ing experiment was set up: together with the mitogen, dibutyryl cyclic AMP (db cAMP) was added to the incubation medium in a concentration which is able to inhibit thymocyte transformation[6]. In a separate experiment, it was found that db cAMP, at the concentration used (1×10^{-5} *M* 1 ml^{-1} of medium), decreased the incorporation of ^{3}H-thymidine to 9% of the control (Con A without db cAMP), which corresponds to the value for the non-stimulated thymocyte population. The results obtained in these experiments (Figure 2) indicate that inhibition of the cell activation does not change the proportion of rosette-forming thymocytes. Therefore we can conclude that rosette formation by mouse thymocytes after incubation with mitogens is not dependent on the changes in the cell membrane linked to cell activation, but on the character of the cell surface at the moment when the cell makes contact with a mitogen, and on the presence of the mitogen bound to the cell surface. Since this conclusion contradicts that of POLITIS et al.[7], we examined the effect on rosette formation of specific inhibitors[8] of the binding of mitogen to the cell surface. We found (Figure 1) that washing with these inhibitors thymocytes previously exposed to a mitogen caused a significant decrease in the number of rosette-forming thymocytes, which showed that our conclusion was correct.

The difference between the mouse thymocytes in ability to form rosettes could, however, be connected with a different distribution of the surface receptors for PHA and Con A because all the thymocytes bind these mitogens[9]. Perhaps the different distribution of the surface receptors is also responsible for the difference among thymocytes in responsiveness to the mitogens[10,11].

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Lack of correlation between structural features and function of synthetic agents tested for leukocyte chemotaxis[1]

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Summary. A number of proteins, poly-L-amino acids, oligopeptides and lipids were tested for neutrophil, eosinophil and macrophage chemotactic activity. One myoglobin preparation was active. Based on the negative findings for all other substances, primary structure, secondary structure, degree of hydrophobicity, size and charge of a molecule, could be ruled out as structural features recognized by chemotactically responding phagocytes.

Numerous leukocyte chemotactic factors have been described recently[3] but it nonetheless remains unclear how phagocytes are able to discriminate chemotactic agents and by what mechanisms they respond to a vectorial signal. Phagocytes could carry substrate-specific receptors for chemotactic recognition or they could recognize various factors by a basic common perception mechanism. The great diversity of cytotaxins has thus far precluded establishment of the characteristics which specify a molecule as a leukocyte attractant.

In the present work, this problem was explored by using an array of compounds of known structure for evaluating their chemotactic activity in vitro. Agents were selected on the basis of criteria which might contribute to chemotactic recognition:

a) specific amino-acid forming the N-terminal or C-terminal end of an active peptide, b) defined primary structure (sequence) forming the active center of a cytotaxin, c) defined secondary structural conformation (e.g. random coil[4], d) the overall charge of a molecule as expressed by its isoelectric point[5], e) the degree of hydrophobicity of a molecule[3,6].

Material and methods. Most of the 28 compounds tested were synthetic di-, tri- and poly-L-aminoacids and lipids obtained from Sigma, St. Louis, Mo., USA. Myoglobin was obtained from Sigma (M 1882) and Serva, Heidelberg, BRD (29895). Test materials were dissolved in Gey's balanced salt solution to which 0.1% ethanol and 0.01% $NaHCO_3$ were added for dissolving lipids.

Test cells were rabbit neutrophils, guinea-pig neutrophils, rabbit macrophages and guinea-pig eosinophils. Tests on lipids were made only with rabbit neutrophils. Preparation of cell suspensions, chemotaxis chambers and other details of methodology were as described previously[7]. Positive controls were immune complex-activated heated (30 min, 56 °C) rabbit serum (5% v/v) and casein (1% w/v) for rabbit neutrophils, casein (1–0.1%) for rabbit macrophages, dextran-activated heated pig serum (10 and 5%) for guinea-pig eosinophils and the latter as well as casein (1% and 0.5%) for guinea-pig neutrophils.

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Only experiments with 100–500 granulocytes per field or 25–150 macrophages respectively and a weak background (0–10 granulocytes, 0–5 macrophages) have been considered. Each substance was tested at least twice in duplicate.

Results and discussion. As a first approach, proteins of known structure and available at a high degree of purity were tested. It soon became evident that agents obtained from different sources, although highly purified, varied considerably in the activity displayed. This is documented with the example of myoglobin in the figure. Myoglobin (Serva) was, with the exception of the highest concentration tested, devoid of chemotactic activity whereas the same agent obtained from Sigma was reproducibly as active as strong positive controls and showed a dose response curve typical for chemotactic factors. No difference between the 2 products was detected by differential spectrophotometry. Presumably, this activity is attributable to contamination or to partial denaturation.

