiltration of the upper dermis with an ascent of mononuclear cells from a superficial capillary into the epidermis. Note vacuoles escorting the infiltrating cells. (Magnification, $\times 400$.)

cells, accompanied by vacuoles of varying sizes (Figs. 5 to 10). Epidermal cells at the points of the mononuclear incursion seem only passively involved, having become disunited from their close cohesion. In some instances an intercellular edema spreads centrifugally into the surrounding epidermis (Figs. 7 and 8).

Fig. 5.

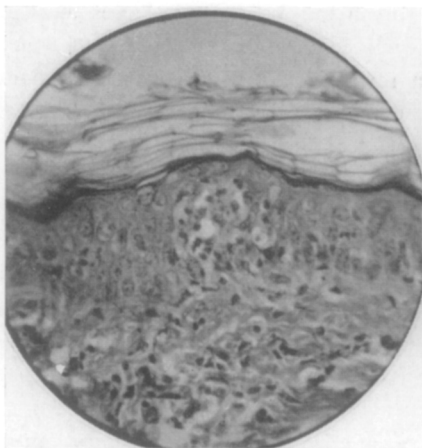


Fig. 6.

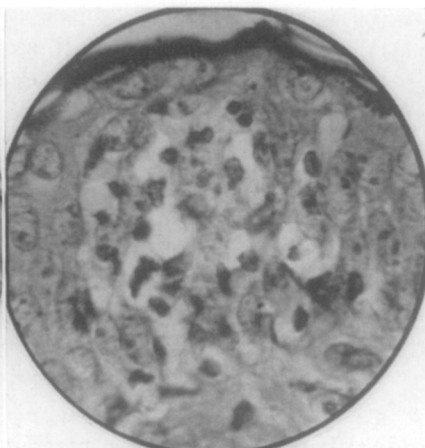


Fig. 7.

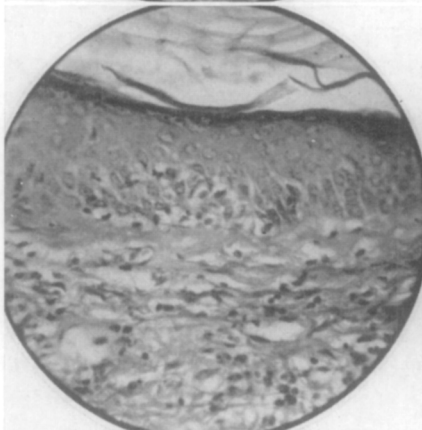


Fig. 8.

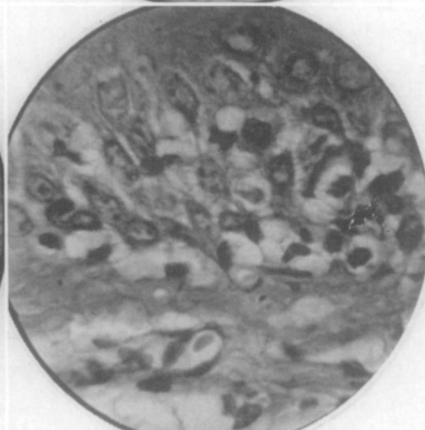


Fig. 9.

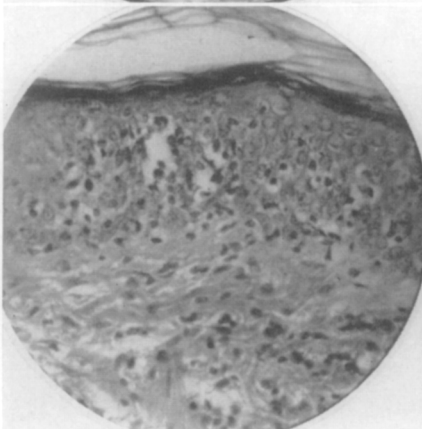
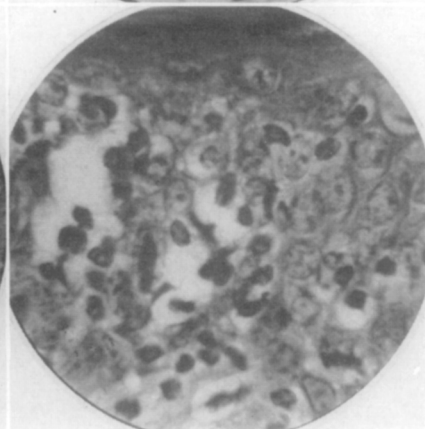


Fig. 10.



Figs. 5 and 6.—Nine-hour lesion; 0.01 per cent DNCB. A circumscribed invasion of the epidermis by mononuclear cells and formation of vacuoles. (Magnification: Fig. 5, $\times 400$; Fig. 6, $\times 1,000$.)

Figs. 7 and 8.—Twelve-hour lesion; 0.1 per cent DNCB. A localized invasion of the epidermis by mononuclear cells. Formation of vacuoles at basal membrane and spread of intercellular edema in the epidermis. (Magnification: Fig. 7, $\times 400$; Fig. 8, $\times 1,000$.)

Figs. 9 and 10.—Twelve-hour lesion; 0.1 per cent DNCB. An epidermal region invaded by a number of mononuclear cells. Note varying stages of vacuole formation. (Magnification: Fig. 9, $\times 400$; Fig. 10, $\times 1,000$.)

In the twenty-four-hour lesions a formation of microvesicles was noted (Fig. 11). Examination of serial sections yielded the impression that the vesicles had developed at the points of a massive mononuclear cell invasion in a relatively small area of the epidermis (Figs. 12 to 14). Histologic changes in the periphery of the vesicles resemble the changes viewed in the nine- and twelve-hour lesions, that is, incursion of the epidermis by mononuclear, formation of vacuoles, and centrifugally spreading intercellular edema-spongiosis (Fig. 15). The tangential arrangement of the flattened epidermal cells (Fig. 14) seen in some sectors of the vesicles suggests that high central pressure is the mechanism of vesicle formation. On the other hand, spongeliike multilocular sectors in the same vesicles (Fig. 11), formed by septa from flattened epidermal cells between individual vacuoles, indicate fusion of the vacuoles as the mechanism of vesicle formation.

