Histopathology of experimental eczema (allergic contact-type eczematous dermatitis) in man*

A study by the technics of silver impregnations of Rio Hortega with special reference to the early microscopic lesions

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Towards the end of the past century, Unna and Besnier published their studies on histopathology of eczema (now often called “allergic eczematous contact-type dermatitis” a name coined by Sulzberger to distinguish this entity from atopic dermatitis and circumscribed lichen simplex). These descriptions are now classics and have been extensively quoted in most well-known textbooks of dermatology.

These authors considered that intercellular epidermic edema—described as “status spongoides” (Unna) and “spongiosis” (Besnier)—was the fundamental lesion in eczema. On the basis of this spongiosis the vesicles would be formed by the confluence of those small foci of edema.

Gans (1), Kreibich (2), MacLeod (3) and MacCarthy (4), among others, have developed this classic description in their well-known books.

In 1925, Civatte (5) in France, published a revision on the subject and sustained that in eczema, a primary microscopic vesicle (“vésiculette primordiale”) appeared in the stratum mucosum before the spongiosis. Spongiosis does not always follow the primary lesion. The “vésiculette primordiale” is formed by lysis of two or three malpighian cells, through cytoplasmic alteration. This primary lesion is frequently followed by spongiosis which tends to enlarge the cavity by the current of serum that comes from the papillary body. This process gives place to a larger cavity that occupies the greater depth of the prickle cell layer. Therefore, according to Civatte, the process appears to be originally of intracellular nature, followed by intercellular edema. In spite of the lack of confirmatory reports abroad, Civatte insisted in his findings in 1936 (6) and 1937 (7).

In 1937, Darier supported Civatte’s opinion (8).

Percival and coworkers (9), without mentioning Civatte or discussing the “altération cavitaire” describe the process of eczema as follows: “multiple localised foci of epidermal necrosis are produced with, or as a result of, the allergic process” mentioning “liquefied epidermal cells in a commencing vesicle”.

Sachs, Miller and Gray (10), in 1944, studied the histopathology of eczematoid dermatosis in their typical aspects but omitted the early alterations.

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Received for publication Feb. 18, 1949.

1 Gans and Mac Carthy also mentioned the early appearance of the “altération cavitaire” (Leloir) in the cells of stratum mucosum.

2 Civatte summarizes (6) the histopathologic process of vesiculation in three phases: 1: death of the malpighian cell with “exocytose” and minimum “éxosérose” and the appearance of the “vésiculette primordiale”; 2: spongiosis and 3: definite vesiculation.
Miller (11) employing biopsies obtained from patch tests, describes spongiosis saying that "this type of formation of vesicles is known as primary vesicular formation".

Ginsberg, Stewart and Becker (12), in a study on sensitiveness to poison ivy considered that histologic lesions of the guinea pig resembled those of man at 24–48 hours intervals after contact; and also that the spongiosis is the typical alteration.

PRESENT STUDY

Since 1940, one of us (A.M.) has studied the experimental eczema (contact-type allergic eczematous dermatitis) in man by a simple technic that consists in sensitizing human skin through contact with a drop of 2–4 dinitrochlorobenzene, 10% in acetone (13) and with inunctions of p-phenyldiamine, 10% in petrolatum (14). Five to twenty days after this preparatory phase, the same sites are touched with 1:100 concentrations of the same substances. In this manner we obtain erythema and vesicles that begin to appear after 6–8 hours and last between 4–8 days. Histological findings in these experiments show that these lesions are identical to those found in clinical eczema (15).

These experiments helped to obtain biopsies in series at 6, 12, 24, 48, 72, 96 hours. In all, 136 specimens were studied, 28 of which were impregnated with ammoniated silver carbonate, according to the technics of Rio Hortega.

With this material we tried to confirm the results of the studies of Civatte. This paper is part of a series of investigations in progress on the mechanism of vesiculation in eczema.

TECHNIC

Biopsies were fixed in 10% formalin (Merck) and the frozen sections were later stained with the silver impregnations of Rio Hortega (ammoniated silver carbonate, nuclear variant, for prickle cells bridges—"epiteliofibrillas"—and for reticulin).

MICROSCOPIC DESCRIPTION

The earliest histologic lesion observed in our material emanates from biopsies taken 6 hours after the application of the test with 2–4 dinitrochlorobenzene. Careful search of the epidermis at this period revealed small round clear zones that with low magnification seemed to correspond to the disappearance of a few cellular elements. These small lesions always appeared at the top of stratum mucosum. With greater magnification, we established that in these sites 1, 2 or 3 cells (Fig. 2) were beginning to lose the tinctorial affinity of their cytoplasm, which appears to be undergoing destruction, becoming granular, more clear and contracted around the nuclei. The latter sometimes appear to be normal and other times show beginning pyknosis.

