BASOPHILIC LEUKOCYTES IN ALLERGIC CONTACT DERMATITIS*

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(Received for publication 13 August 1971)

Delayed hypersensitivity has generally been regarded as a homogeneous group of reactions including reactivity to proteins such as tuberculin, contact allergy, resistance to infection with viruses and other microorganisms, and allograft rejection (1). Recently, however, we have found that several forms of delayed-onset cell-mediated hypersensitivity in animals are characterized by an extensive infiltration of basophilic leukocytes (2). Such reactions have been designated cutaneous basophil hypersensitivity or CBH to distinguish them from true delayed hypersensitivity (DH) in which basophils are infrequent or altogether absent. In many instances, both forms of reactivity may be elicited to the same antigen, depending on the mode of immunization, and CBH is favored when sensitization to protein antigens (2–4), contact allergens (4, 5), a virus (6), or allografts (7) is accomplished without the use of complete Freund’s adjuvant. While CBH reactions to protein antigens are often transient and of the Jones-Mote type (3), in other instances, as with contact allergy and viral immunity, the characteristic basophil response persists for weeks or months (4, 6). In addition to differences in morphology and requirements for immunization, CBH may be distinguished from DH by virtue of certain immunologic and immunochemical properties (8) and by differences in the response of lymphocytes from such animals to specific antigen in tissue culture (9).

Prompted by animal studies, we wished to determine whether a similar heterogeneity of the cellular immune response could be demonstrated in man. Preservation of human basophils during fixation and tissue processing is particularly difficult. However, we have been able to establish conditions for reliable identification of these cells in tissue sections at the light microscopic level and report here the participation of basophilic leukocytes in the lesions of allergic contact dermatitis. In serial biopsies available from one individual, it was possible to describe the evolution of the microscopic pathology as it unfolded at successive intervals after patch test. In addition to basophils and the characteristic epidermal changes, a number of other microscopic features in-

*This work was supported by US Public Health Service Grants AI-10496 and AI-09529.
§Advanced Clinical Fellow No. 204, American Cancer Society.
The abbreviations used in this paper: CBH, cutaneous basophil hypersensitivity; DH, delayed hypersensitivity.
including fibrin deposition, vascular compaction, and a significant late eosinophil infiltration set this lesion apart from classic descriptions of tuberculin hypersensitivity in man (1). A preliminary communication of portions of this work has been reported (10).

**Materials and Methods**

The principal experimental subject was one of the authors (H. F. D.), a 33 yr old white male who first noted hypersensitivity to poison ivy 6 months before the start of this investigation. Lesions of allergic contact dermatitis (Fig. 1) were elicited by application of a 1:100 dilution of urushiol (oleoresin) in peanut oil (Hollister-Stier Laboratories, Spokane, Wash.) to the upper arms in the form of 2-cm in diameter patch tests, and patches were kept in place for 2 days except when biopsy was performed earlier. Lesions were scored and 4-mm punch biopsies were obtained after infiltrating the circumference of the reactions with Xylocaine. A total of 12 biopsies was obtained from multiple patch tests at 10 hr and at 1, 2, 3, 4, 6, and 11 days after application of allergen, at 1 day after application of vehicle (peanut oil), and at 6 and 48 hr after production of nonspecific inflammation by exposure of the skin to liquid nitrogen for 15

Figs. 1-7 and 9-21 illustrate the gross and microscopic pathology of allergic contact dermatitis to poison ivy as elicited with 1:100 urushiol extract. Except for Fig. 3, all photomicrographs were made of 1-μ sections stained with alkaline Giemsa.

**Fig. 1.** Gross appearance 50 hr after patch test. Numerous vesicles stud the surface of the erythematous and edematous patch test site which is irregularly surrounded by a 2-3 mm flare. × 1.5.

**Fig. 2.** Superficial epidermal vesicles in the 72 hr lesion containing basophils (metachromatic granules), an eosinophil (blue-green granules), and mononuclear cells (upper) which apparently have phagocytosed eosinophil granules. Cells are situated in a menstruum of proteinaceous fluid. × 1130.

**Fig. 3.** Touch preparation of ruptured epidermal vesicle from a 4 day reaction illustrating the various types of granulocytes (two each, basophils [B], eosinophils [E], and neutrophils [N]) and mononuclear cells present in the infiltrate. Wright's stain, × 1070. (Eosinophil granules stain red in air-dried touch preparations and blue-green in fixed, embedded tissue sections; basophil granules stain metachromatically with both techniques.)