Induration of the dermis reaches a maximum in the twenty-four-hour lesions. Interstitial edema and mononuclear cells diffusely distributed among dissociated collagen fibrils account for the two- to threefold thickening of the dermis (Fig. 16).

Histologic examination of biopsies made with a punch key or of those obtained from lesions folded and pinched between the fingers always revealed minute hemorrhages and a small number of polymorphonuclear cells. Apparently, this poor technique caused vascular injury and distortion of the infiltration. However, in the biopsies obtained by the scissor-snip method,³² neither hemorrhages nor polymorphonuclears are seen.

The localized changes in the epidermis described above were not present in all twenty-four-hour lesions. They were observed in nearly one-half of the biopsies induced at this interval and were reproduced in the same animals weeks later. The change in the remaining biopsies was a rather general invasion of large regions of the epidermis by mononuclear cells accompanied by vacuoles, with or without formation of microvesicles (Fig. 17).

Epidermal changes reached a maximum development in forty-eight-hour lesions. All of the challenged epidermal surface is extensively invaded by mononuclear cells. Distinction between epidermis and dermis, easily distinguished in the stained sections, is difficult in black and white reproductions as a result of the density of the mononuclear infiltration and the total disruption of the epidermal structure by countless vacuoles. Marked vacuolization pervades the entire thickness of the epidermis (Fig. 18) and the upper portion of the hair follicles (Figs. 19, 20, and 21). Microvesicles were found in about one-half of the biopsies and were most prevalent in specimens with the greatest acanthosis. (No acanthosis was found in control biopsy specimens tested with plain olive oil.)

The changes observed in twenty-four-hour lesions were of two kinds—a circumscribed invasion and a general invasion of the epidermis by mononuclear cells. The changes in forty-eight-hour lesions largely resemble one another, being of one type only, an intensive general mononuclear invasion of the epidermis. It seems in order to assume that the varying epidermal changes observed

Fig. 11.

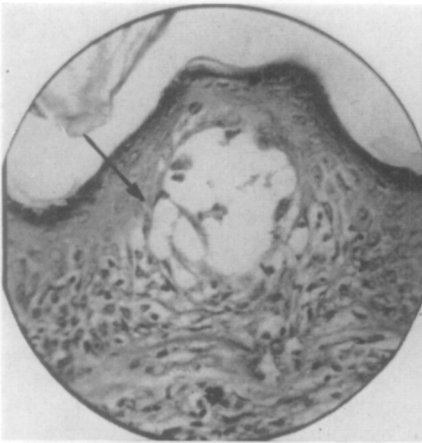


Fig. 14.

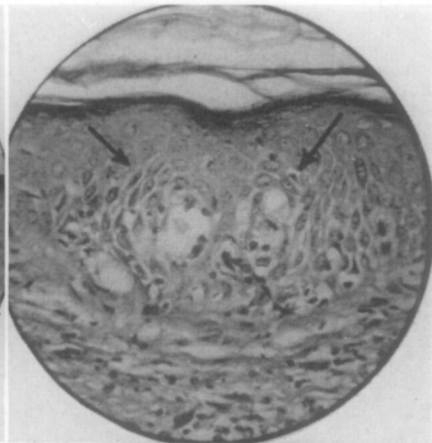


Fig. 12.

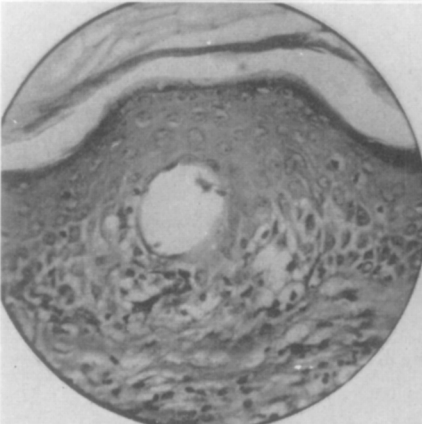


Fig. 15.

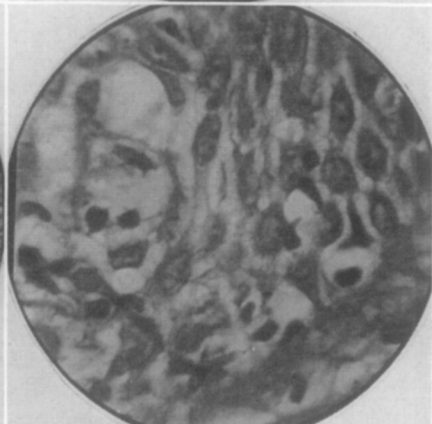


Fig. 13.

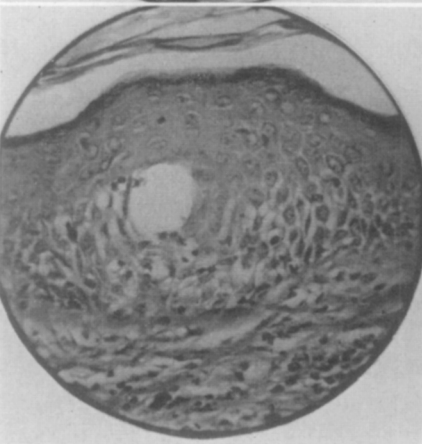


Fig. 16.

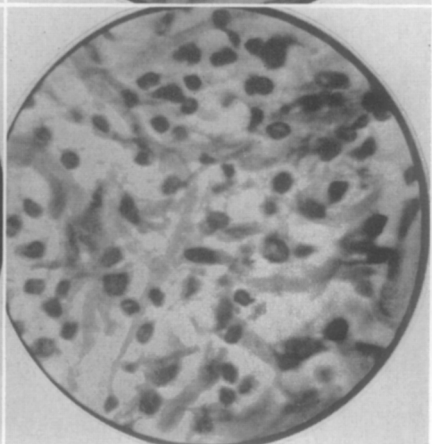


Fig. 11.—Twenty-four-hour lesion; 0.1 per cent DNCB. Central section of a microvesicle. Note spongelike, multilocular sector, formed by septa from flattened epidermal cells between individual vacuoles. (Magnification, $\times 400$.)

Figs. 12, 13, and 14.—Twenty-four-hour lesion; 0.1 per cent DNCB. Peripheral sections through the microvesicle shown in Fig. 11. Note the tangential arrangement of epidermal cells surrounding the vesicular cavity. (Magnification, $\times 400$.)

