

Morphology of Delayed Type Hypersensitivity Reactions in Man

I. Quantitative Description of the Inflammatory Response

HAROLD F. DVORAK, M.D., MARTIN C. MIHM, JR., M.D., ANN M. DVORAK, M.D., RICHARD A. JOHNSON, M.D., ELEANOR J. MANSEAU, B.A., ELLEN MORGAN, B.A.,
AND ROBERT B. COLVIN, M.D.

Departments of Pathology and Dermatology, Massachusetts General Hospital, Boston, Massachusetts 02114, and Department of Pathology, Tufts University Medical School, Boston, Massachusetts 02111

A comprehensive, semiquantitative morphologic study of allergic contact dermatitis and delayed hypersensitivity skin reactions to various allergens and microbial antigens was undertaken on 180 biopsies obtained from 91 volunteers and patients. In addition to the mononuclear cell infiltrates of classic descriptions, a number of important new features were found to characterize these reactions, including infiltration and piecemeal degranulation of basophilic leukocytes; degranulation and replication of fixed tissue mast cells; increased vascular permeability leading to dermal and epidermal edema, vascular compaction, and erythrocyte extravasation; microvascular alterations affecting endothelial cells and pericytes, with compromise of vessel lumina and basement membrane thickening; and activation of the clotting system, with deposition of fibrin in a characteristic intervascular pattern in the reticular dermis. The frequency of these new findings was documented and their evolution was described in volunteers sensitized and serially biopsied following skin testing with dinitrochlorobenzene. In man as in the guinea pig, basophils were more numerous in allergic contact dermatitis (91 per cent of fully developed positive reactions) than in classic delayed hypersensitivity reactions elicited with microbial antigens (61 per cent). The frequency of basophils, however, was regularly lower than in corresponding reactions of cutaneous basophil hypersensitivity in the guinea pig, but fixed tissue mast cells apparently had a more important role in human delayed reactions. These findings are in accord with the view that basophils and mast cells, closely related and functionally similar cells, may supplement or replace each other in delayed reactions elicited in various species. Dermal fibrin deposition, vessel permeability, and the changes affecting the vascular endothelium and pericytes were far more striking than in guinea pig reactions and were nearly always present in mature delayed reactions in man. The new morphologic findings reported here alter and complicate current conceptions of delayed hypersensitivity. We have advanced a tentative model which incorporates these new findings in the pathogenesis of delayed reactions.

Additional key words: Lymphocytes, Basophils, Mast cells, Fibrin, Clotting, Degranulation, Endothelium, Pericytes.

Cell-mediated hypersensitivity has generally been regarded as a homogeneous group of reactions characterized by perivascular infiltrates of mononuclear cells (2, 4, 6, 7, 42, 45, 47). Recently, however, employing improved light microscopic techniques as well as functional immunologic criteria, we have established that at least two discrete forms of cellular immune response exist in experimental animals (3, 17-23, 26, 44, 51). These are (1) classic delayed hypersensitivity, an evolving, long lasting state of hypersensitivity mediated by lymphocytes with relatively high avidity for antigen and characterized by extensive mononuclear cell infiltrates and (2) cutaneous basophil hypersensitivity (CBH), a state of reactivity mediated by lymphocytes of relatively

low avidity and characterized by extensive infiltrations of basophilic leukocytes. Generally, animals are primed for delayed hypersensitivity following immunization with antigens incorporated in mycobacterial adjuvants, whereas immunizing regimens which avoid such adjuvants lead to the induction of CBH. Both types of immune reaction are thought to have a similar pathogenesis, involving interaction between appropriately sensitized T lymphocytes (13) and antigen at a local site, followed by an outpouring into the tissues of bone marrow-derived cells, lymphocytes and monocytes in delayed hypersensitivity, and, in CBH, also including abundant basophils. Recognition of CBH as a discrete entity has some significance in that many biologically

important expressions of cellular immunity are characterized by a basophil-rich infiltrate. These include contact allergy, skin allograft and tumor rejection, and viral hypersensitivity (17, 21, 23, 25, 26).

In view of these animal studies, we wished to determine whether a similar heterogeneity of the cellular immune response could be demonstrated in man. A preliminary investigation involving a small group of patients with naturally acquired allergic contact dermatitis suggested that basophils did, in fact, participate in human delayed type reactions (25). However, a number of additional and quite unexpected features were observed as well, including piecemeal basophil and mast cell degranulation; striking microvascular changes affecting endothelial cells, the basement membrane, and pericytes; and deposition of fibrin-like deposits in the dermis (11, 25). Evaluation of these findings, and of their possible significance in the pathogenesis of delayed hypersensitivity, required the study of a much larger group of patients, including some who had been sensitized under defined conditions and who were biopsied sequentially after skin testing so that the morphologic evolution of delayed reactions could be determined. In addition, it was necessary to study patients tested intradermally with classic bacterial, fungal, and viral antigens. We here present a comprehensive morphologic description of delayed type hypersensitivity skin reactions as they occur in man, employing 1- μ m. Epon-embedded sections studied by light microscopy, immunofluorescence, and electron microscopy.

MATERIALS AND METHODS

IMMUNIZATION AND SKIN TESTS

One hundred eighty biopsies from 91 volunteers and patients were studied:

1. Twenty-one normal male volunteers, ages 18 to 51, were sensitized to dinitrochlorobenzene (DNCB) by application of 0.1 ml. of a 10 per cent acetone solution to the volar surface of the forearm. Twelve to 14 days later, contact reactions were elicited by application of 25 μ l. of 0.1 or 0.2 per cent DNCB in acetone or methyl Cellosolve to the lateral aspect of the upper arms. As many as six separate test sites were applied simultaneously to facilitate sequential biopsy of undisturbed lesions. Reactions were read and biopsied at various intervals from 4 hours to 13 days. As a control, each subject received a skin test at the time of sensitization which was biopsied at 48 hours, well before active immunity had developed.

2. Spontaneously acquired contact reactivity to various environmental allergens was studied in 30 male and female outpatients, ages 17 to 56, of Dr. Normand Olivier. These patients had symptomatic contact dermatitis to a variety of allergens but were otherwise healthy and receiving no known medication at the time of testing. Allergens included nickel (seven patients); formaldehyde (six patients); 1,4-benzenediamine (three patients); lanolin (two patients); epoxy (two patients); rubber (two patients); copper (two patients); and kerosene, acrylic, Urushiol, olive oil, and commercial skin creams (Medicreme, Neutraderm) were studied in one patient each. Each received a skin test of the appropriate

allergen applied as a patch test in the standard concentrations recommended by Fisher (28). Patches were removed at 48 hours, and reaction sites were scored and biopsied at 72 hours.

3. Forty male and female volunteers, ages 20 to 49, were tested intradermally with four standard skin test antigens, including Old Tuberculin (1/1000), *Candida albicans* (1/1000), streptokinase (5 units)/streptodornase (1.25 units), and mumps (0.1 mg.). Tests were read at 4, 24, and 48 hours. Biopsies were taken at 48 hours from 36 clinically positive reactions (>10 mm. of erythema and induration), from five negative intradermal test sites, from four sites that had received injections of isotonic saline, and from seven untreated sites.

The 21 volunteers sensitized to DNCB, 12 of the patients with contact reactivity to environmental allergens, and 14 of the volunteers tested with microbial antigens were subjects in an earlier report describing the deposition of fibrin in delayed type hypersensitivity reactions (11).

Delayed reactions elicited by epicutaneous application of contact allergens were scored using the following scale: 0, no reaction; $\pm$, patchy erythema; 1+, confluent erythema; 2+, erythema and induration; 3+, erythema, induration, and vesiculation; 4+, bullae in addition to the other findings. In the case of delayed hypersensitivity elicited by intradermal injection of protein antigens, the diameters of erythema and the extent of induration were measured and reactions were classified as follows: 0, no reaction; $\pm$, <10 mm. of erythema and induration; 1+/2+, 10 to 15 mm. of erythema and induration; 3+/4+, >15 mm. of erythema and induration.

BIOPSY AND PROCESSING OF TISSUE FOR MICROSCOPIC EXAMINATION

Biopsies were obtained with a Keyes 4-mm. cutaneous punch, using field anesthesia with 1 or 2 per cent Xylocaine without epinephrine. Xylocaine was injected intradermally around and subcutaneously below, but never directly into, the site to be studied. Hence, artifacts due to needle trauma or to anesthetic were avoided. Tissue for light or electron microscopy was placed in a pool of fixative composed of 2 per cent paraformaldehyde, 2.5 per cent glutaraldehyde, and 0.025 per cent CaCl_2 in 0.1 M cacodylate buffer, pH 7.4 (31). This fixative has been shown to preserve human basophils and other tissue structures more reliably than some 20 others tested (25). After appropriate trimming, tissues were allowed to fix for 5 hours at room temperature and then were transferred to cold 0.1 M cacodylate buffer. For preparation of 1- μ m. sections, tissues were postfixed in osmium tetroxide, dehydrated, and embedded in Epon (22). Sections were stained with Giemsa's reagent and were evaluated under the light microscope, by at least two observers, at magnifications up to $\times 1000$.

For electron microscopy, tissues were processed in duplicate according to two protocols. For routine work, specimens were postfixed for 2 hours in cold 2 per cent s-collidine-buffered osmium tetroxide, dehydrated in a graded series of alcohol, and were embedded in a propylene oxide-TAAB (TAAB Laboratories, Reading, England) sequence. Some specimens were stained *en*

bloc with uranyl acetate before dehydration (27, 31). Other specimens were processed according to a new osmium-potassium ferrocyanide (OPF) schedule (15) involving postfixation for 2 hours at 4° C. in 2 per cent aqueous osmium tetroxide and 1.5 per cent potassium ferrocyanide in 0.1 M sodium phosphate buffer (pH 6.0) before processing as above. Sections were cut with diamond knives, lightly stained with lead citrate, and studied in a Philips 300 electron microscope.

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The unfixed half of 97 biopsies was embedded in 7.5 per cent gelatin and frozen in a Dry Ice-acetone slurry. Cryostat sections were cut, washed in phosphate buffered saline, and stained for 30 minutes with monospecific fluoresceinated goat or rabbit antiserum (Cappel Laboratories, Downingtown, Pennsylvania) to human fibrinogen/fibrin, IgG, IgM, IgA, IgE, β_{1c}/β_{1s} (C'3) and albumin (human serum albumin) diluted 1:4 or 1:8 as previously described (11). Because antifibrinogen antibodies have specificity for fibrinogen, fibrin, and certain large fibrinogen and fibrin fragments, the antigens detected with this antiserum are collectively termed Fib, even though, as discussed below, fibrillar deposits in tissue that bind this antiserum certainly represent fibrin. After washing in phosphate-buffered saline, sections were mounted in Elvanol (E. I. duPont de Nemours and Company, Wilmington, Delaware) and examined in a Zeiss dark-field fluorescence microscope (Carl Zeiss, Inc., New York, New York) equipped with a UG3 excitor filter and a 44 barrier filter to provide optimal color contrast between the green fluorescence of fluorescein and the blue autofluorescence of the dermal elastic fibers. The extent and over-all intensity of Fib staining was graded 0 to 4+. Small but definite focal deposits were scored +; intermediate deposits, 1+ or 2+; and extensive, intense deposits 3+ or 4+. The activity and specificity of the fluoresceinated antisera were confirmed by double gel diffusion, immunoelectrophoresis, and inhibition by unlabeled specific antisera. Further, a washed fibrin clot, made from bovine thrombin-clotted human fibrinogen (Miles Laboratories, Inc., Kankakee, Illinois), removed all activity from the rabbit anti-Fib antiserum but did not diminish the activity of the anti-IgG antiserum.

SCORING OF LIGHT MICROSCOPIC SECTIONS

Sections were evaluated semiquantitatively with regard to some 20 variables on a scale of 0 to 4+ (Tables 1 to 4). For each variable, a score of + indicates trace pathology, focally distributed in the dermis or epidermis. Intermediate changes were scored 1+ or 2+ and extensive changes 3+ or 4+.

