Electron microscopy of experimental uveitis

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The fine structural features of the early pathological changes induced in uveal tissue by single intravitreal injections of three different quantities of bovine serum albumin (BSA) in rabbits were studied. Small quantities of antigen (BSA) did not produce clinical signs of uveitis but a mild leukocytic infiltration (mainly monocytes) was found in the base of the ciliary body. The occasional basophilic leukocytes in the reaction site and choroidal mast cells were not degranulated and no alteration in the blood-aqueous barrier was found. Larger quantities of BSA produced mild uveitis and an impressive mononuclear infiltration in the base of the ciliary body, the root of the iris, the pars plana, and the peripheral choroid. The infiltrating cells included some which appeared to be intermediate between small lymphocytes and plasma cells, as well as monocytes. Intercytoplasmic connections between monocytes and lymphocytes were observed, suggesting that immunological information was transferred by this means. Emigration of all types of mononuclear cells from blood vessels, and their passage through the ciliary epithelium into the posterior chamber, was frequently noted; however, in these regions, little alteration was observed in the structures associated with the blood-aqueous barrier. Small lymphocytes passed through, and larger mononuclear cells between, the endothelial cells when leaving the vascular system. Small islands of polymorphonuclear (PMN) heterophilic leukocytes were also seen in both the iridial and ciliary processes. In these regions, pronounced alteration was found in the blood-aqueous barrier and both basophilic leukocytes and mast cells showed degranulation. Injection of a still larger amount of BSA provoked severe uveitis with massive PMN heterophilic infiltration in the base of the ciliary body and in localized areas in the iridial and ciliary processes. Significant hemorrhage, thromboses, and necrosis of blood vessels were observed in the ciliary body. The structures associated with the blood-aqueous barrier showed marked changes. In addition to such vascular alterations, mononuclear cell infiltration was seen in the pars plana and the peripheral choroid.

Key words: allergic uveitis, albumin, intravitreal injection, epithelium of ciliary processes, blood-aqueous barrier, antigen-antibody reaction, histopathology, electron microscopy.

A single injection of an antigen into the vitreous humor of rabbits has repeatedly been shown to produce a nongranulomatous uveitis following a latency period of some days. It is well known that this inflammatory reaction is immunological in nature, connected with antigen-antibody reactions taking place in the uveal tissue and resulting in its injury,1,6 but the pathogenetic mechanism is not clearly understood. To date, the fine structural changes

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which occur during the inflammation have not been studied. Therefore, a series of electron microscopic observations on the cellular events produced in uveal tissue by single intravitreal injection of an antigen, bovine serum albumin (BSA), was made.

Particular attention was paid: (1) to the effect of mononuclear, heterophil, and basophilic leukocytes and of mast cells on the eye tissue, (2) to the process of degranulation of basophils and mast cells, (3) to morphological evidence of transfer of immunological information between cells, (4) to the route taken by cells in leaving blood vessels, and (5) to the pathology induced in the ciliary processes.

Materials and methods

Three groups of chinchilla rabbits weighing about 3 kilograms each were used in this experiment. All eyes were examined with a slit lamp and ophthalmoscope for abnormalities before experimentation. Crystallized BSA (Nutritional Biochemical Corporation, Cleveland, Ohio), was dissolved in isotonic saline and sterilized by passing through a Swinny filter immediately prior to its use. One tenth, or 0.15 ml. of the BSA solution was injected slowly into the vitreous humor of each rabbit, in the same manner employed by Zimmerman and Silverstein,2 Fernando,5 and Larsen3 in their experiments. Care was taken to avoid damage to the lens capsule.

In group I, 0.5 mg. or 2.0 mg. of BSA were injected in one eye, and in group II, a 10 mg. dose of BSA was given in one eye of each rabbit. The second eye of these animals was not injected. In group III, however, 45 mg. of BSA were injected simultaneously in both eyes. As controls, 0.15 ml. of isotonic saline was also injected in one eye of each of a fourth group in the same manner as the antigen injections. After injection, all eyes were examined daily with a slit lamp. Both the control and group I rabbits showed no clinical signs of uveitis a week to ten days after disappearance of the traumatic inflammation which was due to trauma of the injection. At this time the eyes were enucleated and prepared for electron microscopy.

The eyes of group II showed clinical signs of a mild uveitis one week to ten days after disappearance of the traumatic inflammation. Some eyes were enucleated one day, and other eyes seven days following the onset of the uveitis. The uveitis produced in group III was more severe than that in the second group, and occurred about seven days after disappearance of the inflammation due to trauma. These eyes were enucleated one day after the onset of the uveitis. The normal, noninjected eyes were also used as controls.

