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Histologic studies of chronic idiopathic urticaria

**Sheila Farkas Natbony, D.O., Mildred E. Phillips, M.D., Jules M. Elias, M.A.,
H. P. Godfrey, M.D., Ph.D., and Allen P. Kaplan, M.D. Stony Brook, N.Y.**

Skin biopsy specimens were obtained from 43 consecutive patients with chronic idiopathic urticaria and from seven normal controls. Of 43 patients, 42 had a non-necrotizing perivascular infiltrate composed primarily of mononuclear cells. There was no evidence of damage to vessel walls, of nuclear debris, or of extravasation of red blood cells, and most cells were seen around vessels rather than within the vessel wall. One patient had vasculitis with a neutrophilic infiltrate, nuclear debris, and positive immunofluorescence. Quantitative cell counts revealed four times the number of mononuclear cells and 10 times the number of mast cells in urticaria biopsy sites vs normal skin. Thus chronic urticaria is characterized by an accumulation of mononuclear cells and mast cells with mast cell degranulation presumably associated with hive formation. In our series, the characteristic lesion is not vasculitic. The stimulus responsible for the infiltration of skin with these cells is unknown. (J ALLERGY CLIN IMMUNOL 71:177, 1983.)

Chronic urticaria is a relatively common disease for which there is no known etiology. It does not appear to be an allergic reaction in the classic sense, since IgE antibody is not involved, and no external allergen is needed to initiate or perpetuate the process. Chronic urticaria also differs from allergen-induced skin reactions or physically induced urticarias in that histologic studies reveal a cellular infiltrate predominantly seen about small venules,¹ while no significant infiltrate is

seen in these other disorders. A better definition of the cells that infiltrate the tissue in a population of patients with chronic urticaria would allow further characterization of the disease histologically and would also suggest molecular mechanisms that could be responsible for the recruitment of such cells and for hive formation. Although there is indirect evidence to suggest that histamine is involved in causing the pruritus and increased vascular permeability seen in chronic urticaria,^{2, 3} the mechanism by which it is released is unknown. The most likely source of histamine would be dermal mast cells, unless significant numbers of basophils were found to be recruited into the reaction site.

In reviewing the literature, we have found few detailed histopathologic studies on the lesions of idiopathic chronic urticaria. The infiltrate seen in chronic urticaria has been qualitatively described^{4, 5} and immunofluorescent studies and complement component analysis have been carried out,^{6, 7} but no attempt

From the Department of Medicine, Division of Allergy, Rheumatology, and Clinical Immunology, and the Department of Pathology, The State University of New York at Stony Brook, Stony Brook, N. Y.

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Reprint requests to: Allen P. Kaplan, Division of Allergy, Rheumatology, and Clinical Immunology, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, NY 11794.

Abbreviation usedCH₅₀: total hemolytic complement activity

has been made to quantitate the various cells present and to determine which ones predominate. Our study was undertaken to address this question.

PATIENTS

We have studied the skin biopsy specimens of 43 consecutive patients who presented with histories of recurrent idiopathic urticaria and seven normal controls. The minimal duration of urticaria was 8 wk and the maximum was 4 yr. The chronic urticaria patients were screened in an attempt to discern an underlying etiology such as drug reaction, food allergy, food-additive sensitivity, infection, endocrinopathy, malignancy, and connective tissue disease. A patient's urticaria was considered to be idiopathic when no etiologic agent or underlying disease could be shown to be related to the urticaria. Table 1 summarizes our patient data. The 43 patients studied had an age range of 14 to 71 yr, with a mean of 41.3 yr. There was a 2.5:1 female to male ratio. Of these patients, only one showed evidence of vasculitis, with distinct immunoglobulin and complement deposition seen upon immunofluorescence. This was primarily seen at the dermal-epidermal junction and not within vessels and consisted of deposition of IgG, IgA, C3, and fibrinogen. This patient also had marked hypocomplementemia: CH₅₀, C4, and C3 were each approximately 30% of normal. Sixty-five percent of the dermal infiltrate was neutrophils and nuclear debris was evident. The remaining 42 patients had evidence of a non-necrotizing, mononuclear, perivascular infiltrate. Five patients had an elevated sedimentation rate, with a range of 23 to 36 mm/hr. Four patients showed a decrease in serum levels of C3, C4, or CH₅₀; none had increased complement levels. Three patients had an increase in the C1q binding assay for immune complexes (>9% binding), and nine patients had positive immunofluorescent staining. One of these nine was the patient with vasculitis. The other eight patients had faint linear staining of either C3, C4, or fibrinogen at the dermal-epidermal junction or the upper dermis. One patient had a positive test for antinuclear antibody, with a speckled pattern; all had negative tests for rheumatoid factor, cryoglobulins, and cold agglutinins. Seven patients had other associated diseases: three patients had histories of hypothyroidism and one had hyperthyroidism, but all were euthyroid during the period of assessment and had had the onset of their thyroid disease several years prior to the onset of urticaria. One had pernicious anemia and two had adult-onset diabetes.