**Fig. 4.** Venule of papillary dermis in a 50 hr lesion illustrating three basophils and several neutrophils (no granules) within the lumen. A basophil (B) is also present in the extravascular space as is a cluster of basophil granules (arrow), presumably derived from basophil degranulation or death. × 840.

**Fig. 5.** Reticular dermis (intervascular zone) of a 50 hr reaction demonstrating at least five intact basophils and fragments of other basophils and eosinophils (blue-green granules, EG). There is considerable edema (pale areas) with strands of fibrillar material (arrows), presumed to represent fibrin. Mononuclear cells are also present some of which represent fixed tissue histiocytes. × 1130.

**Fig. 6.** Perivascular zone of dermis of 6 day lesion illustrating mononuclear cells of various types, one of which is in mitosis. A mast cell (M) with small, dark-purple cytoplasmic granules is present at the left. A fragment of granule-containing mast cell cytoplasm, possibly representing a process extending from the mast cell illustrated, is adjacent to the right. Compare the mast cell morphology with that of the basophil (B) (upper right) which also shows focal vacuolation of the cytoplasm. × 1130.

**Fig. 7.** Interverascular reticular dermis of a 96 hr reaction illustrating a basophil (B) and several intact eosinophils (E) as well as fragments and free granules of other eosinophils (EG). A small lymphocyte (lower right) and two fixed tissue histiocytes are also present. × 1130.
In addition, a biopsy was obtained 2 days after application of 1:100 urushiol to an unsensitized individual.

As part of a continuing investigation, single biopsies were also obtained from a number of patients studied together with Doctors Normand Olivier and Maury Goldman. These individuals, all young or middle aged adults (four females, three males), had clinically significant allergic contact dermatitis of weeks to years duration; all were patch tested with the appropriate allergen for 2 days and were biopsied on day 3.

Histologic Methods.—In man, as in animals, basophils could not be identified in routine histologic sections. Hence, biopsies were cut into smaller pieces and fixed for 5 hr in a solution composed of 2% paraformaldehyde, 2.5% glutaraldehyde, and 0.025% CaCl₂ in 0.1 M cacodylate buffer pH 7.4 (11). This fixative preserved basophils more reliably than some 20 others tested, including other dilutions of the Karnovsky fixatives, various concentrations of glutaraldehyde alone in several buffers with or without sucrose supplementation, Dalton’s chromo-osmium solution, and various forms of primary osmium fixation (12). Tissues were postfixed in osmium tetroxide and 1-μ Epon-embedded sections were prepared and stained with Giemsa diluted 1:10 in 0.05 M phosphate buffer, pH 7.5 (alkaline stain), or 0.05 M acetate buffer, pH 4–6 (acid stain), as described previously (3). This technique is ideal for quantitative analysis of cellular infiltrates since it affords optimal light microscopic morphology on large blocks of tissue; structural detail normally reserved for electron microscopy is preserved while the sampling problems inherent in the latter method are avoided.

With either alkaline or acid Giemsa stain, basophils (Figs. 2–7) were identified as polymorphonuclear cells with prominent, brightly metachromatic granules and were readily distinguished from fixed tissue mast cells (Fig. 6) which had larger unilobed nuclei with less dense peripheral chromatin clumping, more numerous and smaller metachromatic granules, and often a complex cell surface with granule-containing dendrite-like processes. Eosinophils (Figs. 2 and 7) generally had bilobed nuclei and numerous cytoplasmic granules that stained green-blue with the alkaline stain and bright red with the acid stain. Neutrophils (Fig. 4) had multilobed nuclei and blue-green cytoplasm when stained at pH 7.5. With the acid stain, small numbers of minute, red cytoplasmic granules were visible.

Cover slip touch preparations of fluid from ruptured epidermal vesicles were made on 3- and 4-day contact reactions and these were air-dried and stained with Wright’s stain (Fig. 3). It was thus possible to examine the inflammatory cells infiltrating epidermal vesicles by a routine hematologic method, affording an independent check on the nature of the cells observed in microscopic sections.