Electron microscopic procedure. The enucleated eyes were opened by an equatorial incision, and the two halves fixed immediately with cold 2 per cent osmium tetroxide in Millonig's buffer at pH 7.3 to 7.4. Portions of the iris, ciliary body, and the choroid were cut into small pieces while in the fixative, and fixation was continued. Some eyes were initially fixed with cold 4 per cent glutaraldehyde in phosphate buffer at pH 7.3 followed by post fixation in 2 per cent cold phosphate buffered osmium tetroxide at pH 7.3. The pieces were then dehydrated with acetone or alcohol, and embedded in Epon 812 in the usual way. One-micron sections were made with an LKB-ultramicrotome, and stained with 0.1 per cent toluidine blue (pH 8.0) and the site of inflammatory reactions was identified with the light microscope. Thin sections of these areas were then cut on the same microtome, stained with uranyl acetate and lead citrate, and observed under Siemens Elmiskop IA or Hitachi 11A electron microscopes. In order to confirm the presence of small lymphocytes within the endothelium of blood vessels, more than 100 thin serial sections were mounted on grids with 1.5 mm. diameter apertures.

Results

Cell types. The many cell types mentioned in this report were identified on a morphological basis by means of electron micrographs. Accepted definitions of these types were adopted. Therefore, a detailed description of them is not needed except for the basophilic leukocytes and mast cells which have not been thoroughly studied in this species. A description of the "blast" cells is included because definition of this cell type may not always have been precise.

Basophilic leukocytes. The fine structure of these cells has many characteristics in common with other blood granulocytes and is quite different from that of mast cells in spite of the similar staining reaction of the granules in light microscopy (Fig. 1). The cells were usually 5 μ to 5.5 μ round or oval, with few cytoplasmic protrusions. Some had lobulated nuclei, but others had a single eccentric nucleus with peripherally clumped chromatin. Mitochondria, unlike the elongated ones of mast cells, were usually round. A well-developed Golgi complex was frequently...
Fig. 1. A basophilic leukocyte in the base of the ciliary body, 24 hour stage, Group II experiment. Note the eccentric nucleus as well as the peripheral clumps of chromatin. Golgi complex (Gc), glycogen (gl), basophilic granule (g), early stage of basophilic granule (eg). (x16,000.) Throughout, the line on each figure represents one micron.

Fig. 2. A choroidal mast cell. The specimen is from a 24 hour stage in the Group II experiment. The fine structure, however, is normal. Mast cell granule (g), elongated mitochondrion (m), cytoplasmic processes (cp). (x25,000.)
found, in which were numerous small vesicles containing electron-dense 40 m\(\mu\) particles suggestive of early stages of granule formation. Glycogen was often present. Cytoplasmic granules were quite different from those of neutrophilic or eosinophilic leukocytes, and rather resembled mast cell granules. Their average size was about 0.25 \(\mu\); they were always surrounded by a single membrane, and consisted of electron-dense material, similar to the small particles seen in the vesicles in the Golgi area.

**Mast cells.** These were similar to, or somewhat larger than, basophilic leukocytes, usually oval or elongated, and always possessed several cytoplasmic protrusions from their surface (Fig. 2). Their nucleus was relatively large and oval, and always centrally located. The nuclear chromatin showed slight condensation at the periphery. A nucleolus was rare, and mitochondria were few in number but elongated and large. A few sacs of rough-surfaced endoplasmic reticulum were found, but a Golgi complex and glycogen were rarely seen. The mast cell granules consisted of three types. The most common type, “A,” was round or oval, frequently indented, 0.4 to 1.0 \(\mu\) in diameter, and consisted of coarsely particulate material, i.e., they were composed of a less electron-dense matrix and of more electron-dense particles. They were surrounded by a single membrane, outside of which were electron-dense particles (about 30 \(\mu\) in diameter) and several small tubular structures radiating from the granules. A less common type, “B,” was an almost homogeneous, electron-dense granule similar in size and shape to the “A” type, but in many instances without a surrounding membrane. Occasionally, a small, empty area was seen within the granule. A rare granule, type “C,” usually round, 0.6 to 1.2 \(\mu\) in diameter, was always surrounded by a single membrane and consisted of fine particles which were moderately electron dense in the center of the granule and occasionally electron dense in the periphery.

**Blast cells.** These were in transition or development and were large (8.0 \(\mu\)) rounded cells (Figs. 3 and 11) characterized by a large unindented nucleus, with homogeneously distributed chromatin and a prominent nucleolus. Ribosomes were abundant, and their fine structure, and that of the rough-surfaced endoplasmic reticulum and mitochondria, was very similar to that of large lymphocytes. The blast cells were of two types, one comparatively poor in rough-surfaced endoplasmic reticulum, and the other having an abundance of this component scattered irregularly throughout its cytoplasm. The former is the immunoblast, and the latter the plasmablast type as described by Movat and Fernando. Both types occasionally showed mitotic activity.

**Experimental data.**

**Group I.** These eyes, which received the smallest amount of antigen (0.5 to 2.0 mg. of BSA), showed no clinical signs of inflammation after the initial reaction to the injection had subsided. However, when enucleated 7 to 10 days after that time, a mild cellular infiltration in the base of the ciliary body near the anterior chamber was found histologically. It was almost purely mononuclear, but a few metachromatic granule-containing cells were occasionally noted. The mononuclear cells consisted of lymphocytes, histiocytes, and, predominantly, monocytes. The monocytes were both extra- and intravascular, but the histiocytes were always extravascular in the connective tissue. The few lymphocytes were small or medium in size. The metachromatic cells were basophilic leukocytes. Some metachromatic cells were also seen in the choroid, but they were mast cells. Neither the basophils nor mast cells were degranulated. Morphological evidence indicating a breakdown of the blood-aqueous barrier was not found.

