

Histamine release in skin monitored with the microdialysis technique does not correlate with the weal size induced by cow allergen

L.HORSMANHEIMO, I.T.HARVIMA, R.J.HARVIMA, J.YLÖNEN,* A.NAUKKARINEN†
AND M.HORSMANHEIMO

Departments of Dermatology, *Clinical Microbiology and †Clinical Pathology, Kuopio University Hospital, University of Kuopio, Clinical Research Unit, PO Box 1627, 70211, Kuopio, Finland

Accepted for publication 17 March 1995

Summary

The purpose of this study was to monitor histamine release in immediate-type hypersensitivity reactions in the skin of 10 atopic patients, sensitive to cow, by using the microdialysis technique. Three healthy subjects, without any atopic features or background, served as the control group. The probe inserted into the forearm dermal skin was perfused with isotonic saline solution. Samples were collected at 15-min intervals. After the first allergen challenge of four prick tests close to the probe with cow allergen extract, the skin was similarly repricked again in five patients and three healthy subjects, and in five other patients, 25 µl of 10 µmol/l substance P (SP) was injected intracutaneously. The samples were analysed for histamine by radioenzyme assay. The patients were clinically evaluated for allergic symptoms, prick- and scratch-patch test reactivity and for serum cow-specific, and total, IgE levels.

The baseline histamine concentration was 7.5 ± 4.0 nmol/l (mean $\pm$ standard deviation: SD; $n = 10$). After the allergen challenge, the histamine concentration in the consecutive samples was 11.9 ± 11.0 nmol/l, 91.1 ± 127.3 nmol/l, 61.0 ± 94.2 nmol/l and 33.7 ± 53.7 nmol/l. The peak concentration was detected in the 15–30 min fraction, and it varied between 0 and 406 nmol/l regardless of the weal size. The second allergen challenge was unable to induce marked additional histamine release, but SP induced extensive histamine liberation in those patients who did not exhibit histamine release during the preceding prick tests. In three healthy subjects, the baseline histamine concentration was 6.2 ± 3.9 nmol/l. After the allergen challenge, no additional histamine liberation could be measured.

Surprisingly, the histamine release was not related to the size of the cow-induced weal nor was it related to any specific allergic symptoms or IgE levels. The results suggest that, in some patients, mast cell mediators other than histamine play a significant part in immediate-type allergic reactions of skin.

The microdialysis technique has been used for many years in experimental animal models to study metabolic events in brain tissue.¹ Lately, it has been applied also to human subcutaneous adipose tissue² and skin to measure interstitial levels of glucose³ and percutaneous ethanol absorption.⁴ Recently, studies on histamine release during skin challenge have also been performed.^{5–7}

It is well established that mast cells play a critical part in immediate-type hypersensitivity reactions. Vasoactive and chemotactic mediators released from activated mast cells in skin are involved in the pathophysiology of urticaria and probably also in atopic dermatitis.

Oedema, erythema and pruritus, which are typical symptoms of cutaneous hypersensitivity reactions, result from the rapid action of mediators released from mast cells. Histamine released by cutaneous mast cells is considered to be the main mediator in the evolution of the weal and flare in skin prick tests. However, other mediators released by mast cells, such as prostaglandin D₂ (PGD₂)⁸ and leukotriene C₄ (LTC₄),⁹ can also have a significant impact on these reactions. The local intracutaneous injections of LTC₄ and PGD₂ into human skin can result in long-lasting weal and flare responses.^{10,11}

The *in vivo* measurement of inflammatory mediators has been restricted to suction blister and skin chamber fluid models.^{12–16} However, the removal of the overlying epidermis may interfere with allergic reactions as keratinocytes have been recognized as being active mediator-releasing cells.¹⁷ Kinetic measurements of histamine studied in the skin chamber fluid models have revealed maximal concentrations of histamine during the first 30 min after allergen challenge.¹³ This has also been confirmed by the microdialysis method. However, only a few subjects were studied, and the total histamine released was found to be similar between the subjects.⁷ In skin chamber and blister fluid methods, the correlation between histamine release, weal formation, and skin sensitivity to allergens, has been variable, and no definite conclusion can be drawn.^{14–16} Studies comparing the prick-test size with serum-specific IgE levels are also available. With animal danders, a moderate relationship between the allergen/histamine weal ratio and serum IgE has been found, but a poor correlation existed between the mean weal diameter and serum IgE.¹⁸

In the present study, we have used the microdialysis technique to collect histamine released in skin prick-test reactions induced by cow allergen extract, and have evaluated the relationship between clinical weal formation and histamine release.

