

Cutaneous histamine metabolism in chronic urticaria

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Impaired metabolism of histamine in the skin of patients with chronic idiopathic urticaria (CIU) might explain the observed enhanced¹ and prolonged² skin responses to intradermal histamine. Histamine metabolism was measured in homogenates from unaffected forearm skin in nine patients with CIU and in skin of age- and sex-matched control subjects with a radiochromatographic assay, and the results are expressed as nanograms of histamine metabolized per milligram of protein per hour. Endogenous histamine content was determined by RIA. There was a highly significant increase in endogenous histamine content in the skin of patients with urticaria (407.8 ± 188.3 ng/mg of protein) compared with that in skin of control subjects (240.0 ± 73.0 ng/mg of protein) (mean $\pm$ 1 SD; $p < 0.02$), which suggests either an increase in mast cell number or histamine concentration per cell. No significant difference was observed in the metabolism of histamine between patient and control group; therefore, an alternative mechanism may underlie differences in skin reactivity to histamine. (J ALLERGY CLIN IMMUNOL 1992;89:944-50.)

Key words: Skin, urticaria, histamine, metabolism

More than 70% of patients with chronic urticaria who present to dermatology clinics fall into the idiopathic category in which no specific allergic, physical, or other underlying cause is evident.³ Histamine is believed to be one of the major mediators involved in the pathogenesis of chronic urticaria because elevated levels have been identified in both tissue extracts⁴ and skin-blister fluid⁵ overlying urticarial lesions. Furthermore, H₁ antihistamines are an effective treatment in most patients.⁶ Enhanced¹ and prolonged² wheal-and-flare reactions to both intradermal histamine and the nonspecific mast cell degranulator, codeine,⁷ have been documented in CIU, and an increased histamine level in skin-blister fluid in response to compound 48/80 has also been reported.⁸ An impaired clearance of histamine might explain these experimental findings and would be important in the pathogenesis of CIU. We have therefore measured the metabolism of histamine in skin homogenates from patients with CIU.

Abbreviations used

CIU:	Chronic idiopathic urticaria
S-AM:	S-adenosyl methionine
TLC:	Thin-layer chromatography
R _f :	Mobility of a substance relative to the solvent front
ImAA:	Imidazole acetic acid
MelmAA:	Methyl imidazole acetic acid
HMT:	Histamine methyltransferase
DAO:	Diamine oxidase

SUBJECTS AND METHODS

The study was approved by the Guy's Hospital Ethical Committee and involved only subjects who had given informed consent.

Nine patients with CIU and individually age- and sex-matched control subjects were recruited into the study. CIU was defined as the appearance of recurrent wheal and flare for at least 3 months, occurring at intervals of not less than a week. Patients with physical urticaria or urticaria clearly related to, and not occurring in the absence, of a specific food or drug were excluded. All patients had had a trial of an additive and preservative-free diet without improvement. Antihistamines were stopped for 48 hours before the day of study and, in the case of astemizole, for 6 weeks before the study day. A 4 mm punch biopsy specimen was obtained under local anesthetic without adrenaline from nonlesional skin on the inner aspect of the forearm. The sample was then snap frozen, stored at -70°C , and assayed within a week.

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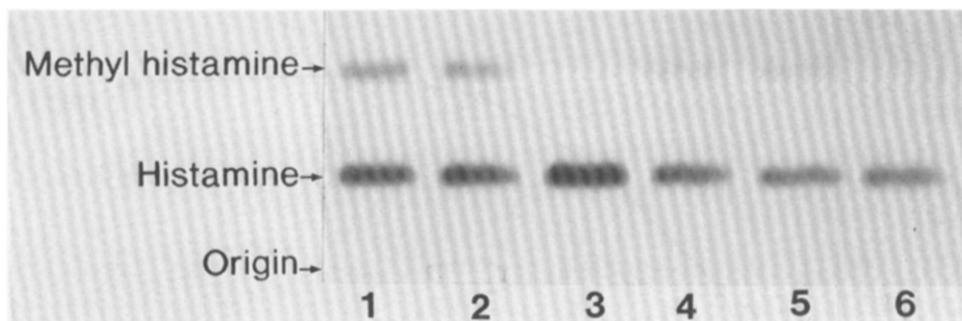


FIG. 1. Autoradiograph of TLC plate from two subjects with urticaria. Each sample was performed in duplicate; *subject 1*, lanes 1 and 2; *control sample*, lane 3; similarly, *subject 2*, lanes 4 and 5; *control sample*, lane 6.