**Group II.** These rabbits, which received 10.0 mg. of BSA in one eye each, developed a mild uveitis a week to ten days after the disappearance of the inflammation due to trauma. It consisted mainly of a perilimbal injection, hyperemia of the iris,
Fig. 3. Cellular infiltration in the peripheral choroid. The infiltrate consists of a monocyte (M), small (SL), medium (ML) and large (LL) lymphocytes, blast cells (B), and plasma cells (PS). This is an early (24 hour) stage of inflammation of Group II. (x4,200.)

Fig. 4. Mononuclear cell response in the base of the ciliary body. Small (SL) and medium (ML) sized lymphocytes and a monocyte (M) containing a phagocytic vacuole (pv). Note the lysosomes accumulated around and making contact with this vacuole. The phagocytic vacuole contains moderately electron-dense material and lysosomes (Is) are very near it. Early stage of inflammation of the Group II experiment. (x13,000.)
and cellular infiltration and a proteinaceous exudate in the anterior chamber.

The Group II experiments were the most extensive, and the report of the results is divided into sections with respect to the cell type involved and the structures affected.

1. The Mononuclear Cell Response. In the eyes enucleated one day after onset of the uveitis, pronounced cellular infiltration was found not only in the base of the ciliary body but also in the root of the iris, the pars plana, and the peripheral choroid. It was almost entirely mononuclear in character. The infiltration was generally composed of a few histiocytes, many monocytes, numerous lymphocytes of various sizes, and a small number of blast and plasma cells (Fig. 3). The proportion of these cells varied within one eye depending upon the region examined. An occasional monocyte was seen (Fig. 4) containing phagocytic vacuoles which were surrounded by a single membrane and filled with a moderately electron-dense homogeneous material. An accumulation of electron-dense lysosomes was found around these vacuoles, some of which appeared to contact the vacuolar membrane.

The route taken by cells when leaving blood vessels. Venules and capillaries, within or near the site of cellular infiltration, were frequently filled with mainly small or medium-sized lymphocytes and a moderate number of monocytes, although large lymphocytes, blast cells, and plasma cells were also present. Emigration of mononuclear cells from vessels into the connective tissue was frequent. Monocytes, large lymphocytes, blast cells, and plasma cells always passed through the intercellular junctions of the vascular endothelial cells (Figs. 5 and 11). However, small lymphocytes never took this route, but always appeared to pass through their cytoplasm via membrane-bound vesicles. In the first step of this process they made contact with the endothelium and became enveloped by its cytoplasm (Fig. 6). In the second stage the lymphocyte was found entirely within the cell (Figs. 7, 8, and 9). In the final step it appeared in the space between the endothelial cell and its basement membrane (Fig. 10). Some cells seemed to pass through both the endothelial cell and the basement membrane at one time, but others apparently did this in stages and penetrated the basement membrane after having left the endothelial cell and paused, perhaps momentarily, in the space between them. Before and after this emigration, no fine structural alteration was visible in either the cell or the basement membrane.

Intercellular Transfer of Information. Intercytoplasmic connections were found between monocytes and lymphocytes on several occasions. As seen in Figs. 12 and 12a, the cell membranes of the cells fused, forming a definite cytoplasmic continuity.

Numerous monocytes, a moderate number of lymphocytes, and a few blast cells and plasma cells were found between the epithelial cells of the ciliary processes, presumably en route to the posterior chamber (Fig. 13). Only a few disengagements of the epithelial interdigitations or disruptions of their desmosomal linkages were noted. The ciliary epithelial cells themselves adjacent to the infiltrating cells showed no fine structural change, nor was there any disruption of the internal limiting membrane. This is very different from the condition attending the passage of polymorphonuclear heterophilic leukocytes, as will be described later. The majority of monocytes in the posterior chamber had phagocytic vacuoles (Fig. 14). The membrane surrounding these showed discontinuities where lysosomes were very close to them.

In eyes taken 7 days after the onset of the uveitis, numerous plasma cells, monocytes, a moderate number of blast cells, and a few lymphocytes were found in the same region as described above in the one-day stage. However, neither accumulation of these cells in the blood vessels nor emigration into the connective tissue
Fig. 5. Passage of a monocyte (M) from a blood vessel lumen (BVL) into the extravascular space. Endothelial cell (EC), Schwann cell (SC). Early stage (first day) of inflammation, Group II. (x10,000.)

Fig. 6. A small lymphocyte (SL) is seen passing through the cytoplasm of an endothelial cell (EC) of a blood vessel. A part of the lymphocyte is enclosed with the endothelial cytoplasm but the other part is still in the blood vessel lumen (BVL). Endothelial junction (EJ). Early stage of inflammation, Group II. (x8,000.)