Fig. 15.—Twenty-four-hour lesion; 0.1 per cent DNCB. Detail of the microvesicle section shown in Fig. 14. Note mononuclear cells, varying stages of vacuole formation and the intercellular edema-spongiosis. (Magnification, $\times 1,000$.)

Fig. 16.—Twenty-four-hour lesion; 0.1 per cent DNCB. Mononuclear infiltrating cells and edema of the upper dermis. (Magnification, $\times 1,000$.)

Fig. 17.

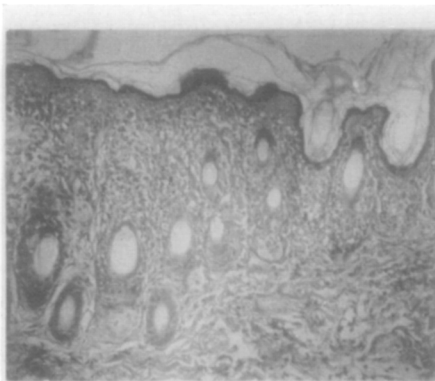


Fig. 20.

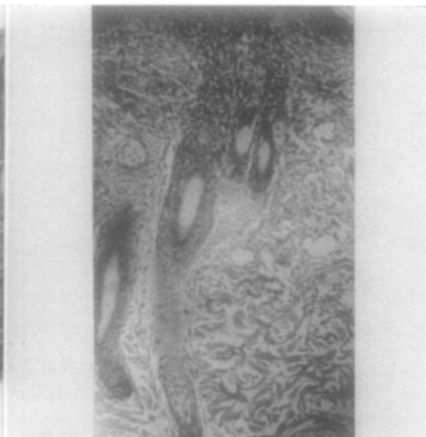


Fig. 18.

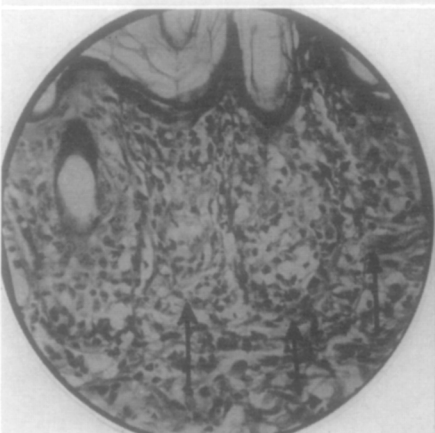


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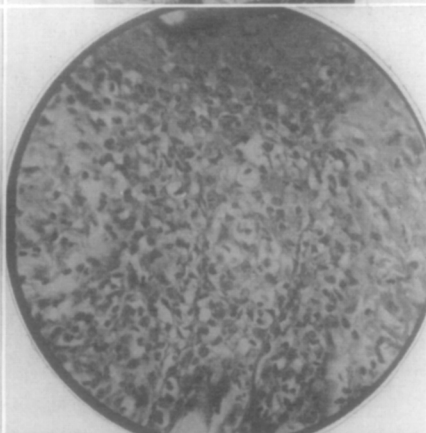


Fig. 19.

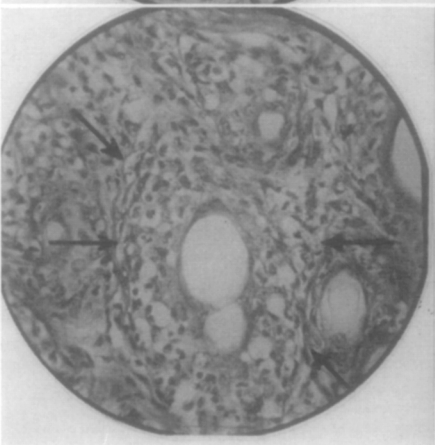


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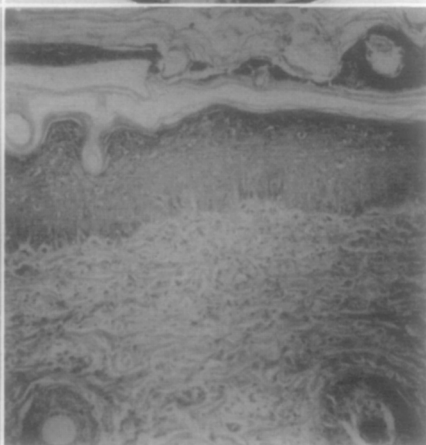


Fig. 17.—Twenty-four-hour lesion; 0.1 per cent DNCB. Generalized invasion of the epidermis and hair follicles by mononuclear cells and vacuoles. (Magnification, $\times 100$.)

Figs. 18 and 19.—Forty-eight-hour lesion; 0.1 per cent DNCB. Massive mononuclear infiltration and marked vacuolization pervading the entire thickness of the epidermis (Fig. 18) and the upper portion of the hair follicles (Fig. 19, in cross section) totally disrupting the cohesion of epidermal cells. (Magnification, $\times 400$.)

Fig. 20.—Forty-eight-hour lesion; 0.01 per cent DNCB. Hair follicle in longitudinal section. Invasion of the epidermal lining by mononuclear cells and vacuoles. (Magnification, $\times 100$.)

Fig. 21.—Forty-eight-hour lesion; 0.01 per cent DNCB. Detail of Fig. 20. Massive mononuclear infiltration and marked vacuolization of the upper portion of the hair follicle lining. (Magnification, $\times 400$.)

Fig. 22.—Five-day lesion; 0.1 per cent DNCB. Regenerated epidermis with hyperplasia of stratum granulosum and stratum malpighii, covered by a thin scale of cast-off epidermis containing mononuclear cell detritus. (Magnification, $\times 100$.)

in twenty-four-hour lesions reach a uniformity and a maximum in forty-eight-hour lesions. In causal relationship, it is noteworthy that in the twenty-four-hour lesions the cellular infiltration has reached a maximum and is uniformly diffuse throughout the dermis. In the forty-eight-hour lesions the infiltrating cells crowd the epidermis and the upper portion of the dermis, whereas the deeper layers of the dermis now show only a moderate infiltration. The observed shift in density of cellular infiltration is a result of a transmigration of the infiltrating cells toward and into the epidermis. Therefore, the two types of changes seen in the epidermis of twenty-four-hour lesions seem to be related to the velocity of locomotion, the distance which the infiltrating cells must travel, or both. In the lesions of following days the number of infiltrating cells in the dermis remains constant, whereas the accompanying vasodilatation and interstitial edema gradually disappear.