Materials and methods

Patients

Ten atopic patients (five male, five female, age 28–60 years, mean age 44.8 years), with a previously documented type I allergy (positive prick tests) to cow allergen, were included in this study. All 10 patients had

allergic rhinitis, five patients had occasional urticaria, six patients had atopic eczema and, of these, three had atopic eczema and urticaria. The patients with occasional urticaria did not recall having had any recent outbreaks of urticaria prior to investigations. Two patients had occupational cow-induced asthma (Table 1). Three healthy subjects, without any atopic features or background, served as the control group. All the subjects studied did not show dermatographism. The patients had not received any systemic anti-histamine or anti-inflammatory medication or local treatment for at least 2 weeks before entering the study. The methods used in this study were approved by the Ethics Committee of Kuopio University Hospital, Kuopio, Finland.

Skin tests

Skin prick tests were performed on the medial aspect of the forearm with a diluent series of crude cow dander extract 1:1 (10 mg/ml), 1:10 and 1:100 prepared, as described earlier,¹⁹ together with a commercial ALK cow antigen, histamine (10 mg/ml) acting as a positive control, and with a negative control (Soluprick, ALK Laboratories, Hørsholm, Denmark). At the same time microdialysis experiments were performed on the other forearm. The longest and shortest dimensions of the weal were measured at 15 min when the weal and flare reactions were maximal and the area was calculated by using the ellipse formula. The weal and flare reactions after prick tests disappeared within 1 h. Skin scratch-patch tests²⁰ were also performed with the same cow allergen dilutions and cow hair applied on to the lateral aspect of the arm. Two of the patients had positive scratch-patch reactions. Total serum IgE levels (U/ml)

Table 1. The basic clinical details on the 10 atopic patients allergic to cow undergoing microdialysis

	Sex/age	Urticaria	Eczema	Rhinitis	Asthma	Specific serum IgE (U/ml)	Total serum IgE (U/ml)
1	M/28	+	+	+	+	3.06	125
2	M/46	–	–	+	–	5.27	118
3	M/60	+	–	+	–	<0.34	25
4	F/47	–	+	+	–	98.24	361
5	M/58	–	+	+	–	2.71	145
6	F/41	+	–	+	–	1.02	88
7	F/57	+	+	+	–	1.24	26
8	F/42	–	–	+	–	16.28	176
9	F/54	+	+	+	+	5.84	26
10	F/42	–	+	+	–	<0.34	ND

ND: not determined.

and specific IgE for cow (U/ml) were measured by radioimmunoassay (RIA) (Pharmacia IgE RIA and Pharmacia CAP System RAST RIA, Kabi Pharmacia Diagnostics AB, Uppsala, Sweden).

Microdialysis equipment

The basic equipment used in microdialysis, which included a pump (CMA/100), a probe (CMA/20) and a fraction collector (CMA/140), was purchased from CMA Microdialysis (Stockholm, Sweden).

Isotonic saline solution (Ringersteril[®], Orion Corporation, Medipolar, Oulu, Finland) was perfused through the system by a microinjection pump at a perfusion rate of 3.3 µl/min. In this study, a probe with a shaft length of 25 mm and a membrane length of 10 mm was selected. The diameter of the membranous part of the probe was 0.5 mm. The semipermeable polycarbonate membrane allows passive diffusion of small molecular size substances up to 20 kDa from the extracellular space into the perfusing isotonic saline solution without removing liquid from the tissue. Once a molecule crosses the dialysis membrane, no enzymatic degradation or protein binding occurs, which is one of the advantages of this technique. Plastic tubing carries the perfusate to the microfraction collector and, in this study, 49.5 µl samples were collected at 15-min intervals. The samples were stored at -20°C prior to histamine analysis.

