CHARACTERIZATION OF THE CHOROIDAL MAST CELL*

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INTRODUCTION

MANY ETIOLOGIES HAVE BEEN PROPOSED FOR INFLAMMATORY DISEASES OF THE eye. The specific mechanisms, however, remain obscure. When tissue-damaging infections involving parasitic, bacterial, and viral agents are excluded, there remains a group of inflammations of the anterior and posterior uvea that involve unknown immunologic reactions. Histologic study of pathologic uveal tissue confirms the presence of inflammatory changes but reveals little to suggest how the inflammation developed. Study of normal eyes discloses little to clarify the origins of ocular immunopathology. Conditions such as inflammatory macular edema probably have a locally initiating mechanism, but how it occurs is not apparent.

The presence of the mast cell in the anterior and posterior uvea in the normal eye, with particular localization in the ciliary body and posterior choroid, coincides with the clinical anatomic division of anterior and posterior diseases seen in some inflammatory conditions. The literature on mast cell products establishes them as effective multipotential inflammatory mediators. Histamine has been the most widely studied of the mast cell granule products. However, these products are not limited to immediate hypersensitivity reactions, and they may participate in many immunologic processes. Thus, the mast cell may have a central role in intraocular inflammation, which opens a new avenue for the understanding of local modulation of inflammatory and immunologic reactions.

Histologic examination reveals that the mast cell is about 9 to 16 μm in diameter, is round or oval, and has cytoplasmic granules that stain metachromatically. These granules contain histamine, sulfated glycosaminoglycans (including heparin, and chondroitin sulfate), proteases, and chemotactic factors. Mast cells have high-affinity IgE receptors on their membrane surface, which appears to act as a trigger for mast cell degran-

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TR. AM. OPHTH. SOC. vol. LXXXV, 1987

ulation in IgE-mediated hypersensitivity reactions. The nucleus of the mast cell resembles that of the plasma cell. It occasionally appears bilobed but lacks the nuclear peripheral chromatin usually seen in basophils. Electron microscopy does not show the cytoplasmic glycogen aggregations that are seen in basophils, and the cell membrane reveals more evenly distributed folds and processes. The mature mast cell does not migrate but is a relatively fixed resident of the tissue in which it is found.

Although mast cells have been observed in tissue for many years and have been recognized for nearly a century for playing some role in inflammation, this role has been thought to be limited to immediate hypersensitivity. Compared with other inflammatory cells, mast cells were considered insignificant in other types of inflammation. Recently, however, the mast cell has been shown to play a role in altering immune reactions through modulation of various aspects of inflammation by immunologic stimuli.

The choroidal mast cell has not been characterized in any definite way, and the mechanisms of its role under physiologic or pathologic conditions are unknown. However, sufficient mast cells are present to produce a significant physiologic or pathologic impact on the tissue.

A large amount of recent literature suggests that mast cells are not the same in all areas of the body. Compartmentalization appears to occur, and mast cells apparently have different functions and characteristics in various anatomic locations. One such mast cell compartment where a specialization of function has been found is the intestinal mucosa. The concept of compartmentalization and regional differentiation of mast cells implies that mast cells may respond differently in certain areas. The possibility of regional specialization occurring in the uvea merits serious consideration.

The clinical puzzle of severe intraocular inflammatory disease with no detectable systemic involvement calls for a search for local regulatory mechanisms of the inflammatory process. In a highly vascular tissue such as the choroid, blood-borne influences on tissue inflammation should occur frequently. A local modulating effect on inflammation and circulation would be helpful and important in reducing local tissue damage.

It is appropriate to study the characteristics of the choroidal mast cell and to review the current understanding of the role of the mast cell in modulating inflammation, in the immune response, and in the physiologic process. Worth study is the hypothesis that these mast cells must play a significant role in the pathophysiology and physiology of the posterior pole. Increasing the understanding of the choroidal mast cell should give better insight into inflammation and diseases of the choroid and associated tissues.

REVIEW AND DISCUSSION OF THE LITERATURE

OVERVIEW OF MAST CELLS IN INFLAMMATION: HISTORICAL PERSPECTIVE

The mast cell was originally described by Ehrlich in 1877.[1] It was observed as a deeply staining metachromatic cell with granules, having a nucleus similar to that of the plasma cell and a characteristic tissue location near lymphatics, blood vessels, and inflammatory sites.

