affect sebum excretion rate when compared with placebo. However, a similar preparation of 6.25% aluminium chloride hexahydrate in absolute alcohol has previously been shown to reduce sweat production by forehead skin (4).

Sweat alters both the physical state of sebum and the absorbent properties of the collecting vehicle (2). Thus changes in surface sweat may mask changes in sebum excretion rate. Despite this, it is unlikely that the clinical improvement in acne noted by Hurley & Shelley was related to any change in sebum excretion rate, as small reductions in SERdo not appear to be associated with clinical improvement of acne (5).

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# The Chloroacetate Esterase Reaction for Mast Cells in Dermatopathology: A Comparison with Metachromatic Staining Methods

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*Abstract.* This paper compares standard metachromatic methods with Leder's chloroacetate esterase reaction on mast cells in paraffin wax-embedded tissue of urticaria pigmentosa and a variety of inflammatory and benign neoplastic cutaneous conditions. Our conclusions are that Leder's method allows easier identification of mast cells and can be a useful adjunct to conventional metachromatic methods. The technique could be conveniently adopted in routine dermatopathology for mast cell identification.

*Key words:* Mast cells; Metachromatic stains; Naphthol ASD chloroacetate; Histochemistry

Identification of cutaneous mast cells is capricious because of the readiness of degranulation under traumatic influences such as skin biopsy. Metachromatic staining of the granules with basic aniline dyes such as toluidine blue and methylene blue has been the most popular method for the recognition of mast cells in paraffin wax-embedded sections.

Leder (3) devised a method that is not dependent on metachromasia. Reactivity of a specific esterase in mast cell granules with naphthol ASD chloroacetate was used to identify mast cells in paraffin wax-embedded sections.

We compared Leder's method with standard metachromatic staining methods for identification of mast cells in urticaria pigmentosa, melanocytic naevi, benign connective tissue tumours and a variety of inflammatory skin conditions.

## MATERIALS

Biopsy material obtained from cases of urticaria pigmentosa, melanocytic naevi, neurofibroma, dermatofibroma, pyogenic granuloma, keloidal folliculitis, psoriasis, Zoon's balanitis, pemphigoid and actinic keratosis was fixed in 10% formol saline and embedded in paraffin wax. The clinical diagnosis of each patient was confirmed by examination of haematoxylin/eosin-stained

Table I

| Diseases | No. of cases | Average number of mast cells in 10 high-power fields (×400) | |
|---|---|---|---|
| | | Chloroacetate esterase method | Metachromatic method |
| 1. Urticaria pigmentosa | 4 | Numerous | Numerous |
| 2. Melanocytic naevi | 4 | 55 | 50 |
| 3. Neurofibroma | 3 | 76 | 68 |
| 4. Dermatofibroma | 3 | 50 | 43 |
| 5. Pyogenic granuloma | 2 | 40 | 40 |
| 6. Keloidal folliculitis | 4 | 47 | 45 |
| 7. Psoriasis | 2 | 30 | 30 |
| 8. Zoon's balanitis | 5 | 65 | 56 |
| 9. Pemphigoid | 2 | 20 | 18 |
| 10. Actinic keratoses | 3 | 22 | 23 |

sections. For each condition unstained sections were stained with (*a*) Leder's method, and (*b*) the metachromatic method.

(*a*) *Leder's method*

Reagents: 1) 10 mg Naphthol AS-D chloroacetate in 1 ml, *N.N*-dimethylformamide; 2) 20 mg sodium nitrite in 0.5 ml of distilled water plus 0.5 ml of pararosaniline hydrochloride; 3) Veronal acetate buffer, pH 6.8.

The tissue specimens were deparaffinized by soaking twice in xylene and subsequently rehydrated through graded alcohols. Reagent 2 was added drop by drop to 30 ml of Veronal acetate buffer (Reagent 3) until the pH fell to 6.3. Reagent 1 was then added and the resulting mixture filtered. The sections were incubated in the clear red filtrate for 30 min at 20°C. The slides were subsequently rinsed with tap water and counterstained with Mayer's haemalum for 10 min. After further washing in tap water the sections were dehydrated in graded alcohols. Counterstaining with saffron alcohol was then carried out for 2 min. After rinsing with 100% alcohol, the specimens were cleared in xylene before mounting in DPX.

(*b*) *Metachromatic method*

Rehydrated unstained sections were stained with 0.5% toluidine blue in aqueous solution for 5 sec or 0.5% polychrome methylene blue (Sigma) for 10 sec. Dehydration was then carried out through graded alcohols before mounting in DPX.

Sections treated by both methods were examined by light microscopy. The numbers of intact mast cells were counted in 10 consecutive high-power (×400) fields by two independent observers and the average figure calculated.