Relative recovery

The concentration of histamine in the microdialysis perfusate vs. the concentration of histamine outside the dialysis membrane of the probe is called the relative recovery, which is expressed as a percentage. In this study, the relative recovery was determined to be 35% under experimental conditions using 20 and 200 nmol/l histamine standards. According to previous experiments by Anderson *et al.*, the probe depth in the skin measured with the ultrasound device Dermascan A does not influence the histamine levels.⁶

Sterilization of the probes

As the microdialysis probes are relatively expensive, a sterilization procedure was developed. The probes were perfused with and soaked in 80% ethanol overnight followed by soaking in 1.3% hypochlorite containing 0.2% NaOH (17% Erikloori[®], Erisan Hospital Hygiene, Orion, Helsinki, Finland) for 1 h. No effect on the relative

recovery of histamine could be observed when this procedure was repeated three times.

Skin challenge during microdialysis

All the skin challenges were performed in the same windowless examination room with an even room temperature and humidity. The probe was inserted into the dermis of the lateral forearm, using a plastic guiding cannule together with a needle as described by the manufacturer, as superficially as possible so that it made a clear eminence in the skin. Also, on some patients, the location of the probe in the dermis was confirmed with the Dermascan A ultrasound device. Previous reports by Anderson *et al.*⁶ have shown, in eight patients, that the mean histamine levels in the first 10-min sample following probe insertion is 39.4 nmol/l, because the trauma of the probe insertion causes release of histamine. The mean histamine value fell down with successive 10-min samples (8.8, 4.6, 2.3 nmol/l). An equilibration period of 40 min following probe insertion is suggested for histamine studies, where allergen provocation of the skin is to be performed.⁶ One hour after probe insertion, the baseline sample was collected for 15 min. Seventy-five minutes after probe insertion, the skin was challenged. The skin was pricked four times, 3 mm from the probe, two on each side, with a standard lancet (ALK Laboratories, Hørsholm, Denmark) through a drop of 10 mg/ml extract prepared from cow dander.¹⁹ This was shown to be a fully saturated allergen concentration giving maximal weal. Four prick stings as close to the probe as possible were chosen, instead of one or two, to ensure an even histamine release around the probe. The skin reactivity to prick tests around the microdialysis probe was the same as on the contralateral forearm. Samples were collected for 1 h at 15-min intervals. Thereafter, for five patients and three healthy controls, the second set of four prick tests were performed at the same locations as described above with the cow extract. For other five patients, 25 µl of 10 µmol/l substance P (SP) (Cambridge Research Biochemicals Ltd, Cambridge, U.K.), a neuropeptide which is known to rapidly release histamine from skin mast cells,²¹ was injected intracutaneously adjacent to the probe followed by sampling for an hour.

To clarify the reliability of the microdialysis method, two probes were inserted into the dermis of the lateral forearm at least 3 cm apart from each other in three patients. Skin prick tests were performed around both probes as described above with cow allergen¹⁹ in two patients and with commercial ALK cat antigen

Table 2. Allergen-induced histamine release in skin of three individuals undergoing microdialysis with two adjacent probes simultaneously

Subjects	Probe	Histamine released (pmol)	Allergen
1	1	22.9	Cow
	2	22.9	
2	1	1.5	Cow
	2	2.4	
3	1	11.5	Cat
	2	12.7	

Released histamine (pmol) reflects the total amount of histamine measured in four consecutive microdialysis fractions after allergen challenge.

(Soluprick, ALK Laboratories, Hørsholm, Denmark) in one patient (Table 2).

Analysis of histamine

Histamine was analysed in duplicate by using the radioenzyme assay²² with the detection limit of 0.5–1.0 nmol/l. Histamine-N-methyltransferase was purified from rat kidneys as described previously.²³ ³H-Methyl-S-adenosyl-L-methionine (15 Ci/mmol) and ACS[®] aqueous liquid scintillation counting solution were obtained from Amersham (Amersham, U.K.). K₂-EDTA was purchased from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade from Sigma (St Louis, MO, U.S.A.) or E. Merck (Darmstadt, Germany). Deionized water was used throughout this study. The radioactivity was measured using a LKB 1215 Rackbeta liquid scintillation counter with automatic quench correction (Wallac Oy, Turku, Finland).

Statistical analysis

Statistical analysis with correlation matrix table was performed by using StatView 4.0 FPU Program from Abacus Concepts, Inc. (Berkeley, CA, U.S.A.).