Mast cell degranulation following the intraperitoneal injection of substances such as egg white was shown to produce generalized acute hyperemia and edema, as in anaphylaxis, but without previous sensitization. The observation that mast cell degranulation occurred in urticarial reactions finally linked the mast cell degranulation with these immediate hypersensitivity reactions.[2-4]

The presence of heparin and the role of heparin in producing the metachromatic staining of the granules was noted in 1937.[5] The relation of the mast cell and histamine to allergic reactions was established by the ability of compound 48/80, a known membrane activator, to degranulate mast cells and produce effects usually associated with histamine,[6] and by the close correlation between tissue histamine and mast cell number in many tissues in several species.[7,8]

Passive transfer of anaphylactic properties was demonstrated by Prausnitz and Küstner in 1921. They showed that serum from a sensitized subject transferred to a normal, unsensitized subject's skin induced an allergic reaction when contact with the antigen was made. This passively transferred sensitivity would persist for some time.[9] The transferred factor, called reagin, was later identified as IgE in the serum. Later it was shown to be on the surface of mast cells and to participate in triggering the mast cell degranulation with the release and synthesis of a number of factors, including histamine, eosinophil and neutrophil chemotactic factors, proteases, other enzymes, and arachadonate metabolites.[10] This allergic or anaphylactic reaction is now known as immediate hypersensitivity.[11]

OVERVIEW OF THE PHYSIOLOGIC AND IMMUNOLOGIC ROLE OF THE MAST CELL AND MAST CELL PRODUCTS

Recently, the mast cell and its release products have been shown to have important physiologic and immunologic roles.[12] This is best demonstrated by histamine, which has a regulatory action on the microvascular system. It also affects cells involved in humoral and cell-mediated immunity in certain steps in the immune response. Regulatory effects have been shown in vitro to occur with concentrations of histamine that are present

in tissues where reactions are taking place. Histamine therefore may act as a local feedback modifier of certain physiologic reactions and of inflammation. The mast cell is the major source of histamine in almost all the body tissues, and through determining the release of histamine, the mast cell can modulate many reactions.[12]

At least two histamine receptors have been identified. Bronchoconstriction and gut contraction are mediated by one type of histamine receptor on effector cells, designated the histamine type 1 (H_1) receptor. H_1 antagonists include diphenhydramine and chlorpheniramine.[13] Another receptor has been designated histamine type 2 (H_2) receptor. One effect that is dependent on the type 2 receptor is gastric acid secretion. This effect cannot be blocked by type 1 antagonists but can be blocked by some special antagonists such as cimetidine.[14] Some effects of histamine, such as vascular dilation, are manifested only when both H_1 and H_2 receptors are present and stimulated. Blocking this effect seems to require both H_1 and H_2 antagonists.[15,16] These mechanisms are not totally understood, but much investigation is being done. Some of the evolving research concerning H_1 and H_2 receptors suggests they may be responsible for regional adjustments and control over many processes other than those directly involved in inflammation.

The effects of H_2 receptor stimulation with histamine have been shown to cause elevation of intracellular cyclic adenosine 3′,5′-monophosphate in many tissues. It has been suggested that this elevation can inhibit cellular proliferation in mouse fibroblasts and lymphocytes. Histamine receptors are detected on most leukocytes. The ratio of H_1 to H_2 receptors on normal T cells was reported to be 1.5:1 by one technique.[12]

In vitro studies show that histamine blocks the release of granule histamine from basophils. This inhibition of histamine release by exogenous histamine is blocked by H_2 antagonists but not by H_1 antagonists; H_2 agonists mimic the suppressive effect of exogenous histamine on histamine release.[12] If this inhibition occurs with mast cells, it would provide evidence for H_2-related locally mediated control of histamine release.

Modulation of polymorphonuclear leukocytes by histamine has been demonstrated. Furthermore, histamine may inhibit the migration of basophils under certain circumstances but does not alter macrophage migration response to complement component C5a. H_2 antagonists block the histamine-mediated inhibition of basophil migration.

Histamine affects eosinophil migration, development of complement receptors, and elevation of intracellular cyclic nucleotide levels in the responsive cells. The chemotactic effect of histamine can be blocked only by a combination of H_1 and H_2 antagonists. Histamine H_1 agonist in-

creases complement factors C3b and C4 receptors on eosinophils. Factor C3b enhances the eosinophil-dependent killing of certain parasites. Parasitism is associated with high levels of IgE, which may be directed against the parasite. Sensitization of mast cells with IgE may lead to specific antigen-induced degranulation and subsequent histamine release. Histamine release may lead to attraction of eosinophils to the site of the reaction and may potentiate their ability to destroy the parasite.[12]

Histamine effects on neutrophils have been reported to include alteration of enzyme release, chemotaxis, chemokinesis, adherence, superoxide anion production, and hydrogen peroxide formation.