Preparation of skin homogenate

Samples were homogenized at 4° C with a Polytron PT3000 homogenizer (7 mm head PT/DA 3007/2, Phillip Haris Scientific, London, England) in 0.75 ml of phosphate-buffered saline, pH 7.4, at 7000 rpm for 30 seconds, and the preparation was then spun at 3000 g at 2° C for 15 minutes (MSE Mistral 3000i, Fisons Scientific Equipment, Loughborough, Leicestershire, England). The resulting supernatants were aspirated, 50 µl aliquots were stored at -70° C for later histamine and protein estimation, and the remaining volume was used immediately for measuring histamine metabolism.

Endogenous histamine was measured in patient and control samples with an RIA kit (Serotec, Oxford, England). Samples were assayed in duplicate.

Protein content was determined according to the Coomassie blue dye method,⁹ and samples were assayed in triplicate.

Assay for histamine metabolism

The method according to Francis et al.¹⁰ was modified as follows: [³H]Histamine dichloride (25 to 50 Ci/mmol) (Amersham International plc, Amersham, Buckinghamshire, England) was added to skin homogenate and incubated with the methyl donor, S-AM (Sigma Chemical Co., Dorset, Poole, England). Metabolites formed were separated and quantified by TLC, as described below. Preliminary work was performed with skin from mastectomy samples to determine the time course of the reaction (*n* = 2) and the optimum concentrations of S-AM (*n* = 3) and [³H]histamine (*n* = 3). Each incubation mixture contained [³H]histamine dichloride, 2 pmol, 0.1 µCi S-AM, and 0.02 pmol and 0.2 ml of skin supernatant in a total volume of 0.24 ml. The samples were incubated at 37° C for 1 hour, cooled rapidly in ice, and were then freeze-dried. Negative controls were processed in the same manner except S-AM was omitted. Each skin sample was assayed in duplicate.

TLC

Reference solutions of 1-methyl histamine, histamine dichloride, MelmAA and ImAA (Sigma Chemical Co.) were made up at 2 mg/ml concentrations. Histamine me-

tabolites in each freeze-dried sample were extracted with 0.8 ml of methanol. Samples were left at room temperature for 2 hours before they were centrifuged at 3325 g for 10 minutes. Supernatants and standards (40 µl) were loaded onto plastic-backed, silica gel TLC sheets (Phase Separations, Ltd., Deeside, Clywd, U.K.), which were chromatographed in chloroform-methanol-ammonia (35% NH₃), 70:29:4.3, vol/vol, respectively (Analar grade, Merck Ltd., Dagenham, Essex, England). The section of sheet containing standards was cut and developed in iodine, and *R_f* values of each standard were determined. The remaining plate was coated with ENHANCE spray (Dupont UK Ltd., Stevenage, Herts, England), dried, mounted against an x-ray film (Fuji Photofilm Ltd., London, England), and exposed for 24 hours at -70° C. The film was developed and used to precisely locate sites of radioactive metabolites on the TLC plate. Each radioactive spot was cut out, placed in a scintillation vial with 2 ml of methanol, and left to stand at room temperature for 2 hours. Liquid scintillant Supersolve-X (10 ml) (New Brunswick Scientific, Ltd., Herts, England) was then added, and radioactivity was counted in a liquid scintillation counter (Packard 1900, Canberra Packard, Pangbourne, Berkshire, England). The amount of metabolite formed was expressed as a percentage of total recovered counts after subtraction of corresponding negative control values.

Quality control

Aliquots (0.2 ml) of breast-skin homogenate were freeze-dried and stored at -70° C. Each time an assay was performed, activity in one of these aliquots was also measured as a quality control.

