CUTANEOUS BASOPHIL HYPERSENSITIVITY

II. A Light and Electron Microscopic Description*, ‡

By HAROLD F. DVORAK,§ M.D., ANN M. DVORAK,‖ M.D., BLANCHE A. SIMPSON, HAL B. RICHERSON, M.D., SIDNEY LESKOWITZ, Ph.D., and MORRIS J. KARNOVSKY, M.B.B.Ch.

(From the Department of Pathology, Massachusetts General Hospital and Harvard Medical School, and the Departments of Medicine and Bacteriology and Immunology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114)

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Delayed-onset erythematous skin reactions elicited in adult guinea pigs sensitized with protein antigens in *incomplete* Freund's adjuvant have been called "Jones-Mote" reactions to differentiate them from classic tuberculin type delayed hypersensitivity (1). However, this distinction has depended on relatively minor gross and microscopic differences and on the fact that Jones-Mote reactions, in contrast to delayed hypersensitivity, may be elicited only at early intervals after sensitization (2, 3). It is not surprising, therefore, that many workers have regarded Jones-Mote reactivity as a weak expression of delayed hypersensitivity (4).

We have previously presented immunochemical and biologic data which permit a further distinction between Jones-Mote and classic delayed hypersensitivity reactions (5, 6). The two categories of reaction were shown to have different carrier requirements for hapten-specific sensitization but similar requirements for the expression of skin test reactivity. It was suggested that both reactions involve sensitized lymphoid cells, and that Jones-Mote reactions do not represent a combination of antigen with preformed antibody.

We here demonstrate that Jones-Mote reactions have a unique light and electron microscopic morphology which is characterized by the accumulation of large numbers of basophilic leukocytes in the dermis. We prefer to designate

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§ Fellow, the Medical Foundation, Inc., Boston, Massachusetts, to whom communications should be directed.

‖ Post Doctoral Trainee, National Institutes of Health, United States Public Health Service.

reactions having this appearance *cutaneous basophil hypersensitivity* (CBH), a descriptive term which emphasizes the principal morphologic feature of the lesion. The reactions studied by Jones and Mote (7) were elicited in man at late intervals in the course of immunization, their morphology was not examined, and their possible relation to the reaction described here is, therefore, unknown.

We also demonstrate that accumulations of basophils occur in skin test reactions performed at early intervals after sensitization with protein antigens in *complete* Freund's adjuvant in addition to the infiltrate of mononuclear cells comprising classic delayed hypersensitivity. Skin test reactions elicited at later intervals in the course of sensitization with incomplete adjuvant had a quite different time course and histology and have been designated *late* reactions.

Materials and Methods

Antigens—Preparation of arsanilic acid (ABA) conjugates with *N*-acetyltyrosine, bovine serum albumin (BSA), and with the synthetic polypeptides L- and D-GAT was described in the preceding paper (6). Egg albumin (EA), horseradish peroxidase, human serum albumin (HSA), and keyhole limpet hemocyanin (KLH) were obtained from commercial sources.

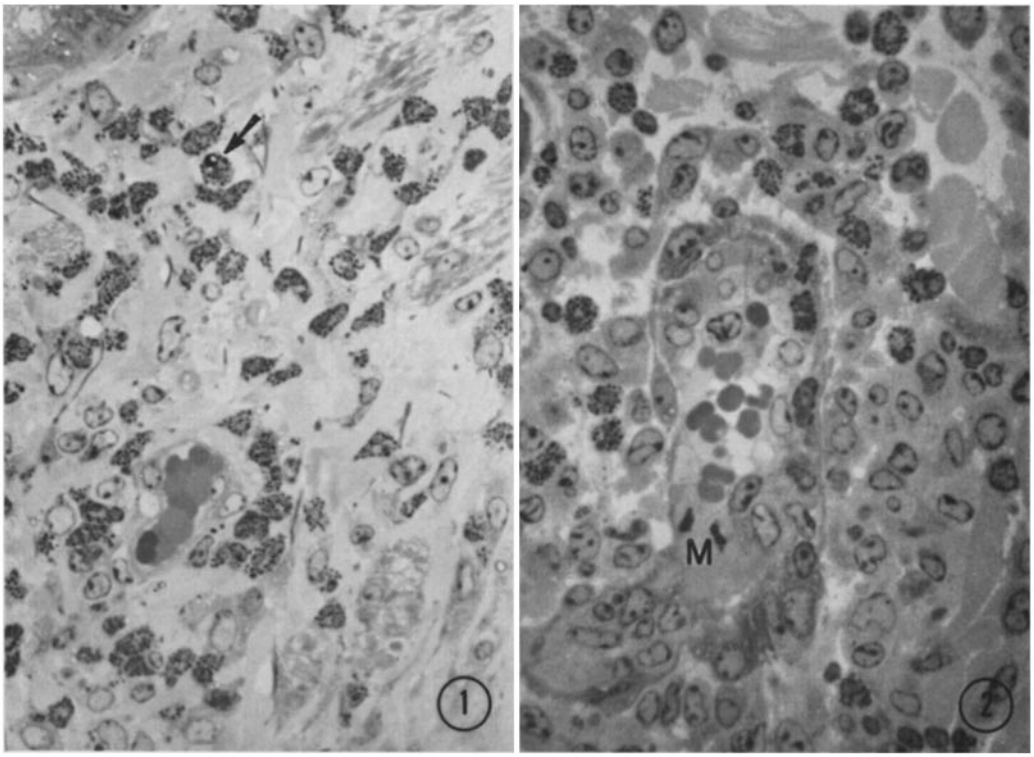
Immunizations and Skin Tests—400–500g English short hair guinea pigs were sensitized with 10 or 100 μg of the protein antigens or with 2×10^{-6} moles of ABA-L-tyrosine in the four foot pads emulsified in complete Freund's adjuvant (8.5 parts mineral oil, 1.5 parts Arlacel A, 3 or 5 mg/ml of killed mycobacteria), incomplete Freund's adjuvant, or saline. Skin tests were performed with 10 or 50 μg on depilated flank skin 1, 2, 3, 5, or 6 wk later. Reactions (one to four per animal) were read, and biopsies were taken at intervals of 6 hr to 16 days after skin test. Control sites were studied in normal animals or in animals sensitized to an unrelated antigen.

Fixation and Processing of Tissues—Animals were sacrificed by a blow to the head or were anesthetized with ether, and skin reactions were immediately excised, cut into thin strips, and for light microscopy fixed in 5% phosphate buffered glutaraldehyde, pH 7.3. Tissues were postfixed in osmium tetroxide, and 1 μ Epon embedded sections were prepared. Sections were stained for 1 hr in Giemsa diluted 1:10 in 2% sodium borate solution (alkaline stain), or in Giemsa similarly diluted in 0.05 M acetate buffer, pH 4.0–6.0 (acid stain). Routine hematoxylin and eosin paraffin sections were also prepared. A total of 326 reactions from 120 guinea pigs were studied microscopically. Touch preparations were made of skin lesions in some instances, and these were air dried and stained with Wright's stain as were blood smears obtained from the venous plexus of the ear.

For electron microscopy, skin reactions were cut into small pieces and fixed for 5 hr at room temperature in a mixture (8) containing 2% paraformaldehyde, 2.5% glutaraldehyde, and 25 mg $CaCl_2$ in 0.1 M sodium cacodylate buffer, pH 7.4; they were then washed overnight in 0.1 M sodium cacodylate buffer, pH 7.4, and postfixed in 1.5% collidine-buffered osmium tetroxide. Preceding dehydration in alcohol the specimens were stained en bloc with uranyl acetate according to the method of Farquhar and Palade (9) as modified by Karnovsky (10). Tissues were embedded in Epon 812.

In order to minimize sampling difficulties, only those lesions which showed a dense cellular infiltrate with the light microscope were examined further. 10 48-hr and two 24-hr hapten-specific lesions were examined by electron microscopy. In addition, two 48-hr lesions were examined from animals sensitized and tested with horseradish peroxidase. Blocks were cut

with glass knives on an LKB II ultratome, stained with lead citrate (11), and examined in an RCA EMU 3F microscope by Dr. A. M. Dvorak. Two animals bearing 48-hr hapten-specific lesions received 0.5 ml dialyzed Pelikan (Spezialtusche, C11/143a; Günther-Wagner, Hanover, Germany) colloidal carbon intravenously 40 min before sacrifice to assess vascular permeability in the reaction site.