Quantitative Analysis of Cell Infiltrate.—In order to quantitate the various types of inflammatory cells participating in the lesions of contact allergy and to determine the order of their arrival, detailed cell counts were performed on the sequence of biopsies obtained from H. F. D. Differential cell counts were performed on all cells infiltrating the epidermis. The entire length of epidermis available from each biopsy was measured with an ocular micrometer (distance A—A in Fig. 8) and counts were expressed in terms of cells per millimeter of epidermal surface studied. The dermal infiltrate was analyzed by counting all cells encountered in a series of swaths taken perpendicular to the skin surface and followed to the deep resection margin of the biopsy. Swaths, each 100 μ in width, were defined with the aid of an ocular micrometer. This method was followed in a systematic fashion (Fig. 8) until all available tissue had been viewed or until at least 3000 cells had been registered, in which case counting was terminated after completion of the swath containing the three-thousandth cell. The number of swaths counted was recorded so that cell counts could be expressed in absolute terms of cells per milliliter of linear surface as well as in the form of a percentage of the total cell infiltrate (Tables I and II). Because the cellular infiltrate of allergic contact reactions occupied mainly the superficial portions of the skin, the deep resection margin of each biopsy always included a zone of uninvolved connective tissue.
RESULTS

Gross Appearance of Patch Test Sites.—Patch test of the subject sensitized to poison ivy (H.F.D.) elicited typical delayed-onset, erythematous, and vesicular lesions of contact allergy (Fig. 1). At $10^{1/2}$ hr the test site exhibited scattered faint white circular or oval areas, averaging 0.5 mm in diameter. Some of these were slightly raised but there was no erythema. At 25 hr blotchy erythema and scattered 1 mm pink to opalescent-white papules were observed. The reaction was now sharply demarcated as a round area conforming exactly to the distribution of urushiol application. Surrounding this zone was an irregular flare which extended as far as 5 mm into the surrounding skin. Numerous papulovesicles, 0.25–1.0 mm in diameter, were scattered over the lesion and a red flare extended irregularly up to 1.7 cm around the test site. At 72 hr the edema had increased further and numerous vesicles, averaging 0.5 mm in diameter, covered the patch site. Rare small erosions, exuding a thin clear fluid, were present and the flare had largely subsided. At 96 hr confluent vesicles, averaging 2.5 mm in diameter, covered the greater part of the test site, interrupted by tiny, dry, yellow-brown crusts. The lesion gradually subsided and by 11 days
consisted of a sharply demarcated, pink-fawn colored area which was irregularly raised. Several 1-2 mm papules were surmounted by gray-tan scales. No flare, ulcerations, or weeping were present.

Patch test reactions similar to the above description and with a similar time course were elicited in five of seven patients with standard concentrations (13) of a variety of allergens (see below). However, two patients developed only mild erythema without edema or vesiculation.

Patch test of H.F.D. with peanut oil (vehicle) or of an unsensitized subject with urushiol elicited no gross lesion. Freeze-burn of H.F.D. with liquid nitrogen caused immediate erythema followed within a few hours by blister formation; this lesion had a similar appearance at 48 hr.

**Microscopic Pathology of Allergic Contact Reactions to Urushiol in H.F.D.—** Quantitative counts were performed on the inflammatory cells infiltrating the epidermis and dermis of each biopsy obtained from H.F.D. and are tabulated in Tables I and II. A detailed description of the microscopic pathology at each time interval follows.

10½ hr: At 10½ hr there was very focal, mild intercellular edema involving the lower epidermis and the hair-follicle epithelium with occasional rupture of intercellular bridges. Occasional intraepithelial lymphocytes were observed in affected areas of the hair follicle but not in the epidermis itself. In the papillary layer of dermis there was a focal lymphocytic infiltrate about the venules of the

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### TABLE I

**Absolute and Relative Counts of the Inflammatory Cell Infiltrate in the Dermis at Various Intervals after Patch Test of H.F.D. with 1:100 Urushiol in Peanut Oil and at 2 Days after Patch Test with Peanut Oil**

<table>
<thead>
<tr>
<th>Interval</th>
<th>Total cells counted</th>
<th>Absolute cell counts expressed as cells/linear mm surface</th>
<th>Mononuclears</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>Basophils</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>10½ hr</td>
<td>376</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>97.6</td>
</tr>
<tr>
<td>1 day</td>
<td>431</td>
<td></td>
<td>96.3 (98.6%)</td>
<td>1.0 (1%)</td>
<td>11.7 (9.4%)</td>
<td>0.3 (0.2%)</td>
<td>123.4</td>
</tr>
<tr>
<td>2 days*</td>
<td>3901</td>
<td></td>
<td>471.6 (82.2%)</td>
<td>19.3 (3.3%)</td>
<td>56.5 (9.8%)</td>
<td>26.3 (4.5%)</td>
<td>573.7</td>
</tr>
<tr>
<td>3 days</td>
<td>2448</td>
<td></td>
<td>610.2 (54.4%)</td>
<td>70.1 (6.2%)</td>
<td>153.9 (13.7%)</td>
<td>287.2 (25.6%)</td>
<td>1121.4</td>
</tr>
<tr>
<td>4 days</td>
<td>2995</td>
<td></td>
<td>892.9 (73.7%)</td>
<td>20.2 (1.6%)</td>
<td>118 (9.7%)</td>
<td>179.5 (14.8%)</td>
<td>1210.6</td>
</tr>
<tr>
<td>6 days</td>
<td>3456</td>
<td></td>
<td>931.7 (61.9%)</td>
<td>12.2 (0.8%)</td>
<td>244.5 (16.2%)</td>
<td>315.4 (20.9%)</td>
<td>1503.3</td>
</tr>
<tr>
<td>11 days</td>
<td>1460</td>
<td></td>
<td>454.0 (70.2%)</td>
<td>2.2 (0.3%)</td>
<td>25.2 (3.9%)</td>
<td>165 (25.5%)</td>
<td>646</td>
</tr>
<tr>
<td>1 day control†</td>
<td>233</td>
<td></td>
<td>74.9 (98.2%)</td>
<td>1.0 (1.3%)</td>
<td>0</td>
<td>0.3 (0.3%)</td>
<td>76.2</td>
</tr>
</tbody>
</table>