Histamine metabolism was expressed as nanograms of histamine (endogenous plus exogenous) metabolized per milligram of protein per hour.

RESULTS

Optimum assay conditions

The only metabolite detected was methyl histamine that had an *R_f* value of 0.53 (Fig. 1). *R_f* values for histamine and ImAA were 0.29 and 0.04, respectively; MelmAA did not reliably separate from ImAA.

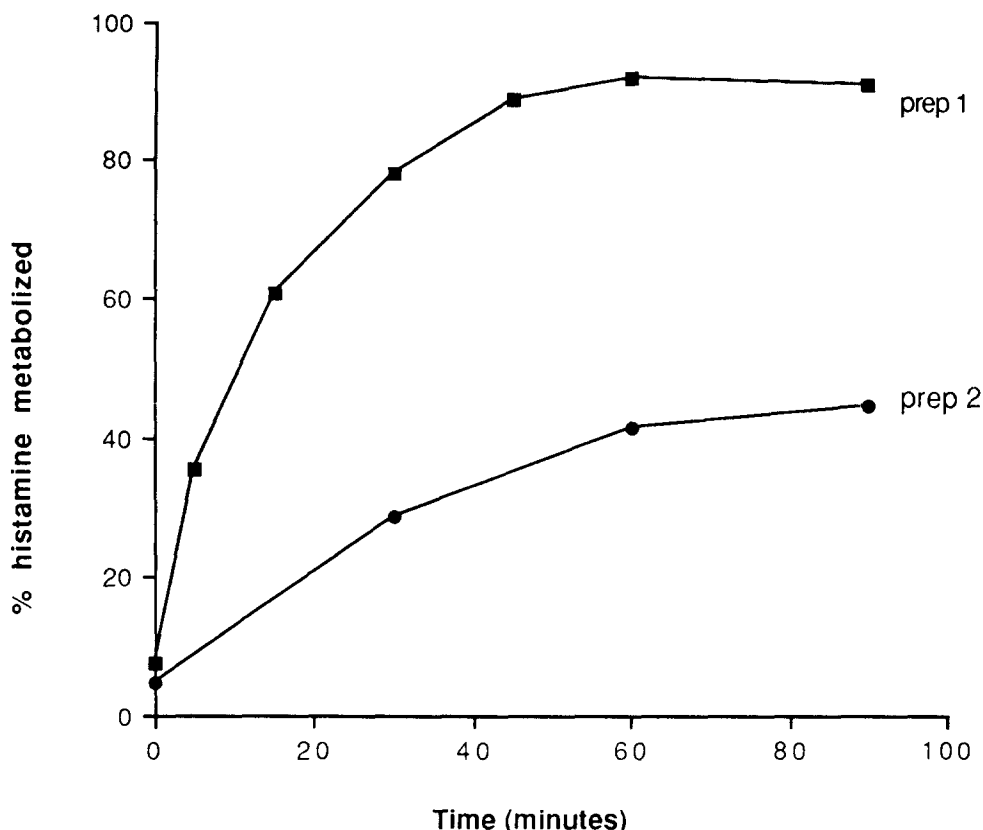


FIG. 2. Time course of histamine metabolism.

When homogenates of skin were incubated for times of up to 90 minutes, methyl histamine formation continued until a plateau was reached at 60 minutes (Fig. 2). An incubation time of 1 hour was chosen for all experiments since this time allowed maximal sensitivity of the assay. The amount of radiolabeled histamine added (0.2 to 5 pmol) was found to have no overall effect on percent histamine metabolized, since the endogenous histamine concentration was between 30-fold and 40-fold greater than that of radiolabeled histamine (Fig. 3). However, at the lowest dilution of label, spots of radioactivity were only just visible on the autoradiograph after a 36-hour exposure, making it difficult to precisely locate the site of metabolite. This led to underestimates of metabolism and poor reproducibility. At high concentrations of label, the autoradiograph was overexposed after 12 hours, and background counts increased dramatically. The mid-point-specific activity of 0.04 $\mu\text{Ci}/\text{pmol}$ was therefore chosen. Saturation of the enzyme system occurred at a final concentration of 0.1 nmol/L of S-AM, and significant inhibition did not occur until between 1 and 10 nmol/L (Fig. 4). The reaction was demonstrated to be linear down to a 1:4 dilution of skin supernatant and a protein range of 0.1 to

