Direct Histochemical Demonstration of Histamine in Cutaneous Mast Cells: Urticaria Pigmentosa and Keloids

A conspicuously high level of histamine has been reported in certain skin lesions; the highest amount of histamine occurs in urticaria pigmentosa[1-4]. In urticaria pigmentosa the number of mast cells correlates well with the amount of histamine measured[2].

Recently, a histochemical method was developed for the cellular localization of mast cell histamine by fluorescence microscopy[5-7], based on the condensation reaction with *o*-phthaldialdehyde (OPT) introduced by SHORE et al.[8]. The technique as used in the present laboratory also permits the visualization of non-mast cell histamine[7,9,10].

This study is part of an investigation to clarify the specificity and applicability of the histochemical method for demonstrating cellular histamine. For this purpose, human tissues rich in histamine, or mast cells, such as urticaria pigmentosa[2] and keloids[11,12], were studied.

Material and methods. Small pieces of skin from the arms and abdomen were excised in local anesthesia from a 60-year-old woman suffering from urticaria pigmentosa with generalized cutaneous lesions and increased serum histamine. The material also includes skin from the corresponding regions of 2 healthy women, 47 and 69 years old. Keloids were removed from cicatricial skin in 4 male patients, 2 of them negroes, aged 40–65 years.

Histamine was demonstrated by the histochemical OPT method on cryostat sections[7] or on freeze-dried, paraffin-embedded material[9]. Mast cells were identified by their metachromasia upon toluidine blue staining[13]. Chemical assay of histamine was performed according to SHORE et al.[8]. The identity of histamine in the tissue extract was secured by analysis of the excitation and emission spectra of the histamine-OPT fluorophore.

Results and comments. Fresh cryostat sections taken from the urticaria skin lesions and treated with OPT gas contained a large number of yellow-fluorescent cells (Figure 1) identified as mast cells by their metachromasia after subsequent staining of the sections in toluidine blue (Figure 2). The mast cells were most numerous in the corium and around the blood vessels in the subcutis. Specimens removed from adjacent non-pigmented areas, or from healthy individuals, contained only scattered mast cells.

Chemical assay demonstrated very high histamine concentration in the pigment lesions (42.5–89.6 μg/g). In non-pigmented areas, the patient's skin histamine concentration ranged from 6.0–7.4 μg/g; skin from healthy subjects contained 4.8–6.2 μg/g histamine.

A large number of cells with an OPT-induced fluorescence (Figure 3) and identified as mast cells by toluidine blue staining occurred in the keloids. The histochemical results were the same whether cryostat sections or sections from freeze-dried, paraffin-embedded material was analyzed.

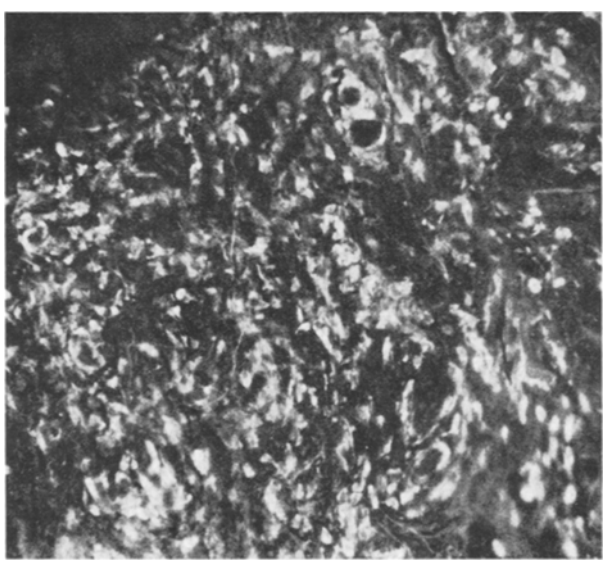

Fig. 1. Urticaria pigmentosa, transverse section through lesion. Extremely large amount of OPT-fluorescent cells in corium (upper half) and subcutis (lower half). × 100.

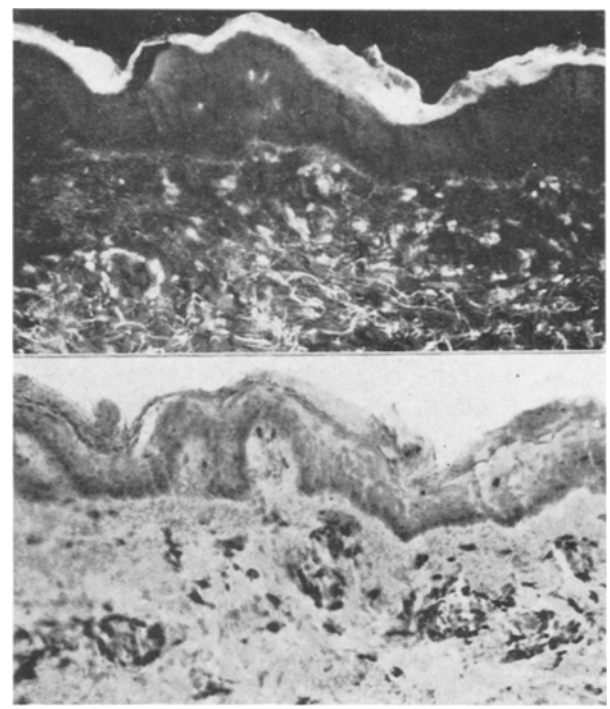

Fig. 2. Urticaria pigmentosa, transverse section through a region of the lesion with somewhat fewer histamine cells. Above: Fluorescence micrograph showing fluorescent cells in corium. Stratum corneum exhibits intense autofluorescence, remainder of epithelium is black. Autofluorescent connective tissue fibres in corium. Below: Same section stained in toluidine blue. Metachromatic mast cells (black) in corium correspond to the fluorescent cells. × 100.