* Two separate biopsies were performed at the 2 day interval and the results averaged. At all other intervals counts were made on sections obtained from a single 4 mm punch biopsy.

† Patch test with peanut oil alone.

§ Relative cell counts, expressed as per cent of total infiltrate, are included in parentheses.
superficial vascular plexus. Fixed tissue mast cells were fully granulated and present in normal numbers at this and at all subsequent intervals.

1 day (25½ hr) (Figs. 9 and 10): Discrete, minute intercellular vacuoles (Fig. 9) were present between keratinocytes of the mid- and lower epidermis. Hair follicles showed patchy, intercellular edema as well as a focal infiltrate of lymphocytes and occasional basophils but there was no inflammatory cell involvement of the epidermis.

About the venules and veins of the superficial vascular plexus there was a somewhat greater inflammatory infiltrate (Fig. 10), composed predominantly of lymphocytes (81.4%) but including basophils (9.4%) and rare eosinophils. Basophils, along with other granulocytes and mononuclear cells, were observed both within vessel lumens and in the surrounding tissue whereas neutrophils were largely confined within vessels. Some of the superficial capillaries and venules draining dermal papillae were engorged with tightly packed red blood cells which had lost their rounded contours and assumed a polyhedral configuration; such compacted vessels contained little plasma and, together with the dermal edema, suggested increased vascular permeability with loss of vascular fluid and reduced blood flow (14) (Figs. 16 and 17 illustrate these changes in a 4 day lesion).

2 days (Figs. 1, 4, 5, 11-15): At 50 hr prominent spongiotic vesicles had developed in the mid- and upper epidermis (Figs. 11-14). These intraepidermal spaces were lined by keratinocytes, both intact and ruptured, and contained

### TABLE II

<table>
<thead>
<tr>
<th>Interval</th>
<th>Total cells counted</th>
<th>Absolute cell count expressed as cells/linear mm surface$\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mononuclears</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>10½ hr</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 day</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 days*</td>
<td>429</td>
<td>45.9 (93.2%)</td>
</tr>
<tr>
<td>3 days</td>
<td>286</td>
<td>42.3 (68.1%)</td>
</tr>
<tr>
<td>4 days</td>
<td>160</td>
<td>30.1 (67%)</td>
</tr>
<tr>
<td>6 days</td>
<td>181</td>
<td>26.1 (71.3%)</td>
</tr>
<tr>
<td>11 days</td>
<td>15</td>
<td>4.7 (86.9%)</td>
</tr>
<tr>
<td>1 day control†</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Two separate biopsies were performed at the 2 day interval and the results averaged. At all other intervals counts were made on sections obtained from a single 4 mm punch biopsy.
† Patch test with peanut oil alone.
§ Relative cell counts, expressed as per cent of total infiltrate, are included in parentheses.
lymphocytes and basophils in a menstruum of amorphous proteinaceous material (Figs. 13 and 14). The adjacent epidermis exhibited marked but patchy intercellular edema (Fig. 12) with vacuolated keratinocytes and a

diffuse mild infiltrate of inflammatory cells, mainly lymphocytes. Similar epidermal changes affected the epithelium of sebaceous glands and of hair follicles but sweat glands and their ducts remained uninvolved at all intervals studied.