1 mg/ml (Fig. 5). All skin supernatants studied in patients with urticaria and in control subjects fell within this range. Percent histamine metabolized in quality control samples demonstrated a coefficient of variation of <10%.

Skin of patients and control subjects

No significant difference in histamine metabolism between skin of patients with urticaria and skin of normal control subjects was observed, 61.2 ± 16.2 and 46.1 ± 23.7 ng/mg of protein per hour, respectively; $p > 0.5$ (mean $\pm$ 1 SD). However, there was a significant increase in the amount of endogenous histamine in the nonlesional skin of patients with urticaria compared with that of skin of control subjects, 407.8 ± 188.0 and 240.0 ± 73.0 ng/mg of protein, respectively; $p < 0.02$ (mean $\pm$ 1 SD) (Fig. 6).

DISCUSSION

We have developed a reproducible assay for measuring histamine degradation in very small samples of skin based on modifications of existing radiochromatographic methods.^{10, 11} EN³HANCE spray¹² enabled precise location of metabolite(s), and use of the plastic-backed TLC plates allowed relevant areas to

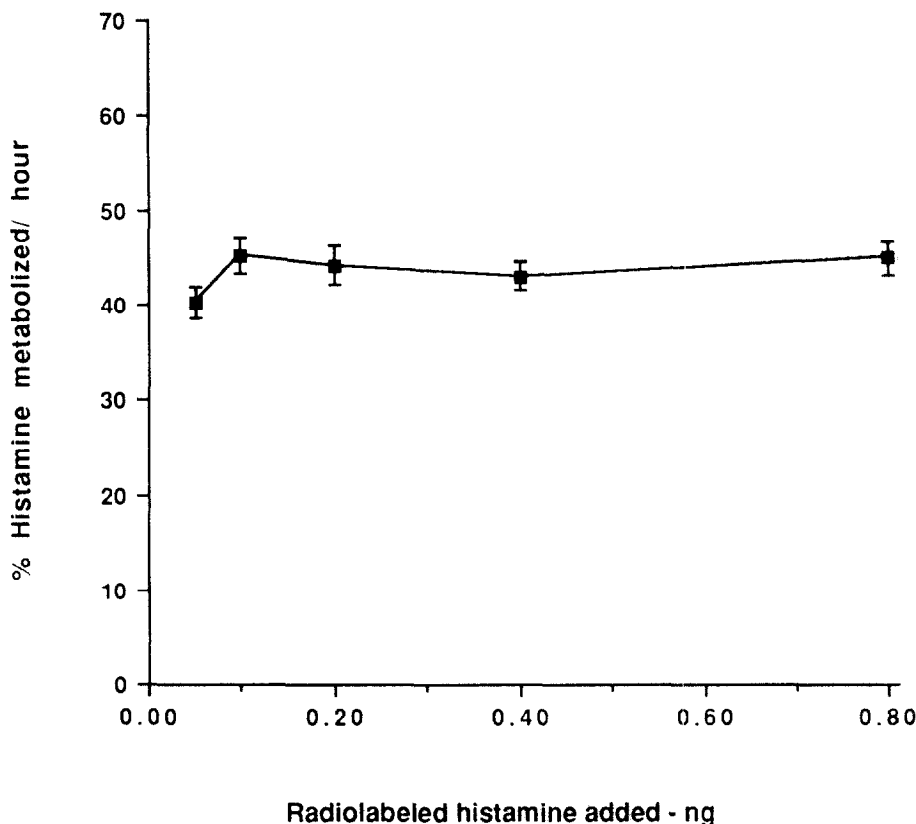


FIG. 3. Effect of increasing concentrations of exogenous histamine on histamine metabolism; error bars, mean $\pm$ 1 SEM; $n = 3$.