Fig. 7. For legend, see Figs. 8 to 10.
Figs. 8, 9, and 10. Part of a series of sections (see also Fig. 7), showing a small lymphocyte (SL) within the cytoplasm of an endothelial cell (EC) of a blood vessel. The lymphocyte is always enclosed by the endothelial cell cytoplasm. Endothelial junction (EJ), blood vessel lumen (BVL). Early stage of inflammation, Group II. (×8,000.)

Fig. 10. Small lymphocytes (SL) appear in a space between the endothelial cells (EC) of a blood vessel and their basement membrane (BM), presumably after having passed through the endothelial cell cytoplasm. Blood vessel lumen (BVL), pericyte (P). Early stage of inflammation, Group II. (×8,000.)
Fig. 11. A blast cell (B), a large lymphocyte (LL), and a plasma cell (PS) in a blood vessel lumen (BVL). A part of the blast cell is passing through a junction of endothelial cells (EC) between the lumen into the extravascular space. In a space between the endothelial cells and the basement membrane (BM), small (SL), and medium (ML) sized lymphocytes and a monocyte (M) are seen. Early stage of inflammation, Group II. (x6,600.)

Fig. 12. Intercytoplasmic connection between a monocyte (M) and a small lymphocyte (SL), in the base of the ciliary body. Early stage of inflammation of Group II. (x16,000.)

Fig. 12a. Higher magnification of the intercytoplasmic connection seen in Fig. 12. (x38,000.)
Fig. 13. Monocytes (M) penetrating the ciliary epithelium. Nonpigmented epithelial cell (NE), pigmented epithelial cell (PE). Early stage of inflammation, Group II experiment. (×4,500.)

Fig. 14. Monocytes (M) in the posterior chamber. Note that the surrounding membrane of the phagocytic vacuoles (pv) is interrupted, and lysosomes (ls) are very near the opening. Early stage of inflammation, Group II. (×8,800.)

Fig. 15. Plasma cells (PS) penetrating the ciliary epithelium and lying in the posterior chamber (PC). Nonpigmented epithelial cell (NE), pigmented epithelial cell (PE). Late stage of inflammation, Group II. (×4,000.)
Fig. 16. Cellular infiltration in the root of the iris. The infiltrate consists of monocytes (M) and plasma cells (PS). The plasma cell contains Russell bodies (Rb). A monocyte (M) phagocytizes a plasma cell (PS) which is undergoing lysis. Late (7 day) stage of inflammation, Group II. (×4,500.)

Fig. 17. The ciliary epithelium consists of only portions of pigment epithelial cells (PE) as a result of desquamation of the epithelium. Note PMN leukocytes (L) and fibrin (F) in the posterior chamber (PC) and red cells (R) in the connective tissue stroma (CTS). Monocyte (M), blood capillary (BC). Early stage of inflammation, Group II. (×3,200.)
Fig. 18. Precipitates (PT), part of a PMN leukocyte (L) and fibrin (F) in the posterior chamber. Early stage of Group II. (×16,000.)

Fig. 18a. High magnification of zonula fibrils, showing periodicity. (×35,000.)

Fig. 18b. High magnification of fibrin, showing periodicity. (×35,000.)

Fig. 19. Polymorphonuclear leukocytes in the posterior chamber. The cells contain phagocytic vacuoles (pv), some closely associated with, or containing PMN leukocyte granules (g). Precipitate (PT), fibrin (F). Early stage of Group II. (×22,000.)
was found. Most plasma cells were mature and frequently contained Russell bodies, and phagocytosis of plasma cells by monocytes was often observed (Fig. 16). The fine structure of some of the phagocytized plasma cells was almost normal, but others were undergoing lysis. Numerous plasma cells and a moderate number of monocytes were seen in the posterior chamber and traversing the ciliary epithelium (Fig. 15). However, there was again no evidence suggestive of a breakdown in the blood-aqueous barrier.

2. POLYMORPHONUCLEAR LEUKOCYTE RESPONSE. Several small, localized areas of polymorphonuclear (PMN) leukocyte infiltration (accompanied also by some mononuclear cells) were found, mainly in the iridal and ciliary processes in early stages (approximately 24 hours) of inflammation. In many instances, the accumulation was more evident in the posterior chamber than in the connective tissue.

Fibrin and a "precipitate" were frequently found among the cells in the posterior chamber (Fig. 18). The precipitate consisted of small (about 1 μ), variably shaped, homogeneous masses of a moderately electron-dense material. Its fine structure was different from that of zonula fibers or fibrin (Figs. 18a and 18b). Many of the leukocytes (mostly heterophils) had phagocytic vacuoles containing material similar to this extracellular precipitate (Fig. 19). The leukocyte granules were frequently clustered around these vacuoles, some of which had fused with, and released their content into, them.