The histamine effect on the T lymphocyte function is not clear, but in mice there appears to be an inhibition of T lymphocyte-mediated cytotoxicity. This effect is consistent and shows a similar effect on the same cell populations, but it varies widely with different cell populations and appears to be mediated by H_2 receptors. Natural killer cell function also seems to be decreased by histamine. Concanavalin A-induced lymphocyte suppressor cells are stimulated with histamine through the H_2 receptor in animal experiments as well as human lymphocytes. Cimetidine blocks histamine-induced inhibition of T-cell proliferation in mixed lymphocyte culture, but chlorpheniramine does not. This suggests that the inhibition of mixed lymphocyte culture proliferation by histamine is mediated by the activation of suppressor T cells through their H_2 receptors.[12,17-19]

In lymphokine-producing cells, histamine partially reduces the expression of delayed hypersensitivity. This effect is blocked by H_2 antagonists. Several histamine-induced lymphokines that influence lymphocyte motility have been identified. Inhibition of macrophage migration-inhibition factor (MIF) production has been detected with histamine. Inhibition of histamine MIF production seems attributable to H_2 receptor-bearing lymphocytes.[12,16-18] Suppressor cell activity has been induced by histamine in animal and human studies and seems related to a histamine-induced suppressor factor (HSF) produced by the T-lymphocytes—but not by macrophages or B cells.[17] This production seems related to H_2 receptors. The action of HSF seems dependent also on the presence of macrophages, inducer T-lymphocytes, and suppressor cell precursors. The activation of suppressor cells seems to be a complex process involving several factors that act as checks and balances. The response to histamine stimulation seems variable from one individual to another. Inhibition of antigen-specific suppressor cell activity by histamine has been described and can be blocked by H_1 receptor antagonists. The complex interaction among the various subsets of T cells suggests many modulating effects.

Although these interactions are not well clarified at this point, there is ample evidence to support their important role in modulating the immune response at the cellular level.[12]

B cell immunoglobulin production is also affected by histamine. A T-suppressor cell with a histamine receptor appears to have a regulatory effect on antibody production.[18-20] Histamine also affects antibody production at other levels, but again, the mechanisms are not yet clear. The release of histamine can be effected not only by antigen IgE mechanisms from sensitized mast cells but also by antigen stimulation of T-effector lymphokine-producing cells. A histamine-releasing factor may act independently of IgE-mediated reactions.[12]

Blood vessel function is directly affected by histamine. Other, more physiologic means for the release of histamine or the production of histamine locally have been suggested. For example, histidine decarboxylase activity has been implicated as a local tissue enzyme that can produce histamine in and around the cells of the vessels and act as a regulator of the microvasculature.[21,22] However, almost all tissue histamine is found in the mast cell. Neurotensin, a vasoactive tridecapeptide from the brain and gut, can stimulate histamine release from mast cells.[23] Both H_1 and H_2 receptors have been shown to play a role in postprandial hyperemia and vasodilation in the intestine in response to histamine.[24] Cerebrovascular blood flow seems more sensitive to H_2 receptor stimulation. Significant dilation of cerebral vessels occurs with low concentrations of histamine.[25] Renal blood flow is also altered significantly by histamine, and both H_1 and H_2 have been identified in outer and inner cortices. Variations in renal function can be demonstrated by separate H_1 or H_2 receptor stimulation.[26] Kidney studies imply a local modulation of blood flow as part of the normal physiologic regulation in various tissues, but the interaction of H_1 and H_2 receptors seems different in different tissues.[27]

Histamine has been more extensively studied than any of the other mast cell products, and its complex interactions are becoming more manifest. Much remains to be explained, however, before its multiple interactions will be fully appreciated. Nonetheless, it is clear that it has significant implications.

Serotonin, or 5-hydroxytryptamine, is found in rodent mast cell granules but not in human mast cells.[28] It is, however, present in both rodents and humans in the enterochromaffin cells in the gastrointestinal tract, platelets, and central nervous system (CNS) tissue.

The anticoagulant heparin has been detected in the mast cell granules for many years and has been a subject of extensive investigation. It is composed of glycosaminoglycan side chains attached to a peptide core.

Heparin increases the inhibition by antithrombin III, thrombin, and several other coagulation serine proteases.[28] It also affects kallikrein and plasmin activity. However, its functional role as a mast cell product is not yet clearly defined. There is little evidence that it is released by mast cell degranulation, as histamine is. It forms the matrix of the secretory granule, and many of the granule products are attached to the heparin molecule. Heparin is retained on the cell surface as the granule is exposed. Chymase, tryptase, and carboxypeptidase bind heparin. Heparin alters the activity of these proteases not only in the granule but probably also after degranulation.[28]

Chondroitin sulfates also are glycosaminoglycans. There are four types: chondroitin-4-sulfate (CS-A); dermatan sulfate (CS-B); chondroitin-6-sulfate (CS-C); and chondroitin-4, 6-disulfate (CS-E). (The numbers show the position of o-sulfonation on the galactosamine portion of chondroitin sulfate.) Chondroitin sulfate can be present in varied amounts in most cell granules.