Since the degree of purity of commercially available proteins is not sufficient for studying the relation between structure and chemotactic activity, synthetic peptides of known structures were tested: Polymerized L-aspartic acid, L-glutamic, L-histidine, L-lysine, sarcosine, L-tyrosine, glycine, L-valine, L-tryptophane, L-alanine, L-serine and DL-phenylalanine. More over the dipeptides glycyl-L-hydroxyproline, glycyl-L-proline and the tripeptides glycyl-L-prolyl-L-alanine, glycyl-L-prolyl-L-hydroxyproline and L-prolyl-glycyl-glycine were evaluated. Also several lipids such as palmityl-palmitate ester, D-panthotenic acid, lysolecithine and cerasine were investigated. These agents were tested over a wide range of concentration (10^{-3} to 10^{-10} M in most instances).

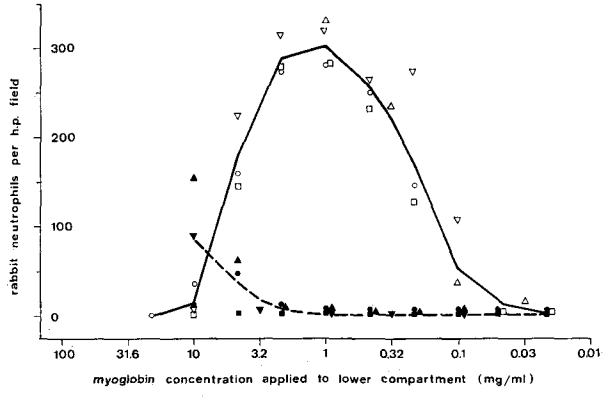
All these substances were all devoid of chemotactic activity. With the exception of poly-L-lysine up to a concentration of 10^{-4} M, no toxic effect was noted. It was therefore concluded, that none of the criteria listed serve as chemotaxis recognition signals.

These studies refute an earlier proposal of Wilkinson[4] that neutrophils recognize random coil structures since peptides with this conformation (e.g. poly-lysine and poly-glutamic acid) are inactive. Wilkinson and McKay[6] have come to the same conclusion. Other secondary structures such as helical conformation or beta-sheat structure are equally ruled out as leukotactic signals by the present data.

Frimmer and co-workers[5,8] tried to correlate the extent of the basic character of thymus-derived basic histones with their chemotactic potency. In this study, even strongly basic peptides such as poly-L-arginine and poly-L-lysine were inactive. Moreover, recognition of a specific N-terminal or C-terminal amino acid can be excluded, since poly-amino acids found to be inactive for phagocytes include those which carry the same amino acids in the N-terminal or C-terminal position as known peptidic cytotaxins (e.g. arginine in N-terminal position of hog C5a, serine in human C3a, lysine in the C-terminal position of hog and human anaphylatoxin II and arginine in human C3a[9–11]).

It is known that low molecular weight split products of collagen are chemotactic[12]. Due to the peculiarity of its structure, it was of interest to test oligopeptides of the amino acids glycine, proline, hydroxyproline for their chemotactic activity. The oligopeptides which have been tested over a broad concentration range failed to exert any chemotactic activity for the cells tested. These results suggest that properties other than a defined primary structure render collagen split products chemotactic. The fact that inactive molecules can acquire chemotactic properties without change of the primary structure[3,6,13,14] corroborates this view. It is noteworthy, that in a recent study[15] some oligopeptides were found to be chemotactic and chemokinetic depending on the constituent amino acids and their position in a di- or tripeptide. However, it remains unclear whether other cytotaxins are recognized through the same receptors.

Wilkinson and McKay[3,6] suggested that the number of hydrophobic groups exposed at the outside of a protein molecule is the parameter to which chemotactic properties are attributable. Although unfolding of an inactive precursor molecule could be related to the acquisition of chemotactic properties in many cases[12,14,16–18] and an increase in hydrophobicity normally parallels such an unfolding process, it seems questionable whether hydrophobicity is directly related to chemotactic recognition. Peptides with non-polar residues only, such as poly-L-tyrosine, poly-L-valine, poly-DL-phenyl-alanine proved to be chemotactically inactive. In addition, a few randomly chosen lipids were also inactive for neutrophils.



Neutrophil chemotactic activity of 2 commercially available myoglobin preparations. Whereas myoglobin obtained from Sigma (—○, □, △, ▽) is strongly chemotactic, a similar preparation from Serva, Heidelberg (---● ■, ▲, ▼), is only weakly chemotactic in the highest concentration. Identical signs refer to the same individual experiment. Similar results were obtained with human monocytes and rabbit macrophages as test cells and with other Sigma myoglobin preparations.

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This, however, does not imply that all lipids are devoid of chemotactic activity, as Wilkinson and colleagues reported very recently on lipids with weak chemoattractive properties in low doses[19].