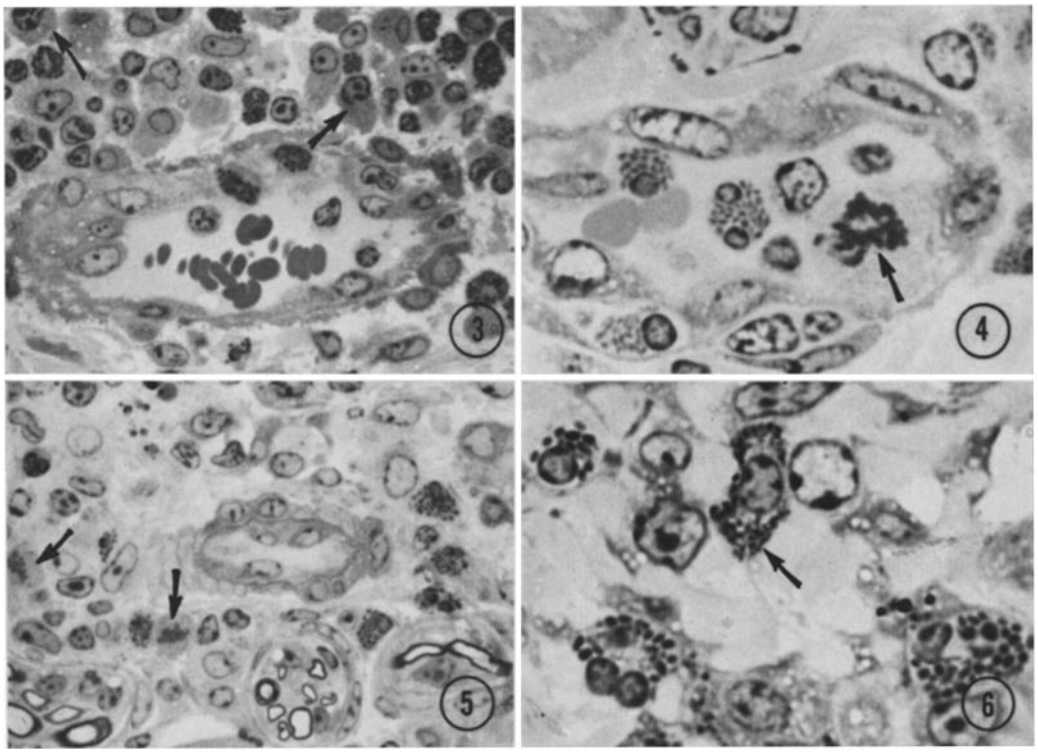
Figures 1–7 and 9 represent photomicrographs of 1 μ sections of Epon-embedded reactions stained with alkaline Giemsa.

FIG. 1. Superficial dermis of a 24 hr CBH (cutaneous basophil hypersensitivity) reaction to ABA-L-GAT. Note overwhelming predominance of basophilic leukocytes, one of which (arrow) is undergoing necrosis. × 600.

FIG. 2. Lower hypodermis of the same reaction as in Fig. 1. Emigration of basophils from a large vein and mitosis of a mononuclear cell in the vessel lumen (M). Mononuclear cells are relatively prominent here as compared with the papillary dermis. × 600.

Light Microscopic Appearance of Guinea Pig Granulocytes and Mast Cells—Guinea pig basophils appeared in 1μ Epon sections as round or irregular cells with the size and multilobed nuclei of polymorphonuclear leukocytes (Figs. 1–9). The cytoplasm contained large oval or round granules, which stained blue with Giemsa at alkaline pH but poorly or not at all below pH 5.0. Basophil granules stained metachromatically in Wright's stained touch preparations

of skin reactions and in blood films, a property which was lost after aqueous fixation. Basophils were readily distinguished from tissue mast cells in the guinea pig in that the latter have larger, oval shaped and unilobed nuclei with smaller and more numerous cytoplasmic granules which retained metachromatic staining properties after aqaueous fixtion and tissue processing



FIGS. 3–5. Hypodermis of 48-hr CBH reactions to ABA-L-GAT. Note blast cells (arrows) in Fig. 3 included in the infiltrate around a small vein from which there is a continued efflux of basophils. Fig. 4 illustrates another such vessel containing basophils and a dividing mononuclear (arrow) at higher power. In Fig. 5, mononuclear cells derived from the circulation are shown to be dividing in the tissues (arrows). Figs. 3 and 5, × 600; Fig. 4, × 1450.

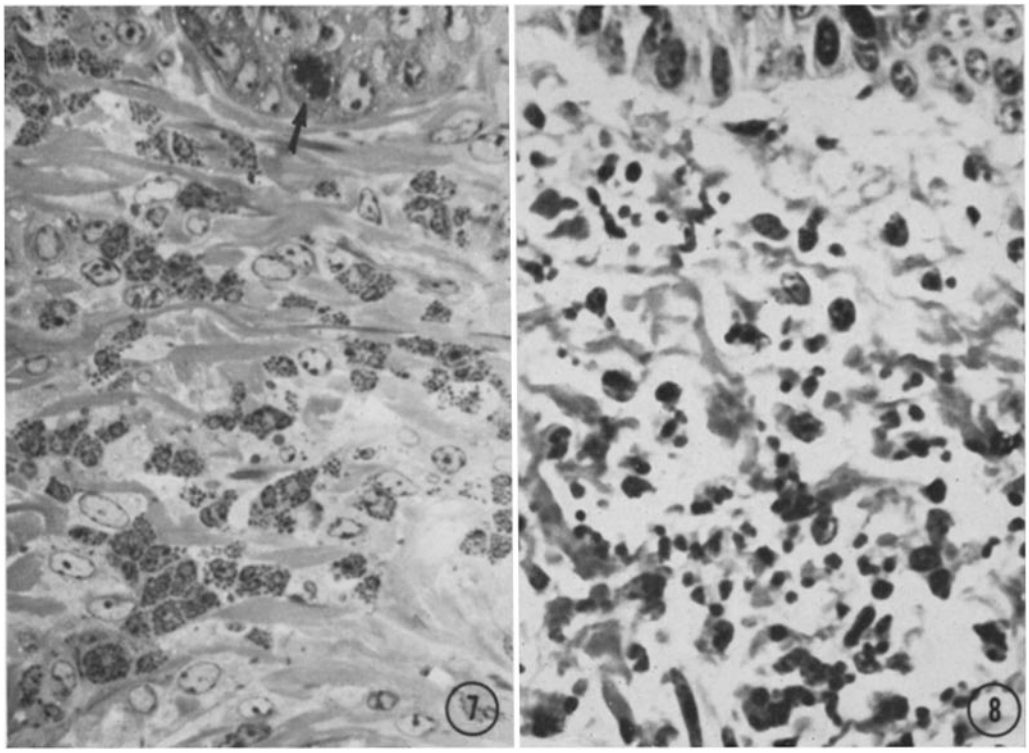
FIG. 6. Papillary dermis of a 48 hr CBH reaction to ABA-L-GAT demonstrating the distinguishing morphologic features of a fixed tissue mast cell (arrow) and three surrounding basophils (larger granules). Mast cell granules are smaller than basophil granules and retain their metachromasia in tissue sections (not illustrated in this black and white photomicrograph). Moreover, basophil nuclei are multilobed, whereas mast cell nuclei are larger and unilobed. × 1280.

(Fig. 6). Basophils were not readily appreciated in routine paraffin sections (compare Figs. 7 and 8) since the characteristic granules were not stained with hematoxylin and eosin.

Neutrophils had multilobed nuclei and scattered small cytoplasmic granules which stained bright red with acid Giemsa. Eosinophils had bilobed nuclei and a cytoplasm crowded with

oval granules nearly as large as those of the basophil. These, however, stained blue with alkaline and brick red with acid Giemsa.

Performance of Cell Counts—A major objective of this study was to differentiate cutaneous basophil (CBH), delayed (DH), and *late* hypersensitivity reactions on the basis of strict morphologic criteria. Delayed and CBH reactions differed little in diameter or erythema, but



FIGS. 7 and 8. Papillary dermis of a 48 hr CBH reaction to ABA-L-GAT, one-half prepared for 1 μ Epon-embedded sections (Fig. 7) and the other half for routine histology with paraffin embedding and hematoxylin and eosin staining (Fig. 8). Basophils, readily identified in Fig. 7, are not recognizable in the hematoxylin and eosin section. Note epidermal cell in mitosis (arrow) in Fig. 7. Both $\times$ 600.

the former were indurated and hence considerably thicker than the latter. In order to compare the cellular composition of these reactions in an objective manner, the methods illustrated in Fig. 10 were devised. Differential cell counts were performed on all infiltrating cells encountered in two randomly selected swaths (each 100 μ wide) taken vertically through the entire thickness of the dermis and subjacent fat and muscle under oil immersion with an ocular micrometer (method A in Fig. 10). Thus, it was possible to arrive at values for the numbers of each of the various cell types and of the total infiltrating cells per unit surface area in each of the three types of reaction.