Scattered superficial capillaries and venules of the papillary dermis continued
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to show compaction, a feature of biopsies taken through 6 days. Edema in the
dermis was now diffuse, involving the entire papillary layer and extending to
the superficial portion of the reticular layer. Prominent deposits of fibrillar
material, having the morphology of fibrin, were noted in the edema fluid, at-
testing further to altered vascular permeability. The cellular infiltrate had
increased approximately fourfold from 1 day and was distributed in the form

![Image](https://example.com/figure11)

**Fig. 11.** Overview of 50 hr reaction. Epidermis shows intercellular edema with so-called
spongiotic vesicle formation and a focal cellular infiltrate. Papillary dermis is edematous and
there is a predominantly perivascular inflammatory response, disposed about the vessels of the
superficial vascular plexus. × 100.

**Fig. 12.** Epidermis of 50 hr reaction. The intercellular edema is striking and has progressed
to the formation of small vesicles which contain protein-rich fluid. Note the absence of an asso-
ciated inflammatory-cell infiltrate. × 1000.

of perivascular cuffs (Figs. 11 and 15) surrounding venules and veins of the
superficial plexus and around venules extending to the middle portion of the
reticular dermis. In addition, inflammatory cells were scattered diffusely, but in
lower concentration, in the intervascular connective tissue of the papillary
layer and upper portion of the reticular layer of the dermis (Fig. 11). While
the overall composition of the infiltrate had changed little from that at 24 hr, it
was apparent in this and in subsequent biopsies that the inflammatory cells
disposed about venules and veins were largely lymphocytes and other mono-
nuclear cells, whereas the intervascular infiltrate included a higher percentage
of basophils and at later intervals eosinophils.
Fixed tissue histiocytes were prominent and appeared "activated." These cells, occasionally binucleate, had enlarged, deeply staining, and often vacuolated cytoplasm and a complex cell surface with elongate, dendrite-like, cytoplasmic processes. Endothelial cells of the venules and veins of the dermis

Figs. 13 and 14. Epidermal vesicles in a 50 hr reaction illustrating basophils (B), (Figs. 13 and 14), admixed with mononuclear cells (Fig. 13). Note extensive intercellular edema (spongiosis) composed of protein-rich fluid. Both × 1000.

Fig. 15. Perivascular infiltrate in the dermis of a 50 hr reaction. Various types of mononuclear cells, a plasma cell (P), and a neutrophil (N) are present. A typical mast cell (M) (center) exhibits dendrite-like cytoplasmic processes containing granules. × 1000.
were also activated, exhibiting an enlarged, variably vacuolated cytoplasm and enlarged nuclei with prominent nucleoli.

50-hr biopsies were obtained from H.F.D. on two separate occasions, several months apart, and both showed an identical histology, indicating that the passage of time and multiple patch tests had not altered the character of the reaction.

3 and 4 days (Figs. 2, 3, 7, 16, 17): At these intervals the epidermis was generally spongiotic and edematous (Figs. 16 and 17). In accordance with the gross description, intraepidermal vesicles were prominent, and many of these were subcorneal (Fig. 2). A few vesicles had ruptured and contained only proteinaceous debris and remnants of the various types of inflammatory cells. The cellular infiltrate in the epidermis was maximal on day 3 and included rela-
Fig. 18. 6 day reaction illustrating dermal infiltrate, maximal at this interval, and persistent epidermal changes including patchy intercellular edema and a large intraepidermal vesicle. The perivascular dermal infiltrate which has a nodular, pseudogranulomatous appearance, is associated with an extensive intervascular infiltrate. × 80.

Fig. 19. Higher power view of a perivascular accumulation of cells illustrated in Fig. 18. Note the pseudogranulomatous appearance. Mononuclear cells of various types predominate and are commonly observed in mitosis (arrow). Basophils and eosinophils are more numerous in the intervascular zones (see Figs. 4, 5, 7, 20, and 21), while mononuclear cells but not granulocytes remain in the vicinity of vessels. × 400.
tively fewer lymphocytes and more numerous basophils (16%). Intraepidermal
eosinophils and neutrophils were observed for the first time (Table II). The
majority of infiltrating cells was present in vesicles but inflammatory cells
were also scattered elsewhere in the edematous epidermis. In these biopsies as
well as in those taken at other intervals there was not an invariable association
between inflammatory cells and edema. Thus, in some portions of the epidermis
edema was present and there were few or no associated intraepidermal inflam-
matory cells (Figs. 9, 11, 12, 14, 16, 17); in other portions, inflammatory cells
of all types were identified wedged between adjacent epithelial cells in the
absence of significant intercellular edema. Some keratinocytes in the lower
epidermis had pyknotic nuclei and clumped, irregularly staining cytoplasm,
but the great majority appeared viable.