be cut out so that loss of counts was minimal compared with that of glass-backed plates. Methyl histamine was the only detectable metabolite. This result is consistent with previous work that suggested that HMT is the major histamine-metabolizing enzyme found in human skin.^{10, 13} HMT activities in skin have been reported to range from 22 ng/mg/hour¹⁴ to 500 ng/mg/hour¹³ with a modified method according to Snyder et al.¹⁵ in which radiolabeled S-AM was added to the incubation mixture containing histamine and the source of enzyme. The [¹⁴C]methyl histamine formed was then extracted into an isopentanol/toluene mixture, and the organic phase was counted in scintillant fluid. Two aspects of the current assay are likely to have resulted in failure to measure maximal rates of HMT activity. First, the incubation period of 1 hour extends beyond the linear part of the reaction-time curve. Second, because endogenous histamine concentrations ranged from 0.5 to 1.2 $\mu\text{mol/L}$, which approximate to the reported Michaelis constant, 4.2 $\mu\text{mol/L}$ of skin HMT (although this measurement was made with unpurified enzyme preparation),¹³ the enzyme may not have been measured under saturation conditions.

With the method described, the capacity of skin homogenates to metabolize histamine in subjects with urticaria and in control subjects was found to be similar. Substrate inhibition of HMT occurs at histamine concentrations of 25 to 100 $\mu\text{mol/L}$ ¹⁰; although higher histamine levels were observed in subjects with urticaria, they remained well below this reported inhibition level and were therefore unlikely to have led to falsely low measurement of HMT activity. Methyl histamine is metabolically inactive, but in vitro studies suggest that it may block histamine receptors on vascular endothelium until further metabolized by DAO to MelmAA.¹² The major source of DAO is the gut,¹⁶ but measurable amounts are also present in circulating neutrophils and eosinophils.¹⁷ Theoretically therefore, impaired circulating levels of this enzyme could also delay the clearance of skin histamine. This theory appears unlikely, since in patients with cold urticaria, in which histamine is also a major mediator,^{18, 19} DAO levels in circulating eosinophils are normal and then increase during ice-cube challenge.²⁰ A recent study²¹ of reduced levels of jejunal DAO in a subgroup of patients with CIU and abdominal pain may be relevant, although a subsequent study measuring posthep-