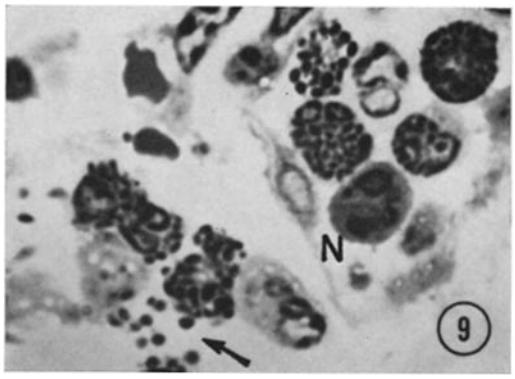


FIG. 9. Hypodermis of a 48 hr CBH reaction to ABA-L-GAT. Basophil granules lie free in tissue (arrow), adjacent to two normal appearing basophils and a mononuclear cell (probably a macrophage). Free granules of this type were not uncommon and may reflect basophil death or degranulation. Vessel to right contains basophils, a mononuclear cell, and a neutrophil (N). × 1450.

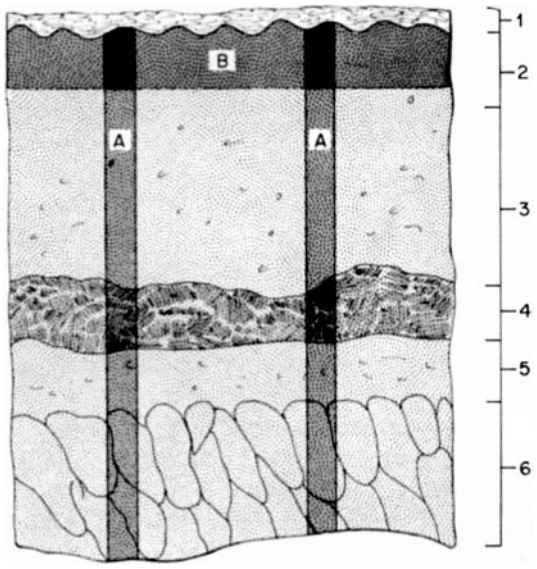


FIG. 10. Schematic representation of guinea pig skin including: 1, epidermis; 2, papillary or upper dermis; 3, hypodermis; 4, panniculus adiposus (fat); 5, loose connective tissue; 6, panniculus carnosus (muscle). Differential cell counts were performed as in A by counting all infiltrating cells encountered in two vertical swaths, each 100 μ wide, through the entire thickness of the skin. Differential counts performed as in B involved analysis of the first 200 cells encountered in the papillary dermis within 150 μ of the epidermal junction.

During the course of this work it became clear that basophils were not randomly distributed in the skin but were preferentially concentrated in the upper portion of the papillary dermis. In order to analyze this region, differential cell counts were performed on the first 200 cells encountered within 150 μ of the epidermal junction (method B in Fig. 10).

RESULTS

Three distinct histologic patterns of skin test reaction were recognized in guinea pigs immunized with the various antigens studied, depending on the vehicle in which the sensitizing antigen was incorporated, the size of the skin test dose, and the interval after sensitization at which testing was performed. These are cutaneous basophil (Jones-Mote) hypersensitivity (CBH), delayed hypersensitivity (with or without a basophil component), and a late appearing inflammatory reaction, possibly mediated by antibody.

General Morphologic Description of Cutaneous Basophil Hypersensitivity (CBH)

These reactions occurred in animals sensitized with antigens in adjuvants lacking mycobacteria or in saline and skin tested at early intervals (1–2 wk) after sensitization. Hapten-specific reactions to the arsanilic acid group were studied in greatest detail because antibody with specificity for this determinant, which if present might complicate interpretation, was not detectable within the first few weeks after sensitization. Such reactions were elicited in animals immunized with ABA-BSA in incomplete adjuvant by skin testing with ABA-L-GAT. However, similar results were obtained with a variety of protein antigens in appropriately sensitized animals.

The CBH reactions studied were grossly identical with those described previously (5, 6) and consisted of circumscribed or ill-defined erythematous macules with little or no induration. They first became visible at 6 hr, reached a maximum at 24 hr, and had faded significantly at 48 hr after skin test.

Scattered round cells, neutrophils, and occasional basophils in and around thin-walled veins and venules were noted microscopically as early as 6 hr after skin test. Normally granulated mast cells were present, and lymphatic channels were dilated.

With time this process became more extensive, and basophils and mononuclear cells in abundance were identified within vessel lumens and passing through the walls of capillaries and small venules of the superficial dermis and larger venules and veins of deeper tissues (Figs. 2–5, 11, 14, 17, and 18). Mononuclear cells were commonly noted to be dividing within vessel lumens and as they passed through vessel walls into the interstitium (Figs. 2, 4, 5). Junctions between endothelial cells in these vessels were invariably closed (Fig. 14), except during cell migration, and there was no evidence of intercellular gaps to suggest an histamine-like effect (12). Moreover, colloidal carbon injected intravenously 40 min before sacrifice showed insignificant leakage from vessels in 48-hr lesions as observed in glycerol-cleared whole skin lesions or in 1 μ sections.

The cellular infiltrate of mature (24–48-hr) lesions was consistently located in two areas of the cutis, each having a somewhat different distribution of cell types. The superficial dermis and periadnexal zones (Figs. 1 and 7) were massively infiltrated with basophilic leukocytes and with smaller numbers of mononuclear cells described in greater detail below. Mononuclear cells and rare basophils invaded the epidermis focally, and there was loosening of contacts among epidermal cells with resulting subepidermal (and more rarely intraepidermal) clefts or vesicles (Fig. 12).

A second and more focal zone of cellular infiltrate was present in all but the weakest reactions in the lower dermis and subcutis in relation to large, thin

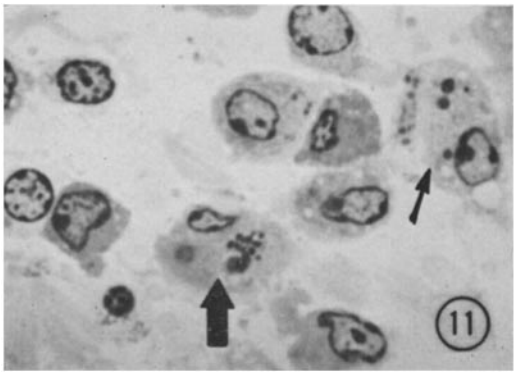


FIG. 11. Hypodermis of a 48 hr CBH reaction showing lymphocytes and macrophages, two of which (arrows) contain phagocytosed material. Basophil granules and pyknotic nuclear material are visible within one macrophage (larger arrow). × 1450.

walled venules and veins (Figs. 2–5). This infiltrate contained many mononuclear cells but included moderate numbers of basophils as well.

Frequent mitoses were observed among epidermal and hair follicle cells (Fig. 7), and there was progressive acanthosis of the epidermis over the course of several days. Tissue fibroblasts, normal inhabitants of the dermis, appeared activated with elongate nuclei containing one or two prominent nucleoli. There was abundant cytoplasm filled with strands of dilated rough endoplasmic reticulum containing flocculant electron-opaque material as well as prominent Golgi areas and numerous mitochondria. These large cells with prominent dendritic processes were commonly seen closely apposed to and surrounded by infiltrating cells including basophils. Myocytolysis with focal necrosis and secondary acute inflammation of isolated muscle fibers of the panniculus carnosus was present in some lesions, a finding that has been described in classic delayed reactions (13).

The cellular infiltrate was generally maximal at 24–48 hr after skin test, even

though the grossly observed reaction of erythema faded significantly after 24 hr. In typical lesions, basophils comprised 50–90% of the papillary dermal infiltrate and a smaller per cent of the deep dermal infiltrate. Exudate cells of all types including basophils and the various types of mononuclear cells formed

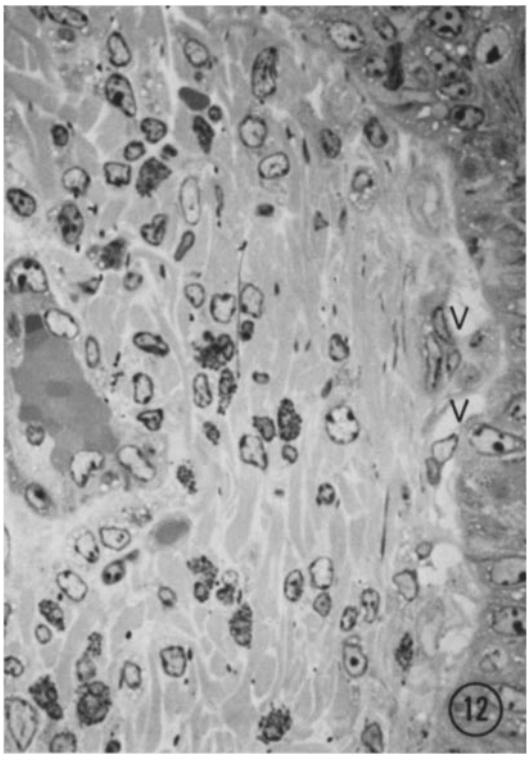


FIG. 12. Papillary dermis of a 24 hr reaction to 50 μg EA in an animal immunized previously with 10 μg EA in complete Freund's adjuvant. Note the prominent basophil infiltrate and subepidermal vacuoles (V). Reactions of this type were regarded as a mixture of delayed and cutaneous basophil hypersensitivity. × 600.

varied sized aggregates in close approximation to each other (Figs. 1, 7, 19). Close associations of basophils and macrophages were particularly common (Fig. 19). Basophils were never seen in mitosis, although dividing mononuclear cells were frequently encountered. A prominent feature in 48-hr lesions that was also evident to a lesser extent at earlier intervals was phagocytosis of fragmented basophils with pyknotic nuclei by macrophages (Figs. 11 and 20). Degranulation of basophils was thought to occur occasionally (Figs. 9 and 16, and see below).