THE PATHOLOGY OF THE CILIARY PROCESSES. The internal limiting membrane in the reaction site was frequently discontinuous or irregular in thickness, and both the nonpigmented and pigmented epithelial cells were frequently separated by leukocytes. PMN leukocytes, mainly in the nonpigmented layer, occasionally contained phagocytic vacuoles. However, the "precipitate" was not detected between the epithelial cells. In areas where PMN leukocytes penetrated the epithelium, in contrast to regions where mononuclear cells were between these cells, interdigitations frequently had disappeared and large intercellular spaces formed, especially between the nonpigmented cells. These often contained fibrin. Desmosomal linkages between pigmented and nonpigmented cells were often disrupted by intervening leukocytes and, in some cases, the pigmented and nonpigmented layers were completely separated (Fig. 17). In several instances, even the pigmented epithelial cells themselves began to desquamate leaving only the basement membrane lining the posterior chamber (Fig. 20). Disruption of the basement membrane was not detected, but passage of fibrin through it was frequently seen. Despite considerable disruption of the intercellular junctions, few significant alterations within the ciliary epithelial cells themselves were noted, even if the cells were detached from each other and the basement membrane.

CONNECTIVE TISSUE SPACE AND VESSELS. Many of the PMN leukocytes seen in the connective tissue space had no phagocytic vacuoles, but those which did were quite similar to those in the posterior chamber. In areas where vacuole-containing PMN leukocytes were present in close proximity to blood capillaries, discontinuities of the vascular basement membranes were observed. In such areas, small, round granules, similar in appearance to PMN leukocyte granules, were frequently found scattered between endothelial cells and pericytes, or in the space external to the pericytes and its portion of the basement membrane. There were frequent gaps in the junctions of the endothelial cells, almost always accompanied by disruption of the capillary basement membrane (Fig. 21). Erythrocytes were often seen passing through such openings into the connective tissue space (Fig. 22). Except for the cellular disjunctions and some vacuole formation, few alterations were found in the endothelial cells. In the seven-day stage only slight PMN leukocyte infiltration and
Figs. 20, 21, and 22. For legends see opposite page.
a few small hemorrhages were seen, and no thrombosis or necrosis of vessels. In contrast to the condition found in the ciliary body, no disjunction of endothelial cells was found in the choroidal vessels.

The endothelial cells of venules and capillaries in the ciliary body and processes which were not infiltrated with mononuclear or heterophilic leukocytes also occasionally showed disjunctions. This, however, was not accompanied by disruption of the basement membrane, and diapedesis was not observed.

3. BASOPHILIC LEUKOCYTE-MAST CELL RESPONSE AND PROCESS OF DegrANULATION. In the early (24 hour) stage of inflammation, cells with metachromatic granules were found, not only in the choroid, but also in the anterior uvea where they are normally absent. These were basophilic leukocytes and were relatively numerous, although not in comparison with mononuclear and heterophilic leukocytes. The metachromatic cells in the inflamed choroid consisted of mast cells, and a few basophilic leukocytes in the periphery. Most mast cells showed little fine structural abnormality, but in some, especially in the peripheral choroid, the granules showed signs of disintegration (Fig. 23) and others complete degranulation. The process of degranulation was quite similar to that of basophilic leukocytes. Vacuoles formed around each granule, enlarged, and fused, and the granule contents “disappeared.” Expulsion of intact granules into the surrounding tissue was never seen. In completely degranulated cells a well-developed Golgi complex, glycoprotein, and relatively numerous sacs of rough-surfaced endoplasmic reticulum were often observed. Many small, moderately dense granules of a finely particulate (50 mμ) material were frequently found in the Golgi area, and also among degranulating granules.

In contrast to the choroid, all metachromatic cells seen in the anterior uvea were basophilic leukocytes. They were most frequently found in the ciliary body but were also scattered sparsely elsewhere. Most basophilic leukocytes showed some stages of granule disintegration (Fig. 24), especially in the ciliary body processes. However, no intact granules were seen extracellularly in the connective tissue. Stages in the process of disintegration of the granules were very nearly the same as in mast cells. A clear empty vacuole appeared to be the final stage. After disappearance of the dense material, numerous vesicular components (about 40 mμ) were frequently seen in the Golgi area and among the degranulated “granules” as was also observed in the mast cells. In the later (7 day) stages of inflammation, no basophilic leukocytes were detected in the anterior uvea, although mast cells were seen in the choroid.

Group III. These rabbits, which received 45 mg BSA in each eye, developed a severe uveitis, demonstrated by chemosis, perilimbal injection, hyperemia, and hemorrhage in the iris, and fibrinous, cellular, and proteinaceous exudate in the anterior chamber, 7 days after disappearance of the postinjection inflammation.

In some eyes enucleated one day after onset of the uveitis, massive cellular in-
Fig. 23. A part of a mast cell showing disintegration of its granules. The number by the granules indicates the stage in the process of granule disintegration from early (g1) to late (g4). Early stage of Group II. (×14,000.)