METHODS

Two 4 mm punch skin biopsies of two adjacent sites that were part of the same urticarial lesion were performed. The most recent fully developed urticarial lesion was selected for biopsy, which was done with a 4 mm disposable trephine punch (Chester A. Baker Laboratories, Inc., Miami,

Fla.) under local 1% lidocaine anesthesia (without epinephrine). The anesthesia was placed circumferentially about the hive and the biopsy sample was taken at the center. The samples were wrapped in damp, saline-soaked gauze and brought immediately to the histopathology laboratory, where each specimen was then divided in half by cutting at right angles to the epidermis. One-half of the first sample was fixed in neutral 10% buffered formalin and embedded in paraffin, and 5 μ m thick sections of this block were cut. One section was stained with hematoxylin and eosin and another with chloracetate esterase, an enzyme stain that specifically marks mast cells and neutrophils. The remaining half of this first sample was quick frozen and used for immunofluorescence analysis. One half of the second sample was placed in Karnovsky's fixative overnight, washed, dehydrated, embedded in glycolmethacrylate plastic, and stained with a Giemsa reagent, and 2 μ m thick sections were cut. The remaining half of the second sample was quick frozen and stored for future studies.

Each Giemsa-stained specimen was examined by light microscopy, and a detailed grid counting of mast cells, basophils, neutrophils, and mononuclear cells was performed with a 10 by 10 mm reticule that fit into the eye piece of the microscope. Five grids of adjacent areas of the specimen were counted, and the total counts per five reticules were reported. The slides were first screened under low power to locate the zone or zones of infiltration, and a representative area was then examined and counted under oil immersion. If there was more than one zone of infiltration (e.g., involvement of both superficial and deep venous plexus), five reticules of each were read and the values were averaged separately.

RESULTS

Histologic results indicated that normal skin from our controls contained a small number of cells infiltrating the dermis, with minimal accumulation about small venules (Fig. 1, A). By comparison, the patients with chronic urticaria had an infiltrate evident throughout the dermis, which was more prominent in the superficial region beneath the epidermis. There were also obvious areas of infiltration surrounding small venules (Fig. 1, B).

Cellular infiltrates of chronic urticaria consisted of mononuclear cells, mast cells, eosinophils, and neutrophils. Fig. 2 demonstrates a field in which a mast cell and an eosinophil are visible. Mast cells were identified as large cells containing many small violet granules. These granules were often found free, surrounding the mast cell or scattered throughout the dermis as evidence of mast cell degranulation. The mast cell nucleus is large, symmetric, and nonlobulated. Basophils were not seen in chronic urticaria. They are distinguished from mast cells because they are a smaller cell with lobulated nuclei and purple black granules that are few in number but larger than those seen in mast cells.