After 48 hr, reactions were seldom visible grossly, but the cellular infiltrate persisted and disappeared slowly over the course of 2 wk with death and phagocytosis of basophils. Deeper components of the reaction persisted longest, and scattered round cells and basophils remained as late as 16 days.

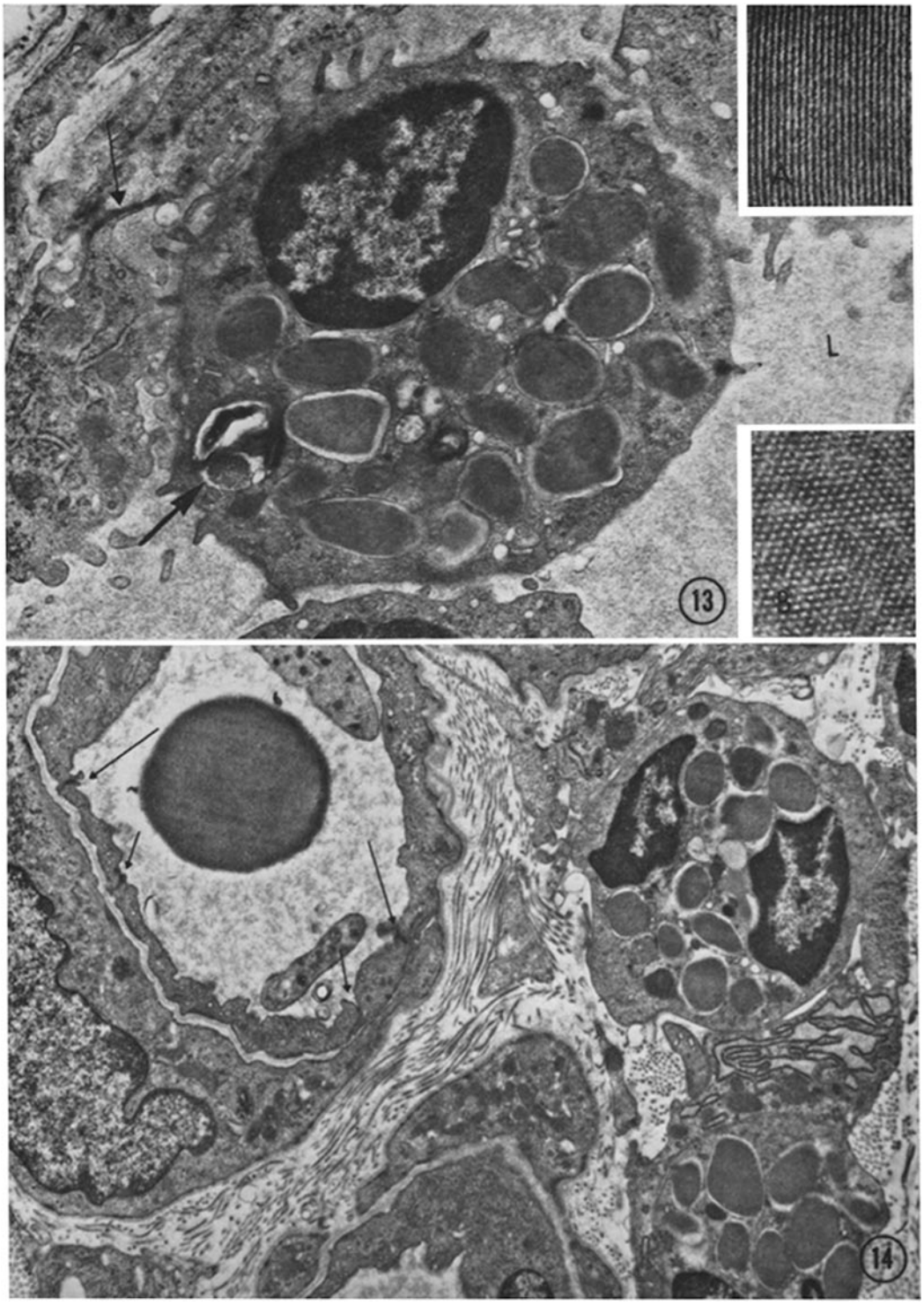
The above description was generally true for all antigens tested. Animals immunized with the ABA-BSA conjugate reacted as strongly to the carrier (i.e., azobenzoate-BSA) as to the hapten (i.e., ABA-L-GAT). Reactions elicited with the homologous immunizing conjugate, ABA-BSA, were qualitatively similar but more intense. Typical reactions were observed in animals sensitized with 10–100 μg of egg albumin, human serum albumin, or peroxidase in incomplete adjuvant or with 100 μg of KLH in saline and tested with 10 or 50 μg of the corresponding protein at 1 wk. Reactions were stronger with the higher skin test dose. Immature blast cells and plasma cells were more common in animals sensitized and skin tested with protein antigens.

Injection of any of the agents used for skin testing into normal or nonspecifically sensitized animals resulted in no reaction or in trivial gross or microscopic reactions.

Detailed Description of Infiltrating Cell Types

Basophilic Leukocytes.—The guinea pig basophil was readily identified by its characteristic large, oval, membrane-limited granules (Fig. 13) whose ultrastructure is unique to this speceis (14). In confirmation of other authors (14–16), we observed the following patterns: alternation of light and dark parallel bands, with a periodicity of approximately 130 A (Fig. 13A), the most common type, and a hexagonal lattice (honeycomb arrangement, Fig. 13B) possibly representing an exact cross-sectional cut of the first pattern. A third pattern, described by Terry et al. (14), the rectangular lattice, may represent a grazing section of the hexagonal lattice and was not observed in our material. Rather, we often found a granular pattern which occasionally showed irregular periodicity and was felt to represent oblique sectioning of the more regular patterns.

The basophils observed in skin lesions (Figs. 13, 14, and 19) were mature granulocytes, exhibiting multilobed nuclei (generally two or three, but occasionally four lobes). The nuclear chromatin was densely clumped at the nuclear membrane and was more dispersed centrally. Mitochondria were present in small numbers. Most cells had a small Golgi apparatus; however, some showed a prominent Golgi area with centrioles. The cytoplasm contained few free ribosomes and only an occasional strand of rough endoplasmic reticulum but numerous small membrane-limited vacuoles of varying size (Figs. 13 and 19). Many such vacuoles were located peripherally and were probably derived from infoldings of surface villi, which were prominent on the surface of circulating basophils (Fig. 13). Larger membrane-limited multivesicular bodies (Fig. 19) were present in nearly every cell. Occasional cells contained large vacuoles with



Figures 13–20 are electron photomicrographs.

FIG. 13. A small venule in the superficial dermis containing a basophil. Note the surface villae on the basophil as well as the large cytoplasmic vacuole with central dense and granular material (large arrow). This may represent an autophagic or phagocytic vacuole. Also note the closed intercellular junction between endothelial cells (small arrow). Vessel lumen (L). × 17,000. A, Portion of mature granule showing the characteristic parallel banded pattern with a periodicity of approximately 130 A; × 85,000. B, Portion of mature granule showing the honeycomb pattern; × 85,000.

FIG. 14. Another small venule in the superficial dermis with a basophil located adjacent to it. The lumen contains a red blood cell and two platelets. Notice four (arrows) intercellular junctions in the endothelium, all of which are closed, supporting lack of an histamine-like