The disappearance of part of the cytoplasm forms a cavity occupied by small argyrophilic granules (cytoplasmatic and cromatinic residues). This cavity cor-
Fig. 1. Stratum mucosum of normal human skin. Technic of Rio Hortega for prickle cells bridges: pyridine-ammoniated silver carbonate-gold chloride.

Fig. 2. "Vésiculette primordiale" (Civatte) 6 hours after contact with 2,4 dinitrochlorobenzene. Ammoniated silver carbonate of Rio Hortega, nuclear variant.
Fig. 3. "Vésiculette primordiale" (Civatte) 6 hours after contact with 2.4 dinitrochlorobenzene. Technic of Rio Hortega for prickle cells bridges: pyridine-ammoniated silver carbonate-gold chloride.

Fig. 4. "Vésiculette primordiale" (Civatte) 12 hours after contact with 2.4 dinitrochlorobenzene. Ammoniated silver carbonate of Rio Hortega, nuclear variant.
responds to the one that Civatte considered the "vésiculette primordiale". The prickle cells surrounding this round or oval cavity did not appear to be altered.

Employing the technic of Rio Hortega for prickle cells bridges ("epiteliofibrilllas") on the same material (6 hours) we were able to note an interesting fact: the two or three cells that by destruction of their cytoplasm made way for the appearance of the small cavity (Fig. 3) did not break their connections, since these could be seen clearly even if they did not present the regularity found in normal tissue (Fig. 1). With this technic we could see the "vésiculette" criss-crossed by the prickle cell bridges that still united the cells of the periphery to the two or three cells that were loose in the cavity (Fig. 3), which thus had the appearance of a small honeycomb.

At 12 and 24 hours after application of the eczematogenous allergen the number and size of "vésiculettes" has increased and now the more simple technics show a neat separation between the perivesicular cells. This last aspect, when it is analyzed by the technic for prickle cells bridges, shows intercellular canals—in the spongiosis—with borders formed by prickle cells whose bridges are stretched to their limit (Fig. 4–5).

At 24 and 48 hours after application of the eczematogenous allergen the process increases, the canals broaden, the bridges stretch yet more and break and finally the cells lose their connections and great confluent lakes are formed which contain loose cells surrounded by edema (Fig. 6).
Fig. 6. Broad canals of spongiosis and confluent lakes which hold loose cells, 24 hours after contact with 2.4 dinitrochlorbenzene. Technic of Rio Hortega for prickle cells bridges.

Fig. 7. Prickle cells and spongiosis far from the sites where the process began. The intercellular bridges are still thick and resistant, 24 hours after contact with 2.4 dinitrochlorbenzene. Pyridine-ammoniated silver carbonate-gold chloride technic.
Fig. 8. Spongiosis and vesicles, 48 hours after contact with 2,4 dinitrochlorobenzene. Ammoniated silver carbonate, nuclear variant.

Fig. 9. Great vesicles holding cells in necrobiosis, neutrophils, lymphocytes and fibrin, 72 hours after contact with 2,4 dinitrochlorobenzene. Note the intense dermic inflammatory reaction. (Reaction in cutis.) Ammoniated silver carbonate, nuclear variant.
The "vésiculettes primordiales" that we described at the beginning are connected by this spongiosis and great cavities are formed through this double mechanism.

The prickle cells far from the sites that we have just described also show spongiosis, but here the intercellular bridges are still thick and resistant. When the process increases these last follow the same course of stretching and rupture (Fig. 7).

Between 48 and 72 hours after application of the allergen the classic lesions described in eczema are seen, that is, the formation of great vesicles—occupying all the epidermis—which hold cells in necrobiosis, neutrophils, lymphocytes and fibrin (Fig. 8–9).

The inflammatory process of the dermis (cutis) began slowly after the first 12 hours of contact. It is only after 24 hours that the dermal process increases with hyperemia and beginning diapedesis (Fig. 10). At this period the perivascular

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**Fig. 10.** Basal cells and papillary body showing dermic (cutis) edema and cellular infiltrate and the mononuclear cells invading the epidermis. Biopsy taken 12 hours after contact with 2,4 dinitrochlorobenzene. Ammoniated silver carbonate, nuclear variant.
infiltrate is very intense with marked mobilization of reticulohistiocytic cells, and with zones of edema and necrobiosis. With the specific stain of Rio Hortega we can see a rich net of reticulin (Fig. 11) which connects the basal “membrane” with the adventitia of the blood vessels.

CONCLUSIONS

From our findings we infer:

1. That the “vésiculette primordiale” of Civatte is the earliest microscopic lesion in human experimental eczema. (Contact-type allergic eczematous dermatitis.)

2. That the spongiosis follows the “vésiculette primordiale” chronologically.

3. That the vesicle is formed by the confluence of “vésiculettes primordiales” and of the canals resulting from intense spongiosis.
4. That the dermic inflammatory process of non-specific type appears later and is probably secondary to the epidermic phenomena.

REFERENCES