The numbers of cells per five reticules in normal

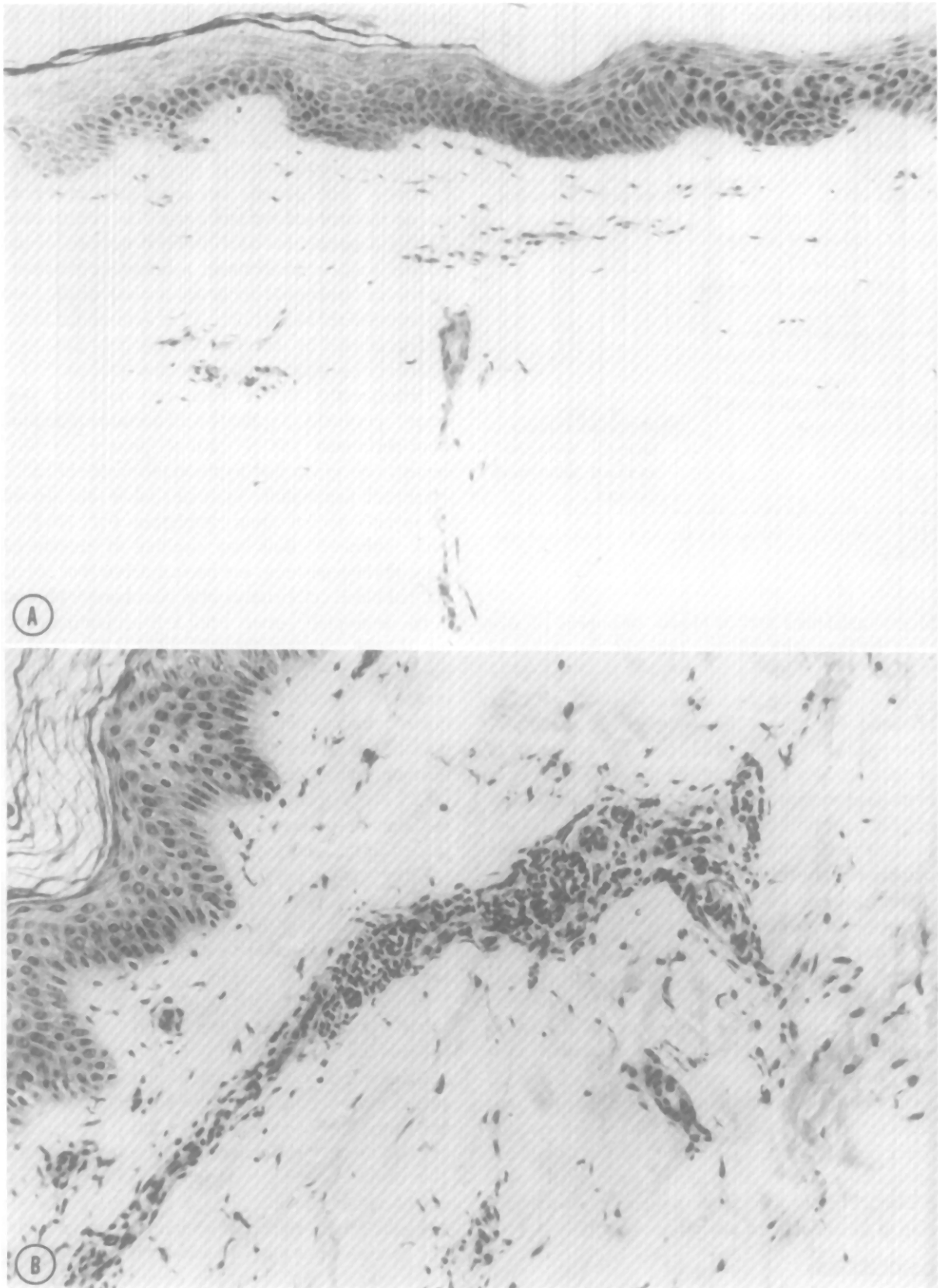


FIG. 1. **A,** Biopsy specimen of normal skin, demonstrating minimal cellular infiltrates consisting of mononuclear cells. **B,** Biopsy specimen of a lesion in a patient with chronic idiopathic urticaria, demonstrating dense perivascular as well as interstitial infiltrates. (Glycolmethacrylate embedment, 2 μ m section, Giemsa stain; original magnification $\times 250$.)

TABLE I. Patient data

No. of patients	43
Age range	14-71 yr (mean 41.3 yr)
Female:male ratio	31:12
Non-necrotizing perivascular infiltrate	42
Vasculitis	1
Elevated ESR	5 (range 23-36 mm/hr)
Positive ANA ($\geq 1:20$)	1
Serum complement changes ($\downarrow$ C3, C4, or CH ₅₀)	4
^a Clq binding assay for immune complexes	3
Positive immunofluorescent staining for C3, C4, fibrinogen, or immunoglobulin (dermal-epidermal junction)	9
Associated diseases	Hypothyroid 3, hyperthyroid 1, pernicious anemia 1, adult-onset diabetes 2

ESR = erythrocyte sedimentation rate; ANA = antinuclear antibodies.