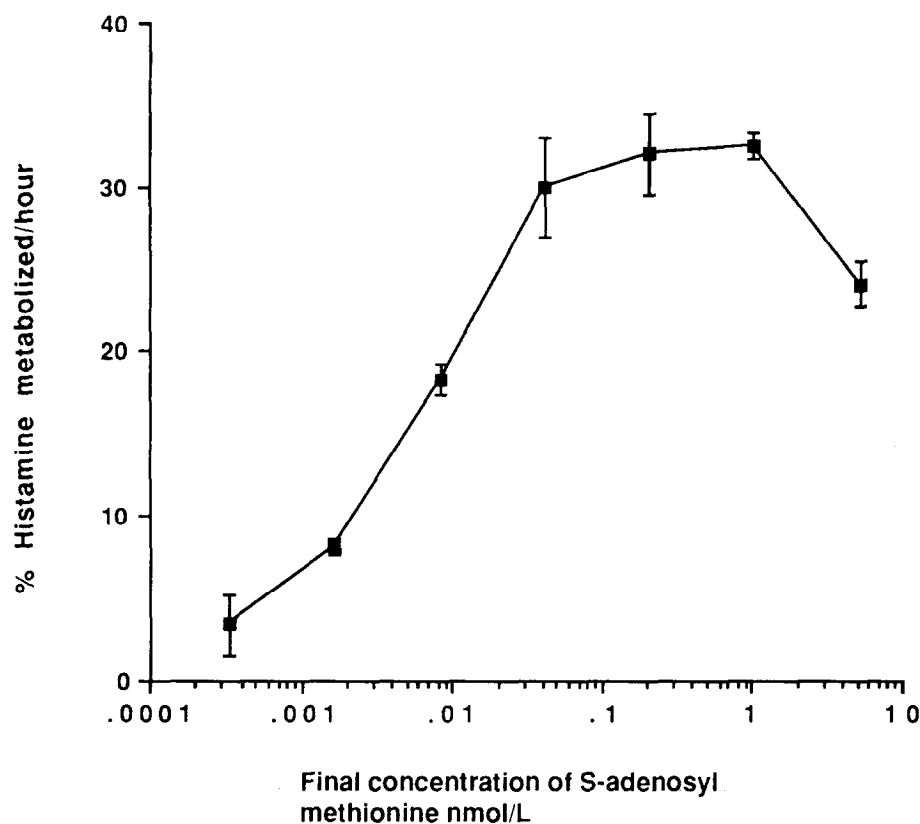


FIG. 4. Effect of increasing concentrations of S-AM on histamine metabolism; *error bars*, mean $\pm$ 1 SEM; $n = 3$.

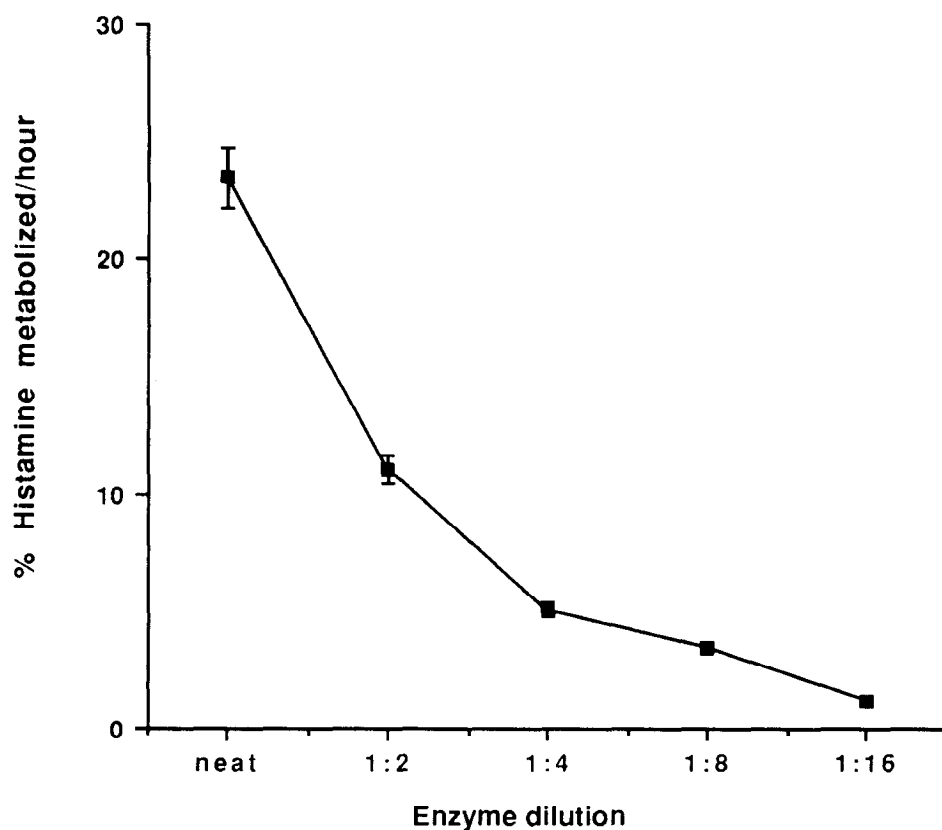


FIG. 5. Effect of doubling dilutions of enzyme on histamine metabolism; *error bars*, mean $\pm$ 1 SEM; $n = 3$.