Superficial capillaries in the papillary dermis (Figs. 16 and 17) were massively
engorged and focal extravasation of red blood cells was occasionally present.
Dermal edema with fibrin deposits (as in Figs. 5 and 21) persisted and the num-
ber of infiltrating inflammatory cells in the dermis had approximately doubled
in comparison to the number present in the 2 day biopsy (Table I). As in the
epidermis, lymphocytes were relatively less frequent and accounted for little
more than half of the inflammatory cells. Basophils and neutrophils increased
moderately while eosinophils showed nearly an 11-fold increase in absolute
number to comprise one quarter of the total infiltrate.

6 days (Figs. 6, 18-21): Discrete, subcorneal vesicles (Fig. 18) persisted
while the epidermal infiltrate continued to decline with a sharp fall in the
number of basophils. The epidermis itself remained edematous and was now
slightly thickened with numerous mitoses evident in the basal cell layer.

A diffuse intervacular and intense perivascular infiltrate, tending to a
nodular, or pseudogranulomatous appearance (Figs. 18 and 19), was present
in the superficial third of the dermis and extended into the deeper portions of
the dermis about the pilosebaceous apparatus. The total dermal inflammatory
cell infiltrate as well as the absolute numbers of basophils and eosinophils
(Fig. 20) were maximal at this interval. Basophils accounted for 16.2% of the
infiltrate and eosinophils, 20.9%, while lymphocytes declined in proportion and
neutrophils were rare. Deposits of dermal fibrin were prominent (Fig. 21).
Mitoses were readily identified in activated fixed-tissue histiocytes (Figs.
6, 19).

11 days: At 11 days intraepidermal vesicles were absent, and only a few
intraepidermal inflammatory cells, either lymphocytes or eosinophils, remained.
Focal, mild, intercellular edema and vacuolation of keratinocytes were noted in
the now irregularly thickened, hyperkeratotic and focally parakeratotic epi-
dermis.

The dermal infiltrate had decreased approximately 60% from the 6 day
biopsy and contained lymphocytes and rare basophils. Eosinophils, many of
them degranulated, accounted for a quarter of the total infiltrate. Occasional plasma cells, both mature and immature, were present, but their significance is uncertain as similar cells were observed in a biopsy from a site at which peanut oil alone had been applied 24 hr previously (see below). Activated histiocytes, some bi- or even trinucleate, were prominent and were diffusely scattered in the superficial dermis. Vascular congestion was focal and less intense than at earlier intervals.

**Biopsies in Patients Patch Tested with Other Allergens.**—A total of seven biopsies was examined from sensitized individuals 3 days after patch test with paraphenylenediamine (2), nickel (2), formalin (2), and rubber (1). While the tempo and intensity of these reactions varied somewhat according to the degree of individual sensitivity, they were in general similar to those elicited to urushiol in H.F.D. and described in detail above. There was moreover a good correlation between the gross description and microscopic appearance of the reaction.
Thus, very weak reactions, one each to paraphenylenediamine and formalin, showed no epidermal changes and only a slight perivascular mononuclear cell infiltrate. The remaining five stronger reactions showed the characteristic mononuclear cell and basophil infiltrate with occasional eosinophils; vascular compaction with dermal edema and fibrin deposition; and epidermal edema with spongiosis, vesicle formation, and inflammatory cell infiltration.

A significant proportion of basophils in the reactions of all individuals studied including H.F.D. showed morphologic changes that may represent physiologic degranulation (Figs. 2, 4–6, 13, 14). Many basophils contained clear cytoplasmic vesicles which sometimes encompassed the cell's characteristic metachromatic granules; occasionally these granules appeared pale and enlarged or swollen, and in some cells seemed to be reduced in number. Changes of this type were never observed in CBH reactions in the guinea pig whose basophils have granules with a unique, crystalline structure (3). Metachromatic granules were also occasionally observed in the extracellular space, adjacent to basophilic leukocytes (Fig. 4). Interpretation of these changes must be cautious because basophils are extremely fragile and because similar alterations may be induced by manipulations such as fixation which do not alter or may actually preserve the structure of other cells. Nonetheless, similar events have been described when living, sensitized basophils are exposed to allergen while being followed continuously under the phase-contrast microscope (15).

Control Biopsies.—No significant infiltrate was observed in a 24 hr biopsy from the site of application of vehicle (peanut oil) on the sensitized subject, although, as noted above, a focus of plasma cells was present. Freeze injury to the skin of the sensitized subject caused an acute inflammatory reaction, maximal at about 6 hr and little changed in quality at 48 hr. Microscopic analysis at 6 and at 48 hr revealed necrosis of the epidermis with bulla formation as well as damage to superficial dermal structures including vessels and appendages. A moderate infiltrate of neutrophils was present with fewer lymphocytes and no basophils. Patch test of an unsensitized subject with urushiol led to a minimal focal perivascular infiltrate of lymphocytes containing no basophils or eosinophils at 48 hr.