biopsy specimens and in biopsy specimens of skin from patients with chronic urticaria are shown in Table II. No difference in the mean number of neutrophils, eosinophils, or basophils was seen when the urticarial and control populations were compared. The major differences noted were that patients with chronic urticaria had a fourfold increased number of mononuclear cells surrounding small blood vessels and the number of mast cells was increased 10-fold. In eight patients there was a marked increase in the number of mast cells (10 or more per five reticules). Thirteen patients showed increases in eosinophils and five patients showed increases in neutrophils (5 or more per five reticules) as compared with normal controls. Of the 18 patients with increased neutrophils or eosinophils, the one patient with true vasculitis had a neutrophil count of 50 per five reticules in her skin specimen, and a second patient who had faintly positive immunofluorescence at the dermal-epidermal junction had an eosinophil count of 12 per five reticules but without vasculitis. The clinical presentation of the remaining 16 patients did not differ from those in whom neither eosinophils nor neutrophils were evident. The histologic pattern seen had no relationship to the duration of urticaria.

DISCUSSION

Studies of chronic urticaria have indicated that the typical lesion consists of a non-necrotizing perivascular infiltrate consisting of mononuclear cells.^{1, 10, 11} However, it has become clear that there are many

types of histopathologic processes that can occur in the skin and manifest as a hive. For example, patients with hypocomplementemia and cutaneous vasculitis can have urticaria and/or angioedema as a manifestation.¹²⁻¹⁴ This concept was further refined by Soter,¹ who described a series of 16 patients who had urticaria as a manifestation of a necrotizing venulitis. Typically, such patients had arthralgias, myalgias, and an elevated sedimentation rate. The criteria used for this diagnosis included fibrinoid necrosis of the venular wall, a predominant neutrophilic polymorphonuclear leukocytic infiltrate, nuclear debris, and extravasated red blood cells. These were similar to the criteria utilized by others in describing urticaria as a potential manifestation of a vasculitic process.¹²⁻¹⁵

Subsequently, further histologic studies of skin biopsy specimens of patients with necrotizing angitis were performed, and two distinct groups were discerned; one group had hypocomplementemia and a prominent neutrophilic infiltrate, while the second group had a normal serum complement and a lymphocytic infiltrate.¹⁶ Both had swelling of endothelial cells, fibrin deposition, and nuclear debris, but edema and red blood cell extravasation was more prominent in the neutrophilic group. Mast cells exhibiting varying degrees of degranulation were noted in both groups. This study did not attempt to correlate the presence of circulating immune complexes or deposition of immune complexes or complement in the skin with the type of angitis as defined histologically. Furthermore, only four of 16 patients had urticaria (the others had palpable purpura), of which two had a neutrophilic infiltrate, while the histologic lesion present in the other two was not reported.

Other studies have focused on chronic urticaria rather than vasculitis as the clinical manifestation to be assessed in a prospective fashion, and different results have been reported by different groups regarding the nature of the vascular insult, the presence of immune complexes, and the deposition of complement. Mathison et al.⁴ studied 72 consecutive patients with chronic urticaria and found 10 with hypocomplementemia. These 10 patients were assumed to have cutaneous vasculitis, and those with evident activation of the classic complement pathway had elevated levels of circulating immune complexes (six patients). Two of these patients were reported to have deposition of immunoglobulin and complement components along the dermal-epidermal junction and vessel walls. The cutaneous histologic results in the other 62 patients who did not have hypocomplementemia were not reported. Their overall incidence of vasculitis in chronic urticaria was 14% if hypocomplementemia and vasculitis are equated.

