# DELAYED-TYPE HYPERSENSITIVITY TO STORAGE MITES OF THE *Tyrophagus putrescentiae* COMPLEX IN EXPERIMENTAL ALLERGIC DERMATOSIS

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**KEY WORDS:** storage mites; allergic dermatosis; spongiosis

Recent years have seen a sharp increase in the prevalence of allergic diseases. An important place among them is occupied by atopic dermatitis [9]. Many workers have noted the important role of storage mites and house dust mites in the genesis of atopic dermatitis [3, 4, 7]. Involvement of B lymphocytes and humoral antibodies of the immunoglobulin E class in the immunopathogenesis of this disease has now been confirmed by many investigators [6, 8]. It has also been shown that a marked proliferative T-cell response to acarine allergens exists in patients with sensitization to mites [7, 10]. A marked T-cell reaction to different fractions of an extract from the mites *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* has been obtained on a model of delayed-type hypersensitivity in mice previously treated with cyclophosphamide [7].

The aim of this investigation was to obtain immunomorphological confirmation of the role of T lymphocytes in experimental acarine atopic allergic dermatoses induced by allergens from storage mites of the *Tyrophagus putrescentiae* complex, belonging to the Acaridae family, a species widely distributed in many enterprises of the food and agriculture industry [5].

## EXPERIMENTAL METHOD

Experiments were carried out on male guinea pigs weighing 150 g, divided into 5 groups (3 in each group; all the animals had light colored hair). The allergen was prepared from mites of the species T. putrescentiae, purified from culture medium (oatmeal + 1/20 yeast). Extraction was carried out by Coca's method in 0.5 M ammonium bicarbonate buffer (pH 8.2) for 48 h at 4°C. The homogenate was centrifuged at 6000 rpm and filtered through fine-pore filters. The filtered extract was dialyzed against 0.05 M ammonium bicarbonate buffer for 12 h at 4°C. After freeze-drying the allergen was kept at −20°C. The allergenic extract was tested for specific activity in the inhibition of the radioallergosorbent test (RAST) method, using FADB AS RAST kits and allergen-specific disks (d-72) to *T. putrescentiae* (Pharmacia, Sweden). The experimental animals were immunized by injection of allergen from mites in a concentration of 0.3 mg/ml into the footpads of all four limbs, in Freund's complete adjuvant, in a volume of 0.1 ml into each footpad. After 3 weeks, i.e., on the 21st day from the beginning of immunization, testing was carried out with intradermal injections of acarine allergen by means of insulin needles, on the backs of the experimental animals. The quantity of allergen injected intradermally was 0.1 and 0.01 ml, with dilutions of 1:1000, 1:100, and 1:10 of the original concentration in physiological saline. Physiological saline alone, and Hanks' solution, the diluting fluid, were used as the control. The state of reactivity of the skin was tested by injection of 0.1 ml histamine in a concentration of 0.01% intradermally. The state of cellular immunity was tested on control animals

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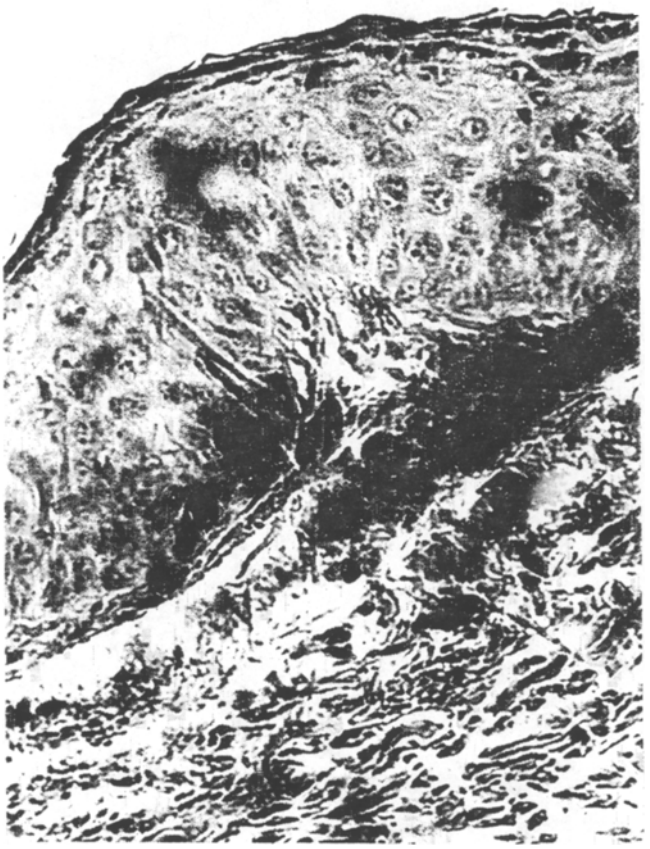