## RESULTS

The results are tabulated in Table I.

(*a*) *Leder's method*

The mast cells consistently appeared brilliant red, standing out strikingly against a pale yellow background. They were usually round or oval in shape in loose connective tissue, but elongated or fusiform in scar tissue and around blood vessel walls. The granules were clearly visible in intact mast cells. The nucleus, readily identifiable by its bluish-black staining, was single, oval or kidney shaped and was centrally placed, giving a "poached egg" appearance to individual cells (Fig. 1).

The only other cell that gave a positive reaction was the polymorphonuclear neutrophil which was differentiated from the mast cell by its polysegmented nucleus. Eosinophils, plasma cells, lymphocytes and fibroblasts remained unstained and appeared bluish grey.

(*b*) *Metachromatic method*

In the sections stained with toluidine blue or methylene blue, the staining of mast cell granules varied from dark blue to purple. In some sections the mast cells could not be easily distinguished from the blue staining of adjacent connective tissue cells. Occasionally, differentiation was possible at high magnification only. The dark blue stained nucleus was often obscured by the metachromatic granules.

## DISCUSSION

The method using naphthol AS-D chloroacetate makes the granules appear larger and stains mast cells consistently. According to Lennert & Pawaresch (4) the chloroacetate esterase reaction is usually positive in mast cells of malignant mastocytosis, even where metachromasia is negative. Results with metachromatic stains, however, show a wide variation in staining intensity, probably due to

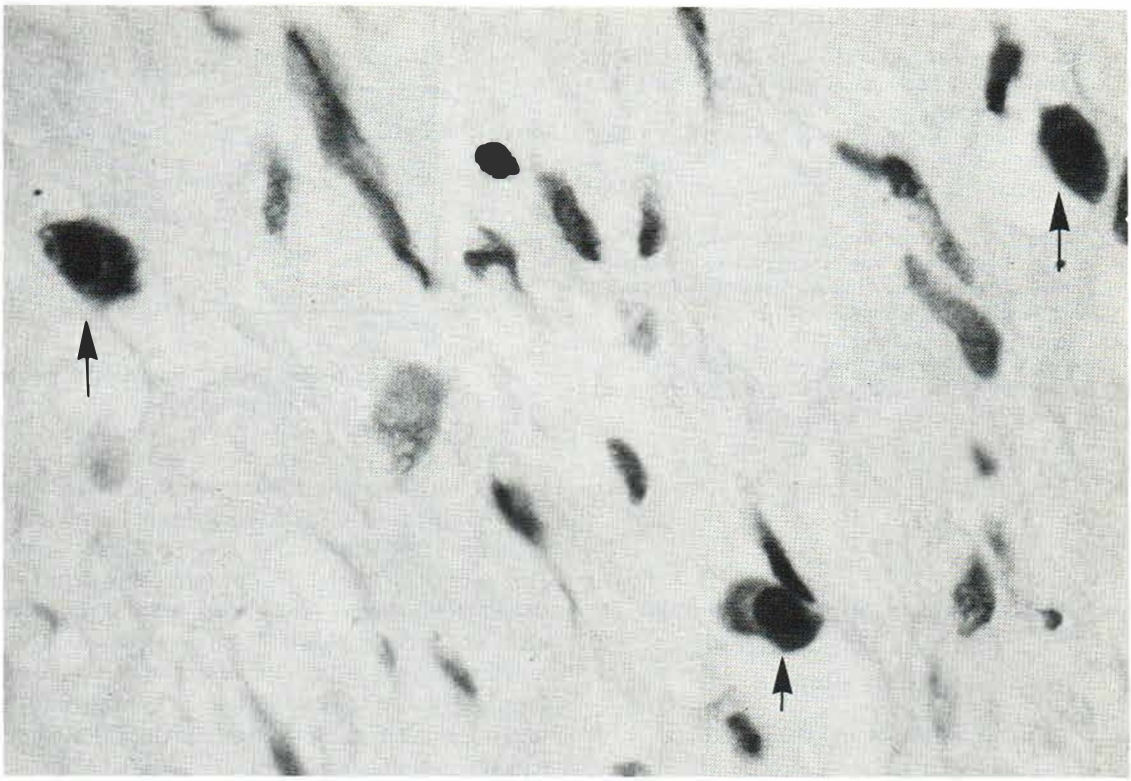

*Fig. 1.* Chloroacetate esterase reaction (×640). Intense positive staining of mast cells (↑) in a neurofibroma.

minor changes in technique of fixation, dehydration, staining and mounting which affect the cell morphology. Staining time varied with reagent concentration, tissue examined, thickness of sections, and pH of solution. Some of the variability of results, however, can be minimized by using frozen sections (1) or semi-thin sections (2).