It is possible to measure the extent of inflammatory cell infiltration in 1- μ m. tissue sections by a fully quantitative method which determines both the absolute number and relative frequency of each cell type (25). However, such detailed counts are impractical when a very large number of lesions is studied. In Table 1 is provided a rough guide for estimating the absolute frequencies (cells per linear millimeter of surface in 1- μ m. sections) of infiltrating cells from the 0 to 4+

TABLE 1. RELATIONSHIP BETWEEN 0 TO 4+ SCORING SYSTEM EMPLOYED HERE AND ABSOLUTE FREQUENCIES OF INFILTRATING CELLS

Infiltrate	Scoring system			
	0	1+	2+	3+ 4+
		cells linear mm. surface		
Dermal				
Mononuclear cells	0	100	100-400	400-1000
Granulocytes	0	10	10-100	100-300
Epidermal				
Mononuclear cells	0	10	10-30	30-50
Granulocytes	0	1	1-5	5-10

scoring system employed here.

RESULTS

ALLERGIC CONTACT DERMATITIS TO DNCB

GROSS APPEARANCE.

All of 17 individuals sensitized with 10 per cent DNCB developed typical, delayed onset, erythematous and vesicular lesions of contact allergy when tested 12 to 14 days later (Table 2). Depending on the degree of individual sensitivity, erythema became evident as early as 4 hours after testing, and by 24 hours all subjects exhibited induration and seven of 13 patients exhibited vesiculation as well. Reactions continued to evolve in intensity over the next 2 days, and at 72 hours all of the 17 men tested had reactions of 3+ or greater. Reactions persisted at nearly maximal intensity for a period of several days and subsided only gradually. By 11 to 13 days they consisted of pink- to fawn-colored, irregularly raised, slightly indurated lesions with scaling.

LIGHT AND ELECTRON MICROSCOPIC PATHOLOGY OF THE DERMIS

Inflammatory Cell Infiltrate.

Mononuclear Cells. In accord with previous descriptions of delayed hypersensitivity in animals and man (4, 18, 25, 45, 47), the initial microscopic finding consisted of a perivenous and perivenular infiltrate of small and medium sized lymphocytes, evident as early as 4 to 8 hours after skin testing in highly sensitized individuals and principally involving vessels in the superficial vascular plexus at the junction of the papillary and reticular dermis. Over the course of the next hours and days, this infiltrate increased progressively in intensity, reaching a maximum on day 3 (Fig. 1). In mature reactions a significant proportion of these cells appeared activated in that they were larger than small lymphocytes, with deeply basophilic and more abundant cytoplasm (Figs. 2 and 5B). In the electron microscope they contained numerous ribosomes and polysomes and had a relatively open nucleus with one or more prominent nucleoli. However, fully developed immunoblasts of the type developing in lymph nodes draining antigen depots or in mitogen-stimulated cultures were rare (13). Although densely packed, the cells comprising lymphoid cuffs did not form specialized associations or attachments with each other.

After reaching a maximum at about 3 days the

TABLE 2. GROSS APPEARANCE AND MICROSCOPIC FEATURES OF ALLERGIC CONTACT DERMATITIS REACTIONS IN INDIVIDUALS SENSITIZED WITH DNCB AND TESTED EPICUTANEOUSLY AT THE TIME OF IMMUNIZATION AND AT 12 TO 14 DAYS LATER, FOLLOWING THE ONSET OF ACTIVE IMMUNITY*

	Score	48 hr.	Interval after skin test					
			4-8 hr.	1 day	2 days	3 days	6 days	11-13 days
Gross appearance	3+/4+	0	0	7	13	17	9	0
	1+/2+	4	3	6	1	0	5	15
	+	0	1	0	0	0	0	1
	0	12	12	0	0	0	0	0
Microscopic features								
Dermis								
Cellular infiltrate								
Mononuclear cells								
Perivascular	3+/4+	0	1	3	8	15	7	3
	1+/2+	7	4	9	6	2	7	13
	+	4	7	0	0	0	0	0
	0	5	4	1	0	0	0	0
Intervascular	3+/4+	0	0	0	3	4	5	0
	1+/2+	0	0	7	7	11	5	5
	+	1	1	3	2	2	4	7
	0	15	15	3	2	0	0	4
Basophils	3+/4+	0	1	3	3	6	4	0
	1+/2+	1	0	2	3	3	2	2
	+	0	1	4	6	6	5	8
	0	15	14	4	2	2	3	6
Eosinophils	3+/4+	0	0	1	0	3	2	1
	1+/2+	0	0	0	2	1	1	2
	+	0	1	1	6	3	5	4
	0	16	15	11	6	10	6	9
Neutrophils	3+/4+	0	0	1	0	2	0	0
	1+/2+	0	0	0	0	0	0	1
	+	0	0	7	4	5	3	0
	0	16	16	5	10	10	11	15
Plasma cells	3+/4+	0	0	0	0	0	0	0
	1+/2+	1	0	0	1	0	0	0
	+	0	0	5	5	5	4	2
	0	15	16	8	8	12	10	14
Vascular permeability changes								
Edema	3+/4+	0	0	1	4	10	6	1
	1+/2+	2	2	8	8	7	8	6
	+	2	6	4	2	0	0	5
	0	12	8	0	0	0	0	4
Compaction	3+/4+	0	1	1	4	4	1	1
	1+/2+	1	2	2	4	5	2	0
	+	0	3	1	0	3	2	3
	0	15	10	9	6	5	9	12
Red cell extravasation	3+/4+	0	0	0	1	6	5	1
	1+/2+	1	1	5	6	5	5	2
	+	0	1	2	2	3	1	5
	0	15	14	6	5	3	3	8
Fibrin deposits								
1- μ m. section	3+/4+	0	0	3	7	11	5	2
	1+/2+	0	0	3	6	5	5	4
	+	0	1	2	0	1	3	4
	0	16	15	5	1	0	1	6
Fluorescent antibody	3+/4+	0	0	7	5	6	2	0
	1+/2+	0	1	3	0	2	3	5
	+	0	6	2	0	0	0	1
	0	9	5	1	0	0	0	2
Microvascular changes	3+/4+	0	1	4	11	15	13	9
	1+/2+	1	2	6	2	1	1	6
	+	3	0	1	1	1	0	1
	0	12	13	2	0	0	0	0
Epidermis								
Epithelial changes								
Hyperkeratosis	3+/4+	0	0	0	0	0	2	4
	1+/2+	0	0	5	8	8	8	11
	+	1	0	1	1	3	1	1
	0	15	16	7	5	6	3	0

TABLE 2—Continued

Microscopic features (cont'd.)	Score	Interval after skin test						
		48 hr.	4-8 hr.	1 day	2 days	3 days	6 days	11-13 days
Necrosis	3+/4+	0	0	1	1	3	0	0
	1+/2+	1	0	0	1	2	0	0
	.	2	3	6	9	6	6	8
	0	13	13	6	3	6	8	8
Hyperplasia	3+/4+	0	0	0	0	1	1	3
	1+/2+	0	0	0	0	6	7	10
	.	0	0	0	0	0	2	1
	0	16	16	13	14	10	4	2
Intercellular edema	3+/4+	0	1	4	10	13	8	2
	1+/2+	2	3	7	4	4	6	11
	.	6	2	1	0	0	0	2
	0	8	10	1	0	0	0	1
Vesicles/bullae	3+/4+	0	0	2	7	6	5	1
	1+/2+	0	0	0	2	6	2	0
	.	0	0	2	1	1	1	0
	0	16	16	9	4	4	6	15
Cellular infiltrate Mononuclear cells	3+/4+	0	0	3	3	6	4	0
	1+/2+	1	3	5	9	10	7	5
	.	3	0	2	1	1	2	9
	0	12	13	3	1	0	1	2
Basophils	3+/4+	0	0	0	1	2	0	0
	1+/2+	0	1	2	3	1	2	0
	.	0	0	0	0	4	0	0
	0	16	15	11	10	10	12	16
Eosinophils	3+/4+	0	0	0	0	2	0	0
	1+/2+	0	0	0	0	0	1	0
	.	0	0	1	3	2	1	0
	0	16	16	12	11	13	12	16
Neutrophils	3+/4+	0	0	0	0	0	0	0
	1+/2+	0	0	1	0	1	1	0
	.	0	0	0	2	1	0	0
	0	16	16	12	12	15	13	16

Individuals were sensitized and skin-tested with DNCB as described in "Materials and Methods."

Interpretation of gross and microscopic scoring is described in "Materials and Methods."

Forty-eight-hour reaction to DNCB test applied at time of initial sensitization.

perivenular aggregates subsided gradually over the course of the next 2 weeks (Fig. 3; Table 2). At their height, they frequently assumed a nodular, pseudo-granulomatous appearance. This pattern was accentuated by the altered appearance of the venules and veins about which these cuffs formed (Figs. 2 and 3). Such vessels exhibited hypertrophy and hyperplasia of endothelial cells and pericytes, obliteration of vessel lumina, greatly thickened basement membranes, and blurring of vascular margins; individual endothelial cell necrosis was also observed. These changes, not previously described, were a regular feature of contact dermatitis and of classic, delayed hypersensitivity reactions in man (Tables 2 to 4) and on occasion interfered with light microscopic identification of affected small dermal vessels (Fig. 2B).

In addition to forming cuffs about venules and veins, lymphocytes and variable numbers of monocytes-macrophages were noted distinctly away from the vessels in intervascular portions of the dermis and the epidermis. The intervascular component of the infiltrate appeared later and was invariably less intense and compact than that surrounding venules and veins in the same biopsy;

it was entirely absent in some weak reactions (Table 2). The intervascular infiltrate reached a maximum on days 3 to 6 and by days 11 to 13 had subsided more completely than the perivascular infiltrate.

At 6 days and later, dermal fibroblasts appeared strikingly enlarged and activated, with large open nuclei and prominent nucleoli (Fig. 4). In the electron microscope the abundant cytoplasm exhibited numerous, dilated cisternae of rough endoplasmic reticulum filled with moderately dense, flocculent material. These cells were commonly observed in mitosis.

Basophilic Leukocytes. Basophilic leukocytes were the first, and frequently the only, granulocyte to participate in the reactions of allergic contact dermatitis (Fig. 5). They were occasionally seen in vessel lumina and in the developing perivascular lymphocyte cuffs at 4 to 8 hours after testing in highly sensitive individuals; they were more commonly evident at 24 hours and reached peak frequency on day 3, being present in 15 of 17 biopsies (88 per cent) studied and abundant ($\geq 3+$) in six of these (35 per cent) (Table 2). Although basophils were generally absent from very weak reactions, their frequency did not exactly parallel reaction intensity, either as measured by

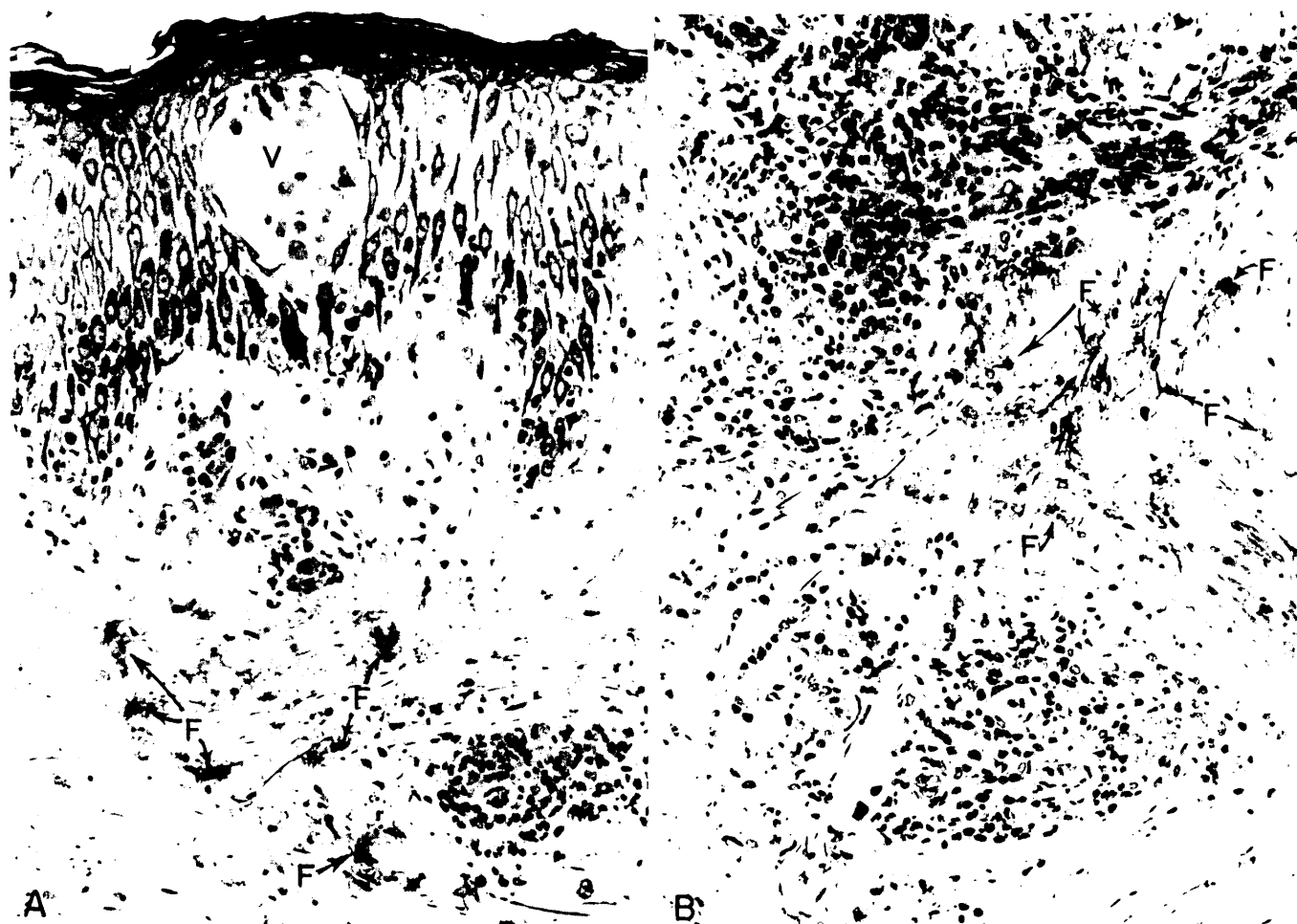


FIG. 1. Photomicrographs of 1- μ m. Epon-embedded sections of 72-hour allergic contact reaction to DNCB in a specifically sensitized subject. In A note spongiotic epidermal vesicle (V) with adjacent severe intercellular edema overlying an edematous papillary dermis. A perivascular inflammatory cell infiltrate is present in the superficial