DISCUSSION

The data presented here indicate that basophilic leukocytes comprise a regular component of the inflammatory infiltrate characteristic of allergic contact dermatitis. Of eight individuals studied, all six that exhibited typical contact reactions had substantial numbers of basophils in their biopsies. Basophils have not been described in previous reports of the histology of contact allergy in man despite the use of electron microscopic methods in some instances (16–22). However, Scandinavian workers (23) have identified these cells in contact reactions in man by analysis of smears of epidermal blister
fluct and skin window cover slips, and our results confirm and extend their observations.

Biopsies of lesions varying in age from $10^{1/2}$ hr to 11 days were available from one individual (H.F.D.) and so it was possible to study the evolution of the cellular infiltrate as it unfolded at successive intervals after patch test. That the results obtained in H.F.D. may be typical of allergic contact dermatitis is suggested by the fact that the gross and microscopic pathology at 3 days after patch test was very similar in H.F.D. and in five other patients developing typical contact reactions to various allergens. In agreement with previous descriptions, the initial dermal lesion consisted of perivascular accumulations of lymphocytes. At 1 day, the absolute number of mononuclear cells had changed little but basophils were now present, accounting for more than 9% of the infiltrate. Over the course of the next days, the absolute number of basophils infiltrating the dermis increased by 20-fold and that of mononuclear cells by 9-fold; at the height of the dermal reaction on day 6, basophils accounted for 16% of the infiltrate and mononuclear cells, 62%. The sequential arrival of lymphocytes and basophils is consistent with the hypothesis that interaction of sensitized lymphocytes with antigen at a local test site is responsible for the attraction of basophils. This mechanism was proposed in animal studies of CBH reactions which could be passively transferred with lymph node cells and which were inhibited by antilymphocyte serum (3, 5, 8). In guinea pigs, however, the skin reactions of CBH evolved too rapidly to ascertain that lymphocytes invariably preceded basophils at the local test site. Eosinophils, rare in the 1st 2 days after patch test, increased greatly both in absolute and relative numbers on day 3 and thereafter accounted for 15–25% of the total infiltrate. The mechanism by which eosinophils are attracted to the lesions of allergic contact dermatitis is unknown, but one or both of the eosinophil-chemotactic substances described in animal systems may be involved (24, 25). Since eosinophils appeared in significant numbers only after the successive earlier arrivals of lymphocytes and basophils, it is possible that eosinophil-chemotaxis is related to the activities of either or both of these cells.

Inflammatory cells in the dermis were arranged in perivascular cuffs, which, at later intervals, came to resemble nodular aggregates, as well as in a more diffuse, intervascular arrangement. This pattern of distribution has significance since the cellular composition of these two zones differed. Cells of the perivascular cuff consisted largely of lymphocytes and other types of mononuclear cells; granulocytes, identified within the lumens and migrating through the walls of these same vessels, did not tend to accumulate locally. By contrast, the intervascular infiltrate was less compact and contained a higher proportion of granulocytes, basophils at early intervals and both basophils and eosinophils at later times, although mononuclear cells and fixed tissue histiocytes were also present. We interpret these results to indicate that the perivascular arrangement of lymphocytes, characteristic of cellular immunity, is not simply a reflection of
the vascular origin of these cells because granulocytes, derived from the same vessels, did not accumulate in the perivascular zones. Rather, we suggest that a fraction of the mononuclear cells (but not of granulocytes) remains adjacent to the vessels from which they have emigrated. The function of the perivascular cuff-cells is unknown but they are ideally situated to regulate the attraction and diapedesis of other cells including monocytes and granulocytes.

A striking feature of all six strongly positive reactions was morphologic alterations in the dermis suggestive of increased vascular permeability, including dermal edema and vascular compaction. Alterations of this sort, which may be elicited by a variety of vasoactive compounds including histamine (14), were evident in H.F.D. at 1 day after patch test and vascular leakage sufficiently severe to result in deposition of fibrin-like material became apparent at 48 hr and persisted through day 6. Thus, in H.F.D. the stigmata of altered vascular permeability correlated roughly with the extent of basophil infiltration in the dermis, and it is possible that histamine released from basophils was responsible for the findings observed. Vascular alterations of this type were not observed in guinea pig reactions of CBH (3). While the clotting system has been implicated in delayed hypersensitivity (26, 27), fibrin deposition has not generally been regarded as a feature of cellular immune reactions.