Fig. 1. Biopsy specimen from guinea pig skin. 280×. Hematoxylin-eosin. Edema of epithelium and spongiosis observed. Waksman's vacuoles. Allergic skin reaction rated at 2 points.

sensitized to tuberculin, by intradermal injection of tuberculin on the 21st day in the usual concentrations by the method in [2]. The animals were killed by decapitation and were exsanguinated. Areas of skin involved in the processof allergic inflammation were subjected to biopsy in absolute alcohol and formalin. This was followed by immunomorphological identification of each specimen by the special treatment and staining methods of Heidenhain, Brachet, Feulgen, and Mallory. The specimens thus obtained were subjected to close study by ordinary light microscopes and also by polarization and interference microscopes.

## EXPERIMENTAL RESULTS

We obtained positive results in respect to reproduction of atopic allergic dermatosis by immunization of guinea pigs with allergen from *T. putrescentiae.* Recording allergic reactions of the T type to intradermal injection of acarine allergens gave the following results. The strongest reaction, due to T lymphocytes, was recorded by us in response to injection of whole allergen and was rated at 3 points (+++). Reactions of 3 points (+++), 2 points (++), and 1 point (+) were observed after injection of the allergen in dilutions of 1:10, 1:100, and 1:1000 respectively. During visual recording we observed an inflammatory skin reaction in the zone of specific allergic inflammation, which was expressed as erythema and hyperemia, and which had the appearance of a round papule 3-4 mm in diameter. On pressure the papule turned pale, but did not disappear completely. No tissue necrosis was observed in the center.

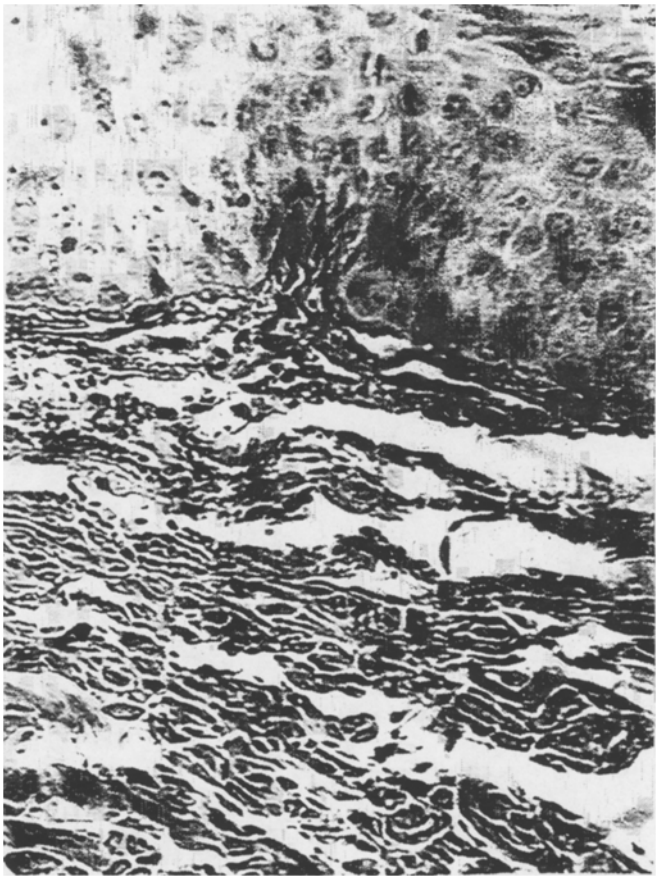

Fig. 2. Biopsy specimen from guinea pig skin. 400×. Hematoxylin-eosin. Allergic skin reaction rated at 3 points. Edema of epithelium, "lane" of T lymphocytes, and spongiosis recorded.