dermis of A and in the mid-dermis of B. In both A and B fibrin (F) lies between collagen bundles and along elastic fibers in the intervascular dermis, removed from inflammatory cell infiltrates. Giemsa's reagent; Figure 1A, $\times 270$; B, $\times 425$.



FIG. 2. Cellular infiltrate surrounding altered small vessels in a 48-hour delayed hypersensitivity reaction to streptokinase/streptodornase (A) and an 11-day contact reaction to DNCB (B). The venule in A has a reduced lumen (L), activated pericytes (P), and variably damaged endothelial cells (E). The basement membrane is irregularly thickened. In B, vessel disruption has proceeded further so that no discrete vascular

channel is visible; arrows point to haphazardly arranged activated pericytes or endothelial cells. Activated lymphocytes predominate in A and are present in B along with other mononuclear cells. Degranulated basophils (B) are present in both parts; variably granulated mast cells (M) are seen in B. 1- μ m. Epon section. Giemsa's reagent; Figure 2A, $\times 840$; B, $\times 780$.

gross scoring or by the extent of the mononuclear cell infiltrate. Moreover, occasional, fully developed reac-

tions, typical of allergic contact dermatitis in every respect, had no (two of 17) or only few (six of 17)

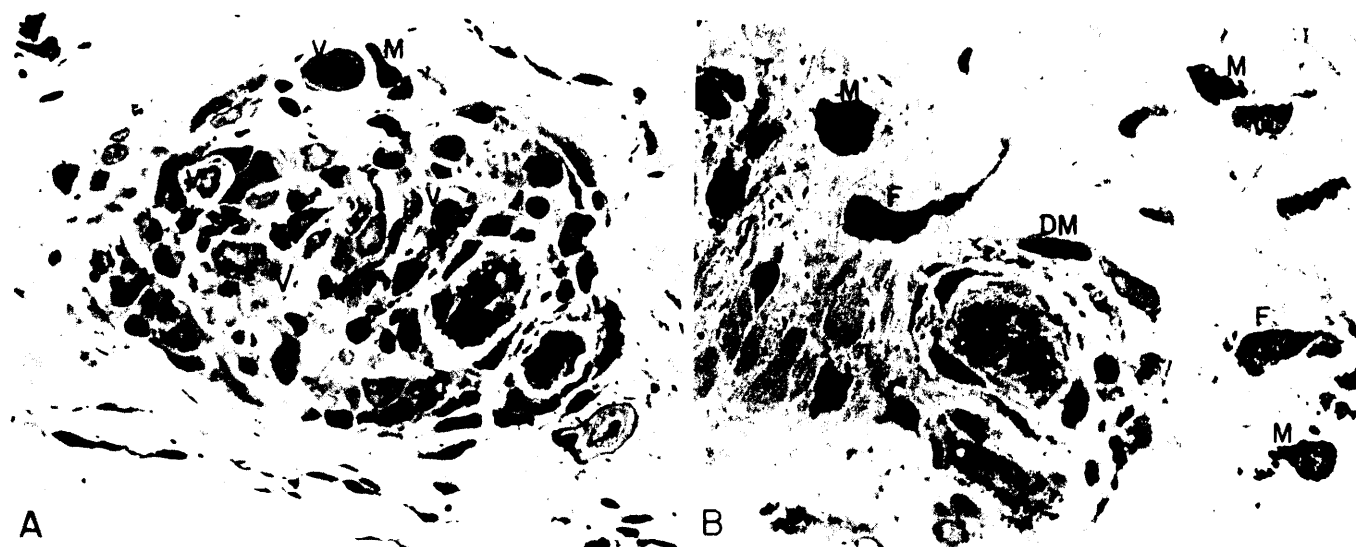


FIG. 3. Vessel changes in 11- to 13-day DNCB reactions. Venules (V) with markedly narrowed or obliterated lumina are surrounded by greatly thickened basement membranes and prominent pericytes. Note the contrast between adjacent activated and damaged endothelial cells

in venule in B. A slight perivascular lymphocyte infiltrate persists in A. Variably granulated mast cells (M), a nearly degranulated mast cell (DM), and activated fibroblasts (F) are present. 1- μ m. Epon section. Giemsa's reagent: Figure 3A, $\times 800$; B, $\times 780$.

TABLE 3. GROSS APPEARANCE AND MICROSCOPIC FEATURES OF STRONG (18) AND WEAK (12) ALLERGIC CONTACT DERMATITIS REACTIONS TO ENVIRONMENTAL ALLERGENS AT 3 DAYS AFTER PATCH TEST*

	Strong reactions				Weak reactions			
	0	+	1-2+	3-4+	0	+	1-2+	3-4+
Gross appearance	0	0	4	14	0	4	8	0
Microscopic features								
Dermis								
Cellular infiltrate								
Mononuclear cells								
Perivascular	0	0	8	10	0	6	6	0
Intervascular	0	5	12	1	7	5	0	0
Basophils	1	5	4	8	11	1	0	0
Eosinophils	8	2	6	2	11	1	0	0
Neutrophils	12	5	1	0	12	0	0	0
Plasma Cells	15	3	0	0	11	1	0	0
Vascular permeability changes								
Edema	0	0	14	4	4	6	2	0
Compaction	7	1	7	3	8	0	4	0
Red cell extravasation	9	2	7	0	12	0	0	0
Fibrin deposits								
1- μ m. section	0	3	6	9	9	1	2	0
Fluorescent antibody								
Papillary dermis	2	3	1	2	2	1	1	0
Reticular dermis	0	1	4	3	2	2	0	0
Microvascular changes	1	1	6	10	6	3	2	1
Epidermis								
Hyperkeratosis	11	1	6	0	8	0	4	0
Necrosis	9	6	3	0	7	2	3	0
Hyperplasia	12	2	4	0	9	1	1	1
Intercellular edema	0	1	8	9	1	4	6	1
Vesicles/bullae	3	3	6	6	11	1	0	0
Cellular infiltrate								
Mononuclear cells	1	4	8	5	5	5	2	0
Basophils	10	2	2	4	12	0	0	0
Eosinophils	13	4	1	0	12	0	0	0
Neutrophils	14	3	0	1	12	0	0	0

* Strong reactions had 1+ or more dermal perivascular lymphocyte cuffing and an intervascular inflammatory cell infiltrate $\geq 1+$. Scoring same as in Table 2.

TABLE 4. GROSS APPEARANCE AND MICROSCOPIC FEATURES OF 36 "POSITIVE" AND EIGHT "NEGATIVE" DELAYED REACTIONS TO INTRADERMALLY INJECTED PROTEIN ANTIGENS AT 48 HOURS

	Positive reactions				Negative reactions			
	0	+	1+/2+	3+/4+	0	+	1+/2+	3+/4+
Gross appearance	0	0	23	13	3	5	0	0
Microscopic features								
Dermis								
Cellular infiltrate								
Mononuclear cells								
Perivascular	0	0	16	20	0	1	4	3
Intervascular	2	10	20	4	2	3	0	3
Basophils	14	8	7	7	4	4	0	0
Eosinophils	21	12	1	2	5	1	1	1
Neutrophils	15	11	7	3	4	1	2	1
Plasma Cells	26	10	0	0	6	2	0	0
Vascular permeability changes								
Edema	2	3	21	10	3	3	1	1
Compaction	21	3	8	4	5	3	0	0
Red cell extravasation	14	9	12	1	5	2	1	0
Fibrin deposits								
1- μ m. section	1	8	8	19	3	1	2	2
Fluorescent antibody								
Papillary dermis	13	5	2	0	5	0	0	0
Reticular dermis	0	0	10	10	3	0	2	0
Microvascular changes	2	4	8	22	3	1	3	1
Epidermis								
Hyperkeratosis	24	3	9	0	7	1	0	0
Necrosis	26	8	2	0	7	1	0	0
Hyperplasia	30	2	4	0	7	0	1	0
Intercellular edema	1	13	18	4	2	2	4	0
Vesicles/bullae	30	5	1	0	8	0	0	0
Cellular infiltrate								
Mononuclear cells	9	15	10	2	4	4	0	0
Basophils	36	0	0	0	8	0	0	0
Eosinophils	36	0	0	0	8	0	0	0
Neutrophils	31	1	4	0	8	0	0	0

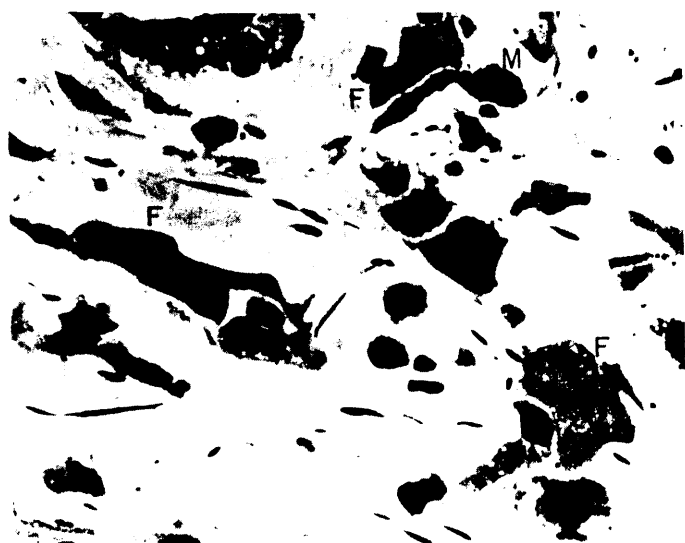


FIG. 4. Activated fibroblasts (F) with abundant cytoplasm, large nuclei, and prominent nucleoli in the intervascular dermis of a 6-day DNCB reaction. A mild lymphocyte infiltrate is also present. M, Mast cell. 1- μ m. Epon section. Geimsa's reagent; $\times 800$.

basophils at the height of reactivity (Table 2).

Basophils were initially localized to the perivascular lymphoid cuffs. However, they and other granulocytes

did not remain in the vicinity of vessels, and at 24 hours and at later intervals the great majority was found in the intervascular dermis and infiltrating into the epidermis. The distribution of basophils in the intervascular dermis was apparently random. No specific associations were detected between basophils and any other cell or cutaneous structure except that basophils were sometimes observed within the perineurium of myelinated nerves of the mid- to deep dermis (Fig. 5C). Although such invasion was observed infrequently, the sampling of nerves in 1- μ m. sections was sufficiently limited (only a few cross-cuts per section) to leave open the possibility that basophil infiltration of a given nerve, at some point along its course, was relatively common. Mast cells were, on occasion, also detected within nerve sheaths, a finding that has been described in normal nerves (39).