The cellular infiltrate in the epidermis was derived from a migration of inflammatory cells from the dermis but had a different time course. Infiltrating cells were not observed in the epidermis until 2 days after patch test and were predominantly lymphocytes, supplemented with sequential waves of basophils (16.4% of the infiltrate on day 3) and eosinophils (≥20% of the infiltrate on days 4 and 6). The epidermal infiltrate, maximal at 72 hr, was concentrated in vesicles and was also scattered diffusely throughout the remainder of the edematous epidermis. The striking epidermal-cell changes of contact allergy are the subject of considerable controversy (10, 12-18, 21). Civatte (16) and Polak and Mom (18) claimed that the primary epidermal lesion was intra- and intercellular edema with subsequent infiltration by lymphocytes. Other authors (19, 20) have stated that the lymphocytic infiltration preceded and was responsible for the epidermal edema which developed. Our own data, in general agreement with that of Carr et al. (21), indicate that some degree of intercellular edema antedated intraepidermal infiltration by inflammatory cells; in fact, the slightly edematous epidermis in H.F.D. remained free from cellular infiltration until 48 hr after patch testing. That the epidermal changes observed can be attributed to toxicity of the allergen is unlikely since an unsensitized subject showed no evidence of epidermal edema after 48 hr of contact with the same concentration of urushiol. Even at later intervals it was difficult to interpret the relationship of epidermal edema to cellular infiltration because edema was observed in the presence or absence of inflammatory cells in all cases studied; moreover, in some instances cellular infiltration was accompanied by minimal or no edema. Thus, no close correlation was found between epidermal edema
and infiltration by any of the various types of inflammatory cells. Possibly inflammatory cells in the dermis secrete mediators responsible for the epidermal changes.

In summary, we have differentiated allergic contact dermatitis in man from classic descriptions of delayed hypersensitivity and have associated it with the parallel reactions of cutaneous basophil hypersensitivity in experimental animals. Further studies will be necessary to determine whether other examples of cell mediated hypersensitivity in man, such as allograft rejection and viral and tumor immunity, have a similar morphology. In any event, our data provide additional support for the hypothesis that "cellular immunity" is composed of a heterogeneous group of reactions mediated by lymphocytes which, while morphologically identical by present methods, are functionally disparate (2, 8, 9). The presence of basophils in certain of these reactions provides further evidence for the cooperation between lymphocytes (presumably of thymus origin) and bone marrow cells and suggests the possibility of a heretofore unsuspected form of synergism between cellular immunity and the forms of immediate hypersensitivity mediated by homocytotrophic antibody which is bound to the basophil surface (27).

SUMMARY

A variety of cell-mediated hypersensitivity reactions in experimental animals include a prominent infiltrate of basophilic leukocytes. This form of reactivity has been designated cutaneous basophil hypersensitivity and is favored when sensitization to several types of antigen is accomplished without the use of complete Freund's adjuvant.

A similar type of hypersensitivity response was sought in man using morphologic techniques which permit identification of basophilic leukocytes. Eight individuals with allergic contact dermatitis to a variety of allergens were studied and six of these developed typical contact reactions with erythema, edema, and epidermal vesicles. The microscopic findings in 3-day biopsies from these individuals differed significantly from classic descriptions of tuberculin hypersensitivity and showed, in addition to mononuclear cells and the characteristic epidermal changes, a substantial infiltrate of basophilic leukocytes and evidence of altered vascular permeability with vascular compaction, dermal edema, and fibrin deposition. Serial biopsies from one individual permitted analysis of the microscopic pathology as it unfolded at successive intervals after patch test. The initial lesion consisted of perivascular accumulations of lymphocytes; this was followed by an influx of basophils and, subsequently, of eosinophils.

These findings associate contact allergy in man with the parallel reactions of cutaneous basophil hypersensitivity in animals and provide further evidence for the heterogeneity of the cellular immune response. The data are consistent with the hypothesis that interaction between sensitized lymphocytes and
antigen, at a local test site, is responsible for the attraction of basophils. They also directly implicate the clotting system in delayed-type reactions and suggest the possibility of a synergistic relationship between cellular immunity and reactions mediated by basophil-bound, homocytotropic antibody.

**Note Added in Proof.**—Immunofluorescence studies with Dr. Robert B. Colvin in our laboratory have confirmed the presence of abundant deposits of fibrinogen-fibrin in several forms of delayed-onset cutaneous hypersensitivity in man, including allergic contact dermatitis.

The authors gratefully acknowledge the skilled technical assistance of Misses Blanche Simpson and Eleanor Manseau.

**REFERENCES**

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