Acta Dermatovener (Stockholm) 50: 327–328, 1970

LYMPH-NODE PERMEABILITY FACTOR AND SKIN HISTAMINE

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Abstract. Analyses of skin histamine in guinea-pigs following intradermal injections of lymph-node permeability factor demonstrated a marked increase 24 and 48 hours after the injection. This rise is similar to the rise in skin histamine found in delayed allergic reactions. The results of this study are in agreement with the suggestion that LNPF may be a mediator of delayed hypersensitivity.

Several attempts have been made to identify mediators involved in delayed allergic reactions. Histamine, plasma kinins and various other permeability factors have been suggested as participants (4, 5, 10, 17). Most of these mediators, however, when administered as an exogenous substance, only reproduce a part of the inflammatory processes typical of delayed allergy. Within recent years, a "lymph-node permeability factor" (LNPF), demonstrated in membrane-free extracts of lymph nodes, has been suggested as a mediator of delayed hypersensitivity (7, 11). This factor increases vascular permeability to plasma protein, causes leucocyte emigration, and brings about deposition of a material resembling connective tissue fibrinoid (9, 11, 12, 13). Additionally, it has been demonstrated (15) that LNPF, when injected into human skin, produces a cellular sequence in "skin windows" similar to the sequence found in various types of delayed hypersensitivity. The present study was undertaken to evaluate skin histamine in guinea-pigs after injections of LNPF. Increased histamine has previously been demonstrated in delayed allergic reactions of the skin (1, 3, 17).

MATERIAL AND METHODS

LNPF was prepared by the technique of Shild & Willoghby (7). Lymph nodes of 10 normal guinea-pig were used. The removed lymph nodes were crushed in Hanks solution. The suspension, containing approx. 250×10^6 lymphocytes per ml, was exposed to alternate rapid freezing and thawing, and thereafter centrifuged (4 000 rpm). Finally, the cell-free supernatant was filtered through a bacteriological filter.

Nine normal guinea-pigs, weighing 300 to 500 g received intradermal injections of 0.1 ml extract on two sites on the back. Twenty-four and 48 hours later, skin samples were removed from the sites of injection by 8 mm punch biopsy. As a control, punch biopsies were also obtained from normal back skin 48 hours after injection. The control biopsies were taken at a distance of at least 4 cm from the sites of the injections. Skin samples from 10 untreated guinea-pigs were also studied. The skin biopsies freed of subcutaneous fat were minced and lyophilized for 48 hours over phosphopentoxide at room temperature under a pressure of 2 mmHg. Defatting was carried out by shaking twice with 15 ml ether for 1 hour. The dried and defatted samples were homogenized in 5 ml of a 0.4 N perchloric acid using a motordriven glass homogenizer. The homogenate was centrifuged, and a 4 ml aliquot of the supernatant was analysed for histamine by the spectrofluorometric method of assay (8).

RESULTS

The results of the histamine determinations are summarized in Table I. Forty-eight hours after injection, the histamine content of LNPF-treated skin was significantly higher than the content of non-treated skin ($p < 0.0025$). Skin histamine of LNPF-treated areas was also significantly increased when compared with skin histamine of untreated controls, 24 hours ($p < 0.01$) and 48 hours ($p < 0.005$) after injection. All histamine values represent microgram histamine base per gram dried defatted skin.

DISCUSSION

In the tuberculin reaction of the guinea-pig an increase of skin histamine has been found to parallel the development of the lesion (3). Simi-

Table I. *Histamine content of LNPF-treated skin of 9 guinea-pigs compared with the histamine content in non-treated skin and skin histamine of 10 non-treated controls*

	Hours after injection	Skin histamine ± S.E. (μg/g)
LNPF	24	11.4±1.6
LNPF	48	12.1±1.5
Non-treated skin[a]	48	5.8±0.4
Controls	—	7.1±0.5

[a] One sample lost.