Monroe et al.⁵ also focused on the incidence of

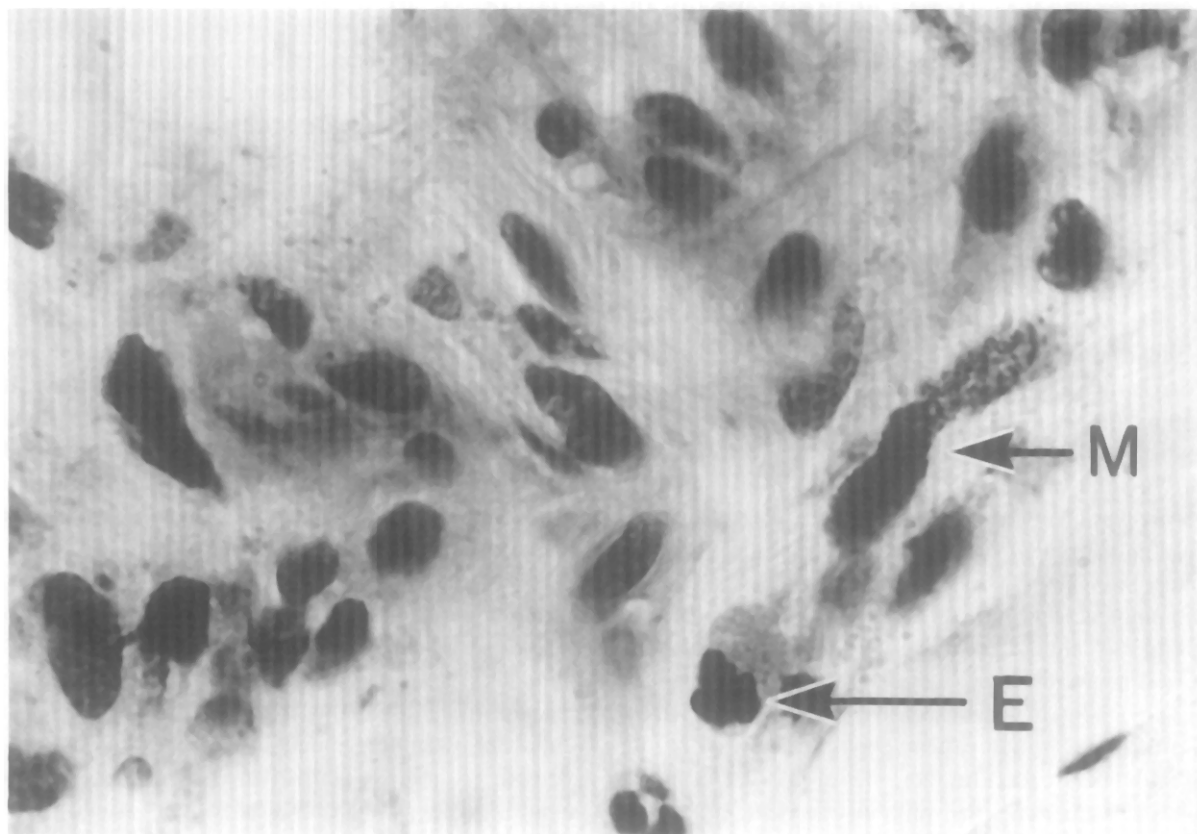


FIG. 2. Biopsy specimen of a lesion in a patient with chronic idiopathic urticaria as seen under oil immersion. Mast cells are identified as large cells containing many small granules with a large symmetric nucleus. *M*, Mast cell; *E*, eosinophil. (Glycolmethacrylate embedment, 2 μ m section, Giemsa stain; original magnification $\times 1250$.)

TABLE II. Number of infiltrating cells in biopsy specimens of chronic urticaria patients as compared with normal controls*

	No.	Eosinophils		Basophils		Neutrophils	
		Mean	Range	Mean	Range	Mean	Range
Normals	7	0		0		0.14	0-1
Chronic urticaria	43	1.2	0-12	0.35	0-2	3.0	0-50

	Mononuclear cells		Mast cells	
	Mean	Range	Mean	Range
Normals	13.4	3-25	0.71	0-3
Chronic urticaria	52.4	16-247	7.6	0-19

*Data are reported as mean and range of cells counted per five reticules.

vasculitis in chronic urticaria and reported on 45 cases. Nine patients showed typical leukocytoclastic angiitis with a neutrophilic vasculitis, with an incidence of 20%. The remaining patients were subdivided into two groups, each of which had a predominantly lymphocytic infiltrate. In 21 patients (47%) there was a sparse perivascular infiltrate with no evident vasculitis. In 15 patients a dense perivascular

infiltrate was seen in which lymphocytes and some eosinophils predominated. These were not considered to be "vasculitis" as defined by the presence of nuclear debris and/or fibrinoid deposits in or around vessel walls. Circulating immune complexes were present in some patients in each category but primarily in the leukocytoclastic angiitis and dense-infiltrate group. Of interest was that the C1q binding assay

detected more positive responses in the former group, while the monoclonal rheumatoid factor assay was more revealing for the dense-infiltrate group. Positive immunofluorescence in vessels was seen in one third of patients with vasculitis and 13% and 9% for the dense- and sparse lymphocytic-infiltrate groups, respectively.