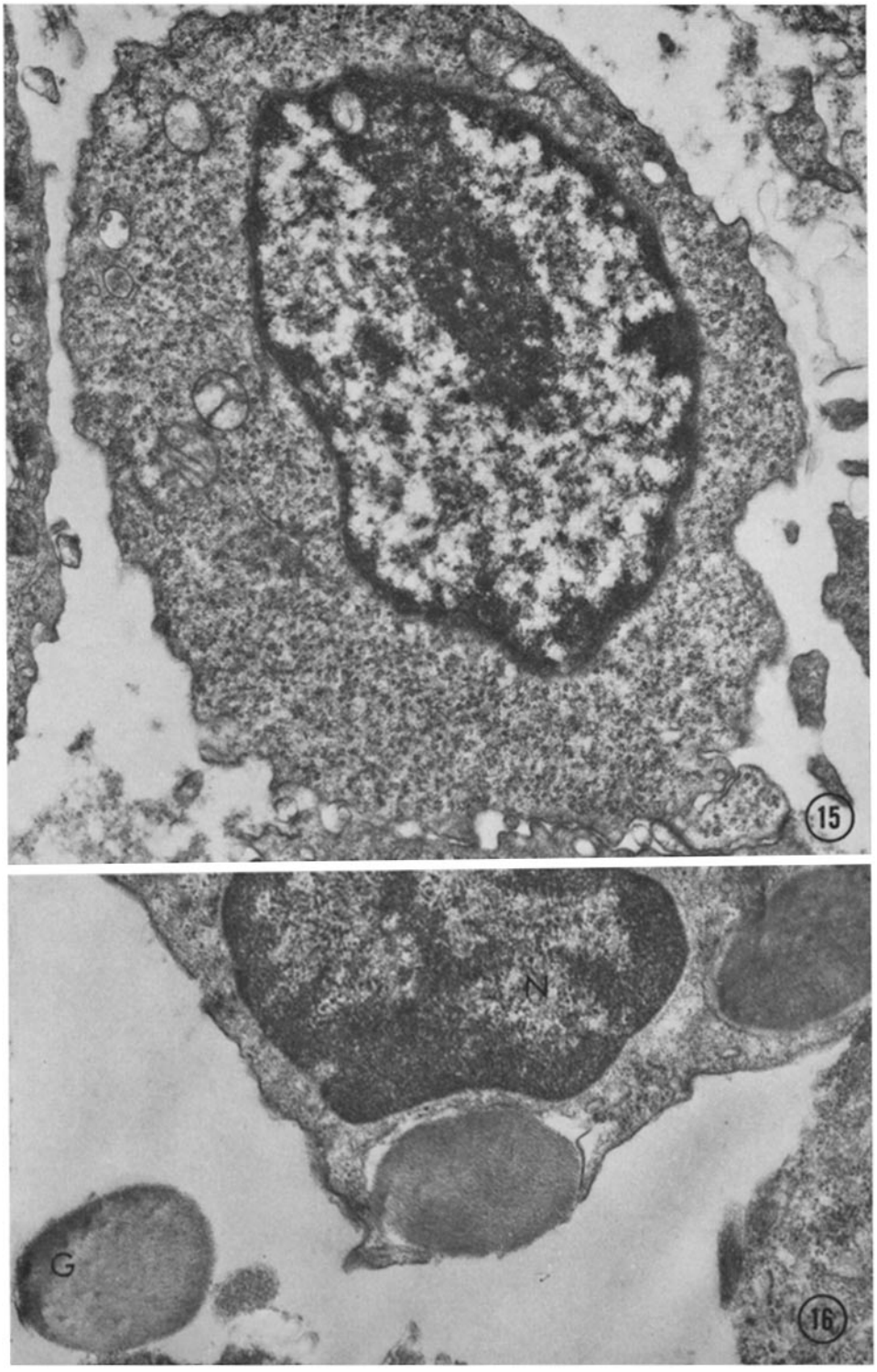
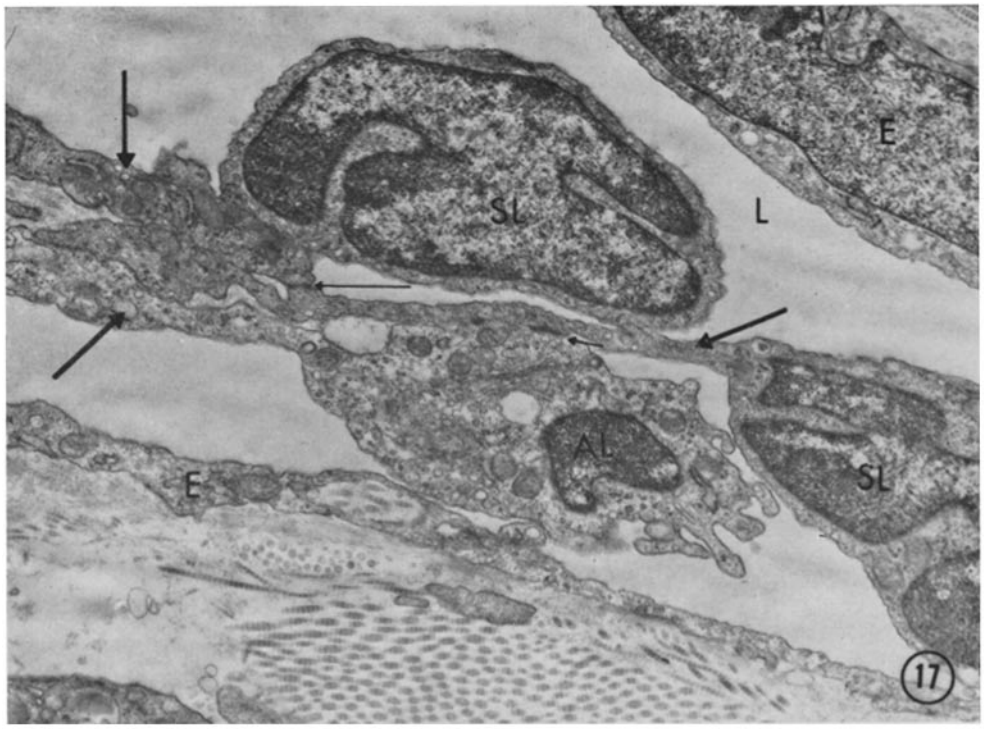


FIG. 15. A blast cell located in the deep dermis which shows a large nucleus with peripherally condensed chromatin and large nucleolus. The cytoplasm is abundant with numerous polysomes, a multivesicular body, and several mitochondria with irregular cristae. Advantageous sections reveal an active Golgi area in these cells. × 17,000.

FIG. 16. A portion of a basophil showing extrusion of a granule through the plasma membrane to the extracellular space. Notice both the granule in the process of extrusion and the granule (G) free in the interstitium retain their characteristic banded pattern. Also, notice the nonpyknotic, viable basophil nucleus (N). × 25,000.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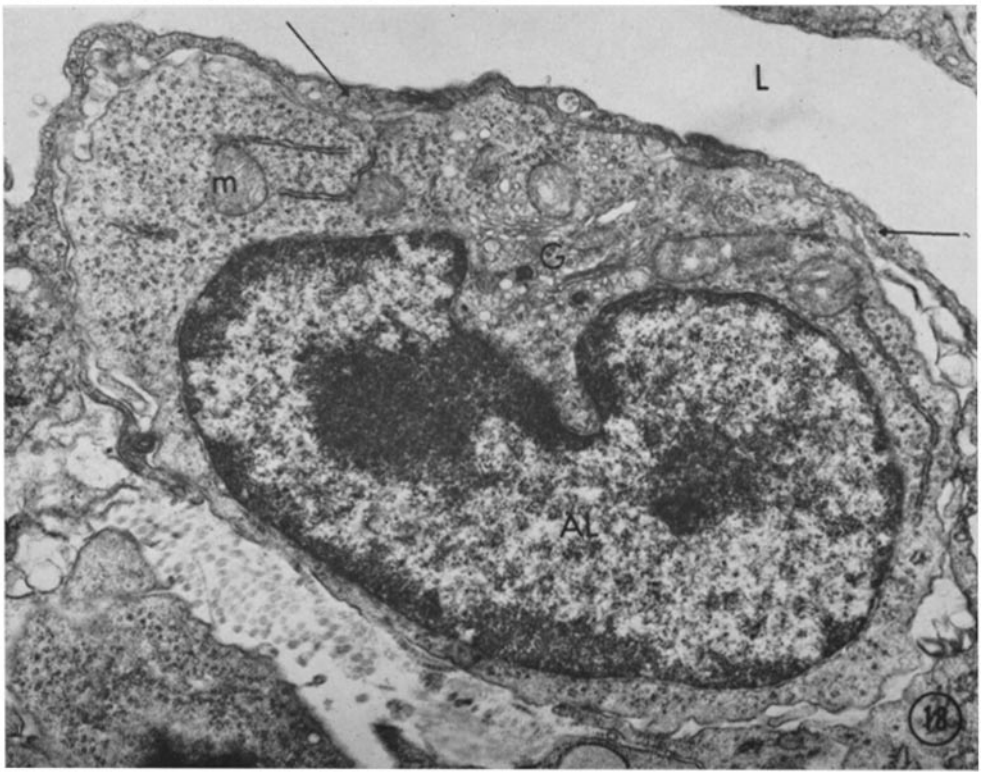
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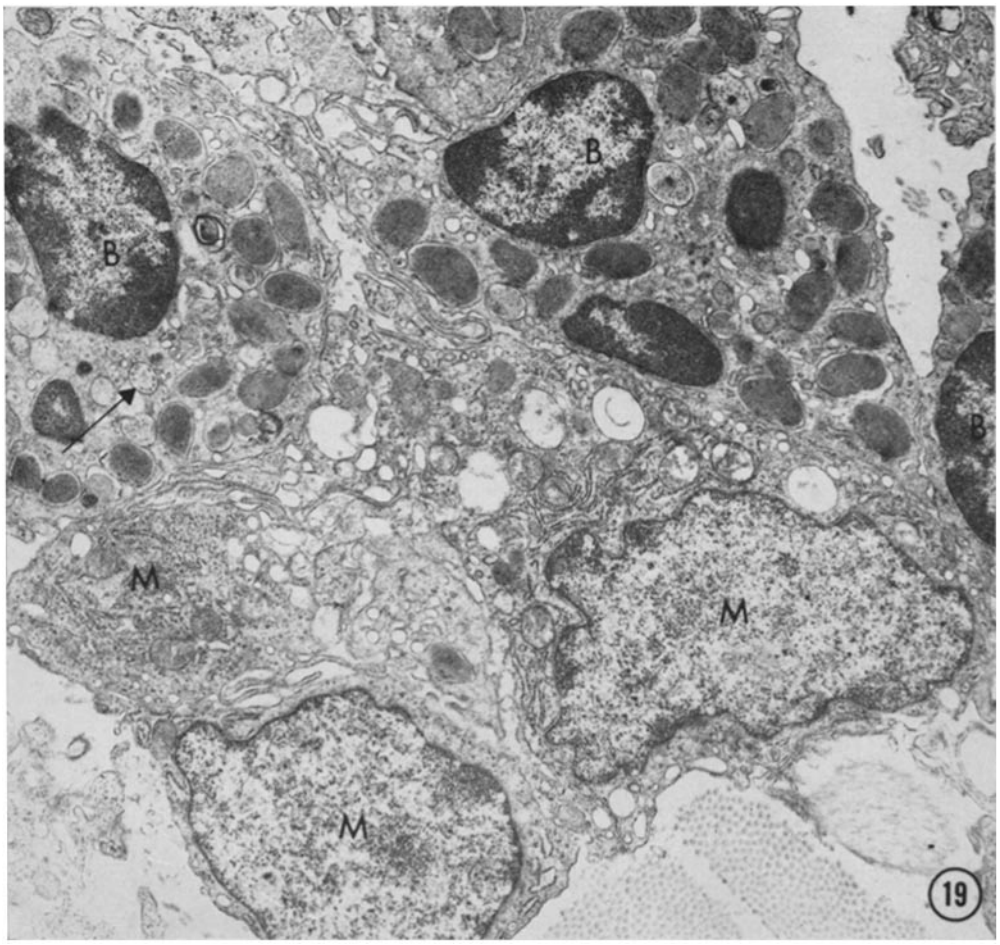