larly, in the dinitrochlorobenzene contact dermatitis of guinea-pigs, the histamine content of the lesion is greater than that in the primary irritant response to the chemical (1). In human studies, an increase in skin histamine has been demonstrated in experimental allergic contact dermatitis (17) and in the normal lymphocyte transfer test (16). The present investigation shows that injections of LNPF into skin produce a rise in skin histamine similar to the rise found in delayed allergic reactions. The change might be brought about by reduced destruction or increased production of histamine. The latter possibility has been suggested by Shayer and co-workers (5, 6), who have found increased histidine decarboxylose activity in delayed allergic reactions. However, the increase in skin histamine could also be due to an immigration of basophilic leucocytes, which are rich in histamine (2). Basophilic leucocytes characterize cellular exudates of the delayed type of hypersensitivity (14) and have been found in skin windows after LNPF (15). The results of the present study are in agreement with the suggestion that LNPF may be a mediator or delayed hypersensitivity.

REFERENCES

1. Fischer, J. & Cooke, R.: Experimental toxic and allergic contact dematitis. J Allergy *29:* 397, 1958.
2. Graham, H., Lowry, O., Wheelwright, F., Lenz, M. & Parish, H.: Distribution of histamine among leucocytes and platelets. Blood *10:* 467, 1955.
3. Inderbitzin, T.: The mechanisms involved in histamine release and histamine increase in allergic skin reactions. Int Arch Allergy *18:* 85, 1961.
4. Juel Henningsen, S., Mickell, J. & Zachariae, H.: Plasma kinins in dinitrochlorobenzene contact dermatitis of guinea-pigs. Acta allerg (Copenhagen). In press.
5. Moore, T. & Schayer, R.: Histidine decarboxylase activity of autografted and allografted rat skin. Transplantation *7:* 99, 1969.
6. Schayer, R. & Ganley, O.: Adaptive increase in mammalian histidine decarboxylae activity in response to non-specific stress. Amer J Physiol *197:* 721, 1959.
7. Schild, H. & Willoughby, D.: Possible pharmacological mediators of delayed hypersensitivity. Brit Med Bull *23:* 46, 1967.
8. Shore, P., Burkhalter, A. & Cohn, V.: A method for the fluorometric assay of histamine in tissues. J Pharmacol exp Ther *127:* 182, 1959.
9. Spector, W. & Willoughby, D.: The effect of vascular permeability factors in the emigration of leucocytes. J Path Bact *87:* 341, 1964.
10. Søndergaard, J. & Greaves, M.: Recovery of a pharmacologically active fatty acid in delayed hypersensitivity. Int Arch Allergy. In press.
11. Willoughby, D., Boughton, B. & Schild, H.: A factor capable of increasing vascular permeability present in lymph node cells. A possible mediator of the delayed reaction. Immunology *6:* 484, 1963.
12. Willoughby, D., Boughton, B., Spector, W. & Schild, H.: A vascular permeability factor extracted from normal and sensitized pig lymph node cells. Life Sciences *1:* 347, 1962.
13. Willoughby, D. & Spector, W.: The production of eosinophilic deposits resembling fibrinoid by injections of lymph node extracts. J Path Bact *88:* 557, 1964.
14. Wolf-Jürgensen, P.: Basophilic leukocytes in delayed hypersensitivity. Thesis. Munksgaard, Copenhagen, 1966.
15. Wolf-Jürgensen, P. & Brændstrup, O.: Lymph-node permeability factor: a mediator in delayed hypersensitivity? Experiments using the skin window technique. Acta Dermatovener (Stockholm). In press.
16. Wolf-Jürgensen, P. & Zachariae, H.: Skin histamine and basophilic leukocytes in the normal lymphocyte transfer test. Acta Allerg (Copenhagen) *21:* 360, 1966.
17. Zachariae, H.: Histamine in delayed skin reactions. J invest Derm *42:* 431, 1964.

Received April 28, 1970

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