Neutral proteases are present in large amounts in the mast cell granules. They are third only to histamine and heparin. Rat mast cells appear to contain chymotrypsin-like protease, and human mast cells contain mostly trypsin-like protease. Chymase and carboxypeptidase A have been found in the rat. They are exposed on the surface of the mast cell following degranulation, still complexed with heparin with altered protease activity, and they apparently are not fully released for some time following degranulation. The physiologic and inflammatory tissue effects of this slowed release are not clearly understood.[28] Hydrolases such as β-hexoxaminidase, β-glucuronidase, β-D-galactosidase, and arylsulfatase are also released with degranulation of the mast cell.[28] Their major effect seems to be in degrading substances in phagolysosomes.

Finally, a somewhat miscellaneous group of mast cell products has been defined. Less is known about the role of these products. They include oxidative enzymes such as superoxide dismutase and peroxidase, which may help modulate the inflammatory response by their enzymatic activity. The role of chemotactic factors in attracting and recruiting other cells to the area of inflammation, including neutrophils, monocytes, and eosinophils, has been established. Prostaglandin D_2 is a major product of both rat and human mast cell activation. Human mast cells have been shown to generate a slow-reacting substance, which is a combination of leukotriene C, D and E and results in variable effects on physiologic processes and inflammation. The platelet activating factor is also a result of mast cell activation. The effects of this factor may be very widespread and include activation of neutrophils, platelet aggregation, and smooth

muscle contraction in pulmonary tissue.[29]

OVERVIEW OF MAST CELL HETEROGENEITY

Studies of mast cells have previously focused on their role in allergy and IgE-mediated immediate hypersensitivity reactions. Most studies have been carried out with mast cells from the peritoneal cavity of rats. It has become evident, however, that mast cells vary considerably in histologic appearance, biochemical composition, and physiologic properties. Not only is there a significant difference between different species, but there appear to be significant differences in mast cell populations among tissues within the same individual. This cautions that mast cell data from one tissue should not be used to interpret mast cell properties of another tissue. Mast cells must be studied in each tissue and characterized for that tissue alone.

In 1905 Maximow[30] proposed that mast cells of the small intestine were different in histologic appearance from other mast cells. In 1966 Enerback[31,32] defined properties of the intestinal mucosal mast cell, and in comparing these properties with those of mast cells found in connective tissue, he showed that the mucosal mast cell has different staining characteristics. In histologic studies, mucosal mast cells show a difference not only in staining characteristics but also in size. Furthermore, they contain different proteases, have a lower age-dependent histamine content, show a thymus-dependent proliferation in immune responses, and have a half-life of < 40 days. The connective-tissue mast cells are larger, have a uniform shape with uniform granules, are nonmigratory, have a high age-dependent histamine content, show no proliferation in immune response, and have a half-life of > 6 months.[33,34]

Galli[35] proposed that the distinction between maturation and differentiation be noted when accounting for mast cell population differences. Mast cells at different stages of maturation may stain differently, and the difference in staining might be mistaken as evidence for differentiation. It has further been shown that mast cells may be able to release products from granules in a gradually slower process than the previously observed rapid "anaphylactic" degranulation. These processes and the regeneration of granules following degranulation may alter the staining properties of the mast cell.[36] These properties can be analyzed by ultrastructural examination with electron microscopy; with conventional light microscopy or histochemistry, however, they may be erroneously interpreted as showing heterogeneity of a mast cell population.[37] Other local factors, such as the mast cell growth factor, p-cell stimulating factor, or interleukin 3, may be present, influencing the mast cells and changing their appearance.[35]

The microenvironment may even help produce heterogeneity, and this as well as other influences may make heterogeneity the rule rather than the exception.[35]

Stevens and associates[38] recently updated and enforced the view that heterogeneity occurs because there are subclasses of substantially different mast cells. There are at least two populations with different proteoglycans. Heparin-containing mast cells are present in connective tissue. Chondroitin sulfate-containing mast cells are found in the small intestine.

Again, each tissue where mast cells are present will need to be studied.[39]

OVERVIEW OF UVEAL MAST CELLS

In 1937 mast cells were noted in the limbal area of normal human eyes by Jorpes and associates.[5] In 1940 Holmgren and Stenbeck[40] reported on mast cells in the ciliary body and iris in cases of glaucomatous iritis; Zollinger[41] made similar observations in 1949. In 1959 Vannas[42] noted a considerable number of mast cells in the uvea. However, his study concentrated on mast cells in the anterior segment of the eye and their role in glaucoma.