In the three-day lesions the vacuoles near the epidermal basement membrane have fused, separating the epidermis and the dermis. The staining property of the epidermal layer is altered, for it stains poorly with hematoxylin and the affinity for eosin is not increased. In the four-day lesions the dead epidermis and the fragments of mononuclear cells are shed. A new epidermal layer (1 to 3 cells thick) covers the dermis. These new cells show proliferation and normal staining property. The probable location of epidermal cells from which the regeneration of the epidermis has begun was not determined.

The five-day lesions show marked thickening of the new epidermis due to hyperplasia of the stratum granulosum and stratum malpighii. The epidermal surface is covered by a thin scale of cast-off epidermis with fragments of mononuclear cells (Fig. 22).

Histologic Changes at the Site of Primary Toxic Dermatitis.—Three hours after a toxic concentration (2 per cent, 3 per cent, and 5 per cent) of DNCB in olive oil has been applied to the shorn skin of normal animals, neither the epidermis nor the dermis shows any histologic change. Epidermal changes occur in a few six-hour lesions but are manifest in all twelve-hour lesions. The histologic findings vary with the concentration of the chemical and also with each animal. To simplify description, the predominant changes are enumerated as observed in lesions induced by the different concentrations of DNCB.

In lesions induced by 5 per cent DNCB the predominant toxic damage is an epidermal necrobiosis, in varying stages of development from incomplete (Fig. 24) to complete (Fig. 23) (loss of cellular integrity and of the distinction between nucleoplasm and cytoplasm). Irrespective of the stage of epidermal necrosis, a separation of the epidermis from the dermis was seen in all specimens. In lesions induced by lesser concentrations of DNCB the epidermal damage was less marked. Basal-cell layer proliferation with simultaneous epidermal separation is prevalent in lesions induced by 3 per cent DNCB (Fig. 25), whereas proliferation without epidermal separation occurs in lesions induced by 2 per cent DNCB. The dermis in the twelve-hour lesion is moderately indurated by interstitial edema and polymorphonuclears and mononuclears in about the same proportion as found in blood.³²

Fig. 23.

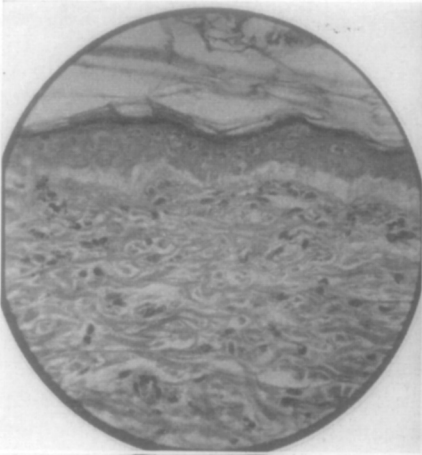


Fig. 26.

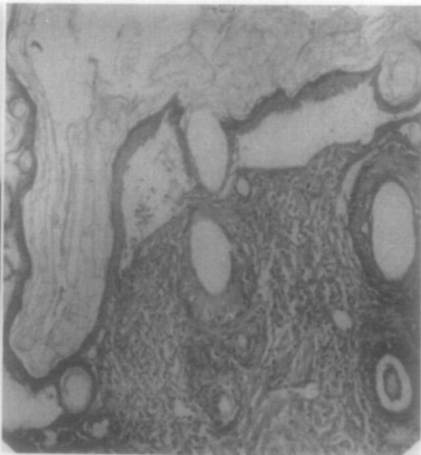


Fig. 24.

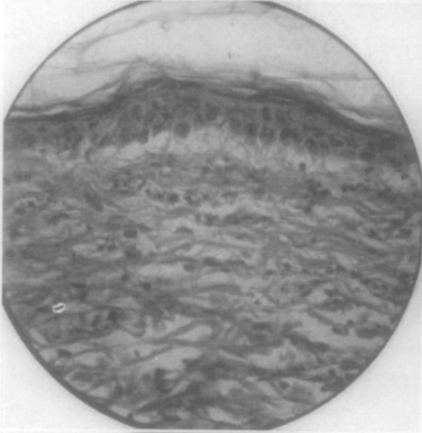


Fig. 27.

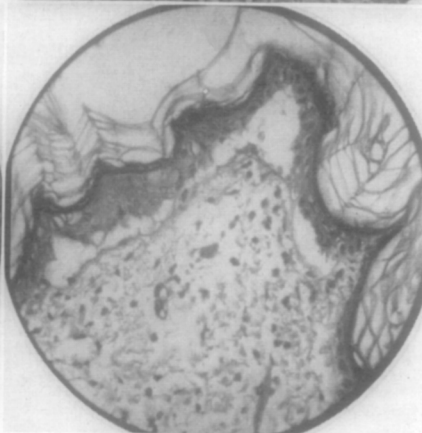


Fig. 25.

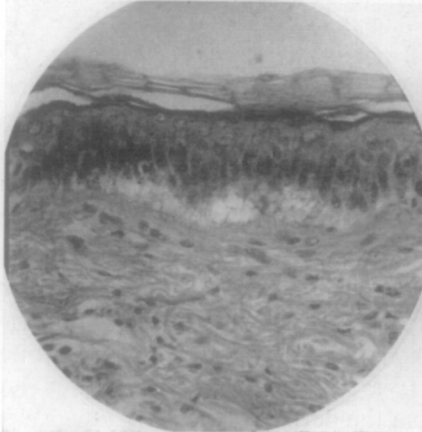


Fig. 28.

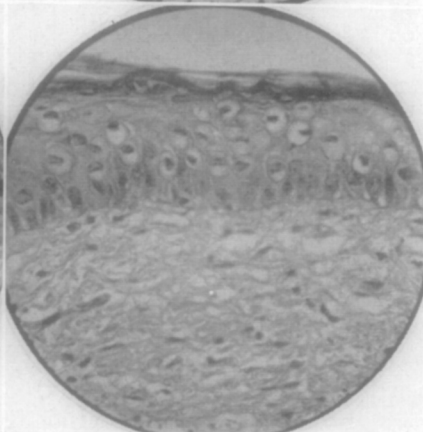


Fig. 23.—Twelve-hour lesion; 5 per cent DNCE. Complete epidermal necrosis (loss of integrity of individual cells and the distinction between nucleo- and cytoplasm) and partial separation from the dermis. (Magnification, $\times 400$.)