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The present study has shown that the basis of leukotactic recognition remains unclear. It is evident that leukocytes do not recognize chemotactic peptides by their primary structure, a specific amino-acid at the N- or C-terminal end, by a defined secondary structure or by its basic or hydrophobic character.

Thermal effects from degranulation of mastcells in cutaneous mastocytosis

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Summary. Small biopsies of the skin were taken from patients with papulo-cutaneous mastocytosis. The mast cell tumours were then degranulated with compound 48/80 (250 µg/ml in saline), and with a Sorption microcalorimeter, relatively strong exothermic reactions were measured, whereas normal skin showed only $^1/_{10}$th the intensity. Di-sodium chromoglicate (1%) had no inhibitory effect on this thermal reaction.

The aim of this investigation was to try to measure thermal effects from the degranulation of mastcells in cutaneous mastocytoma, using microcalorimeters which can determine very small heat quantities (50×10^{-6} to 500×10^{-3} calories) and follow the heat changes during long periods of time.

Urticaria pigmentosa tumours contain accumulations of mastcells, which can easily be degranulated by compound 48/80. The mastcell granulae contain a number of biologically active substances, which could be supposed to give rise to thermal effects when they are released by degranulation and allowed to act on the surrounding tissue. As degranulation of mastcells is a central process in allergic reactions, for example in the skin, it might be of great importance if it is possible to measure thermal effects from the mastcell degranulation, as in vitro tests of allergic reactions on skin biopsies might be an application.

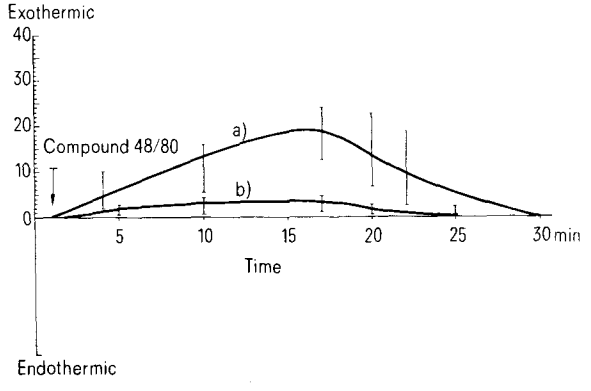
In order to study the thermal effects from degranulation of mastcells in urticaria pigmentosa tumours of the skin, we took peasized biopsies from patients suffering from papulocutaneous mastocytosis. The diagnosis was verified histopathologically. Control biopsies comprizing epidermis, corium and subcutis of the same size and weight as those from the mastcell tumours, were taken from healthy persons or from healthy skin in the patients with mastocytoma. The pieces were at once put in saline. For degranulation of the mastcells we used compound 48/80 in the concentration of 250 µg/ml saline. We also investigated if it was possible to inhibit compound 48/80 with di-sodium chromoglicate (DSCG) solution (1%).

The LKB Sorption Microcalorimeter was used and the tissue pieces were within 2–3 h placed in the microcolumn and saline and compound 48/80 solution continuously pumped in a downward flow through the cell with a flow rate of 20 ml/h. We also used a Batch Microcalorimeter for some trials.

The results of the Sorption Microcalorimetry measurements are shown in the Figure. When the mastcells tumour from 6 different patients was exposed to compound 48/80, relatively strong exothermic reaction was achieved. The reaction gradually returned to the baseline after 25 min. When healthy skin from the same patients was exposed to compound 48/80 there was a slight exothermic reaction lasting for about 20 min. Histopathological investigations of the mastcells tumours before and after the tests showed that they were totally degranulated by exposure to compound 48/80 in the microcolumn. Investigations on the dilution effect when 0.25% 48/80 in saline solution was mixed with saline, showed that no dilution effects occurred in the Sorption Microcalorimeter.

Pre-exposition of the tissue with the inhibition disodiumcromoglicate 1% in saline did not, in these trials, inhibit the exothermic reaction elicited by compound 48/80 solution on mastocytoma as might be expected with 48/80 as degranulating substance.

Our results thus show, that exothermic effects can be measured from skin mastcells tumours when they are degranulated. Also normal skin, in which mastcells were exposed to 48/80, produced heat which was about $^1/_{10}$ th of the intensity from that of the mastcell tumour degranulation (Figure). Exothermic reactions have been reported using the Batch Microcalorimeter when histamine is released from rat peritoneal mastcells sensitized with immunglobulin E[1]. The dilution effects of 48/80 in the Batch Microcalorimeter are however so strong that they mask other thermal effects and can easily be mistaken for



Sorption Microcalorimeter thermogram from 6 mastcell tumours (a), and 6 healthy skin pieces (b) exposed to compound 48/80 0.25% in saline. Temperature 37 °C. Flow rate 20 ml/min – downward flow. Equilibrium time: 6 h. Median and range of the values are indicated in the curves.

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