Basophils appeared as polymorphonuclear cells with prominent, brightly metachromatic cytoplasmic granules. The connections between nuclear lobes were much thicker than is the case with neutrophils. As a result, in favorable planes of section the nucleus assumed an "S" or "J" shape which we have not observed in other granulocytes; this nuclear configuration was useful in the identification of degranulated basophils (see below). The magenta- to light pink-staining, oval to round granules were scattered in the cytoplasm and were less

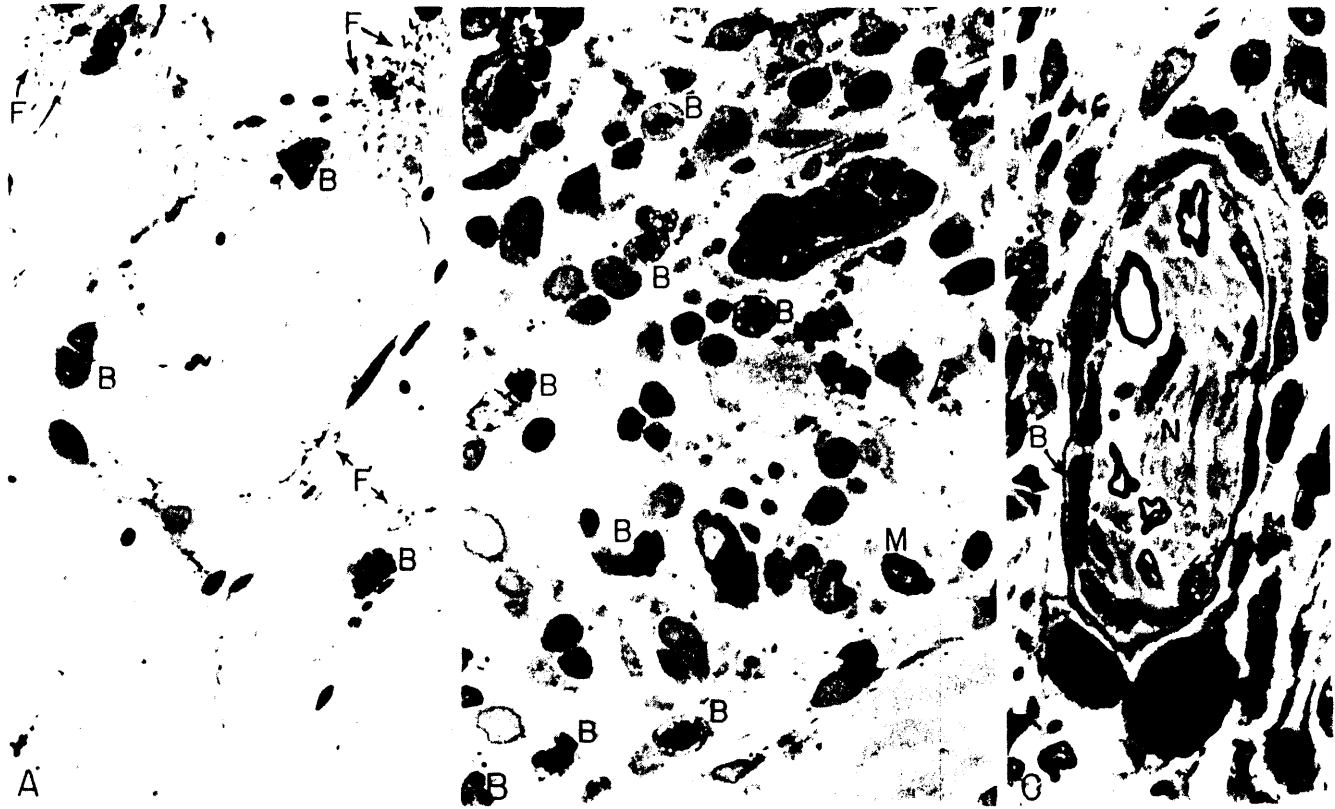


FIG. 5. Basophils (*B*) in delayed hypersensitivity reactions. In *A*, a 72-hour contact reaction to DNCB, three fully granulated basophils are noted in the intervascular dermis. Lacy strands of fibrin (*F*) are interspersed between collagen bundles, distinctly removed from basophils. In *B*, an 11-day contact reaction to DNCB, numerous basophils in varying stages of degranulation are disposed, along with mononuclear cells, about a venule (*V*) with strikingly activated endothelial cells. The nu-

clei of some degranulated basophils appear dark in this print but are not pyknotic. A mast cell (*M*) with an activated nucleus and greatly increased nuclear/cytoplasmic ratio is also present. A basophil (*B*) within the perineurium of a myelinated nerve (*N*) of the deep reticular dermis is identified in *C*. 1- μ m. Epon section, Giemsa's reagent; Figure 5*A* and *B*, $\times 840$; *C*, $\times 940$.

closely packed than are the cytoplasmic granules of eosinophils or mast cells. Clear vacuoles, thought to represent granules which had discharged their contents, were sometimes observed in the cytoplasm.

In the electron microscope basophils appeared as mature granulocytes the cytoplasm of which contained glycogen particles and prominent characteristic granules (Figs. 6 to 8) (53). Granules were membrane-bound structures measuring up to about 1.2 μ m, in largest dimension and were composed of dense particles which varied in size from 113 to 260 Å. Individual granules exhibited variation in electron opacity (Fig. 8), a reflection of variably dense packing of particles within different granules. Some granules were nearly or entirely empty and appeared as membrane-bound vacuoles containing only occasional residual granule or glycogen particles (Figs. 6 and 7). Others exhibited myelin figure-like membrane accumulations (Figs. 7 and 8).

An additional feature of human basophils was the presence in the cytoplasm of a rich supply of 500- to 700-Å membrane-bound vesicles (Fig. 7), similar to those that we have described in guinea pig basophils (14). These vesicles were indistinguishable from pinocytotic vesicles except that some were closely associated with the cytoplasmic granules or were in open communication with them; rarely, vesicles were found within granules (Fig. 8). Some vesicles contained particulate material

similar to that comprising the cytoplasmic granules (Fig. 7). The gradual loss of basophil granule substance occurring in the course of allergic contact dermatitis and its association with cytoplasmic microvesicles has led us to propose a new vesicular transport model for basophil degranulation (16). In this model basophil granules discharge their contents piecemeal by means of microvesicles which bud from the granule membrane and are transported to the cell surface, where they fuse with the plasma membrane and discharge their contents.

Eosinophils. Eosinophils were less numerous than basophils in the reactions of allergic contact dermatitis, were found in a smaller fraction of reactions, and appeared after basophils. Eosinophils were infrequent before day 3, and only on day 6 were even a few present in a majority of reactions. In rare instances, however, eosinophils were very numerous (Fig. 9); in such biopsies (three of 17 on day 3; two of 14 on day 6) they were more frequent than basophils, accounting for 20 to 25 per cent of the total dermal cellular infiltrate (frequencies up to 300 per linear mm. of surface). In such reactions it was not unusual to observe large numbers of eosinophil granules lying free in the dermis (Fig. 9).

Neutrophils. Neutrophils were infrequent at all intervals studied. They were never a part of the earliest cellular infiltrate and were entirely absent from the dermis (except within vessel lumina) in a majority of

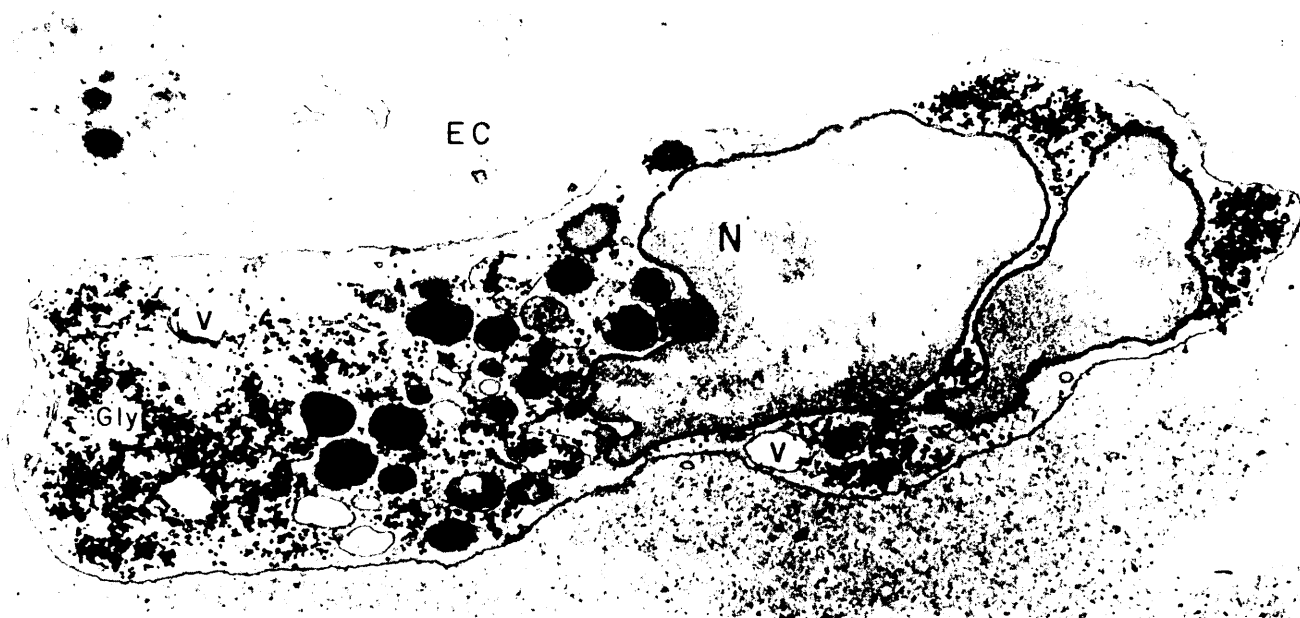


FIG. 6. Electron micrograph showing basophil within the protein-rich epidermal vesicle fluid in a 3-day lesion of allergic contact dermatitis to Urushiol. This elongate cell has a poly-lobed nucleus (N), cytoplasmic accumulations of glycogen particles (Gly), numerous

specific granules, and several vacuoles (V), presumably granules which have lost their contents, and vesicles. The protein-rich extracellular edema fluid (EC) of the intraepidermal vesicle is filled with OPF-positive particles. OPF; $\times 12,000$.

biopsies studied at all intervals except 24 hours. In the rare, unusually intense reaction characterized by extensive necrosis, neutrophils were present in large numbers (four of 106 biopsies), and many of them were dead by morphologic criteria.

Plasma Cells. Never a prominent feature, plasma cells were found in the perivascular lymphocytic cuffs in trace frequency in a minority of reactions, even at 11 to 13 days.

Mast Cells. In fully developed reactions, mast cells of the papillary and superficial reticular dermis exhibited variably reduced numbers of cytoplasmic granules (Figs. 2B, 3B, and 10); moreover, remaining granules frequently appeared smaller than normal and stained with reduced intensity. These changes were subtle and always much less extensive than those observed in cutaneous anaphylaxis of the Prausnitz-Küstner type (24) or in complement-mediated reactions (32); they were, however, obvious when comparisons were made with control biopsies. At late intervals (6 and 11 to 13 days) mast cells were present in increased numbers, and mast cell mitoses were evident.

Consequences of Enhanced Microvascular Permeability.

Edema. Edema was recognized microscopically as decreased over-all staining intensity and spreading or separation of collagen bundles (Figs. 1, 14, and 15). It involved the papillary dermis almost exclusively and extended into the upper reticular dermis only in the severest reactions. The collagen of the papillary dermis is normally composed of single fibers and small groups of fibers that are loosely arranged and irregularly interlaced (52) (Fig. 15A). Smaller fibrils and anchoring fibrils are also present. With edema formation this pattern disappeared, and collagen fibers became diffi-

cult to identify, although stretched, vertically oriented, anchoring fibrils remained visible (Fig. 15).

Trace or moderate papillary dermal edema was evident in one-half of the subjects at 4 to 8 hours after test with DNCB and was well developed in all by 24 hours. Edema continued to develop over the next 2 days, reached a peak at 72 hours, and slowly regressed thereafter. Perceptible edema persisted in 12 of 16 subjects at 11 to 13 days.