A different approach regarding the question of vasculitis in chronic urticaria was taken by Phanuphak et al.,⁴ who defined vasculitis as having significant cellular infiltrate within the wall of vessels rather than criteria of endothelial damage, nuclear dust, or fibrin deposition. With these criteria 52% of patients were reported to have some sort of vasculitis and 48% had a perivascular mononuclear infiltrate. The vasculitic group was then subdivided into types based on the predominant cell type, e.g., neutrophilic, mixed, lymphomonocytic, and eosinophilic. Deposition of immunoreactants was seen in 18% of the vasculitic group and almost exclusively in the neutrophilic or mixed neutrophil-lymphocytic groups.

Our study of 43 consecutive patients emphasizes the fact that in our patient population, vasculitis is a rare occurrence. In fact, 42 of 43 had a non-necrotizing perivascular infiltrate consisting primarily of lymphocytes. In these patients there was no evident damage to the vessel walls and no nuclear debris or extravasation of red blood cells, and most cells were seen around vessels rather than within the vessel wall. In fact, the venules being examined were in most cases so small that it was difficult to evaluate infiltration within the vessel wall. These patients correspond to the 62 of 72 non-vasculitic patients in the study of Mathison et al.,⁶ the sparse- and/or dense-infiltrate group reported by Monroe et al.,⁵ and the "perivasculitis" group of Phanuphak et al.⁴ We have no explanation why our population of patients includes so few with vasculitis. This may be due to differing patient sampling in various parts of the country, different referral patterns, and differing criteria used to determine vasculitis. One of us (A. K.) had considerable prior experience at the National Institutes of Health, where over 150 biopsies were performed over a 6 yr period, and although quantitative cell counts were not performed, over 95% of patients had the qualitative histologic pattern described here.

Our quantitative data reveal that a lymphocytic infiltration represents the main cellular accumulation, although it represents only a fourfold increase over the normal number of lymphocytes present in skin biopsy specimens. However, a 10-fold increase in mast cell numbers was striking and evidence of degranulation was prominent in most specimens. Kaplan et al.² utilized suction blisters and demonstrated a

sixfold increase in histamine release into the blister fluid of chronic urticaria patients compared with normals. Phanuphak et al.³ measured total skin histamine content in chronic urticaria and found it to be increased compared with normals. These data could have been explained by the presence of basophils, an increased number of mast cells, or increased histamine content per cell. Our studies indicate no infiltrate with basophils. Thus the accumulation of mast cells seen may be responsible for these observations. We have no data regarding the histamine content per cell. Since lymphocytes and mast cells accumulate, it is possible that a lymphokine is released, which causes mast cell proliferation (or accumulation) and degranulation. An analogous factor has been reported to be released from activated lymphocytes, which can cause basophil degranulation.^{17, 18}

In this study we did not compare lesional skin to normal-appearing skin in urticaria patients but compared lesional skin with the skin of normals. Previous studies have suggested that normal-appearing skin in chronic urticaria patients is in fact not normal when studies of histamine content and releasability are performed,^{2, 3} and a quantitative assessment of non-lesional skin in chronic urticaria patients would be of interest. We had five patients with an elevated sedimentation rate, four with a decrease in C3, C4, or CH₅₀, three with a positive C1q binding assay for immune complexes, and nine with positive immunofluorescent staining for complement components or immunoglobulin. Except for our one patient with true vasculitis, these abnormalities were not found in the same patients and are of questionable significance. Of particular note is that those with positive immunofluorescent staining generally had no deposition in vessels but had irregular staining of the basement membrane at the dermal-epidermal junction or upper dermis. This may represent evidence of increased vascular permeability rather than immune complex deposition.

We conclude that chronic urticaria is characterized by a non-necrotizing perivascular lymphocytic infiltrate with an accumulation of mast cells. The etiology is unknown and we cannot, as yet, even state whether it is or is not immunologically mediated. Patients with vasculitis and urticaria are considered to be a separate subpopulation in whom the cause and pathogenesis of hive formation is probably different.

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