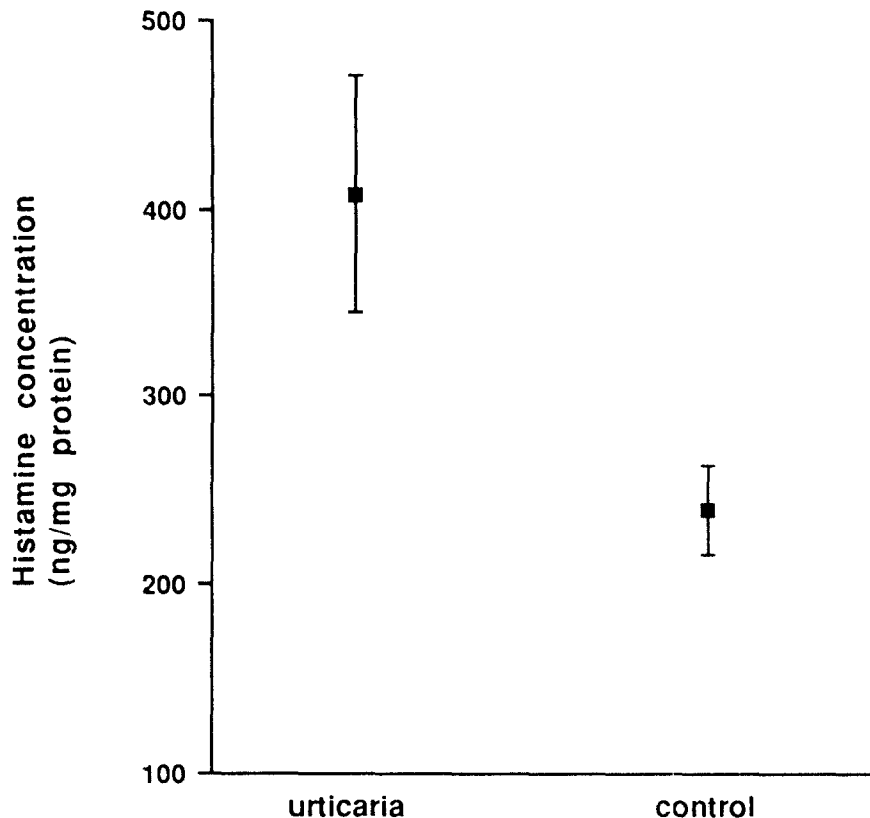


FIG. 6. Histamine content in skin homogenates from subjects with urticaria and control subjects; error bars, mean $\pm$ 1 SEM; $n = 9$; statistically significant increase in skin histamine from subjects with urticaria ($p < 0.02$).

arin DAO activity failed to support these results.²² Observed differences in skin reactivity to histamine may alternatively reflect differences in the rate of diffusion of histamine from its local site of release. However, this hypothesis appears unlikely in view of findings reported by Brunet et al.²³ who compared the profiles of cutaneous histamine release into skin chambers in patients with urticaria and control subjects after challenges of various concentrations of compound 48/80. Although differences were observed in peak histamine release, the rate of histamine clearance was similar in both groups. Thus, the enhanced cutaneous responses to histamine observed in patients with urticaria may result from secondary mediator release rather than a specific defect in the histamine metabolic pathway.

The significant increase in nonlesional skin histamine has been reported by other investigators⁴ and is presumably an indication of increased numbers of mast cells or of an increase in histamine concentration within the mast cell. Great care was taken in selecting the site to obtain a biopsy specimen to ensure exclusion of areas subject to recent whealing, which might have led to falsely elevated tissue histamine levels. Previous studies failed to find any difference in mast cell number in nonlesional skin stained with toluidine

blue,²⁴ although lesional skin is reported to contain tenfold more mast cells than skin of control subjects.²⁵ Mast cells are difficult to quantify because the density of cells varies widely within one biopsy sample, particularly around adnexal structures,²⁶ thus making precise comparison with control skin difficult. In addition, mast cell degranulation may lead to an underestimate of mast cell density,²⁶ and this is particularly likely to be a problem in skin of patients with urticaria in which it is postulated that the mast cells tend to degranulate more readily.^{7, 8} We are currently quantifying mast cell numbers using membrane- and granule-specific monoclonal antibodies that may elicit a more accurate assessment of mast cell number in nonlesional skin.

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