Fig. 24. Part of a basophilic leukocyte showing stages in the disintegration of its granules. The numbers indicate the stages, 1 being the earliest and 4 a late stage. Early stage of Group II (×30,000.)
Fig. 25. Polymorphonuclear heterophil leukocyte infiltration in the base of the ciliary body. Precipitates (PT) and fibrin (F) are scattered among PMN leukocytes which contain phagocytic vacuoles (pv). Early stage of inflammation of Group III. (x3,500.)

Fig. 26. Apparent loss of the cytoplasm of endothelial cells of a venule. Blood vessel lumen (BVL), basement membrane (BM), connective tissue space (CTS). More advanced stage of inflammation of Group III. (x25,000.)
filtration was found in the base of the ciliary body and the anterior surface of the iris near the chamber angle (Fig. 25). This infiltration, unlike that seen in the first or second groups, consisted mainly of PMN heterophil leukocytes. In the reaction sites, PMN leukocytes were observed both extra- and intravascularly. Elsewhere, however, there was no cellular infiltration. Precipitates similar to those seen in the Group II animals, and occasionally fibrin, were scattered in the connective tissue of the reaction sites. The phagocytic vacuoles of the PMN leukocytes contained a material similar to the extracellular precipitate (Fig. 25). In some cells, these vacuoles occupied a large part of the cytoplasm, in which case most of the PMN leukocyte granules were gone. Fusion of the PMN leukocyte granules with the vacuoles was frequent. Many platelets, some of which showed degranulation and lysis, were also aggregated in the lumen of venules and capillaries of the reaction site. Endothelial disjunction, often accompanied by disruption of the basement membrane, and passage of PMN leukocytes and erythrocytes through such openings was frequent.

More complicated inflammatory reaction was found in other eyes of this group. Thrombi, consisting of PMN leukocytes, monocytes, platelets, fibrin, and erythrocytes, were prominent in the ciliary vessels. The majority of venules and capillaries, not only in the ciliary body but also in the iridial and ciliary processes were completely occluded with numerous erythrocytes. In addition, the blood vessel walls frequently showed necrosis; some endothelial cells of others were vacuolated and karyolysis was noted. As a result, regions were found in which the vessel wall was reduced to the basement membrane only (Fig. 26). The connective tissue space in these regions was almost completely filled with erythrocytes. Besides such advanced vascular changes in the ciliary body, mononuclear cell infiltration similar to that found in Group II occurred in the pars plana and the peripheral choroid. Islands of heterophil leukocytic infiltration, similar to, although more exaggerated than those in Group II, were also noted in the iridial and ciliary processes. Mast cells, some of which were degranulated, were observed in the choroid but not in the anterior uvea.

**Group IV. Control animals.** An intravitreal injection of sterile saline was made in one eye of each rabbit of this group, which provoked a transitory inflammation due to trauma. This subsided in a few days, after which the eyes remained quiet until autopsy 7 to 10 days later. No fibrin or "precipitate" was found, as was observed in the eyes with uveitis. No cellular infiltration occurred in these or the uninjected normal eyes. Metachromatic cells were seen only in the choroid and these were fully granulated mast cells. All vessels appeared to be normal and the endothelial junctions were tight.

**Discussion**

Single intravitreal injections of three different doses of the same antigen produced three different types, or perhaps degrees, of inflammatory reaction in the uveal tissue. No such reaction followed saline injections, indicating that they were induced by the antigen. The base of the ciliary body was the principal inflammatory site in all three experiments. This is in the region of the main passageway of aqueous humor, and it has been demonstrated that intravitreally injected antigen leaves the eye largely via the anterior chamber. Therefore, it is believed that the initial interaction of antigen with the uveal tissue takes place in this region. These observations confirm earlier studies. Utilization of electron microscopy permits additional new observations.

The first group, which received a small dose of antigen, did not show clinical signs of uveitis, but minimal cellular infiltration was demonstrated. This was mainly monocytes, but some basophilic leukocytes (with intact granules) were intermingled. In
many immune reactions, phagocytosis and degradation of antigen by macrophages and monocytes have been observed and the monocytes found in these experiments contained occasional phagocytic vacuoles and lysosomes. It could not be proven that the phagocytized material was the antigen, but its appearance was identical with that found in the posterior chamber. There was no morphological or clinical evidence of a damaged blood-aqueous barrier, which suggests that this reaction does not result from the presence of these cells, at least when the PMN leukocytes are not degranulating.

In the Group II experiments the larger dose of antigen produced a frank uveitis and an anterior uveal infiltration, again mainly mononuclear. Some of these were not of ocular origin, since they were seen in large numbers in the vessels, and emigrating into the connective tissue. The present study shows that with one exception, such cells always passed through the junctions of the vascular endothelial cells as they do in normergic inflammation. In contrast, small lymphocytes never took this route but passed from the vessels by entering into, and emerging from, the cytoplasm of the endothelial cells via membrane-bound structures. A similar behavior of small lymphocytes has been observed in the lymph nodes of normal rats. In inflamed lymph nodes, granulocytes and monocytes pass through the postcapillary venule by going between the endothelial cells. Apparently small lymphocytes, unlike other blood cells, have an unusual means of leaving blood vessels.