Evidence of marked vasculitis was found in the deep layers of the dermis. "Waksman's syndrome," pathognomonic of the hypersensitivity reaction of T-cell type, was characterized by the presence of spongiosis and also of disintegration of the walls of the arterioles, venules, and capillaries, accompanied by dense infiltration in the deep layers of the dermis (Fig. 1).

A similar "Waksman's syndrome" was observed in the epithelium at the center of each allergic skin reaction, mediated by T lymphocytes.

Additional signs of the presence of a positive allergic skin reaction were desquamation of the epidermis and disintegration of the desmosomes and their tapering (Figs. 2 and 3).

The following conclusions can be drawn from the above-mentioned facts:

1. T lymphocytes are involved in atopic (noninfectious allergic) reactions induced by storage mites.

2. As regards delayed-type hypersensitivity the allergen from the bodies of the mite T. putrescentiae can be regarded as allergens of weak type according to the classification in [1]. This is confirmed by our use of allergen in high doses and concentrations in order to immunize animals. (When definite allergic skin reactions have been obtained with strong chemical allergens such as dinitrophenol, dinitrochlorobenzene, etc., concentrations 1000 or more times lower are used).

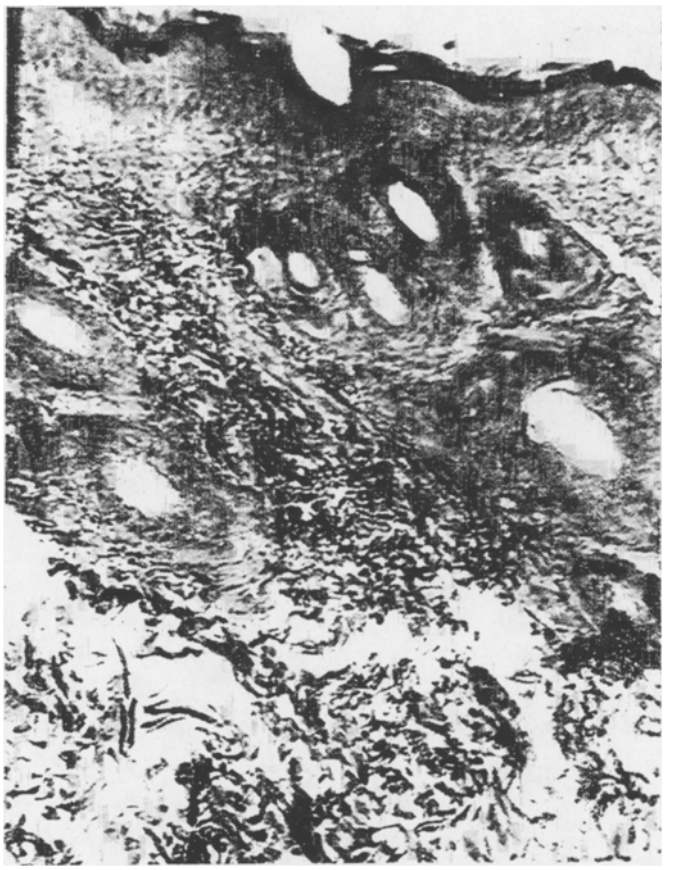

Fig. 3. Biopsy specimen of guinea pig skin. Infiltration of upper layers of skin by T lymphocytes (allergic skin reaction rated at 3 points). Edema of epithelium, spongiosis, and desquamation. 280×. Hematoxylin-eosin.

3. It is natural to suggest the existence of a similar reaction of T-cell type, expressed as occupational allergic dermatoses in food and agricultural workers in close contact with raw materials containing storage mites.

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