FIG. 19. Low magnification photo to show a group of cells in the dermis. Typical macrophages (M) with large, pale, irregular nuclei and a cytoplasm filled with vesicles and vacuoles, mitochondria, rough endoplasmic reticulum, and free ribosomes are adjacent to several basophils (B). These contain multivesicular bodies (arrow) as well as previously mentioned details. × 8000.

FIG. 17. A small venule, cut longitudinally, containing two small lymphocytes (SL) and one activated lymphocyte (AL), all with elongated uropods (large arrows). Notice the two points of attachment (small arrows) between the uropods of these cells and the fine filaments in the cytoplasm at these points. Also note the small amount of cytoplasm with few organelles in the small lymphocytes. Vessel lumen (L); endothelium (E). × 11,000.

FIG. 18. An activated lymphocyte (AL) with an increased amount of cytoplasm containing numerous ribosomes, some grouped in rosettes, a few strands of rough endoplasmic reticulum, an active Golgi area (G), numerous mitochondria (m), and a large nucleus, is located within endothelium (arrows). (L, lumen of a small venule). × 13,000.

central dense material (Fig. 13), indicative of either the residuum of phagocytosis or autophagy, a distinction impossible in the present study.

Basophil granules retaining their characteristic internal structure were seen free in the tissue (Figs. 9 and 16) and were often closely applied to the external surface of basophils and mononuclear cells. Rarely, extrusion of an individual granule from the cell was observed (Fig. 16). While no evidence was obtained to indicate that these cells had a phagocytic function, portions of basophils and

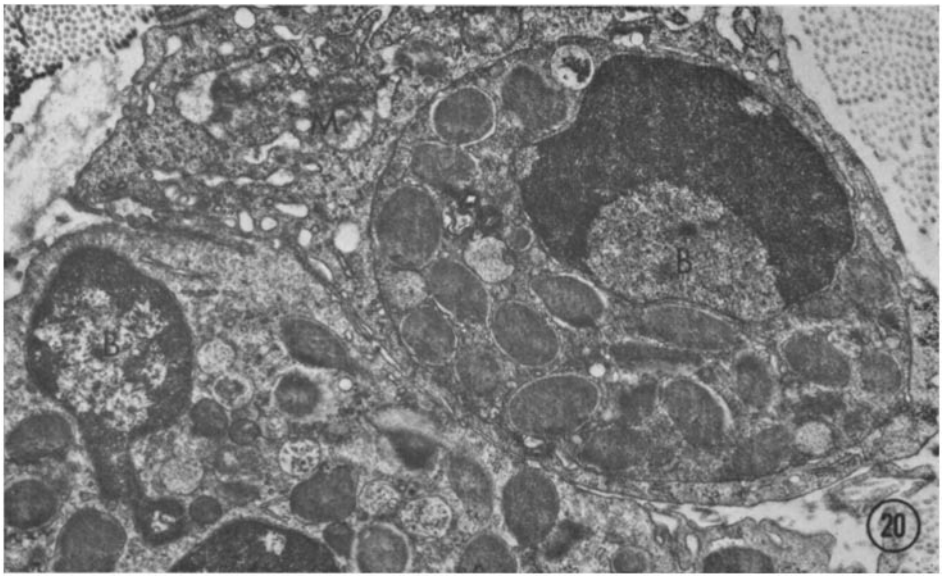

FIG. 20. A macrophage (M) containing a large portion of a dying basophil (B). Another basophil (B) is immediately adjacent to the macrophage. Notice that the nucleus of the phagocytosed basophil is pyknotic, but the granules retain their structure. × 10,000.

their free granules were themselves commonly phagocytosed by macrophages (Figs. 11 and 20). The nuclei of ingested basophils, when observed, were generally pyknotic, and the phagocytosed basophil cytoplasm was vacuolated and degenerated. The granules, however, showed a remarkable resistance to destruction and frequently remained intact with characteristic ultrastructure and limiting membrane (Fig. 20) after their ingestion by macrophages. Some basophils, prior to phagocytosis by macrophages, showed nuclear pyknosis and cytoplasmic degeneration indicative of cell death (Fig. 1).

Other Cells.—Typical small and activated lymphocytes, monocytes, macrophages, and blast cells were observed. In vessels (Fig. 17) or loose connective tissue of the dermis, small lymphocytes had indented nuclei and elongate

uropods which contained fine filaments and served as attachment points to each other, to other cells, and to collagen and endothelium.

The most frequent mononuclear cell was the activated lymphocyte (17). This cell was larger than the small lymphocyte and had a more abundant cytoplasm containing numerous free ribosomes, commonly forming rosettes, and strands of rough endoplasmic reticulum (Fig. 18). Other cytoplasmic organelles including mitochondria and Golgi areas were increased, and multivesicular bodies were common. Occasional membrane-limited dense bodies closely associated with the Golgi area were seen. The nuclei were large, contained one or two prominent nucleoli, and the nuclear chromatin was more dispersed than in small lymphocytes. Advantageous sections revealed prominent centrioles. Activated lymphocytes were observed within small vessels, migrating through vascular endothelium (Fig. 18); and they were prominent in the cell aggregates which formed in the dermis.

Typical monocytes were characterized by their large, pale, irregular nucleus, ample cytoplasm containing many vesicles, and membrane-limited dense bodies but few ribosomes. The classification of individual cells as either monocytes or activated lymphocytes was not always clear-cut since these cells have many features in common.

Macrophages within loose connective tissue had numerous surface villi and commonly occurred in cell aggregates with basophils which they commonly phagocytosed (Fig. 19).

Blast cells were the largest infiltrating cells and were present in small numbers and in close association with the other types of exudate cell described. They had abundant cytoplasm filled with free ribosomes often grouped as polysomes (Fig. 15) and, infrequently, a few strands of nondilated rough endoplasmic reticulum. The cytoplasm contained a prominent Golgi area, numerous mitochondria, often multivesicular bodies, and centrioles. The nuclei usually had peripherally condensed chromatin and one or two large nucleoli. Mitotic figures were observed in these cells. Ultrastructurally similar cells have been identified in the paracortical areas of lymph nodes, draining immunization sites in these animals.[1]

Occasional eosinophils and neutrophils were also observed.

Delayed Hypersensitivity Reactions

Classic delayed reactions were produced in animals sensitized with 2×10^{-6} moles ABA-L-tyrosine, 10 μg of EA or HSA, or 20 μg of peroxidase in complete Freund's adjuvant. Skin tests were performed 1–6 wk after sensitization with 6, 10, or 50 μg of antigen. Typically, erythema appeared at 6 hr after skin test, became maximal with induration at 24 hr, and persisted at 48 hr. Lesions of this description could be elicited at all intervals studied and were more intense at 3–6 wk than at earlier intervals after sensitization.