Counting of cells is also made easier with Leder's method on account of the mast cell staining (red) being in complete contrast to the staining of the other connective tissue cells (blue). Metachromatic staining (varying from blue to reddish purple) did not provide such ready differentiation. Generally, higher mast cell counts were obtained with the esterase method, and this is doubtless attributable to the greater cellular differentiation afforded by the red reaction product (Table I). However, differences in mean counts between the two methods did not reach statistical significance.

Unfortunately the enzyme histochemical method for identification is relatively time-consuming (45 min) whereas metachromatic staining for mast cells can be carried out very rapidly on paraffin wax sections (1–2 min). Cells other than mast cells, e.g. polymorphonuclear neutrophils, also show a positive reaction for chloroacetate esterase. Differentiation between neutrophils and eosinophils in tissue afforded by the chloroacetate esterase reaction revealed that in our cases of pemphigoid, neutrophils were prominent in the blister cavity, which is in contrast to the usual presumption in textbooks of dermatopathology (5). Neutrophil positivity poses little problem in differentiating from mast cells in routine dermatopathology, although in the presence of a dense neutrophil infiltrate, the identification of individual mast cells by this method becomes very difficult.

Despite these relative disadvantages, the merits of the enzyme method are considerable and we suggest, particularly in cases of possible mastocytosis, the routine use of the chloroacetate esterase reaction as an adjunct to metachromatic stains for identifying mast cells in paraffin wax-embedded sections.

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# Impairment of Some Microphage Functions in Recurrent Herpes simplex


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*Abstract.* Chemotactic, phagocytic and intracellular killing activities of polymorphonuclear leukocytes (PMNL) were investigated in vitro in 49 patients suffering from recurrent bouts of herpes simplex. A slight impairment of chemotactic activity and a markedly reduced capacity of killing *Candida albicans* blastospores were revealed. Phagocytic capacity and NADH-dependent oxidase activity of PMNL proved to be normal. The results point to an alteration of some PMNL functions in these patients.


*Key words:* Recurrent herpes simplex; Microphage function; Chemotaxis; Killing test

Polymorphonuclear leukocytes (PMNL), or microphages, resp., are intimately involved, by means of their chemotactic, phagocytic and intracellular killing properties, in the complex organization of the immunological balance and antimicrobial protection of the individual (2, 9). For control of herpes virus hominis infections, the cell-mediated immune response also plays an important part (8). Recent studies on recurrent herpes simplex (RHS) has shown some defects or disturbances of lymphocyte functions, in particular of the T cell line (4, 6). However, the possibility of an additional defect in the PMNL system—which, like a Rapid Deployment Force of the cellular immune apparatus, constitutes the body's 'first line of defence'—is still an open question. The present paper deals with in vitro investigation into the functional activity of PMNL in patients suffering from RHS.

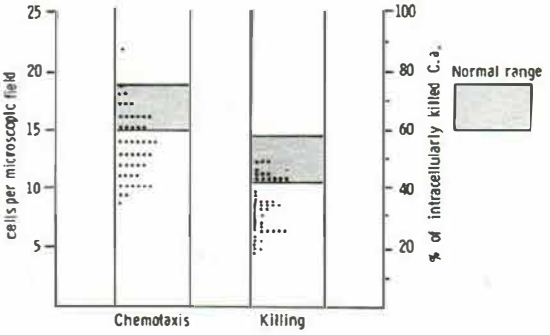


*Fig. 1.* Results of chemotactic and intracellular killing activities of PMNL in RHS ($n = 49$).

## MATERIAL AND METHODS

The study involved 49 patients (38 women and 11 men, mean age 34 years) suffering from RHS, examined in 1978–80. Diagnosis was based in the typical clinical symptoms of labial or genital lesions.

Individual evolution of RHS ranged from 2 to 15 years. Relapses occurred in periods varying from 3 weeks to 6 months. All the data were compared with those of healthy individuals ($n = 100$) in a sex- and age-matched control group examined the same way.

PMNL were separated using the sedimentation method described elsewhere (2). Chemotaxis of PMNL was tested by means of the 'in filter count' technique, by a modification of Boyden's method (1, 2). Intracellular killing activity, phagocytosis of viable and heat-inactivated *Candida albicans* (C.a.) blastospores were assessed by applying Lehrer's method (5), and the NADH-dependent oxidase activity using the nitroblue-tetrazolium test (NBT) *ad modum* Preisig & Hitzig (7).

## RESULTS

Compared with the findings in 100 normal test persons, a marked disturbance of chemotactic activity and intracellular killing of C.a. blastospores was observed in most of the cases (Fig. 1). Phagocytic capacity and the NADH-dependent oxidase activity of PMNL appeared to be regular (Fig. 2.).