Fig. 24.—Twelve-hour lesion; 5 per cent DNCE. Partial epidermal necrobiosis and separation from the dermis. (Magnification, $\times 400$.)

Fig. 25.—Twelve-hour lesion; 3 per cent DNCE. Proliferation of basal-cell layer and partial epidermal separation from the dermis. (Magnification, $\times 400$.)

Fig. 26.—Twenty-four-hour lesion; 5 per cent DNCE. Total epidermal detachment with complete epidermal necrosis. (Magnification, $\times 100$.)

Fig. 27.—Twenty-four-hour lesion; 3 per cent DNCE. Detachment of epidermis with partial epidermal necrobiosis. (Magnification, $\times 400$.)

Fig. 28.—Twenty-four-hour lesion; 2 per cent DNCE. Generalized perinuclear vacuolization of epidermal cells. (Magnification, $\times 400$.)



Fig. 29.

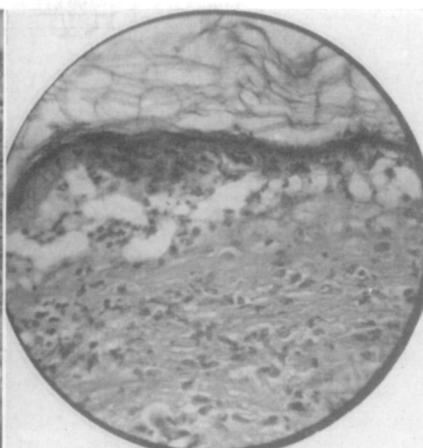


Fig. 32.

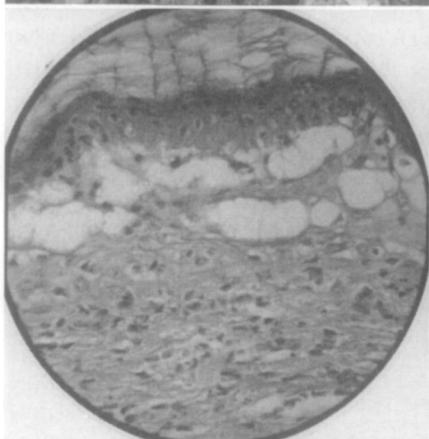


Fig. 30.

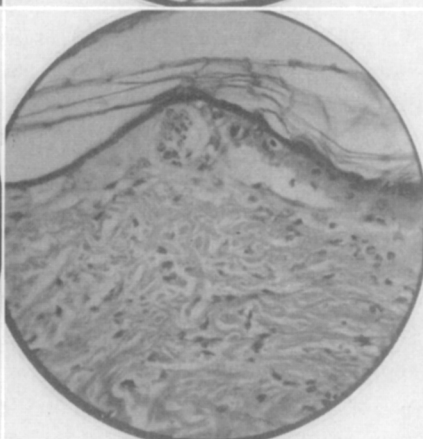


Fig. 33.

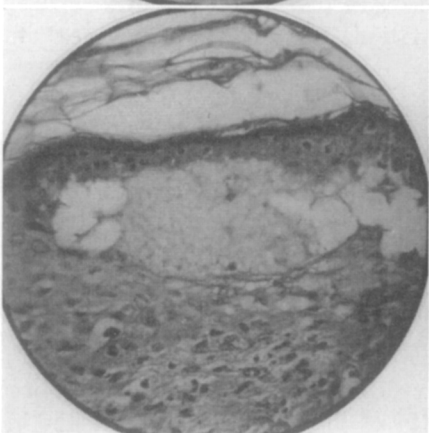


Fig. 31.

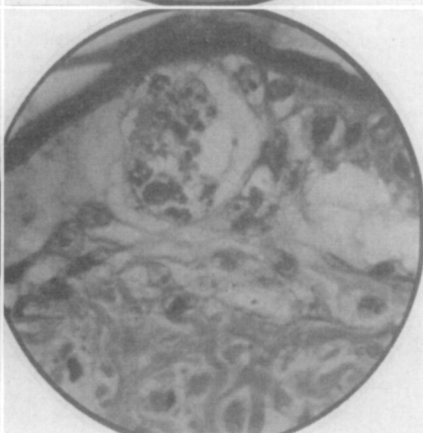


Fig. 34.

Fig. 29.—Twenty-four-hour lesion; 5 per cent DNCB. An intraepidermal cleavage at a junction of complete and partial necrobiosis of the epidermis. Note accumulation of inflammatory cells (polymorphonuclears and mononuclears) in the dermis and in the intraepidermal cleavage. (Magnification, $\times 100$.)

Figs. 30, 31, and 32.—Forty-eight-hour lesions; 5 per cent DNCB. Details of intraepidermal cleavages. Note necrotic surface and surviving basal portion of the epidermis shaping the cavity containing disintegrated epidermal cells (Fig. 30), serous fluid (Fig. 31), and inflammatory cells, predominantly polymorphonuclears (Fig. 32). (Magnification, $\times 400$.)

Figs. 33 and 34.—Forty-eight-hour lesion; 3 per cent DNCB. A circumscribed microvesicle at a junction of complete and partial necrobiosis of the epidermis. Note in the detail (Fig. 34) the content of the vesicle: cellular detritus and polymorphonuclear inflammatory cells. (Magnification: Fig. 33, $\times 400$; Fig. 34, $\times 1,000$.)