In 1959 Larsen[43] noted large numbers of mast cells in the normal uvea of albino animals, including guinea pigs, rabbits, and rats. He recorded the largest number in the posterior choroid. He reported that these mast cells were degranulated by high doses of corticosteroids and by vitamin C-deficient diets. In 1961 Feeney and Hogan[44] found mast cells among the nonpigmented cells of the normal human choroid stroma. With electron microscopy, they identified what they felt were two types of mast cells. One was a large cell (10 to 12 μm in diameter) packed with large, oval homogenous granules and having a rounded nucleus. The other was a smaller cell (9 × 4 μm) with an oval nucleus and clusters of ring-shaped, double membranes forming granules. Hogan,[45] in describing the ultrastructure of the choroid, noted that mast cells were numerous in the human choroid.

In 1961 Larsen[46] witnessed choroidal mast cells increasing in number the first 6 days after a single injection of horse serum into the vitreous of albino rabbits. In this animal model the mast cells decreased markedly from the 6th to the 15th day, indicating degranulation. They were visible in the choroid again, but in decreased numbers, in the subsiding phase of the inflammation and until the experiment terminated at 63 days. Larsen used toluidine blue stains so that mast cells would be visible in the choroidal inflammatory response. Earlier, Silverstein and Zimmerman[47] had utilized hematoxylin and eosin stains in studies of guinea pig cellular

responses in ocular inflammation. They did not note any particular role of the mast cell with these stains.

In 1962 Levene,[48] using histologic sections, made counts of mast cells in the choroid of rats, guinea pigs, rabbits, and monkeys and showed that mast cells were present in significant numbers. He also detected the presence of histamine and serotonin in these tissues. The proof that there was histamine in the choroid helped put into perspective the earlier observations of Halpern et al,[49] Schlaegel,[50] Vilstrup,[51] and Ashton and Cunha-Vaz,[52] whose studies concerned the effect of histamine-induced vascular dilation of the uveal vessels and their increased permeability.

Smelser and Silver[53] showed in 1963 that with flat preparations from the albino rat, rabbit, guinea pig, and ferret, the large numbers of mast cells could be appreciated more readily than with histologic sections. They noted fewer mast cells in the anterior choroid and also noted a tissue distribution along vessels, particularly large veins. No mast cells were seen in the retina, studied under flat tissue mounts. They also showed that intravascularly injected compound 48/80 degranulated nearly all uveal mast cells.

Further description of rat uvea mast cell degranulation with compound 48/80 was made by Kim[54] in 1963. Degranulation began within 1 hour of injection. The most severe changes were noted 7 to 16 hours after injection, when most mast cells were no longer visible because of degranulation and tissue edema.

Lanzieri and Cricchi,[55] working with the guinea pig in 1965, reported a degranulation of mast cells in the choroid, dilation of vessels, and slight edema when the eyes had been injected intravitreally with antigen previously used to sensitize the animal by foot pad injection. The reactions were noted within 3 minutes of the intravitreal injection.

In 1966 Smith and Trokel[56] reported experiments on albino rabbits. They injected the vitreous or artery with compound 48/80 and then examined the blood flow and volume with reflective densitometry or by choroidal flat mounts. They noted a thickening of the choroid 30 to 60 minutes after the injection and felt that the thickening was due to vascular dilation. Histology of flat preparations of choroid showed that mast cells were not visible after injection of compound 48/80.

In 1970 Witmer and Dieterich[57] reported on their ultrastructural studies of ocular inflammation. In 1972 Dieterich[58] reported that ultrastructural characteristics of mast cells in the iris were similar to those of uveal mast cells seen by Feeney and Hogan. He noted a third type of mast cell that he felt might be a premature or regenerating mast cell. Nii and associates[59] in 1974 reported distortion in the granules in mast cells from

the iris of patients with Behçet's disease; however, they were unable to interpret these observations.

In 1973 Price[60] found that the density distribution of mast cell in the albino rabbit choroid was greatest in the posterior pole and dropped as the anterior choroid was approached. In some cases there were no mast cells in the anterior choroid. Price also noted an increase in mast cells while animals were growing. He was unable to evaluate the effects of senescence. He was able to show in 1974 that a single intramuscular injection of cortisone acetate did not alter mast cell numbers in albino rabbit choroids but that repeated daily intramuscular injections for 5 days significantly reduced the observed choroidal mast cell numbers.[61] In 1975 Price[62] showed that the number of visible rabbit choroidal mast cells was reduced as early as 3 days after a single dose (from 2400 to 19,400 rad) of ^{60}Co gamma radiation. The number of mast cells visibly returned to normal within 8 days after the radiation treatment. The higher, 19,000-rad dose did not prevent the return to normal. The ocular mast cells may be very radioresistant.