Compaction. The microvasculature of the papillary and reticular dermis frequently exhibited extreme congestion or compaction with vascular dilation, loss of plasma, and tight intravascular packing of red blood cells. Erythrocytes trapped within such vessels lost their normally discoid shape and assumed a polygonal configuration. Although a common feature of allergic contact dermatitis, compaction was less constant than edema (Table 2). The vessels affected were principally the capillaries and small venules draining the papillary dermis and oriented at right angles to the skin surface; these vessels were not enveloped by cuffs of inflammatory cells. Occasionally, larger vessels of the superficial vascular plexus at the junction of the papillary and reticular dermis were involved as well. Examination of compacted and other superficial dermal venules in the electron microscope often revealed large gaps between endothelial cells through which plasma extravasated into the perivascular space. Similar changes may be induced with a variety of vasoactive materials, including histamine, all of which dilate venules and enhance their permeability so that plasma, but not formed elements, escape (34).

Erythrocyte Extravasation. A less common event than compaction, erythrocyte extravasation involved the same vessels and generally presented as a few scattered red cells in the papillary or upper reticular dermis adjacent to engorged venules.



FIG. 7. Higher magnification electron micrograph of a basophil similar to that in Figure 6. Granules (G) contain variable numbers of particles. G3 has greatly reduced particle content. G1 and G2 and other unlabeled granules contain myelin figure-like intragranular accumulation of membranes which reduplicate the granule membrane or envelop granule particles, or both. Granule G4 exhibits focal complete loss of granule particles associated with several extragranular and intragranular glycogen particles and a vesicular outpouching of the granule mem-

brane. Numerous small vesicles (arrows) at all levels of the cytoplasm contain granule particles; others are apparently empty. One opens to the extracellular space (EC). Several large vacuoles (V), thought to represent granules which have lost their contents, are present. Two are completely empty except for a few residual granule particles; two others contain glycogen particles. The extracellular space consists of epidermal vesicle fluid rich in OPF-positive particles. N, Nucleus; M, mitochondria. OPF: $\times 38,500$.

Fibrin Deposition. Earlier reports (11, 25) involving 47 of the volunteers studied here have established that fibrin deposition is a regular and characteristic feature of delayed type reactions in man. Study of additional subjects has confirmed and extended these observations, and the combined data are presented in Tables 2 to 4. Fibrin was clearly visualized in 1- μ m. Epon-embedded sections as wispy, pale blue-staining, loosely intertwining strands (Figs. 1, 5, and 11). In some instances fibrin subsequently condensed to form a more tightly knit meshwork composed of dense, matted deposits (Fig. 11B). Identification of this material was confirmed in frozen sections by the fluorescent antibody method (Fig. 12). Intensely stained deposits often appeared amorphous under low power but were seen to comprise a delicate fibrillar network at higher magnifications. In the electron microscope this fibrillar material exhibited

a periodicity of 222 Å (Fig. 13), similar to that reported for fibrin (35).

The fibrin observed here was deposited in a characteristic distribution, quite different from that described in antibody-mediated reactions of the Arthus type (37, 40, 41, 48). Thus, fibrin deposits were confined to intervascular portions of the dermis, interspersed between collagen bundles, and frequently coated collagen and elastic fibers (Figs. 11 to 13). Perivascular zones, which contained the highest density of infiltrating cells, were regularly spared, as were blood vessel walls (Figs. 1 and 12). Immunoglobulin and C'3 deposits were not detected in these reactions except in one biopsy (of 94) which had histologic features of a superimposed Arthus component.

Fibrin deposits were clearly related to the inflammatory response and were confined to the upper reticular dermis in allergic contact dermatitis. For reasons that



FIG. 8. Electron micrograph showing two basophil granules at high magnification. The granule in A contains a full complement of typical, dense particles and a focus of closely associated glycogen particles (Gly). Double arrows indicate particle-containing vesicles within the granule. A larger accumulation of membranes is illustrated in the lower portion of the granule. The granule in B has a strikingly depleted particle content which permits visualization of a finely granular matrix. OPF; Figure 8A and B, $\times 75,000$.

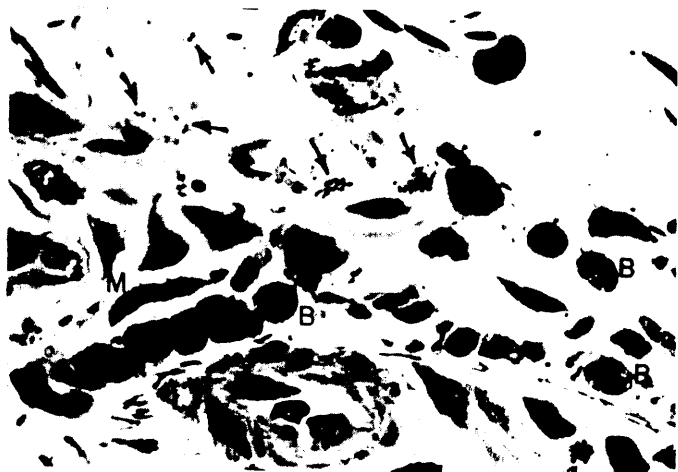


FIG. 9. Unusual finding of numerous eosinophils (multigranulated cells) in a 24-hour contact dermatitis reaction to DNCB. Scattered basophils (B) and a mast cell (M) are also present. Note the free eosinophil granules (arrows) between dermal collagen bundles. 1- μ m. Epon section, Giemsa's reagent; $\times 870$.



FIG. 10. Degranulation of mast cells (M) in delayed hypersensitivity reactions to streptokinase/streptodornase at 48 hours (A) and to DNCB at 11 days (B and C). Cytoplasmic granules persist at the periphery of the mast cells in A and B. Note the striking alteration in the venule (V) in B, with both endothelial cell hypertrophy and damage and apparent luminal obliteration. Only rare granules remain in the cytoplasm of a mast cell in C. 1- μ m. Epon section, Giemsa's reagent; Figure 10A, $\times 940$; B, $\times 870$; C, $\times 800$.

are not apparent, less fibrin was observed in the edematous papillary dermis than in the upper reticular dermis. Fibrin was rarely observed as early as 4 to 8 hours after testing in 1- μ m. sections (one of 16). However, when the more sensitive fluorescent antibody method was employed, a majority of reactions biopsied (seven of 12) exhibited focal Fib deposits at this interval. By 24 hours Fib deposits were much more extensive and could be recognized in eight of 13 biopsies taken for 1- μ m. sections and in 12 of 13 studied by immunofluorescence. At 2, 3, and 6 days, Fib deposition was abundant in all lesions; at 11 to 13 days, Fib continued to be present in a majority of biopsies but in reduced amount. Fib was absent from nine negative control patch test sites.

LIGHT AND ELECTRON MICROSCOPIC PATHOLOGY OF THE EPIDERMIS.

Epithelial Alterations. Epidermal alterations began as focal zones of intercellular edema involving primarily the malpighian and, to a lesser extent, the basal layers of the epidermis (Fig. 14). The corneal and granular layers

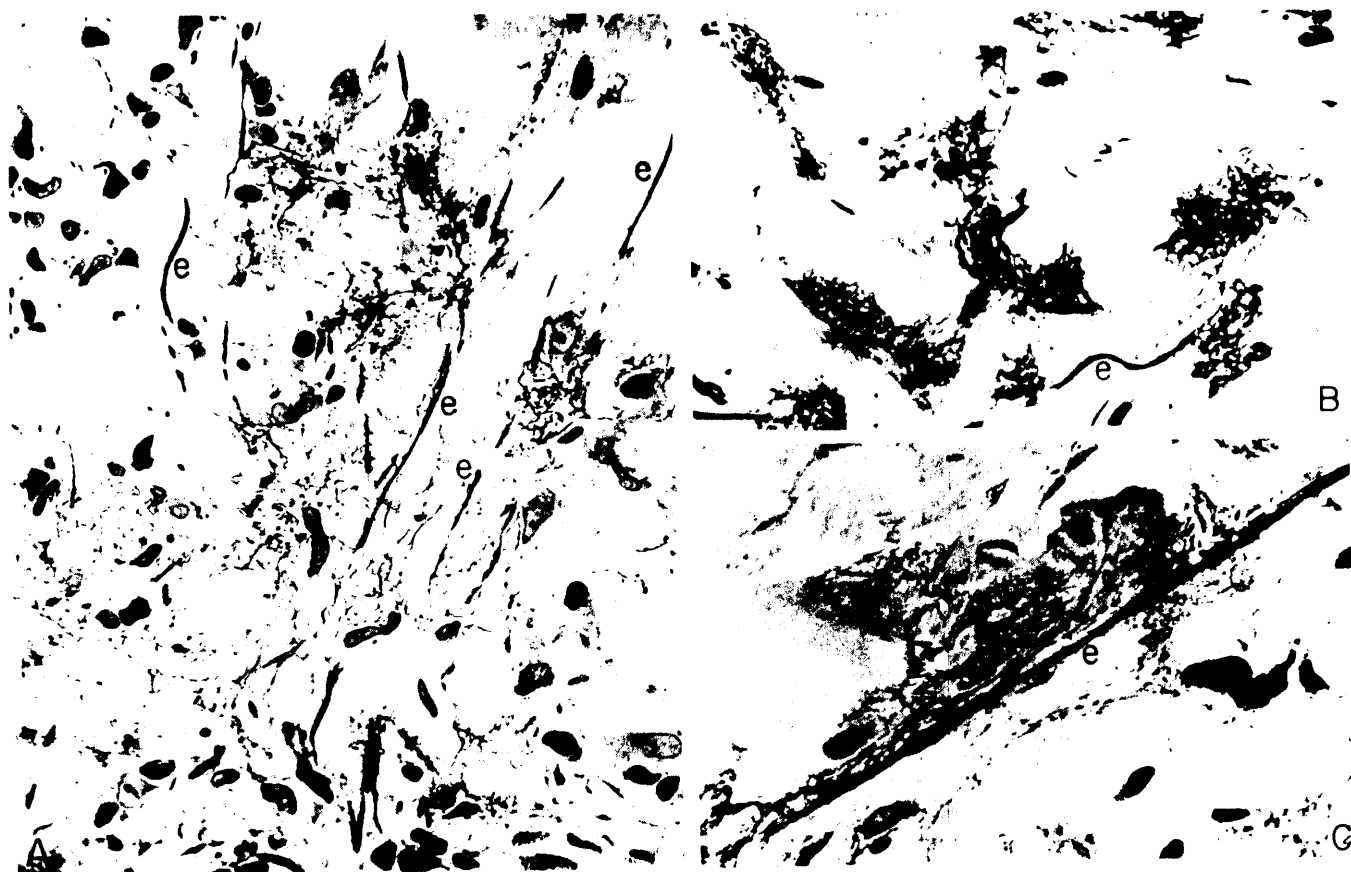


FIG. 11. Fibrin deposition in a 72-hour contact reaction to DNCB. In A and C fibrin is distributed as a loose interlacing meshwork between collagen bundles and along elastic fibers (e). It may also assume a

densely woven and matted appearance (B). 1- μ m. Epon section. Giemsa's reagent; Figure 11A, $\times 630$; B, $\times 790$; C, $\times 780$.