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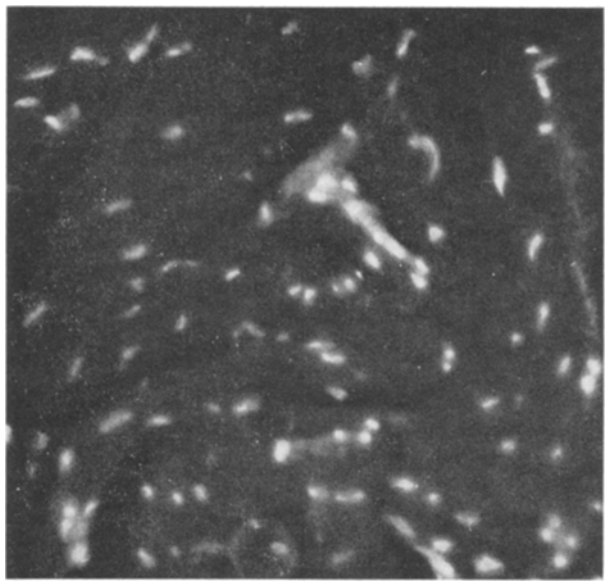

Fig. 3. Keloid. Large number of histamine-containing mast cells are visible after treatment with OPT. × 200.

With the technique used, mast cells were the only tissue component in the skin exhibiting a fluorescence upon OPT treatment. The content of mast cells in the various skin regions was very well correlated with the concentration of histamine as measured chemically[14].

Zusammenfassung. Die Haut von Patienten mit Urticaria pigmentosa und Keloiden wurde mit der histochemischen *o*-Phthaldialdehyd-Technik untersucht. Die Lokalisation von Histamin konnte durch Fluoreszenzmikroskopie festgestellt werden.

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The Effect of Monoamine Synthesis-Inhibitors on the Ovarian Compensatory Hypertrophy

There is growing evidence that hypothalamic catecholamines[1,2], and thus probably serotonin[3], are involved in the neuroendocrine mechanisms that control the secretion of pituitary gonadotrophins. It has been established that drugs modifying the rate of synthesis of catecholamines block ovulation[4,5] and induce pseudopregnancy in rats[5]. According to COPPOLA[6] the brain but not the peripheral catecholamines are responsible for these effects.

In this paper we report experiments showing the effect on the ovarian compensatory hypertrophy of 2 drugs that inhibit the synthesis of brain monoamines. L-α-methyl *p*-tyrosine (MPT) an inhibitor of catecholamine synthesis[7] and L-*p*-chlorphenylalanine (ClPh) that blocks synthesis of serotonin, were used[8]. Experimental and control Holtzman rats were unilaterally ovariectomized concomitant with the first injection. The other ovary was removed 10 days later. The ovarian compensatory hypertrophy (OCH) was evaluated by comparison of the relative weights (absolute weight/g of body weight) of both ovaries. In addition, ovaries were histologically studied in paraffin sections with the Masson thrichromic staining. Vaginal smears were obtained daily in controls and experimentals. Mammary glands were removed at autopsy and analyzed in sections stained with hematoxilin-eosin.

Results. (1) α-methyl *p*-tyrosine (MPT): A continuous i.p. administration of this drug at daily doses of 150 mg/kg[9] completely suppressed the OCH. In the controls, the ovaries which were studied simultaneously, showed a 59% hypertrophy (Table). Figures 1–4 show the histological characteristics of the ovaries. Normal hypertrophied ovaries are characterized in 5 μ sections by the presence of 5–7 large and active corpora lutea interspersed with mature follicles, some of them quite large. On the contrary, sections of ovaries of the group treated with MPT showed just 1 or 2 large corpora lutea and abundant mature follicles. The stroma appeared more densely populated with interstitial cells than in the control ovaries. Vaginal smears, on the other hand, showed for controls a normal estrous cycle whereas the experimental rats presented a constant diestrus. The mammary gland showed the characteristic lobulo-alveolar development reported for rat pseudopregnancy[10]. Treatment with the catecholamine inhibitor reduced the increase of body weight with respect to the controls which showed a 15 g gain in weight. Therefore, no weight losses, as reported after reserpine treatment, which also blocks OCH[11], were found.

(2) Combined administration of α-methyl *p*-tyrosine and DOPA: MPT was injected as described above. Simultaneous treatment with L-DOPA (dihydroxyphenylalanine) at daily doses of 100 mg/kg prevented the blocking effects of MPT on the OCH. No statistical differences were found between the weight of the ovaries of the MPT plus DOPA treated rats and those of the controls. Ovaries, as shown in Figure 3, presented several growing follicles, and 5–7 large corpora lutea. At the stroma, interstitial cells were found in numbers similar to the controls.

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