Results

Monitoring of histamine release

Before the challenge with cow allergen extract, the baseline histamine concentration in the first collected fraction was 7.5 ± 4.0 nmol/l (mean $\pm$ SD, $n = 10$). Following the allergen challenge, the first 0–15 min fraction revealed only 11.9 ± 11.0 nmol/l histamine (Figs 1 and 2), but a rapid increase was observed in

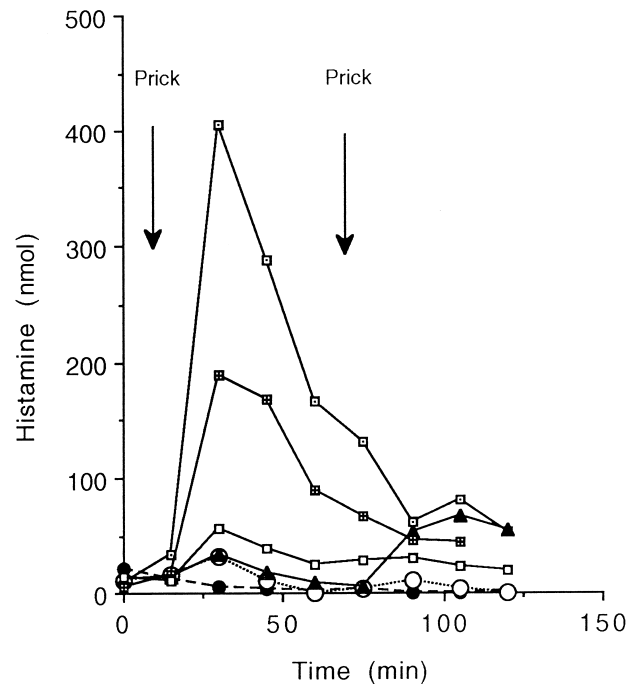


Figure 1. Histamine release in skin prick reactions was measured by the microdialysis technique in five atopic patients sensitive to cow. The skin was pricked four times with cow allergen extract (10 mg/ml) close to the microdialysis probe and samples were collected at 15-min intervals for histamine analysis. The second pricking was performed 1 h after the first challenge.

the 15–30 min fraction reaching a concentration of 91.1 ± 127.3 nmol/l. However, the individual peak values between patients ranged from 0 to 406 nmol/l (Figs 1 and 2). Thereafter, a steady decline was observed in the 30–45 min and 45–60 min fractions (61.0 ± 94.2 nmol/l and 33.7 ± 53.7 nmol/l, respectively). The first allergen challenge was sufficient to release the major portion of the histamine stores, the second allergen challenge, performed in five patients, yielded clearly lower histamine release curves, except in one patient who showed constantly higher histamine release during the second prick test (Fig. 1), a finding confirmed with two probes simultaneously (Table 2). The baseline histamine concentration in three healthy subjects was 6.2 ± 3.9 nmol/l. After the allergen challenge, no additional histamine release could be measured.

To find out whether the microdialysis probe itself was not able to capture the released histamine during mast cell activation resulting in trivial release curves, SP was injected into five patients close to the probe 60 min after the allergen challenge. As demonstrated in Figure 2, SP could rapidly liberate histamine in those patients who responded only weakly to the cow allergen extract (peak histamine value under 50 nmol/l after allergen

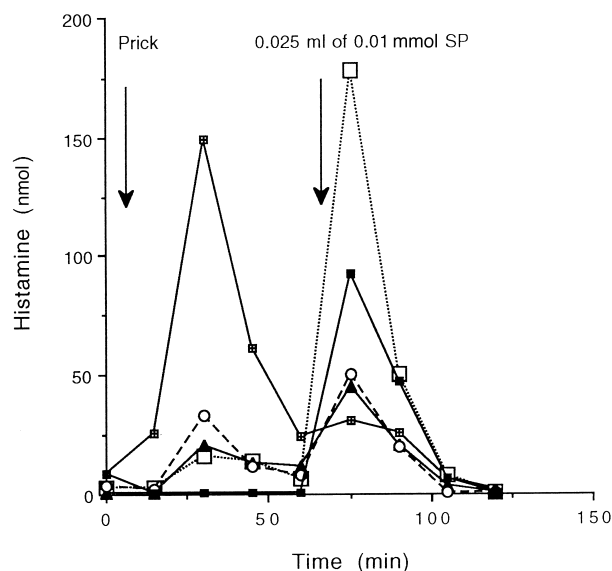


Figure 2. Histamine release in skin prick reactions was measured by the microdialysis technique in five atopic patients sensitive to cow. The skin was pricked four times with cow allergen extract (10 mg/ml) close to the microdialysis probe and samples were collected at 15 min intervals. To ensure that the microdialysis method was able to detect histamine liberation, 25 μ l of 10 mmol/l substance P was injected intracutaneously close to the probe 1 h after the allergen challenge. The samples were analysed for histamine by the radio enzyme assay.