The large lymphocytes appear to be intermediate between smaller lymphocytes and immunoblasts as shown by the presence of ribosomal aggregates and cisternae of rough-surfaced endoplasmic reticulum. Immunoblasts and plasmablasts differ only in degree of development of the rough-surfaced endoplasmic reticulum. Similar graded series of these cells have been found in immunized lymphatic tissue, and are considered morphological evidence of transformation of small lymphocytes into antibody-forming cells, and finally into plasma cells. In fact, these intermediate cell types have been shown to contain antibody, by ferritin and fluorescent tagging, and hemolytic antibody plaque technique. The large lymphocytes, blast, and plasma cells do not circulate in non-immunized animals, but have been found in thoracic duct lymph and blood after antigenic stimulation, and occurred in the present experiments in, and passing through, the walls of uveal vessels. Possibly they are attracted to these areas by the injected antigen or its antibody complex.

Intercytoplasmic connections between monocytes and lymphocytes were found on several occasions. These have been observed also in immunized lymphoid tissue and tuberculin hypersensitivity reaction, and are regarded as morphological evidence that immunological information is thus transferred to small lymphocytes, inducing them to transform into antibody-forming cells.

In late stages, a significant plasmacytosis developed, as described by others. However, the present study shows that, although the infiltrating cells were quite similar in early and late stages, the proportion of different cell types varied. In addition, no intravascular accumulation nor emigration of cells into the extravascular space were seen in late stages of inflammation, suggesting that small lymphocytes transform into antibody-forming and finally into plasma cells locally. At this stage, phagocytosis of plasma cells by monocytes was frequent, and the number and size of their lysosomes increased. This may be a physiological process for removal of dying plasma cells which, because of their short life span, takes place continuously in the reaction site. It has also been observed in immunized lymphoid tissue.

Many features of the uveitis described are striking similar to those seen in lymphoid tissue during antibody production and
indicate, as has been suggested by Silverstein\textsuperscript{6} that ectopic antibody production takes place in uveal tissue during this type of inflammation.

As also noted by others,\textsuperscript{1-4} evidence that the blood-aqueous barrier was broken was found in the present study. No alterations indicative of a breakdown of the blood-aqueous barrier\textsuperscript{27, 28} were found in the regions closely related to the mononuclear cell response, suggesting that they play little or no direct role in this breakdown. However, small islands of PMN heterophilic infiltration occurred, and near them disjunction of vascular endothelium, disruption of the basement membranes and of the desmosomal linkages and of the internal limiting membranes of the ciliary epithelium were found. These observations indicate that the PMN leukocyte, rather than the mononuclear leukocytes, plays an important direct role in the breakdown of the blood-aqueous barrier.

It is believed that the posterior chamber is the primary site of interaction of antibody with antigen in this form of uveitis. Precipitates, presumably antigen-antibody complexes, occur there and it is also the main site of PMN leukocyte accumulation. In both direct and reversed passive Arthus reaction,\textsuperscript{29, 30} the site of leukocyte accumulation is also the same as that of the immune precipitates. According to Silverstein, Welter, and Zimmerman,\textsuperscript{9} clinical signs of this type of uveitis precede the appearance of circulating antibodies, but electron microscopic evidence shows that antibody-forming cells, identified as such on the basis of their morphology, are present early in the reaction sites. This suggests that immune precipitate could be formed by locally produced antibody in this type of uveitis. It has been shown by Cochrane and his colleagues\textsuperscript{31} that the immune precipitate fixing complement brings about chemotaxis of PMN leukocytes. It appears probable that such immune precipitates may attract the PMN leukocytes to the posterior chamber.

The majority of PMN leukocytes in the reaction site possessed phagocytic vacuoles containing material very similar to the extracellular precipitate. A similar but more impressive situation was found in the third group. These observations are in agreement with those of others\textsuperscript{27, 30, 32} made in direct and reverse passive Arthus and in acute anaphylactic reactions,\textsuperscript{33} and suggest that PMN leukocytes phagocytize and digest immune precipitate by means of enzymes contained in their granules (lysosomes). Phagocytosis of immune precipitate by PMN leukocytes has been observed in vitro.\textsuperscript{34}

Recently, evidence has been advanced\textsuperscript{35} indicating that factors associated with PMN leukocytes are essential to the pathogenesis of such inflammatory processes. Injurious agents are released by them, especially during phagocytosis, and have been found in their lysed granules.\textsuperscript{36} Murray and his co-workers\textsuperscript{37} found these substances included cathepsin-like proteases, and Cochrane and Aikin\textsuperscript{38} found enzymes in the PMN leukocyte granule which caused in vitro fragmentation of basement membranes. In electron microscopic observations of reversed passive Arthus reactions the basement membrane of endothelium was frequently seen to be disrupted. Kniker and Cochrane\textsuperscript{39} showed the internal elastic lamina to be affected by PMN leukocytes in arteritis of serum sickness. These findings strongly suggest that substances derived from the PMN leukocytes attack basement membranes and therefore, are responsible for some of the pathology reported here. Disruption of desmosomal linkages and formation of large intercellular spaces may also be caused by them if, as has been suggested, the cement substance between desmosomes is similar to basement membrane material.\textsuperscript{40} Such changes have not been seen in areas infiltrated by mononuclear cells. This is consistent with an observation by Janoff and his colleagues\textsuperscript{41} that PMN leukocyte granule extracts, unlike those of macrophage granules, are phlogistic.

