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].

19 P. C. Wilkinson, personal communication.

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

L. Hellgren and K. Larsson

Department of Dermatology, Sahlgrenska Sjukhuset, S-413 45 Göteborg (Sweden); and Lipid Chemistry Laboratory, University of Göteborg (Sweden), 26 November 1975

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.

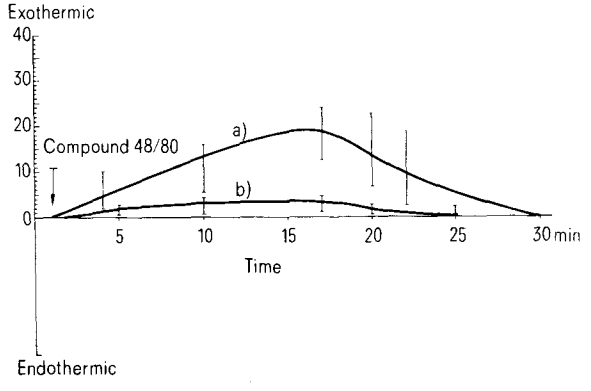
In order to study the thermal effects from degranulation of mastcells in urticaria pigmentosa tumours of the skin, we took peasized biopsies from patients suffering from papulocutaneous mastocytosis. The diagnosis was verified histopathologically. Control biopsies comprizing epidermis, corium and subcutis 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.

[1] Bruges, 1972, quoted by R. M. Sitdall. Conference on Techniques of Microcalorimetric Investigations on Cellular Systems with Special Reference to the Clinical Field. Chemical Center, Lund University, Lund, Sweden, July 9–11, 1973.

a thermal effect from the tissue[2]. It is possible that the histamine released from the mastcells granula might be responsible for the thermal effects measured. Histamine content per gram tissue in solitary mastocytoma has been reported to vary between 0.95-2 mg, whereas 0.08 mg is normal[3,4]. In the Batch Microcalorimeter we have achieved small heat effects when healthy skin biopsies are exposed to compound 48/80 and histamine, and the Warburg studies that we have run parallel indicates that there is an increased metabolism in the tissue caused by these potent substances[5]. Heparin is also bound in the mastcells granulae[6] maybe in relatively weak bound with the amines, for example histamine. Also other of the many substances in mastcells granula may, of course, be responsible for the thermic effects and in further studies we intend to investigate this.

In many allergic conditions affecting the skin, i.e. urticaria, degranulation of the tissue mastcells and the blood basophilics are central processes in the pathogenesis, and this indicates that the present finding that thermic effects can be measured by Sorption microcalorimetry when mastcells in mastocytoma and normal skin are degranulated by compound 48/80, might be of clinical importance in the future.

[2] L. HELLGREN and K. LARSSON, Techniques of Microcalorimetric Investigations on Cellular Systems with Special Reference to the Clinical Field. Chemical Center, Lund University, Lund, Sweden, July 9–11, 1973.
[3] G. B. EST and J. F. RILEY, J. Physiol., Lond. *119*, 44 (1952).
[4] H. ZACHARIAE, Acta derm.-vener., Stockh. *43*, 125 (1963).
[5] L. HELLGREN and J. VINCENT, Personal communication (1975).
[6] J. E. JORPES, H. HOLMGREN and O. WILANDER, Z. mikrosk.-anat. Forsch. *42*, 279 (1937).

Effect of sectioning the prothoracic gland nerves in the larva of the lemon-butterfly, Papilio demoleus L.

K. P. Srivastava, R. K. Tiwari and P. Kumar[1]

Department of Zoology, Banaras Hindu University, Varanasi – 221 005 (India), 4 May 1976

Summary. Sectioning the nerves innervating the prothoracic glands of the larva of the lemon-butterfly has no effect on the development and metamorphosis of the insect. The results are discussed in the light of the known facts.

Introduction. Innervation of the prothoracic glands is known in at least five insect orders[2]. While the function of this innervation has been variously suggested by different authors[2–7], no attempt was made to get a direct clue by denervating the glands themselves. In this paper we present a preliminary report on such an experiment carried out in the larva of the lemon-butterfly, Papilio demoleus.