challenge). Furthermore, the extent of histamine release by SP was related to the preceding prick test, a higher histamine release by allergen challenge was followed by a lower histamine release by SP and a lower histamine release by allergen challenge was followed by a higher histamine release by SP. This finding also rules out the possibility that the mast cells had already released substantial amounts of histamine during the implantation trauma (Fig. 2). This indicates that the probe and the methodology were technically competent to detect histamine release when it occurred.

To clarify the reliability of the microdialysis method, two probes were used at the same time in three patients (Table 2). The probes were inserted at least 3 cm apart from each other and prick tests were performed around both probes the same way as described above. Both probes gave the same histamine values, indicating that this method is reliable in measuring histamine release in skin.

Correlation between histamine release, clinical symptoms and skin tests

As the peak value of the histamine release curve does not reflect the extent of the reaction sufficiently, the total histamine, i.e. curve peak area, was calculated (Table 3) and used to describe the histamine release of each

Table 3. Histamine release *in situ* detected by microdialysis in 10 patients after cow challenge and its comparison with skin challenge results

	Sex/age	Histamine released (pmol)	Cow (10 mg/ml) weal (mm ²)	Histamine control weal (mm ²)
1	M/28	2.3	86	13
2	M/46	1.5	63	20
3	M/60	0.0	13	33
4	F/47	6.5	104	24
5	M/58	22.9	64	38
6	F/41	12.6	44	16
7	F/57	44.3	143	49
8	F/42	2.8	138	38
9	F/54	2.7	28	28
10	F/42	2.0	20	33

Released histamine (pmol) reflects the total amount of histamine measured in four consecutive microdialysis fractions after allergen challenge.

ND: not determined.

patient. This histamine release was correlated to the weal size by 10 mg/ml cow allergen extract. Furthermore, each patient was tested as to whether they show delayed-type hypersensitivity to cow and serum cow-

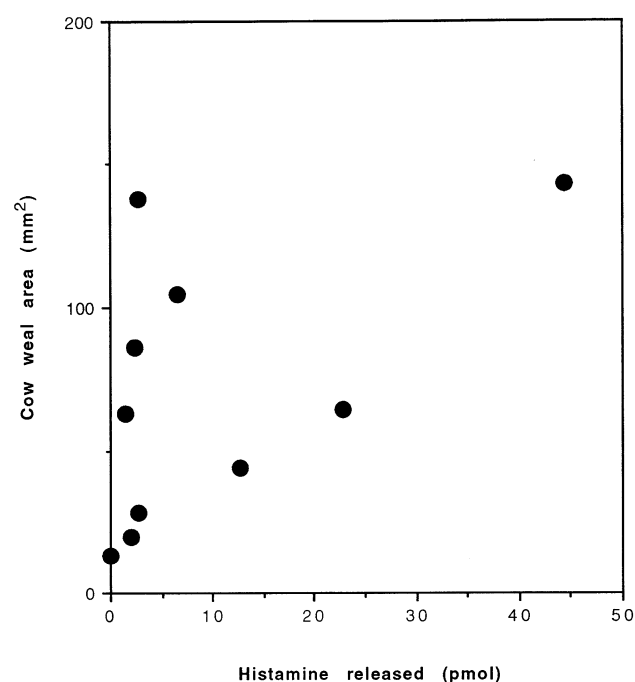


Figure 3. Correlation between the cow weal area (mm²) and histamine release by microdialysis in skin prick reactions in 10 atopic patients sensitive to cow. The histamine release reflects the total amount of histamine measured in four consecutive microdialysis fractions after allergen challenge.

specific IgE. The results have been summarized in Tables 1 and 3.

As demonstrated above, there was a marked variation in the histamine release between patients (Figs 1 and 2). The size of the weal to cow (Fig. 3) and serum IgE levels did not have any significant correlation with the total histamine release detected by microdialysis (scatter analysis). Patients were also examined for their clinical allergic symptoms, but no association between any specific symptom and total histamine release was found nor was there any correlation between scratch-patch test positivity and histamine release (Tables 1 and 3).