In twenty-four- and forty-eight-hour lesions the epidermal changes are as manifold as in twelve-hour lesions. However, more or less definite patterns of epidermal destruction were identified: (1) total detachment of epidermis in specimens with complete epidermal necrosis (Fig. 26), (2) partial detachment with incomplete epidermal necrobiosis (Fig. 27), (3) generalized perinuclear vacuolization of the epidermis (Fig. 28), and (4) formation of intraepidermal cleavages containing inflammatory elements (Fig. 29). The first three patterns seem to evolve from the changes observed in twelve-hour lesions. However, the formation of intraepidermal cleavages seems to be a variation of these changes which arises later (twenty-four to forty-eight hours). Without exception, a necrotic surface portion and a surviving basal portion of the epidermis shape the intraepidermal cleavages (Figs. 30 to 32), within which are found disintegrated epidermal cells (Fig. 30), serous fluid (Fig. 31), and inflammatory cells, predominantly polymorphonuclears (Fig. 32). The dermal infiltration by inflammatory cells reaches a maximum in the same lesions (twenty-four to forty-eight hours). A slow disintegration of the epidermis and migration of inflammatory elements from the dermis seem to be the prerequisites for development of the intraepidermal inflammatory reaction. In some specimens the intraepidermal inflammatory process gives rise to microvesicles (Figs. 33 and 34). A close examination of the vesicle reveals the same basic structure as described above, namely, location at a junction of complete and partial epidermal necrobiosis, containing cellular detritus and inflammatory cells, predominantly polymorphonuclears.

In a few forty-eight-hour lesions, induced by 5 per cent DNCB, the toxic damage extends into the uppermost portion of the dermis. The damage manifests itself with degenerative fragmentation of collagen fibrils next to the necrotic epidermis.

A reparative process first becomes apparent from the histologic changes of three-day lesions. The reproduction of cells is noticeable in the epidermal lining of the upper part of the hair follicles (Fig. 35). The epidermal cells reach the follicular orifice and spread centrifugally under the slough (Fig. 36). In four-day lesions a regenerated epidermal layer, two to three cells thick, covers the entire dermal surface and is overlaid with the dead epidermis (Figs. 37 and 38). In the five-day lesions the new epidermal layer becomes five to eight cells thick (Fig. 40). The basal cells proliferate and the surface cells show degeneration (Fig. 41).

Regeneration of the epidermis is faster than that of the collagen fibrils, first noted in a few forty-eight-hour lesions. A small number of mononuclear cells appear on the fourth day among the fragments of the fibrils (Fig. 39). In the seven- and nine-day lesions the collagen fibrils are largely restored. Residual fragmentation and the presence of pale fibrocytes among the restored fibrils point out the antecedent condition (Fig. 42).

Repair of the collagen is overlapped in time by another histologic reaction, noted first in six- and seven-day lesions. The reaction is characterized by a

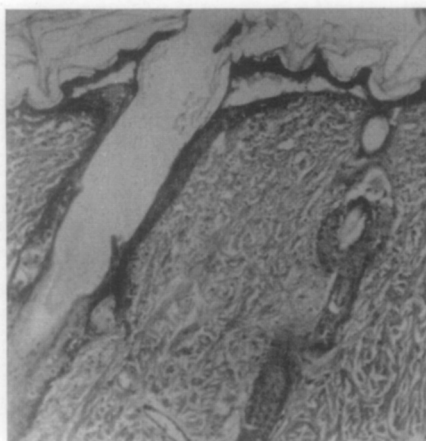


Fig. 35.

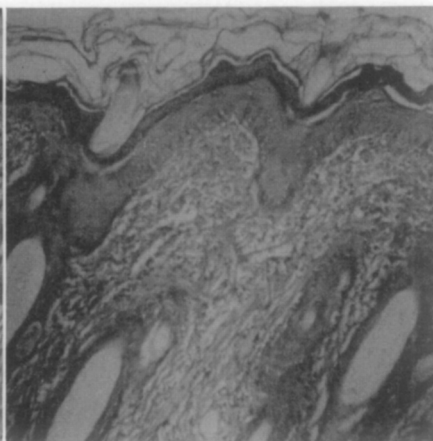


Fig. 37.

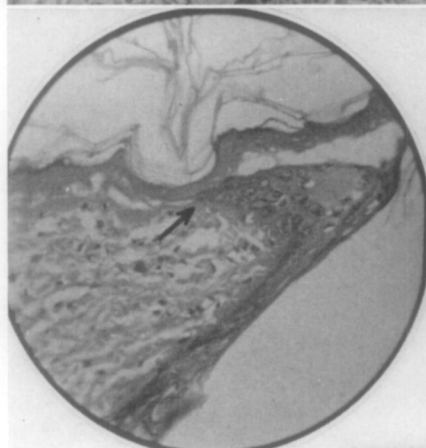


Fig. 36.

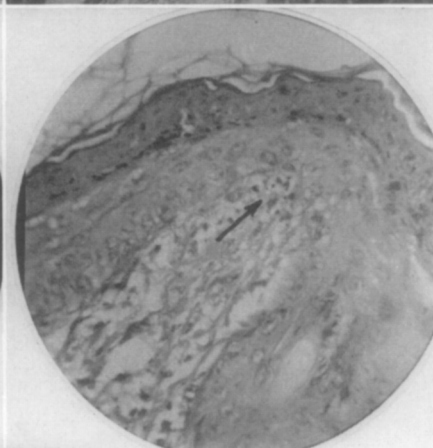


Fig. 38.

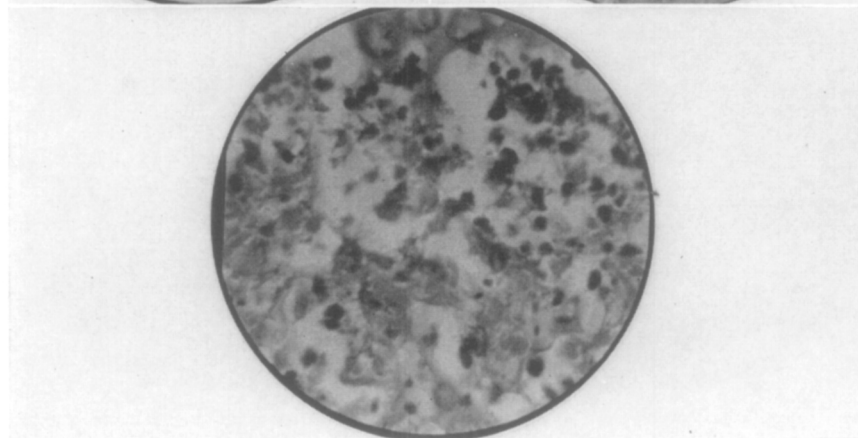


Fig. 39.