In 1978 Friedlander and associates,[63] while demonstrating ocular changes in delayed hypersensitivity reactions in guinea pigs, encountered large numbers of mast cells in the uvea and especially in the choroid. These cells were seen both with Freund's incomplete adjuvant plus ovalbumin and the adjuvant plus bovine gamma globulin sensitization and subsequent challenge. They were also seen when Freund's complete adjuvant was used in place of Freund's incomplete adjuvant. The inflammatory reaction was more intense, with greater thickening of the choroid, in the animals sensitized with Freund's complete adjuvant, but mast cells were less numerous. Results of this study suggest that two patterns of delayed-onset cell-mediated hypersensitivity can be produced by the same antigen, depending on how immunization occurs and on the time between immunization and challenge. Mast cells seem involved in these processes in the uvea, but the exact manner of involvement is not clear.

Allansmith and co-workers[64] in 1979 determined mast cell density in 14 ocular tissue compartments in normal rats and in rats infected for 30 days with *Nippostrongylus brasiliensis*. They calculated 1596 choroidal mast cells/mm^3, or 638 total choroidal mast cells. These counts were from 350-gm Sprague-Dawley rats. Levene[48] calculated 6000 mast cells for 150- to 200-gm Wistar rats. Both calculations were from histologic tissue sections rather than flat choroidal preparation. No significant increase in ocular mast cells was observed in ocular tissues after infection with *N brasiliensis*. In 1980 Allansmith and associates[65] showed the participation of ocular adnexa and globe tissues in anaphylaxis. In anaphylaxis models

using both egg albumin and *N brasiliensis* infection, sensitized animals with appropriate corresponding antigen by intravenous challenge showed an adnexal tissue increased retention of radiolabeled rat serum albumin. The globe tissues showed some increased retention of radiolabeled rat serum albumin in the group immunized with egg albumin.

In another study Allansmith and associates[66] showed extensive degranulation of choroidal mast cells with intravenous injection of 250 μg of egg albumin in previously sensitized rats. This was felt to be the maximum amount of antigen that could be given without producing fatal anaphylaxis. Under these severe conditions, the choroidal mast cells showed more degranulation than other ocular tissue mast cells. The investigators suggested that the greater choroidal blood flow allowed more antigen to reach the choroidal mast cell under these conditions.

Rockey and co-workers[67] in 1981 studied the role of IgE antibodies and mast cells in ocular ascarid infections. In various experiments they injected guinea pigs with ascarid larvae or antigen or anti-IgE antibodies. Fluorescence microscopy of the eyes (which underwent passive systemic anaphylaxis from passive intravenous sensitization with administered IgE antibody, followed 6 to 28 days later by a challenge with intravenous antigen) showed leakage of Evans blue dye in the choroid but not in the iris. A significant (50%) reduction in the number of mast cells in the choroid and ciliary body was found in these eyes. A similar significant reduction in uveal mast cells was noted following primary ocular infection by ascarid larvae after 7 to 10 days, but the number returned to previous levels by day 19. Plasma-cell infiltrates were noted in the infected eyes near the time that IgE antibodies appeared from the infection. Subretinal or intraretinal larvae showed no inflammatory cells in contact with the larvae, but extensive infiltration of eosinophils in the underlying choroid were observed as part of the uveal reaction. These infiltrates were different from granulomas around uveal larvae. Other mechanisms besides the mast cell IgE-released eosinophil chemotactic factor seem to be involved.[68]

In 1981 de Kozak and associates[69] reported seeming increased numbers of choroidal mast cells starting 4 days after immunization in rat foot pads by retinal S antigen with adjuvant and before the clinical appearance of experimental autoimmune uveoretinitis. The number of mast cells peaked at twice the normal number on day 6, then fell to a minimum on day 10, and then slowly returned to normal. The degranulation was at a maximum on day 10. Peritoneal mast cell degranulation was only noted where intraperitoneal immunization was used. The investigators suggested that IgE antibodies may initiate an early change in choroidal

vascular permeability in this model.

Mochizuki and colleagues[70] in 1984 described changes in the choroidal mast cells with experimental autoimmune uveitis and felt that there was a direct correlation between increased choroidal mast cell numbers and increased susceptibility to the development of autoimmune uveitis. They also noted a difference in choroidal mast cell numbers that was strain-related and felt this strain difference was related to increased development of experimental autoimmune uveitis. CAR and Lewis rat strains had significantly greater numbers of choroidal mast cells and showed greater response in the development of experimental autoimmune uveitis than other strains tested. No similar association was found between mast cells of the skin in these strains.

The number of choroidal mast cells previously documented, the known effects of mast cell products, and the possibility that these mast cells may represent a unique functional compartment and may participate not only in the unique pathology of the posterior pole of the eye but also in physiologic processes of the eye all infer that a better characterization and understanding of these cells will give insight into posterior pole physiology and disease processes.