Discussion

It is generally assumed that most, if not all, mediators of allergic type I skin reactions are released from mast cells at the site of allergen challenge. Histamine has been thought to be one of the most important mediators of such allergic reactions. However, other mast cell-derived mediators, such as PGD_2 and LTC_4 , also participate in these processes. Previously, investigations of the *in vivo* mast cell functions at the site of allergen challenge have been mostly restricted to suction blister and skin chamber models. In these models, the removal of the overlying epidermis may interfere with these reactions, as keratinocytes can participate extensively in cutaneous inflammation.¹⁷

In this study, we measured histamine release using the microdialysis method that allows accurate kinetic measurements of mediators in extracellular fluids, released in skin prick tests in atopic subjects sensitized to cow allergen. We found a marked variation in the histamine release between patients regardless of the weal size. Several inherited or constitutional factors may be responsible for this, such as individual variation in mast cell numbers and in the sensitivity to the allergen. It may be possible that other mediators, in addition to histamine, participate in the weal and flare reaction. The subjects with low histamine release but a large weal (histamine non-releasers) could liberate large amounts of PGD_2 and LTC_4 which account for the weal.^{8–10} One further explanation could be that there are variations in the H_1 -receptor density, i.e. relatively small amounts of histamine could induce a large weal reaction. Also, the histamine non-releasers did show extensive histamine release when challenged with SP, which is a potent histamine-liberating agent. This indicates that the cutaneous mast cells do contain histamine, but it is not released in significant amounts regardless of the

extensive introduction of cow allergen into the skin in these patients. It has also been proposed that neuropeptides, like SP and vasoactive intestinal peptide, participate in weal and flare reactions so, not only mast cell-derived mediators, but also neural factors could explain, in part, the observed variations.

The microdialysis technique opens up new possibilities enabling chronological and quantitative studies of inflammatory mediators in intact human skin. In contrast to suction blister and skin window models, the epidermal–dermal interaction is preserved and enzymatic degradation of the samples is minimized. Also, the mediators are caught at the site of formation, thus avoiding dilution due to diffusion. Until now, no method has allowed *in situ* measurement of inflammatory mediators during allergic anaphylactic reactions. However, several aspects must be considered when performing microdialysis experiments. First, care must be taken to ensure that the microdialysis probe is inserted into the dermis and not the subcutis. The probe must be inserted as superficially as possible so that it makes a visible eminence in the skin. Second, the insertion trauma may affect the relative recovery if a large haematoma develops around the probe. In our experiments, no visible haematomas were seen and also the rapid release of histamine by injection of SP confirmed that the probe was reliable in measuring histamine release in skin. Also, with this method it is not possible to determine exact mediator concentrations in the skin from measurements of mediator in dialysates. For such purposes, knowledge of *in vitro* recovery of each mediators is required. In the present study, the method allows semiquantitative measurements of histamine, as the recovery of histamine was shown to be constant in a wide range of histamine concentrations (20–200 nmol/l). Occasionally, the probes may break and leak the perfusion fluid into the surrounding dermis, this can be easily noted as the sample volumes become smaller. Care must be taken that no air bubbles enter the probe, as the diffusion surface of the probe becomes smaller, thus diminishing the substance recovery.

The present results suggest that, in some patients, allergic mediators other than histamine, for example LTC_4 , PGD_2 , can account for the allergic symptoms. Further information on mediators in immediate-type allergic reactions is needed to design patient-specific treatments with new anti-allergic drugs. The microdialysis method used in this study allows accurate measurements of low molecular size substances in skin inflammatory reactions.

Acknowledgments

The authors wish to thank the Medical Council of the Academy of Finland for financial support (grant no. 1311006), and Ms Anne Koivisto for her excellent technical assistance.