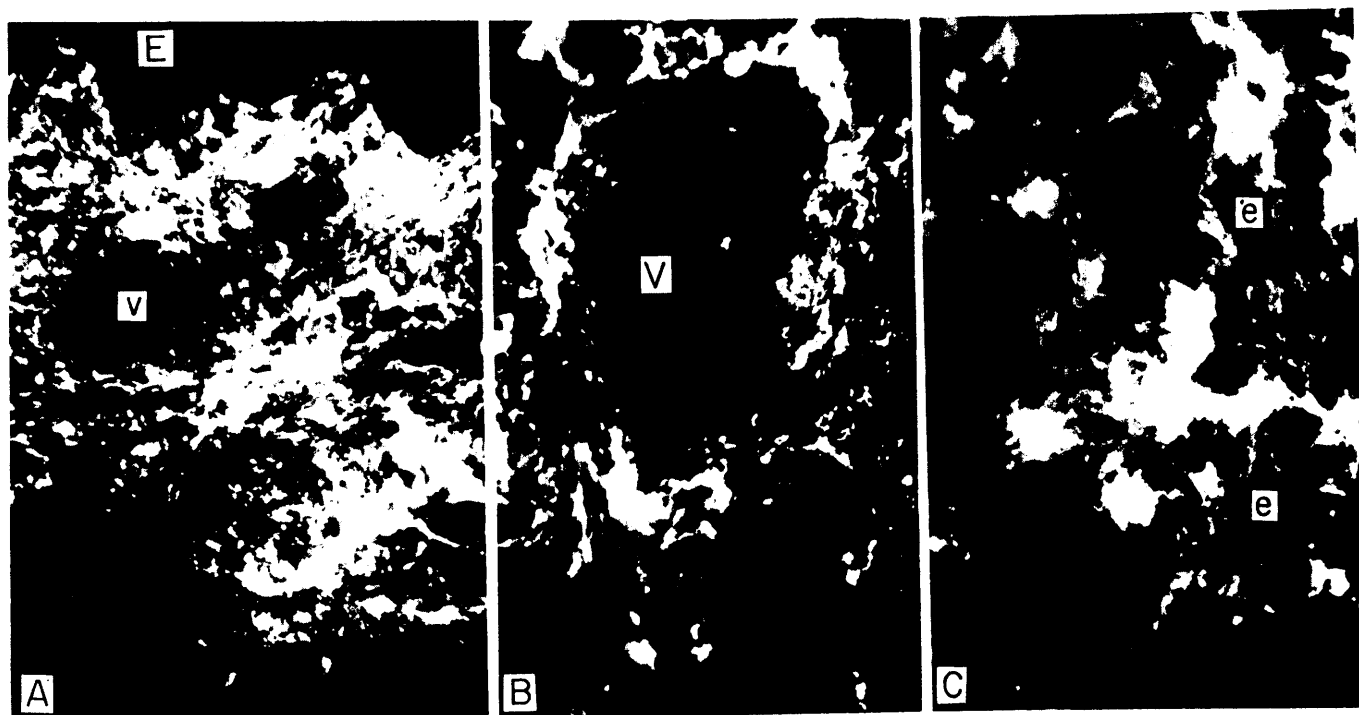


FIG. 12. Immunofluorescence demonstration of fibrin (Fib) deposits in 24-hour (A, B) and 72-hour (C) reactions of allergic contact dermatitis to DNCB. At low power (A) there are extensive deposits of Fib in the upper reticular dermis, with sparing of vascular zones (V) and their surrounding cuffs of lymphocytes. More extensive than usual Fib deposits are present in the papillary dermis. The epidermis (E) and

the deep reticular dermis are spared. At higher magnification (B) a vessel (V) and its associated lymphoid cuff are unstained, but the surrounding reticular dermis contains extensive Fib deposits. At still higher magnification (C) the fibrillar interlacing meshwork pattern of Fib is evident. Note that Fib coats an elastic fiber (running vertically between the two letters e). Figure 12A, $\times 250$; B, $\times 390$; C, $\times 750$.

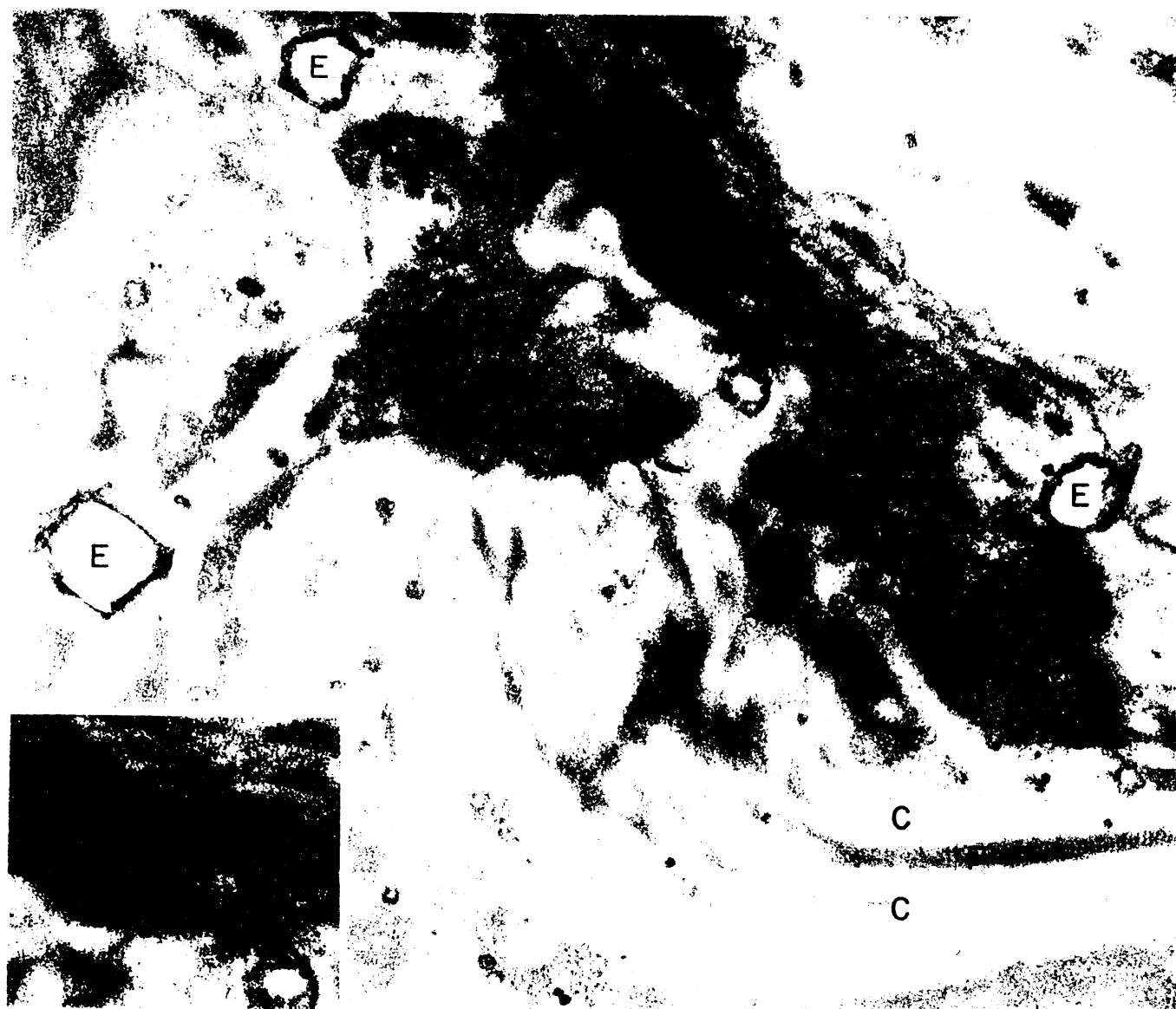


FIG. 13. Electron micrograph illustrating fibrin (*F*) deposition between collagen (*C*) bundles of the reticular dermis. Note intimate associations between fibrin, collagen, and elastic fibers (*E*). Elastic fibers have light, dense centers and thin, very dense borders with the

OPF staining technique. Arrows point to fibrin with 222-Å banding pattern, illustrated at higher magnification in the *inset*. OPF: $\times 45,000$; *inset*, $\times 76,000$.

were largely spared. Changes were evident in nearly one-third of subjects within 8 hours of testing with DNCB and were the earliest and most characteristic epidermal feature of contact dermatitis. Hair follicle epithelium was similarly involved, but changes generally appeared earlier and were more severe than in the epidermis. Patchy edema increased progressively over the next days and frequently became confluent so as to form intraepidermal vesicles filled with protein-rich fluid (Fig. 14*B*). By 48 hours, edema was present in all biopsies, and, by 72 hours, 13 of 17 exhibited vesicles or bullae, or both. Rarely, in the most severe reactions, large subepidermal bullae formed with separation and necrosis of basal cells. Fibrin was commonly found within epidermal vesicles and bullae but was not generally detected in zones of more diffuse epidermal edema. Occasional, and for the most part widely scattered, damaged and even necrotic epidermal cells were found

in some biopsies at all time intervals (Fig. 14*A*).

In confirmation of earlier work (5, 6, 36), intercellular edema was characterized in the electron microscope by the stretching of the normally firm desmosomal attachments between adjacent keratinocytes (Figs. 15 and 16). In some instances this process was sufficiently severe as to result in local elevation and separation of the epithelial cell plasma membrane from areas of underlying cytoplasm. Cells of the malpighian layer were also distinguished by large cytoplasmic accumulations of densely packed glycogen particles, often localized adjacent to one nuclear pole (Fig. 16); smaller but similar aggregates were dispersed throughout the cytoplasm. These deposits appear as clear vacuoles in routinely processed sections and have erroneously been described as foci of "intraepidermal cell" edema (2, 7, 42).

Cells of the basal layer, oriented with their long axes perpendicular to the basement membrane, were also

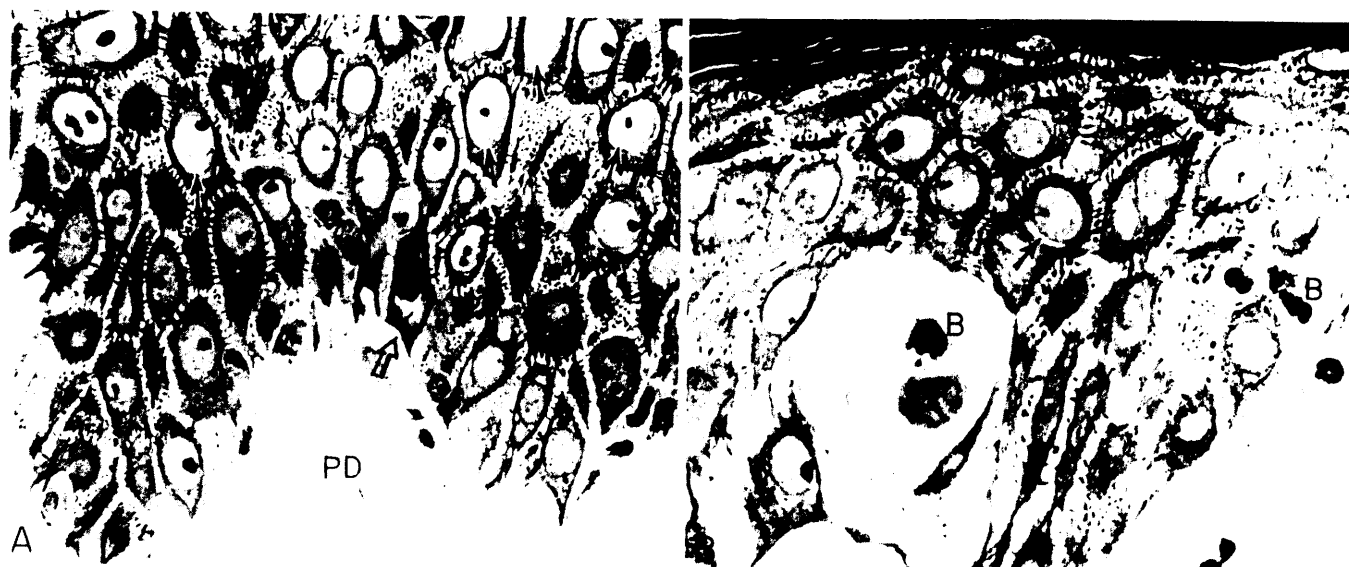


FIG. 14. Epidermal changes in allergic contact dermatitis to DNCB at 24 hours (A) and to paraphenylenediamine at 72 hours (B). In A, mild intercellular edema is associated with perinuclear vacuolation (arrows) and damage to an isolated keratinocyte (open arrow). A slight mononuclear cell infiltrate is also present. There is striking

edema of the papillary dermis (PD). In B, spongiotic vesicles are present, in addition to extensive intercellular edema which spares the granular cell layer. Arrows indicate perinuclear vacuolation. Two variably degranulated basophils (B) are present. 1- μ m. Epon section. Giemsa's reagent; Figure 14A, $\times 810$; B, $\times 860$.