EXPERIMENTAL RESEARCH

GENERAL INTRODUCTION

The importance of mast cells in the choroid is apparently evident, as a review of the recent literature shows, and hence experimental study of the mast cells in the choroid of the albino rat was undertaken. These experiments were designed to characterize the mast cell in the choroid of this particular animal model. The aims were as follows: (1) define the numbers, localization, and distribution of the mast cells in the choroid of the rat; (2) measure the quantity of mast cell histamine and heparin content in the uveal tissues of rat eyes, specifically in the posterior pole choroid, in both young and old rats; and (3) measure the immunologic and ionophoretic release of anterior uvea and posterior choroidal mast cell contents and compare these properties with mesenteric mast cells in the same animals under similar conditions.

EXPERIMENTAL STUDY I: NUMBERS, LOCALIZATION, AND DISTRIBUTION OF MAST CELLS IN CHOROID OF NORMAL YOUNG AND OLD RAT EYES

Materials and Methods

Sprague-Dawley male rats were used for this experiment except where

noted otherwise. These rats are nonpigmented, which allows visualization of cells in the choroid. The age range was from 43 days to 10 months (retired breeder rats). The rats were anesthetized with ether and exsanguinated. Both eyes were enucleated and placed on ice. Eyes used for cross sections in the initial experimental study were fixed in formalin or iso-osmotic solution containing 0.6% formaldehyde and 0.5% acetic acid. These eyes were then imbedded and sectioned for staining. For flat preparations of the choroid, the eyes was dissected by cutting it with a razor blade anterior to the equator (Fig 1) and quartering the posterior pole. The posterior quarters were also fixed in formalin or IFAA (iso-osmotic solution containing 0.6% formaldehyde and 0.5% acetic acid). Fixation times were 16 to 22 hours in most specimens and the same for comparable groups of choroids. The retina and sclera were removed, and the choroid was mounted on cleaned glass slides as flat tissue mounts (described by Smelser and Silver[53]). Tissues were stained with either toluidine blue or berberine sulfate. One group was stained with toluidine

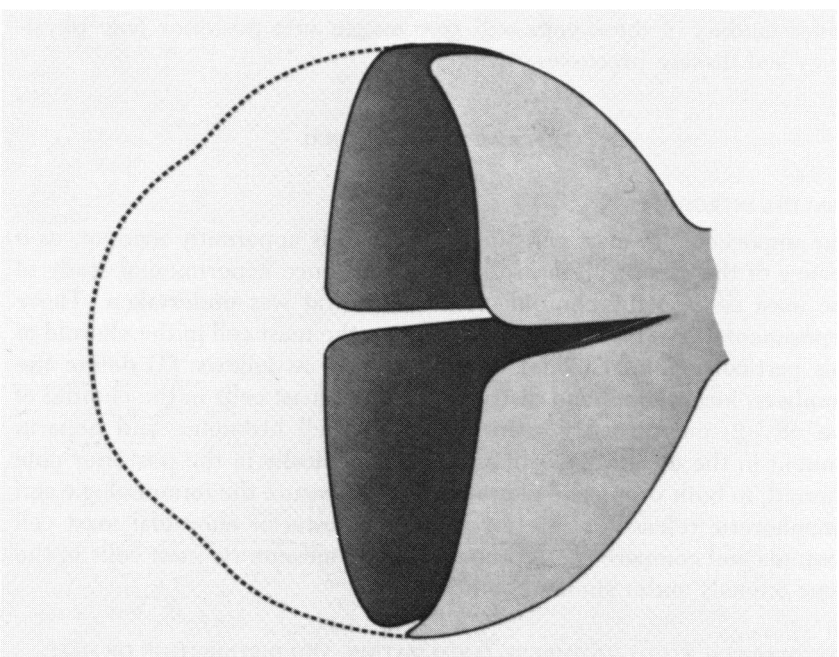

FIGURE 1
Sectioning of eye for preparation of flat histologic mounts of posterior choroid. These quadrants of posterior choroid were mounted flat on glass slide and stained with toluidine blue or berberine sulfate.

blue, counted, and observed and then restained with a more specific heparin stain (berberine sulfate) for a comparison of the same choroidal sections.

Toluidine blue stains were made at pH 1.6, similar to the method described by Enerback.[31] The tissues were washed three times for 10 minutes in distilled water. Two washed for 2 minutes in 95% ethanol were performed, and then the tissues were stained in 0.25% toluidine blue O in 70% ethanol at pH 1.6 for 30 minutes. This was followed by two washes for 2 minutes in 95% ethanol, three washes for 2 minutes in 100% ethanol, and three washes for 2 minutes in xylene. Tissues were mounted with coverslips of Permount and were read by light microscopy (Fig 2).

Berberine sulfate stains were performed according to the method described by Enerback[71] and Dimlich and associates.[72] Tissues were washed three times for 10 minutes in distilled water, then stained for 20 minutes with berberine sulfate 0.02% in distilled water at pH 3. Finally, these tissues were washed for 10 minutes in distilled water at pH 3. They were mounted with glycerol, with coverslips of Permount, and read by fluorescent microscopy (Fig 3).