References

- 1 Robinson TE, Justice JB, eds. *Microdialysis in the Neurosciences*. Amsterdam: Elsevier, 1991.
- 2 Lönnroth P, Jansson P-A, Smith U. A microdialysis method allowing characterization of intercellular water space in humans. *Am J Physiol (Endocrinol Metab)* 1987; **253**: E228–31.
- 3 Petersen LJ, Kristensen JK, Bülow J. Microdialysis of the interstitial water space in human skin *in vivo*: quantitative measurement of cutaneous glucose concentrations. *J Invest Dermatol* 1992; **99**: 357–60.
- 4 Anderson C, Andersson T, Molander M. Ethanol absorption across human skin measured by *in vivo* microdialysis technique. *Acta Derm Venereol (Stockh)* 1991; **71**: 389–93.
- 5 Anderson C, Andersson R, Andersson T, Molander M. Microdialysis: a new bioanalytical sampling technique with relevant applications in skin contact reactions. *Contact Dermatitis* 1990; **23**: 293.
- 6 Anderson C, Andersson T, Andersson RGG. *In vivo* microdialysis estimation of histamine in human skin. *Skin Pharmacol* 1992; **5**: 177–83.
- 7 Petersen LJ, Skov PS, Bindslev-Jensen C, Søndergaard J. Histamine release in immediate-type hypersensitivity reactions in intact human skin measured by microdialysis: A preliminary study. *Allergy* 1992; **47**: 635–7.
- 8 Barr RM, Koro O, Francis DM *et al.* The release of prostaglandin D₂ from human skin *in vivo* and *in vitro* during immediate allergic reactions. *Br J Pharmacol* 1988; **94**: 773–80.
- 9 Talbot SE, Atkins PC, Goetzel EJ, Zweiman B. Accumulation of leukotriene C₄ and histamine in human allergic skin reactions. *J Clin Invest* 1985; **76**: 650–6.
- 10 Soter NA, Lewis RA, Corey EJ, Austen KE. Local effects of synthetic leukotrienes (LTC₄, LTD₄, LTE₄, and LTB₄) in human skin. *J Invest Dermatol* 1983; **80**: 115–9.
- 11 Flower RJ, Harvey EA, Kingston WP. Inflammatory effects of prostaglandin D₂ in rat and human skin. *Br J Pharmacol* 1976; **56**: 229–33.
- 12 Ting S, Dunskey EH, Lavker RM, Zweiman B. Patterns of mast cell alterations and *in vivo* mediator release in human allergic skin reactions. *J Allergy Clin Immunol* 1980; **66**: 417–23.
- 13 Schwartz LB, Atkins PC, Bradford TR *et al.* Release of tryptase together with histamine during the immediate cutaneous response to allergen. *J Allergy Clin Immunol* 1987; **80**: 850–5.
- 14 Talbot SE, Atkins PC, Valenzano M, Zweiman B. Correlations of *in vivo* mediator release with late cutaneous allergic responses in humans. I. Kinetics of histamine release. *J Allergy Clin Immunol* 1984; **74**: 819–26.
- 15 Bedard P-M, Atkins PC, Zweiman B. Antigen-induced local mediator release and cellular inflammatory responses in atopic subjects. *J Allergy Clin Immunol* 1983; **71**: 394–8.
- 16 Dorsch W, Ring J, Reimann HJ, Geiger R. Mediator studies in skin blister fluid from patients with dual skin reactions after intradermal allergen injection. *J Allergy Clin Immunol* 1982; **70**: 236–42.
- 17 Barker JNWN, Mitra RS, Griffiths CEM *et al.* Keratinocytes as initiators of inflammation. *Lancet* 1991; **337**: 211–4.
- 18 Paggiaro PL, Bacci E, Amram DL *et al.* Skin reactivity and specific IgE levels in the evaluation of allergic sensitivity to common allergens for epidemiological purposes. *Clin Allergy* 1986; **16**: 49–55.
- 19 Ylönen J, Virtanen T, Horsmanheimo L *et al.* Affinity purification of the major bovine allergen by a novel monoclonal antibody. *J Allergy Clin Immunol* 1994; **93**: 851–8.
- 20 Van Voorst Vader PC, Lier JG, Woest TE *et al.* Patch tests with house dust mite antigens in atopic dermatitis patients: methodological problems. *Acta Derm Venereol (Stockh)* 1991; **71**: 301–5.
- 21 Lowman MA, Benyon RC, Church MK. Characterization of neuro-peptide-induced histamine release from human dispersed skin mast cells. *Br J Pharmacol* 1988; **95**: 121–30.
- 22 Harvima RJ, Harvima IT, Fräki JE. Optimization of histamine radio enzyme assay with purified histamine-N-methyltransferase. *Clin Chim Acta* 1988; **171**: 247–56.
- 23 Harvima RJ, Kajander EO, Harvima IT, Fräki JE. Purification and partial characterization of rat kidney histamine-N-methyl-transferase. *Biochim Biophys Acta* 1985; **841**: 42–9.