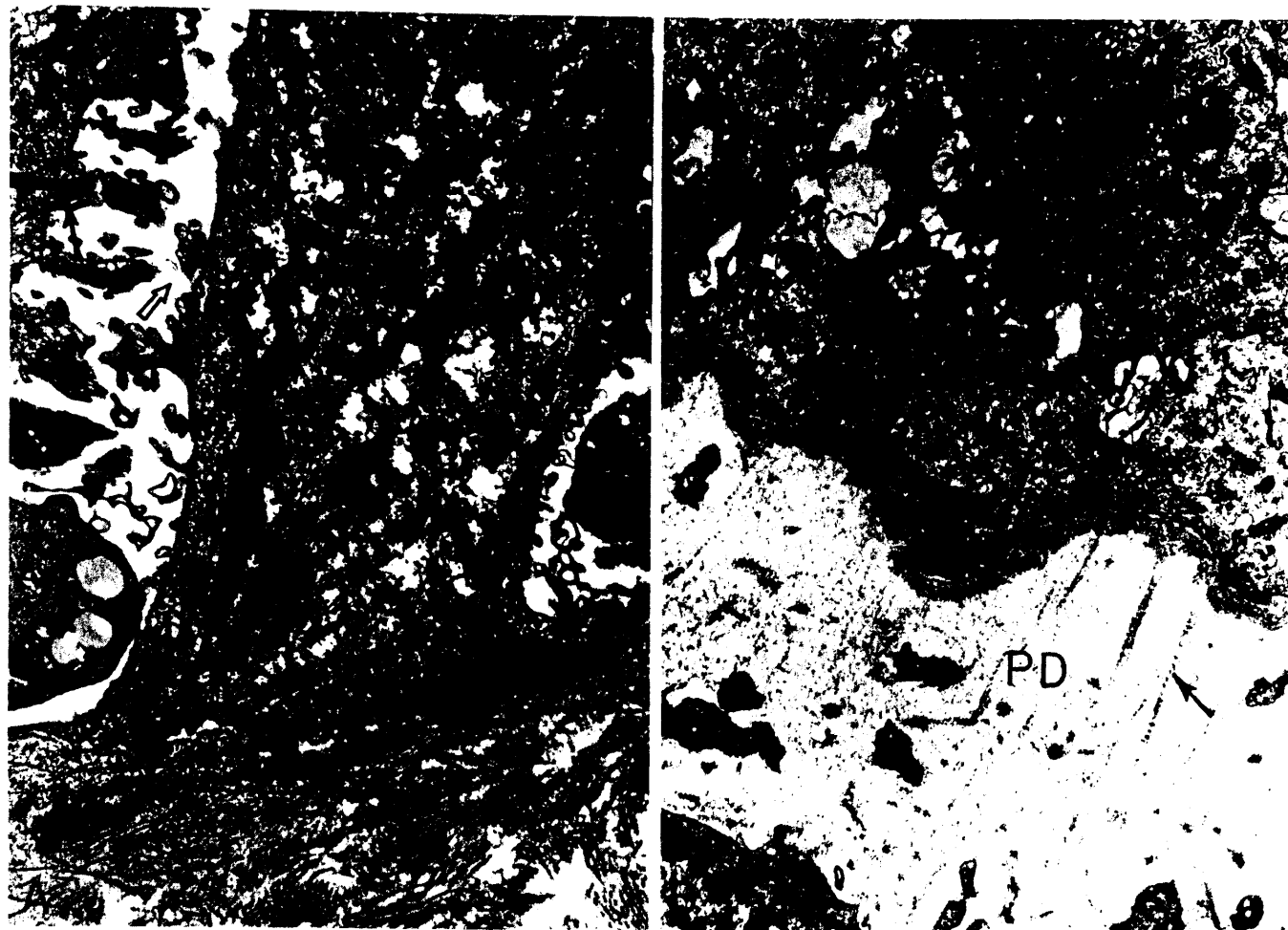


FIG. 15. Electron micrographs of the epidermal-dermal junction in 72-hour allergic contact dermatitis reactions to Urushiol. Basal epidermal cells in A are separated by extensive intercellular edema. Desmosomes (arrow) are intact, but attached portions of cytoplasm have been disrupted (open arrow). Tonofibrils appear normal. The superficial (papillary) dermis (PD) exhibits normal, randomly inter-

lacing pattern of fine collagen fibers. In B there is relatively little epidermal edema but extensive papillary dermal (PD) edema. Collagen bundles are poorly visualized, but vertically oriented anchoring fibrils (arrow) are well illustrated. Sections in both A and B were processed routinely. Figure 15A, $\times 10,000$; B, $\times 7,000$.

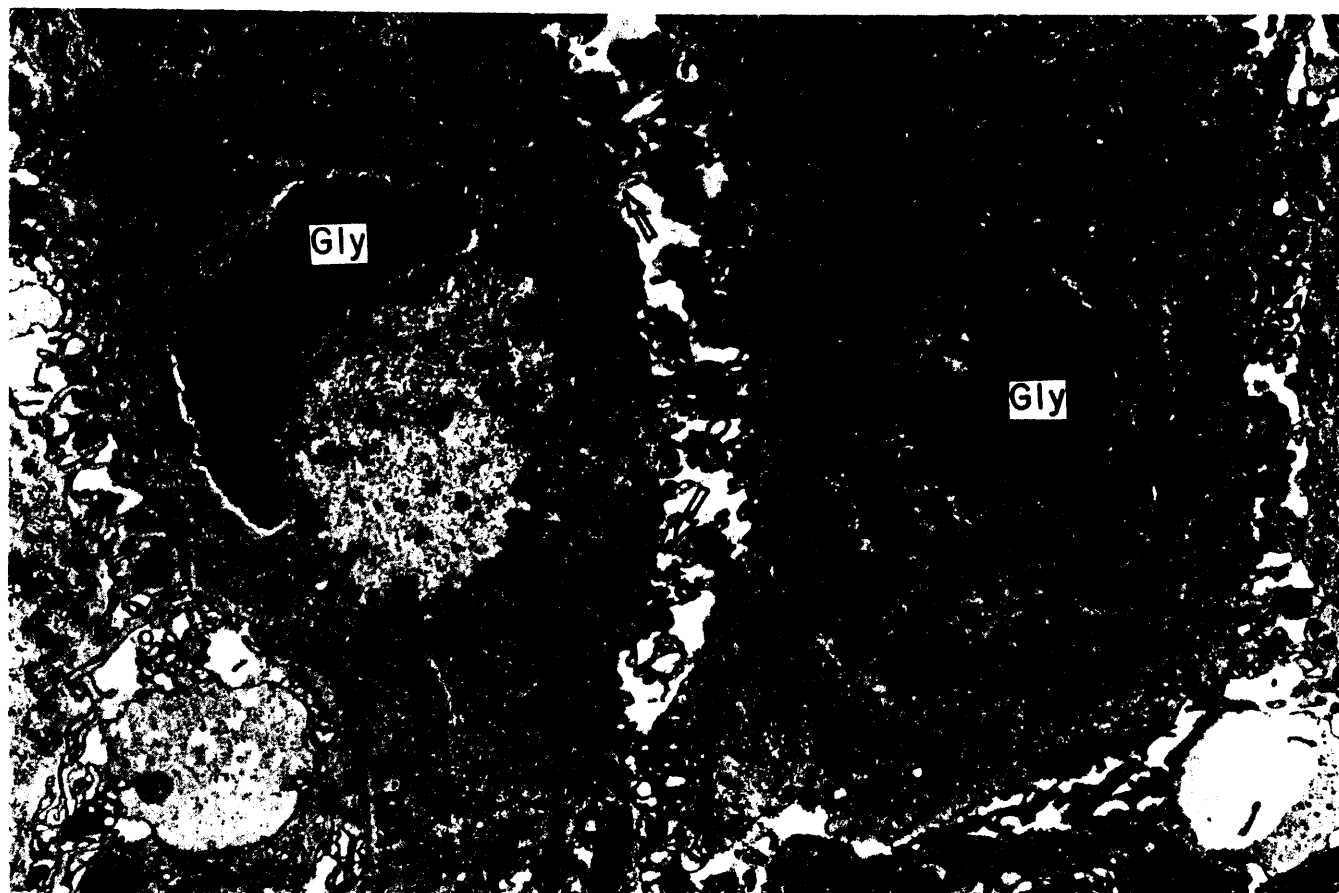


FIG. 16. Low magnification electron micrograph illustrating viable epidermal cells separated by edema. Desmosomes (arrows) are intact, but cytoplasmic processes to which desmosomes attach (open arrows)

extensively separated by intercellular edema. Melanin granules formed a supranuclear cap. Glycogen aggregates were present in the cytoplasm but not in the large masses observed in malpighian layer cells. Although the basal lamina was for the most part intact, it was sometimes disrupted focally, permitting open communication between the dermis and epidermis.

Beginning at about 3 days epidermal mitoses were observed, and some measure of hyperplasia was evident in nearly all biopsies by 11 to 13 days. Hyperplasia assumed either a platelike or psoriasiform configuration and was often accompanied by hyperkeratosis.

Cellular Infiltrate. Mononuclear cells were the first inflammatory cells to be observed in the epidermis. They were nearly all small and activated lymphocytes and comprised the majority of infiltrating cells at all intervals. In highly sensitive individuals, occasional lymphocytes were present at 4 to 8 hours following testing with DNCB, and at 72 hours all DNCB skin tests exhibited some degree of cellular infiltration in the epidermis. Hair follicles and the peripheral cell layers of the lobules of sebaceous glands were more prone than surface epithelium to both cellular infiltration and intercellular edema at early intervals after skin test.

Basophils appeared only after mononuclear cells and were present in seven of 17 biopsies at 3 days (Figs. 6, 7, and 14B). Occasionally they comprised up to 20 per cent of the cells infiltrating the epidermis but more commonly were much less frequent. Eosinophils appeared

are occasionally disrupted. Large perinuclear aggregates of glycogen (Gly) are present and correspond to the perinuclear vacuoles of Figure 14. OPF; $\times 8,400$.

still later and only when preceded by basophils. Neutrophils were exceedingly rare in the absence of significant epidermal necrosis.

Forty-Eight Hour Reactions to DNCB in "Unsensitized" Individuals. The skin tests of individuals at the time of original sensitization elicited weakly positive 48-hour reactions in four of 16 individuals, consisting of erythema and induration but no vesiculation (Table 2). These four individuals had had no known prior contact with DNCB and responded to the immunizing dose of DNCB and to subsequent skin testing in a fashion identical with that of the 12 individuals who exhibited no response to initial skin test.

Microscopically, these four lesions were typical in every way of mild contact sensitivity and were similar to weak reactions elicited to environmental allergens (see below). Moreover, seven of the 12 clinically negative DNCB tests exhibited microscopic features of mild contact dermatitis, particularly perivascular accumulations of mononuclear cells. Certain of the secondary features of contact dermatitis, including basophil infiltration, mast cell alteration, vascular endothelial changes, and epidermal involvement, were sometimes present to a mild degree (Table 2).

ALLERGIC CONTACT DERMATITIS TO ENVIRONMENTAL ALLERGENS

Individuals with a clinical history of allergic contact dermatitis were patch-tested with appropriate environ-

mental allergens. These patients exhibited a less homogeneous response than the volunteers sensitized with DNCB under controlled conditions. Thus, of 30 reactions biopsied at 3 days after skin test, four were + in intensity, 12 were 1+/2+, and 14 were 3+/4+ by clinical inspection (Table 3).

Microscopically, these reactions could be usefully divided into "strong" and "weak" categories, based on the extent of the inflammatory cell infiltrate (Table 3). The gross and microscopic pathology of this group of reactions closely mimicked that of comparable reactions to DNCB elicited in volunteers sensitized under defined conditions (compare Tables 2 and 3). Thus, basophils were identified in the dermis in 17 of 18 biopsies (94 per cent) and were numerous ($\geq 3+$) in eight (44 per cent). Other features of allergic contact reactions, including fibrin deposition, microvascular changes, dermal edema and erythrocyte extravasation, compaction, and epidermal involvement were likewise present. By contrast, all of these features were much less prominent in the 12 reactions graded as "weak" (Table 3).

DELAYED HYPERSENSITIVITY REACTIONS TO INTRADERMALLY INJECTED PROTEIN ANTIGENS IN INDIVIDUALS WITH SPONTANEOUSLY ACQUIRED SENSITIVITY

GROSS APPEARANCE.

Positive reactions elicited with Old Tuberculin (nine), *C. albicans* (nine), streptokinase/streptodornase (eight), and mumps (10), in 36 individuals, consisted of erythematous, raised, indurated lesions that first became visible after a null period of 6 to 12 hours and that achieved maximal intensity at 48 hours (Table 4). In four of five instances, skin testing with mumps virus elicited an early response of erythema and swelling, maximal at 4 hours, that then receded by 8 hours and that was followed subsequently by a typical delayed reaction. None of the other antigens studied exhibited this bimodal pattern of reactivity.

MICROSCOPIC PATHOLOGY.