The basement membrane underlying the
pigment epithelium appeared to be little affected even when severe damage of the epithelial cell layer occurred. However, fibrin passed through it, which suggests that some change may have occurred.

Endothelial cells of capillaries in the reaction sites frequently showed changes unlike those induced by histamine, serotonin, and bradykinin, and in cutaneous anaphylaxis, in that the endothelial disjunctions were almost always accompanied by disruption of the basement membrane. This allowed easy passage of erythrocytes into the connective tissue, and formation of fibrin in the extracellular space which does not occur in inflammation induced by serotonin, histamine, and bradykinin. This suggests a different mechanism in the two situations and that injurious agents released from PMN leukocytes are responsible for the difference. In late stages of inflammation few PMN leukocytes were found. This may be due to early removal of antigen by monocytes, formation of antigen-antibody precipitates, and the subsequent phagocytosis of them by PMN leukocytes, all of which occur early in inflammation. Thus the antigen rapidly disappears and, therefore, immune precipitates are not found in late stages of uveal inflammation despite the presence of large amounts of antibody.

There has been no description of participation of basophilic leukocytes in this type of uveitis, probably because of the difficulty in differentiating them from mast cells under the light microscope. However, relatively large numbers of basophilic leukocytes were found by electron microscopy in these experiments as they have been in the sites of antigen injection in other tissues. The function of basophilic leukocytes is not clearly understood, but it has been shown that these, like mast cells, contain heparin, histamine, and serotonin, and both types of cells react similarly to allergic stimuli. The only metachromatic cells seen in the anterior uvea in early uveitis were basophilic leukocytes. This finding is consistent with Smelser and Silver's observations that the normal anterior uvea, unlike the choroid, lacked mast cells. "Small mast cells," which were observed in the anterior uvea in early stages of uveitis (Smelser) probably were basophilic leukocytes. The basophilic leukocytes in the clinically demonstrable uveitis, Group II, were frequently degranulated. This involved only a disintegration of the granules themselves, without any apparent harm to the cell itself. Such degranulation has been noted also in Plimpton's study, and suggests that physiologically important substances contained in the granules, i.e., heparin, histamine, and serotonin, are released from the degranulated cells into the surrounding tissue space. The degranulation may be caused by the antigen-antibody reaction products, which have been shown to have this property in vitro and/or substances released from the heterophilic leukocytes. Recently, many studies have shown that cationic proteins, which are released during phagocytosis, are mastocytolytic. Studies have shown that both basophilic leukocytes and mast cells react similarly to various stimuli. No damage to the basement membranes was seen near degranulating basophilic leukocytes, as it was near heterophilic leukocytes; endothelial disjunction, however, was present and may indicate that histamine or serotonin released from the basophils were responsible, in part, for the increased vascular permeability. No studies of the fine structure of rabbit mast cells have been reported, although those of other species have been. This may be due to the paucity of these cells in rabbit tissues generally, although they are abundant in the choroid, and for this reason could be studied in this tissue. Their fine structure was similar to that found in some species, but was different from that of others, e.g., man.

In early stages of inflammation, many of the mast cells were degranulated, especially in the periphery of the choroid. The process of degranulation was similar
to that in basophilic leukocytes, but differed, however, from that reported in some other species, caused by compound 48/80 This may be due to the difference in species or in the degranulating agent (e.g., 48/80), in contrast to the inflammatory process.

Disintegration of mast cell granules in the choroid, like that of basophilic leukocytes, is believed due to antigen-antibody reaction. The fact that degranulated cells are relatively few suggests that, in this type of uveitis, the antigen-antibody reaction takes place mainly in the anterior uvea. Failure to find disjunction of endothelial cells in this area may be due to the relative scarcity of degranulated mast cells in the choroid.

Completely degranulated mast cells, unlike the normal and fully granulated, frequently contained well-developed Golgi complexes, considerable rough-surfaced endoplasmic reticulum, and glycogen, plus many small granules, similar in fine structure to the fine particle type. These were found in the Golgi area, and between degranulated vacuoles, suggesting that mast cells regenerate new granules immediately after degranulation.

The uveal pathology in the Group III animals was more severe than in the others, but similar basic processes were involved. Unusual features were greater vascular damage as seen in the form of thrombosis, vessel necrosis, and hemorrhage. These findings are the same as those in direct active and passive Arthus reactions in dermal tissue (of rabbits). Although the pathogenetic mechanism in Group III is believed to be the same as in the others, probably there was a greater early systemic production of antibodies, due to the larger amount of antigen injected into the eye.

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