[1] A. M. Dvorak. Unpublished observations.

The microscopic features of the delayed hypersensitivity reactions studied here were similar to previous descriptions (18). 6-hr lesions were characterized by a perivascular infiltrate of mononuclear cells in the deep dermis, subcutis, and underlying muscle and by a more diffuse infiltrate in the upper dermis. These cells were mostly activated lymphocytes and monocytes with fewer small lymphocytes. Over the succeeding hours cellular accumulations continued to develop, coming to occupy large portions of the dermis and subcutis and choking lymphatic channels. The mononuclear cells occupying the dermis appeared larger than the cells emigrating from blood vessels (presumably their progenitors) with more abundant cytoplasm and a tendency toward a kidney-shaped nucleus. They were thus classified as histiocytes or macrophages. With low skin test doses, neutrophils never comprised more than a minority cell type. Basophilic leukocytes were regularly seen in delayed reactions, but their frequency varied consideraby depending on the size of the sensitizing and skin test dose and the interval between sensitization and testing (see Fig. 12 and below).

Late Reactions

Animals sensitized with ABA-BSA or with HSA in incomplete adjuvant and skin tested 5–6 wk later developed erythematous reactions which reached maximum intensity at 4–6 hr and were somewhat faded at 24 hr. Evans blue dye administered intravenously to such animals shortly after skin test extravasated immediately at the sites of injection of specific antigen, indicating local cutaneous anaphylaxis. Reactions of this type were strongest in animals receiving high sensitizing and skin test doses of antigen.

24- and 48-hr lesions were characterized by extensive infiltration of the dermis and subcutis by neutrophils and eosinophils as well as by mononuclear cells. Basophils were present in the upper dermis but in smaller numbers than in reactions elicited at early intervals after sensitization (Table I). Blast cells were more frequent than in either CBH or in delayed reactions.

Quantitative Comparison of the Cellular Composition of CBH, Delayed, and Late Reactions

The preceding microscopic description has presented qualitative evidence that the three reactions under consideration are morphologically distinct. The data in Tables I–III provide quantitative support for this conclusion.

The cellular infiltrate of CBH reactions included an average of 32–44% basophils (range of individual animals 21–51%), whereas late reactions had less than 10% and delayed reactions less than 2% basophils (Table I). These differences in percentage could have occurred by chance with a frequency of less than 0.1% (significant difference at 99.9% confidence limits). Moreover, the method of cell counting permitted analysis of the absolute numbers of basophils and total infiltrating cells per unit surface area of reaction. Thus, it is possible to state that the total cellular infiltrate in delayed reactions was significantly

greater than that in either CBH or late reactions, an expected finding in view of the increased thickness of delayed reactions.

The increased cellularity of delayed reactions was largely due to increased numbers of mononuclear cells, and it would be possible to conclude that the lower proportion of basophils observed in delayed reactions only reflected an increase in the total number of infiltrating cells (the denominator in a calculation of the proportion of basophils present). However, this is not the case since the absolute basophil count per unit surface area was significantly greater in

TABLE I

*Differential Cell Counts Performed as in Fig. 10A in CBH, Delayed, and Late Reactions**

Reaction	Sensitizing antigen	24 hr skin reaction†	Time of biopsy	Number of animals	Mean cell counts ± SE					% Basophils
					Mononuclear	Neutrophils	Basophils	Eosinophils	Total	
			hr							
CBH	ABA-BSA	14 (0)	24	6	92	6	68 ± 17	3	169 ± 37	40 ± 3
		13 (0)	48	6	204	3	119 ± 18.8	2	328 ± 41.4	36 ± 4
	Peroxidase	12 (0)	48	3	217	4	105 ± 14.8	3	329 ± 26.8	32 ± 7.1
	KLH	10 (0)	48	4	83	<1	68 ± 26.9	2	154 ± 43.5	44 ± 8.9
Delayed	ABA-tyrosine	14 (++−+++)	24	9	491	36	9 ± 2.7	<1	536 ± 52	1.7 ± .6
		13 (+++)	48	2	547	50	6 ± 2.1	0	603 ± 11	1.0 ± .1
	Peroxidase	14 (+++)	24	3	479	74	5 ± .9	3	561 ± 83	0.8 ± .9
Late	ABA-BSA	11 (0)	24	8	126	89	13 ± 5	48	276 ± 55	5 ± 2.0
		11 (0)	48	7	208	20	26 ± 8.6	47	301 ± 45	9 ± 1.7

* CBH reactions were elicited in animals sensitized with 100 μg ABA-BSA or peroxidase in incomplete Freund's adjuvant or with 100 μg KLH in saline, and skin was tested 7 days later with 50 μg ABA-L-GAT, peroxidase, or KLH. Delayed reactions were elicited in animals sensitized with 2×10^{-6} moles of ABA-L-tyrosine or 20 μg of peroxidase in complete Freund's adjuvant and skin tested 2–3 wk later with 50 μg ABA-L-GAT or 6 μg peroxidase. Late reactions were studied in animals sensitized with 100 μg ABA-BSA in incomplete adjuvant and skin tested 5–6 wk later with 50 μg ABA-L-GAT.

† Gross reactions expressed as diameter of erythema (millimeters) and, in parentheses, degree of induration (0−++++).

CBH (68–119 basophils) than in either delayed (6–9 basophils) or late (13–26 basophils) reactions. Similarly, late reactions had significantly more eosinophils than either CBH or delayed reactions, whether based on absolute counts or as a per cent (16–17%) of the total cellular infiltrate.

The data in Table II indicate that the morphologic differences between CBH and delayed reactions were most striking in the papillary dermis. Basophils comprised an average of 58–70% (range of individual animals, 42–90%) of total infiltrating cells in this region in CBH reactions and a considerably lower percentage ($P < 0.001$) in delayed reactions. However, in both lesions such basophils as were present were concentrated in the uppermost dermis. This may explain the frequency with which Wolf-Jürgensen encountered basophils in studies of delayed reactions in man, with the skin window technique (19).

The data in Table III consider the role of the vehicle used for sensitization and the size of the skin test dose in determining the character of delayed-onset reactions elicited 7 days after sensitization with egg albumin. With the small skin test dose typically used to elicit delayed reactions (10 μg), animals immunized with either complete or incomplete adjuvant developed reactions which differed significantly in induration and intensity of erythema but not in cellular

TABLE II

*Differential Cell Counts Performed as in Fig. 10B in the Upper Dermis in CBH and Delayed Reactions**

Reaction	Sensitizing antigen	Time of biopsy	Number of animals	Mean cells counts ± SE					% Basophils
				Mononuclear	Neutrophils	Basophils	Eosinophils	Total	
		hr							
CBH	ABA-BSA	24	6	73	2	116 ± 9	9	200	58 ± 4.3
		48	6	54	2	140 ± 7.5	4	200	70 ± 4.9
	Peroxidase	48	3	71	0	125 ± 3.3	4	200	63 ± 1.8
Delayed	ABA-tyrosine	24	7	167	20	13 ± 6	<1	200	6.5 ± 2.8
		48	2	173	17	10 ± 5.0	0	200	5.0 ± 2.5

* Animals were sensitized and skin tested as in Table I.

TABLE III

*Effect of Vehicle and Skin Test Dose on Cellular Composition of Skin Reactions at Early Intervals after Sensitization with Egg Albumin**

Sensitizing vehicle	Skin test dose	24 hr skin reaction†	Time of biopsy	Number of animals	Mean cell counts ± SE					% Basophils
					Mononuclear	Neutrophils	Basophils	Eosinophils	Total	
	μg		*hr*							
CFA	10	20 (+)	24	4	195	80	21 ± 7.2	5	301 ± 44	7 ± 2.4
		20 (+)	48	3	312	48	29 ± 11.2	5	394 ± 45.8	8 ± 1.9
IFA	10	15 (0)	24	4	110	17	12 ± 3.2	2	141 ± 17.4	8 ± 2.4
IFA	50	16 (0)	24	4	113	5	82 ± 10.4	2	202 ± 7.3	41 ± 5.7
		16 (0)	48	4	151	<1	105 ± 42.5	<1	257 ± 54.5	41 ± 8.1

* Animals were sensitized with 10 μg EA in complete or incomplete Freund's adjuvant, and skin was tested 7 days later with 10 or 50 μg EA in saline. Cell counts were performed as in Fig. 10A.

† Scored as in Table I.

composition. Moreover, the proportion of basophils in animals sensitized with complete adjuvant was higher than would be expected in delayed reactions elicited 2 wk or more after immunization.

A more significant variable was the size of the skin test dose. Reactions elicited with 50 μg EA in animals sensitized with incomplete adjuvant contained a proportional and highly significant increase in the absolute number and per cent of basophils with a smaller increase in the total cellular infiltrate. Reactions elicited with higher skin test doses at early intervals after sensitization with antigens in complete adjuvant also contained increased numbers of basophils.