The delayed lesions elicited with intradermal injections of protein antigens were generally similar to those of allergic contact dermatitis (compare Tables 2 to 4) but were distributed somewhat differently, apparently reflecting differences in antigen distribution within the skin (47). Thus, reactions regularly extended into the deep dermis and frequently involved the subcutis; by contrast, the epidermis was relatively spared. Reactions were characterized by striking, perivascular infiltrates of mononuclear cells, for the most part small and activated lymphocytes but including some macrophages as well. As in contact dermatitis, the intervascular infiltrate was generally less intense than that surrounding vessels. Basophils were included in this infiltrate in 22 of 36 biopsies (61 per cent) and were numerous ($\geq 3+$) in seven (19 per cent). Eosinophils were less frequent than in contact dermatitis, being numerous in only two biopsies. Neutrophils, by contrast, were more frequent than in contact allergy, being present in 21 biopsies (58 per cent) but numerous in only three (8 per cent). Plasma cells were found infrequently. Mast cell hypogranulation, fi-

brin deposition, microvascular alterations and evidence of increased vascular permeability were present as in contact dermatitis. Fibrin deposition extended into the deep dermis and subcutis.

Epidermal changes resembled those in contact dermatitis but were much less well developed. Thus, patchy zones of intercellular edema were found in nearly all instances (35 of 36), and focal slight vesiculation was observed in six. A mononuclear cell infiltrate was present in the epidermis of 27 biopsies but was consistently less intense than that in contact allergy and only rarely included granulocytes.

"NEGATIVE" INTRADERMAL SKIN TEST REACTIONS.

Reactions to the same intradermal skin test antigens were, according to standard convention, regarded as negative if they measured less than 10 mm. of induration. Eight such lesions were biopsied, three being grossly negative, whereas five exhibited mild erythema and induration measuring 3 to 9 mm. in diameter.

Microscopically all of these eight "negative" lesions had cellular infiltrates that did not differ qualitatively from those in positive reactions (Table 4). All exhibited a perivascular mononuclear cell response and six of eight an intervascular infiltrate as well. Granulocytes, particularly basophils and neutrophils, were present in small numbers in one-half of the biopsies and eosinophils in a smaller fraction. Dermal edema was evident in five and compaction and erythrocyte extravasation were evident in three. Fibrin deposits and vascular endothelial alterations were present in five of eight instances. Epidermal changes were minimal, but one-half exhibited trace epidermal mononuclear cell infiltrates and six of eight exhibited some degree of intercellular edema. Several of these lesions could not have been distinguished microscopically from much larger grossly positive reactions.

DISCUSSION

The data presented here provide a comprehensive, semiquantitative morphologic description of delayed type hypersensitivity reactions as they occurred in a large group of human subjects and confirm and extend recent preliminary observations made on a much smaller sample (25). In accord with classic descriptions in both man and animals, reactions began as perivascular collections of lymphocytes which subsequently extended into the intervascular dermis and the epidermis. In addition, however, delayed hypersensitivity in man was characterized by a number of heretofore unsuspected features which at once clarify and complicate current conceptions of this entity and of its pathogenesis. These new findings include the prominent role played by inflammatory cells other than mononuclears, particularly basophils and to a lesser extent eosinophils; degranulation of both basophils and mast cells; increased vascular permeability with consequent dermal and epidermal edema, vascular compaction, and erythrocyte extravasation; mast cell replication; microvascular alterations affecting endothelial cells and pericytes, with compromise of vessel lumina and basement membrane thickening; and activation of the clotting system with deposition of fibrin in

a characteristic intervascular pattern in the reticular dermis.

These new findings varied in their frequency and in the time course of their appearance. Thus, basophils were never observed in the absence of lymphocytes and were rarely present 8 hours after skin testing with DNCB (two of 16 lesions). They attained maximal frequency at 3 days (present in 88 per cent of biopsies; $\geq 1+$ in 53 per cent) and gradually declined thereafter. Similar data were obtained from strong 3 day contact reactions to environmental allergens (basophils present in 94 per cent; $\geq 1+$ in 67 per cent). As in the guinea pig (22), basophils were less numerous in classic delayed hypersensitivity reactions to microbial antigens (present in 61 per cent; $\geq 1+$ in 39 per cent).

Basophils and mast cells exhibited varying degrees of degranulation in all positive reactions studied at 24 hours or later. Mast cell mitoses and hyperplasia were frequently observed at 6 days and at later intervals. Papillary dermal edema, dermal fibrin deposition, and microvascular changes were nearly universal features of mature (2- to 3-day) delayed reactions of all types; these changes persisted to varying degrees as late as 11 to 13 days after testing with DNCB in the great majority of sensitized individuals. Vascular compaction and erythrocyte extravasation were less commonly present but followed a similar time course.

A number of important differences distinguished delayed type reactions in man from those of the animal model. Human delayed reactions had a slower and more prolonged evolution than comparable lesions in guinea pigs. Thus, human delayed reactions were rarely maximal before 48 hours and, in the case of allergic contact dermatitis, did not achieve peak intensity for 3 to 6 days after skin testing; by contrast, delayed reactions in guinea pigs were regularly maximal at 24 hours or earlier and were on the wane by 48 hours. Possibly related to this more prolonged time course, human reactions were also characterized by greater evidence of altered vascular permeability, fibrin deposition, and microvascular changes than the animal model (10). Basophils participated in the majority of all well developed human reactions studied and were particularly prominent in allergic contact dermatitis; however, they seldom exceeded 200 per linear mm. of surface in 1- μ m. thick sections and generally did not comprise more than 15 per cent of infiltrating cells. By comparison, basophils regularly numbered 300 to 600 per linear mm. of surface in guinea pig CBH reactions and accounted for 20 to 60 per cent or more of total cells (22). By contrast, mast cells appeared to have a far more active role in delayed reactions in man, where they regularly underwent loss of granule substance (early) and hyperplasia (late). These data are in accord with a hypothesis we have advanced which suggests that basophils and mast cells are supplementary cells with similar or identical functions and that participation of these cells in delayed reactions is governed by their relative frequency in a given species and by the duration of the inflammatory process (19, 20). Thus, mast cells would be predicted to have a prominent role, relative to basophils, in species such as man, in whom the normal skin contains relatively abundant

mast cells (1) and in whom delayed reactions are prolonged in time.

The new morphologic features reported here substantially alter current conceptions of delayed hypersensitivity as a simple cutaneous infiltration of lymphocytes and macrophages. Although causative relationships have not been rigorously established, it may be useful to interpret the pathogenesis of these new findings on the basis of such evidence as is presently available (Fig. 17). It is generally agreed that delayed reactions are triggered by a primary interaction between specific antigen and small numbers of sensitized lymphocytes (12, 18). This primary interaction initiates a sequence of events beginning with lymphocyte activation (blast transformation) and the elaboration by lymphocytes (or activated lymphocytes, or both) of non-immunoglobulin mediators or lymphokines. Certain of these have chemotactic activity and attract immunologically nonspecific cells (additional lymphocytes, monocytes, and basophils (12, 29, 49, 50)) which together comprise the vast majority of inflammatory cells contributing to these reactions. Other mediators activate and modify the migration properties of macrophages (12). Macrophages were not, however, a prominent feature of these cutaneous reactions, and it is of interest that chemotactic factors, but not migration inhibition factor, have been isolated from delayed skin reactions in animals (9). The intimate associations between lymphoid cuffs and vessels exhibiting endothelial cell alterations suggest that the microvascular changes that we have described are also mediated by lymphocytes or their products.

The relationships between lymphocytes, basophil and mast cell alterations, eosinophils, and altered vascular permeability in delayed hypersensitivity are poorly understood but of particular importance. We propose that lymphocytes or lymphocyte mediators provoke the sustained but incomplete degranulation of basophils and mast cells that characterize these reactions, and that such degranulation liberates a variety of vasoactive agents of which histamine is but one example (19, 20). Such substances, in turn, may cause venous and venular vasodilation (and hence erythema) as well as increased vascular permeability leading to compaction, dermal and epidermal edema, and erythrocyte extravasation. Moreover, vascular leakage of fibrinogen, and presumably other clotting factors, is an essential first step in dermal fibrin deposition. Whether lymphocyte products promote the clotting or fibrinolytic system or both, is uncertain (33, 43, 46); it is known, however, that collagen itself may activate Hageman factor and thus initiate clotting (38). The close anatomical associations between fibrin and dermal elastic fibers suggest a role for these dermal elements in the clotting process as well. Fibrinopeptides released from fibrinogen in the course of clotting are themselves vasoactive in certain species and therefore could augment vessel changes (43). On the other hand, insertion of a meshwork of long chain fibrin polymers in the interstices between dermal collagen bundles may be expected to bind and hold substantial extravascular water; such bound water very likely accounts for the induration characteristic of delayed hypersensitivity (11). Strong supportive evidence for this

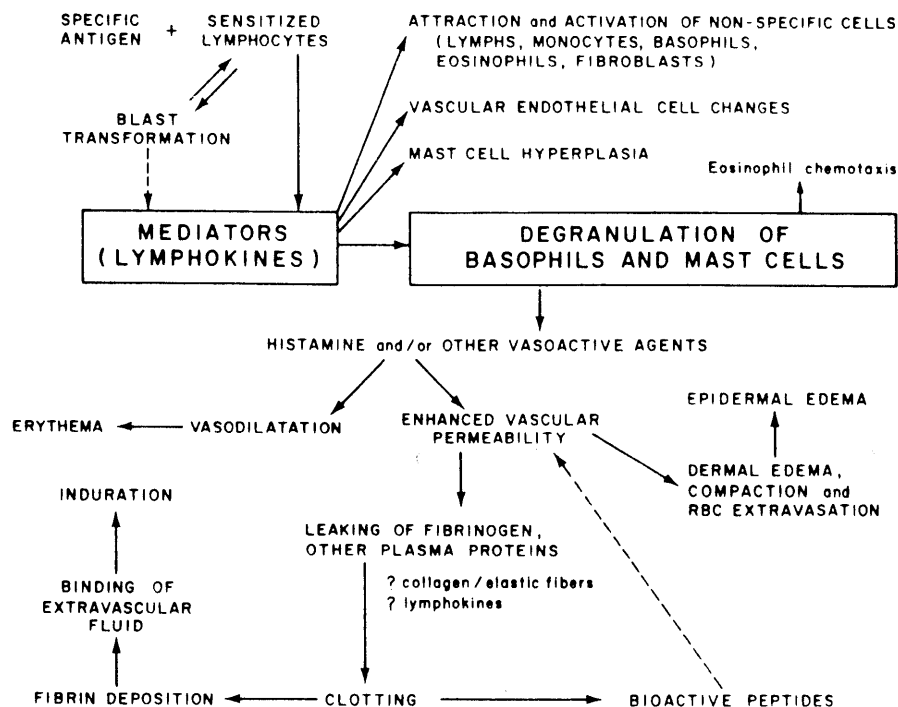


FIG. 17. Flow diagram outlining proposed pathogenesis of delayed type hypersensitivity reactions in the skin.

last possibility comes from studies of delayed hypersensitivity and CBH reactions in guinea pigs; nonindurated CBH reactions have nearly as many inflammatory cells as indurated delayed hypersensitivity reactions but, in contrast to the latter, have insignificant fibrin deposition (10, 22).

Later changes in human delayed reactions included eosinophil infiltration and mast cell replication. Eosinophils appeared irregularly and were invariably preceded by both basophils and lymphocytes. At least two mechanisms have been proposed for eosinophilic chemotaxis, involving either lymphocytes and antigen-antibody complexes or products of basophil/mast cell secretion (8, 30); either or both could be operative in delayed hypersensitivity. The mechanism of mast cell proliferation is unknown, but mast cell hyperplasia is observed in other instances of "chronic inflammation" and may be dependent on the activities of lymphocytes or their products (19, 20).

Weak or grossly negative reactions to protein antigens or contact allergens in unsensitized subjects were not infrequently characterized by perivascular mononuclear cell infiltrates when examined at 48 or 72 hours after skin testing. Although infiltrates were invariably less extensive than those occurring in sensitized subjects, they were sometimes accompanied by some or all of the new features of delayed hypersensitivity that we have described here. By contrast, injections of nonantigenic substances (such as isotonic saline) provoked none of these changes. We interpret these findings to indicate that so-called "unsensitized" individuals in fact possess small numbers of lymphocytes with specificity for antigen, and that sensitization, leading to the development of cellular immunity, primarily induces an expansion of such clones rather than some more fundamental qualitative change. We further infer that the secondary events

characteristic of delayed hypersensitivity and summarized in Figure 17 do not appear until a minimal threshold level of lymphocyte infiltration and function has occurred.

The model of delayed hypersensitivity proposed here represents an attempt to arrange the complex anatomical features which characterize these reactions into some logical order. Morphologic studies alone cannot finally elucidate the pathogenesis of these reactions, but they do provide a useful framework for initiating functional studies. Our model is testable in its various parts and provides a number of leads for further experimentation.

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