Figs. 35 and 36.—Three-day lesion; 5 per cent DNCB. The regeneration of the epidermis begins in the upper portion of the hair follicle. Note an island of epidermal cells around the follicular orifice (Fig. 36) spreading between the dermis and the dead epidermis. (Magnification: Fig. 35, $\times 100$; Fig. 36, $\times 400$.)

Fig. 37.—Four-day lesion; 5 per cent DNCB. Regenerated epidermis, four cells thick, covers the dermal surface and is overlaid by the toxic slough. (Magnification, $\times 100$.)

Figs. 38 and 39.—Four-day lesion; 5 per cent DNCB. Detail of Fig. 37. Note the fragmentation of the collagen fibrils of the uppermost dermis with an accumulation of minute mononuclear cells. (Magnification: Fig. 38, $\times 400$; Fig. 39, $\times 1000$.)

Fig. 40.

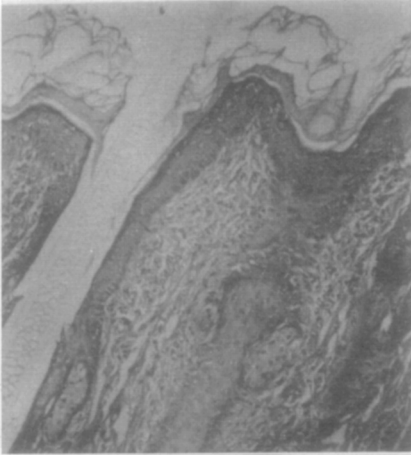


Fig. 43.

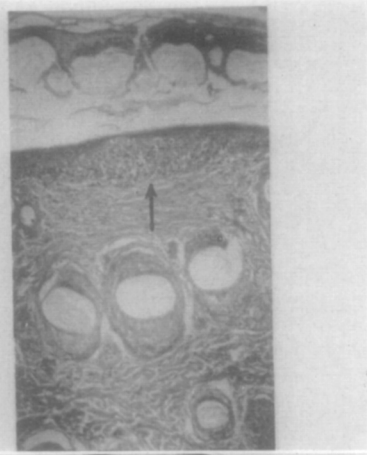


Fig. 41.

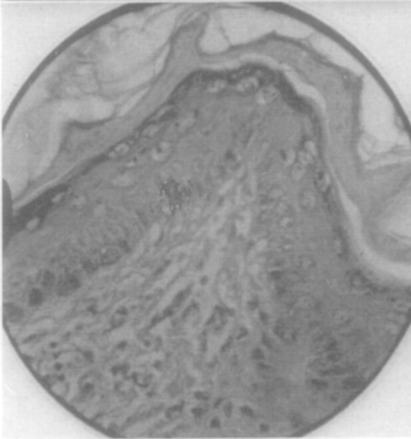


Fig. 44.

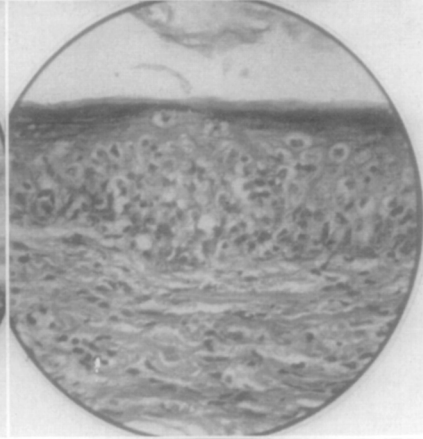
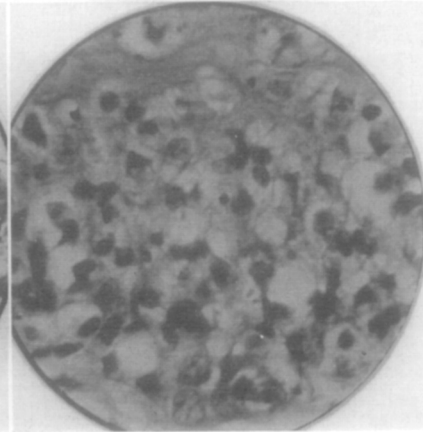


Fig. 42.



Fig. 45.



Figs. 40 and 41.—Five-day lesion; 3 per cent DNCE. Regenerated epidermis, six to eight cells thick. The epidermis is overlaid with a slough. The basal cells proliferate, whereas the upper epidermal strata show dyskeratotic vacuolization. (Magnification: Fig. 40, $\times 100$; Fig. 41, $\times 400$.)

Fig. 42.—Six-day lesion; 5 per cent DNCE. The restoration of damaged collagen fibrils. Note the pale-staining fibrocytes between the collagen fibrils. (Magnification, $\times 1,000$.)

Fig. 43.—Seven-day lesion; 5 per cent DNCE. Regenerated and acanthotic epidermis beneath a slough of toxic reaction. Note invasion of the regenerated epidermis by infiltrating cells. (Magnification, $\times 100$.)

Figs. 44 and 45.—Seven-day lesion; 5 per cent DNCE. Details of Fig. 43. Some epidermal surface cells show a dyskeratotic degeneration. The lower portion of the epidermis is massively invaded by mononuclear leukocytes. Note in Fig. 45 the vacuoles adjacent to mononuclear cells. (Magnification: Fig. 44, $\times 400$; Fig. 45, $\times 1000$.)

flare-up of capillary dilatation, increase of interstitial edema, a fresh extravasation of mononuclear cells into the perivascular tissue, and an invasion of the acanthotic epidermis by mononuclear cells (Fig. 43). The histologic change in the epidermis at the point of invasion of mononuclear cells (Figs. 44 and 45) is identical with that seen in early lesions of allergic contact dermatitis. A formation of microvesicles occurred in most of the nine-day lesions. This histologic change in the six-, seven-, and nine-day lesions coincides with the development of general allergic sensitivity determined by epicutaneous test with 0.75 per cent DNCB in olive oil in a new area of the skin. It seems that the antigen left at the toxic side induced an allergic flare-up at the site of the primary lesion, thus giving rise to histologic changes characteristic of allergic contact dermatitis of extremely sensitive animals. However, the biopsies obtained from the allergic test lesions, induced by 0.75 per cent DNCB in the same animals, revealed only a dense dermal infiltration by mononuclear cells. The epidermal changes seen in the flaring toxic lesions were not reproducible. Various factors present at the site of the original toxic lesion could be responsible for the epidermal reaction. Among these factors are acanthosis of the regenerated epidermis^{15, 17} and vascularization of dermis, both subsequent to toxic injury, which constitute the difference in anatomic structure between the original toxic lesion and the freshly shorn area of skin used for the epicutaneous test.