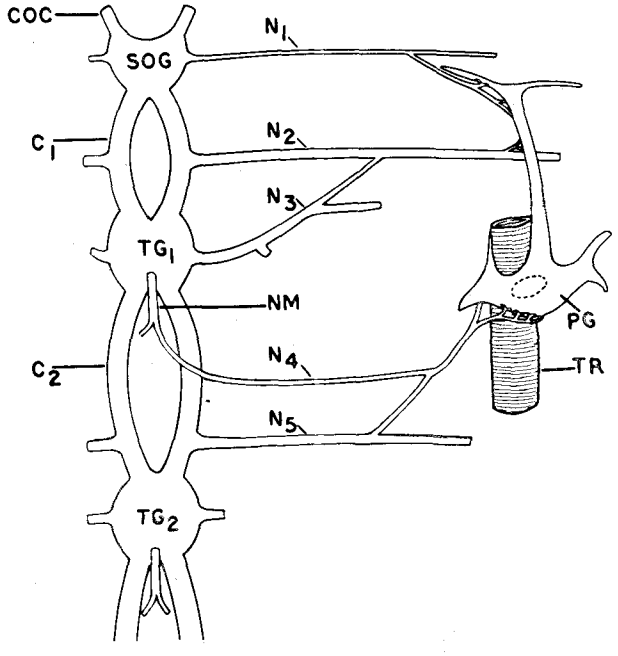


Diagram showing the innervation of the prothoracic gland in the larva of Papilio demoleus. C1, C2, first and second interganglionic connectives; COC, circumoesophageal connective; N1–N5, nerves innervating the different parts of the gland; NM, median nerve; PG, prothoracic gland; SOG, suboesophageal ganglion; TG1, TG2, first and second thoracic ganglia; TR, trachea.

Materials and methods. Young (1-day-old) fifth (ultimate) instar larvae of the lemon-butterfly were water-narcotised[8] and fixed in position with their ventral surface up between 2 crossed pins in a wax dissecting dish. All the nerves (N1 through N5, see below) innervating each gland were sectioned with a pair of microscissors on either side of the ventral nerve cord through incisions made in the intersegmental membranes. An antibiotic-phenyl-thiourea (1:1 by weight) mixture was applied on the wounds and the insects were cooled at 5 °C in a refrigerator for 4–5 h to reduce bleeding[9]. The insects were then transferred to room temperature and kept starved for 24 h to minimize bleeding due to feeding movements. The controls were treated in a similar manner but their nerves were only slightly pulled and not severed.

Results. There are 5 nerves designated as N1 through N5 that contribute to the innervation of each prothoracic gland in this insect (figure). The N1 arises from the suboesophageal ganglion and sends a small branch to innervate the terminal part of the gland. The N2 arising from the first interganglionic connective (C1) lying between the suboesophageal and prothoracic ganglia joins the N3 from the prothoracic ganglion to form a common nerve that sends a branch to innervate the middle part of the gland. Likeweise, the N4 arising from the median nerve of the prothoracic ganglion joins the N5 from the second interganglionic connective (C2) lying between the pro-

1 We wish to acknowledge the financial support from the Atomic Energy Commission, Bombay, and the working facilities provided by Prof. M. S. Kanungo, Head of the Department.
2 W. S. Herman and L. I. Gilbert, Gen. Comp. Endocrin. *7*, 275 (1966).
3 B. Possompes, Arch. Zool. exp. gen. *89*, 203 (1953).
4 K. P. Srivastava and H. H. Singh, Experientia *24*, 838 (1968).
5 Ch. Hintze-Podufal, Experientia *26*, 1269 (1970).
6 N. J. Alexander, J. Insect. Physiol. *16*, 271 (1970).
7 T. H. Hsiao and C. Hsiao, 14th Int. Cong. Ent. Abstr. 176 (1972).
8 K. Sláma, J. Insect Physiol. *10*, 283 (1964).