Circulating Basophil Levels.—It is known that repeated injections of foreign proteins produce a systemic basophilia (20). It was therefore of interest to determine whether animals sensitized to give cutaneous basophil hypersensitivity developed basophilia or whether the accumulations of basophils in skin test sites reflected a local concentration of basophils already circulating. Blood basophil counts were performed by direct (21) or indirect (20) methods on a total of 12 guinea pigs at intervals after sensitization with ABA-BSA, peroxidase, or HSA in incomplete adjuvant. Animals were skin tested on day 7, and all gave typical CBH reactions. There was, however, no significant change in absolute or relative blood basophil counts as late as the 8th day after sensitization.

DISCUSSION

The data presented here provide morphologic evidence that delayed onset reactions elicited in guinea pigs at early intervals after sensitization with protein antigens in saline or in incomplete adjuvant differ qualitatively and quantitatively from the skin reactions of delayed hypersensitivity. Such reactions are characterized principally by massive accumulations of basophilic leukocytes in the papillary dermis accompanied by smaller numbers of mononuclear cells. In contrast to previous reports (3), plasma cells were never a prominent feature. For the reasons given, we have designated lesions with this histology cutaneous basophil hypersensitivity (CBH).

Basophilic leukocytes have not been appreciated in previous descriptions of the so-called Jones-Mote reaction in the guinea pig (3), probably because basophil granules lose their metachromatic staining properties after aqueous fixation and processing and are not subsequently visualized well with ordinary histologic methods (compare Figs. 7 and 8). In standard hematoxylin and eosin sections basophils are easily confused with polymorphonuclear leukocytes, lymphocytes, or macrophages. Aside from the predominance of basophils, CBH has many of the morphologic features of mild delayed hypersensitivity skin reactions, and it is not surprising that others, noting only the mononuclear infiltrate, have regarded Jones-Mote reactions as a weak form of delayed hypersensitivity (4).

Basophils have been described as a minor component of delayed hypersensitivity reactions in man and experimental animals. Wolf-Jürgensen (19), using the skin window technique in human subjects, found increased numbers of basophils in skin sites of individuals possessing delayed hypersensitivity to antigens such as tuberculin, dinitrochlorobenzene, and diphtheria toxoid or undergoing normal lymphocyte transfer reactions. Basophils accounted for 1–7% of total cells although rare values in excess of 20% were recorded. Basophils have also been observed in the subcutaneous tissues of guinea pigs and rabbits receiving large doses of egg albumin or ventriculin (22, 23). Foerster et al. (24) recently reported basophilia in guinea pigs undergoing a systemic graft vs. host reaction and occasional basophils have been described in classic delayed hypersensitivity skin reactions when electron microscopic techniques

were employed (25). We have found that skin reactions rich in basophils may be elicited in guinea pigs tested 1 wk after sensitization with protein antigens in complete Freund's adjuvant, although such reactions have in addition the features of classic delayed hypersensitivity including induration and an extensive mononuclear infiltrate. We interpret such reactions to be a mixture of cutaneous basophil and delayed hypersensitivities. Ironically, it is such early reactions that have often been regarded as the purest form of delayed hypersensitivity since antibody is generally not yet detectable in the circulation.

The function of the basophil in CBH is obscure but is presumably related to the contents of its prominent cytoplasmic granules. The granules of different species are said to contain histamine, an acid mucopolysaccharide (? heparin), glycogen and glycoprotein, proteins and lipoproteins (19). Basophils are thought to be analogous to tissue mast cells, and they might, therefore, carry antibody on their surface and discharge their granules on contact with antigen. Basophil degranulation has been reported in vitro in sensitized subjects on exposure to allergen (26). In mature CBH reactions structurally intact, free basophil granules were commonly observed in the dermal interstitium. Such apparent degranulation could have resulted from an interaction of cell-bound antibody with skin test antigen. Alternatively, these free granules might simply reflect extrusion from damaged or dying basophils. In any case, the majority of basophils comprising the infiltrate of CBH do *not* degranulate, and after a period of residence in the dermis they undergo nuclear and cytoplasmic degeneration with relative preservation of granule structure and are phagocytosed by macrophages.

It is of course possible that pharmacologic agents are released from basophil granules without concomitant degranulation. Cruickshank and Haye (27) have shown that degranulation of human basophils and histamine release are not necessarily coincidental. It is also possible that release of pharmacologic materials from granules, whether extruded or within the cell, is not accompanied by obvious electron microscopic alterations in granule structure. The granules, as seen after aqueous fixation and tissue processing, have lost their characteristic metachromasia and possibly the acid mucopolysaccharide thought to be responsible for this staining property. Therefore, the complex granule arrangement seen with the electron microscope may merely be a naked structural framework to which functionally active molecules are attached.

Whatever the relation of biologically active materials to granule structure, there is at present no evidence, either biologic or morphologic, to suggest significant local histamine release in CBH. Venules and capillaries observed under the electron microscope, though frequently surrounded by basophils, regularly had closed intercellular junctions, and colloidal carbon did not leak from the vessels of mature reactions. Also, Richerson et al. (6) found no evidence of dye extravasation at any interval after skin test. Normal vascular permeability would thus

seem to be another point of differentiation from true delayed hypersensitivity reactions (28). Nothing can be said about the release of other active molecules from basophil granules, and in fact the chemical contents of these granules in the guinea pig are not well defined.

The pathogenesis of cutaneous basophil hypersensitivity is at present unknown. Perhaps basophils have a function analogous to that of tissue mast cells and, carrying homocytotrophic antibody on their surfaces, are attracted to local sites of antigen deposition. However, good evidence exists for the role of sensitized mononuclear cells in the pathogenesis of this reaction. It is possible to transfer cutaneous basophil hypersensitivity reactions with sensitized lymph node cells but not with serum (29), and the reactions in the recipient have the basophil infiltrate described here.[2] The reaction is inhibited by antilymphocyte serum and has antigenic requirements for the expression of skin test reactivity similar to that of delayed hypersensitivity (5, 6). Bast and Dvorak (30) have demonstrated stimulation of DNA synthesis by specific antigen in cultures of lymph node cells from animals with cutaneous basophil reactivity. This stimulation is accompanied by morphologic blast transformation[3] and does not occur in lymph node cells cultured at late intervals after sensitization.

We therefore suggest a mechanism for CBH analogous to that proposed for delayed hypersensitivity in which specifically sensitized lymphocytes interact at the skin test site with antigen and release biologically active substances comparable to *migration inhibition factor*. One such substance could be responsible for attracting basophils. Our hypothesis implies that cellular immunity encompasses a heterogeneous group of reactions presently including at least classic delayed and cutaneous basophil hypersensitivities. Ultimate classification of such reactions will require analysis of the biologically active molecules liberated when specifically sensitized cells are exposed to antigen in vivo or in vitro.

SUMMARY

Delayed onset erythematous skin reactions elicited in guinea pigs *early* in the course of sensitization with azobenzenearsonate-protein conjugates or with protein antigens in incomplete Freund's adjuvant or in saline were found to have a characteristic morphology which sets them apart from delayed hypersensitivity and the classic antibody mediated reactions. The principle feature was massive dermal infiltration with basophilic leukocytes. Mononuclear cells of several types including activated and small lymphocytes, monocytes, macrophages, and blast cells were also present. Such reactions have in the past been designated Jones-Mote hypersensitivity, but we prefer the descriptive term cutaneous basophil hypersensitivity (CBH) for the reasons given.

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[2] Dvorak, H. F., R. C. Bast, Jr., and S. Leskowitz. Unpublished data.

[3] Dvorak, A. M., R. C. Bast, Jr., M. J. Karnovsky, and H. F. Dvorak. Unpublished observations.

Occasional basophils extruded their granules, and individual granules, retaining their characteristic ultrastructure, were commonly seen in the interstitium. However, intercellular junctions between endothelial cells were closed except during cell emigration and there was no morphologic evidence of an histamine-like effect. The majority of basophils, moreover, did not degranulate but underwent nuclear pyknosis and cytoplasmic degeneration and were phagocytosed by macrophages. Phagocytosed basophil granules retained their ultrastructure.

Skin tests performed at late intervals after sensitization had a different time course and morphology. Animals sensitized with protein antigens in complete Freund's adjuvant developed delayed hypersensitivity; however, reactions elicited in such animals at early (but not late) intervals after sensitization contained a prominent basophil component. We interpret such reactions to be a mixture of delayed hypersensitivity and cutaneous basophil hypersensitivity. The function of the basophil in CBH and its relation to the mononuclear cells which accompany it are unknown, and various possibilities are discussed.

We conclude that cutaneous basophil hypersensitivity is a distinct immunologic and morphologic entity, occurring early in the course of sensitization with protein antigens incorporated in any of several vehicles. The mechanism of the reaction is presently unknown, and a general hypothesis to explain its pathogenesis has been proposed.

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