DISCUSSION

The histologic study of allergic and toxic dermatitis was undertaken not only with the object of determining the changes characteristic of both types but also with the thought of finding a clue to the mechanism of the allergic response.

The histologic changes in the toxic and allergic lesions are strikingly different, although the duration of the two reactions is about the same (five to six days) and in both the peak of injury is reached at about forty-eight hours.

The three- to twelve-hour lesions of *toxic dermatitis* are characterized by epidermal injury from withering of the epidermal cells to total necrosis, always accompanied by scant extravasation of polymorphonuclear and mononuclear cells. The three- to twelve-hour lesions of *allergic dermatitis* are characterized by a massive extravasation of mononuclear cells migrating in trails directly into the epidermis. In the toxic reaction the concentrated (5 per cent) chemical caused relatively sparse and scattered cellular infiltration; in the allergic reaction a dilute concentration (0.01 per cent and 0.1 per cent) of the chemical induced prompt extravasation and direct ascent of the mononuclears toward the challenged skin surface. *The most plausible explanation for the behavior of the mononuclear cells seems to be that the sensitization has induced in them a capacity for positive chemotaxis; that is, a faculty to detect a chemical (or its protein derivative) in minute concentrations and to migrate straight into the area of its highest concentration.*³⁶

Nearly all *toxic lesions* of twenty-four to forty-eight hours showed an increase of polymorphonuclear and mononuclear cell infiltration in the dermis following the earlier death of epidermal cells. (In some, in which the epidermis

had partially survived, a formation of intraepidermal cleavages containing infiltrating cells was observed. Partial epidermal necrosis with simultaneous increase of cellular infiltration of the dermis seems to be a requisite condition for this development. It appears that the slow toxic disintegration of the epidermis has promoted the transmigration of infiltrating cells into the epidermis.³⁷) The *allergic lesions* of twenty-four to forty-eight hours, on the other hand, showed a massive invasion of the epidermis by mononuclear cells and formation of vacuoles. Examination of serial sections revealed that each vacuole adjoins at least one mononuclear cell. In some lesions microvesicles develop, presumably at points where vacuoles had fused. The vacuolization and vesiculation cause a disruption of the epidermis which apparently lessens

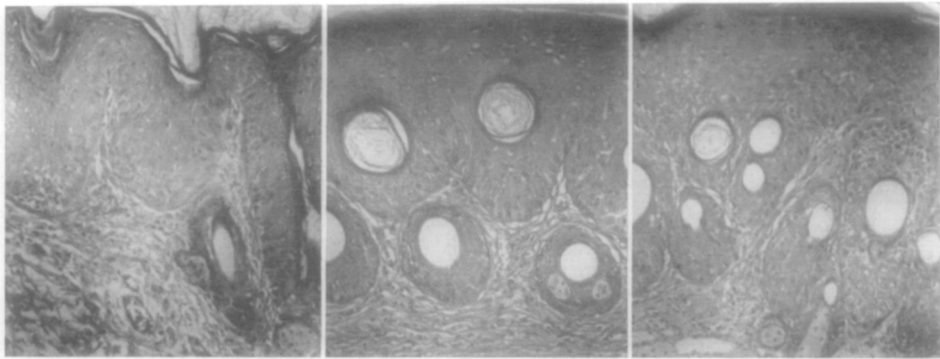


Fig. 46.—Forty-eight-hour lesions; 0.1 per cent DNCB. Three lesions of allergic contact dermatitis of highly sensitive guinea pigs injected with germanin. (100 mg. germanin subcutaneously daily for five days; epicutaneous challenge with DNCB on the third day.) Note the absence of mononuclear-cell invasion into the extremely thickened epidermis. Control sites tested with plain olive oil showed normal histology. (Magnification, $\times 100$; reduced $\frac{1}{10}$.)

its viability and leads to its death and exfoliation. *Mononuclear cell invasion, vacuolization, vesicle formation, and exfoliation of the epidermis are steps in a process characteristic of the allergic response not seen in the toxic lesions.* An excess of histamine accumulates at the site of allergic DNCB contact dermatitis only in the presence of mononuclear cells.^{32, 38} This accumulation results from activation of histamine metabolism by promotion of histidine decarboxylation. Administration of germanin (decarboxylase inhibitor) was found to inhibit the histamine formation.³² Furthermore, it has been shown that the infiltrating cells in allergic DNCB reaction have an increased activity of alkaline phosphatase, which is inhibited by administration of heparin.³⁹ In the toxic lesions there is neither histamine accumulation³² nor increased phosphatase activity.³⁹ *Thus the allergic reactions show not only a specific cellular response but also a characteristic intensity in local metabolic activity, enzymatic in character.*

These results suggest that the reaction of the allergic contact type is dependent on mononuclear leukocytes which acquire specific capacities through sensitization. However, histologic findings at sites of allergic dermatitis of guinea pigs injected with germanin are indicative of a sensitization of epidermal cells as well. In all animals injected with germanin, the forty-eight-hour

allergic lesions reveal considerable dermal infiltration by mononuclear cells. However, the epidermis is extremely thickened and not invaded by the mononuclears (Fig. 46). It seems that germanin has prevented ascent of mononuclear cells in the epidermis. *The extreme epidermal thickening might be regarded as the allergic response of epidermal cells after the participation of mononuclears has been forestalled.*

SUMMARY

Chronologic development of histologic changes at the site of allergic and toxic dermatitis is described in detail.

Mononuclear cell invasion, vacuolization, vesicle formation, and exfoliation of the epidermis are steps in a process characteristic of the allergic response.

A comparison of the histologic and chemical changes in toxic and allergic dermatitis indicates that sensitization induced a capacity in the mononuclear cells for positive chemotaxis, for induction of characteristic inflammatory changes, and for participation in metabolic processes (presumably by increased enzymatic activity).

A sensitization of epidermal cells has not been ruled out. An extreme acanthosis seen in allergic lesions of animals treated with germanin might be regarded as the allergic response of epidermal cells after participation of mononuclear cells has been forestalled.

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