Uveal Mast Cells by Serial Cross Sections

Methodology—In a preliminary pilot study, multiple serial anteroposterior coronal cross sections of eight eyes from 2½-month-old Sprague-Dawley male rates and eight eyes from 10-month-old retired breeder Sprague-Dawley male rats were prepared and stained with toluidine blue. Fifteen sections separated by 15 μm were counted from each eye

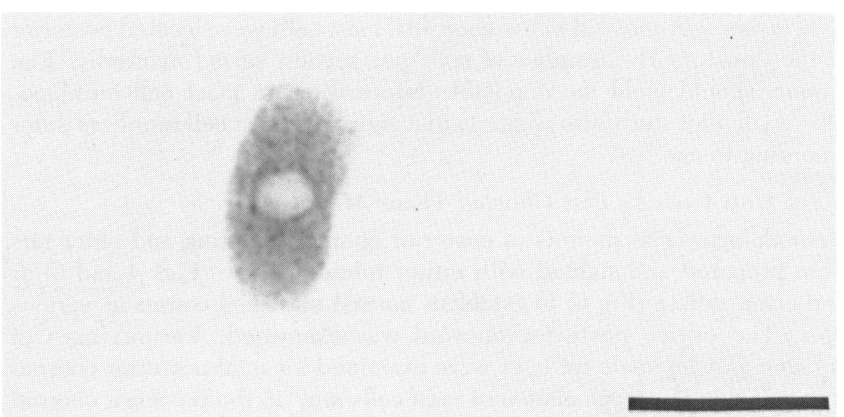

FIGURE 2
Flat choroidal mount with mature rat choroidal mast cell (toluidine blue). Metachromatic granules are seen in cytoplasm (bar = 15 μm).

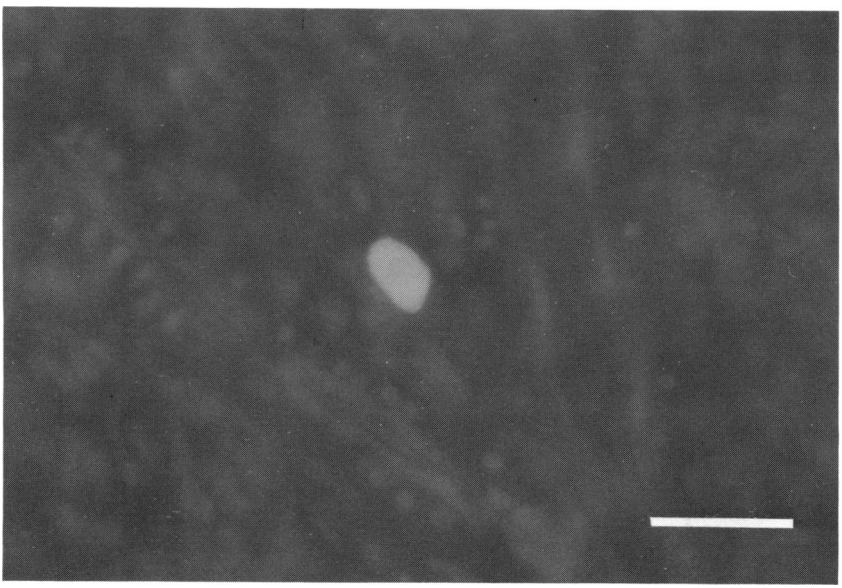

FIGURE 3
Mature rat choroidal mast cell (berberine sulfate). Fluorescence of dense concentration of stained heparin in cytoplasmic granules is seen under fluorescent microscopy. Faint outline of branching vessel is visible on either side of mast cell (bar = 30 μm).

using a similar technique as that described by Allansmith and associates[66] in 1980.

Conclusions—Almost all of the choroidal mast cells were located posterior to the equator. The number of cells per section varied markedly. Flat mounts should yield more reliable information on mast cell numbers. This small pilot study also suggests that choroidal mast cell numbers differ according to age.

Uveal Mast Cells by Flat Choroid Tissue Mounts

Methodology—Flat mounts of posterior choroid of young and older rats were prepared and stained with either toluidine blue (Figs 4 and 5) or berberine sulfate (Fig 6) to establish normal mast cell counts at various ages. The entire posterior choroid was examined. Various ages of Sprague-Dawley male rat eyes were examined for total posterior choroid counts (Table II). A calculation of mast cells/mm^2 in the posterior choroid for these young and older animals is shown in Table III. Lewis male rat eyes and Wister rat eyes were also counted (Table IV). Numbers of mast cells were obtained by counting all mast cells in continuous high-power

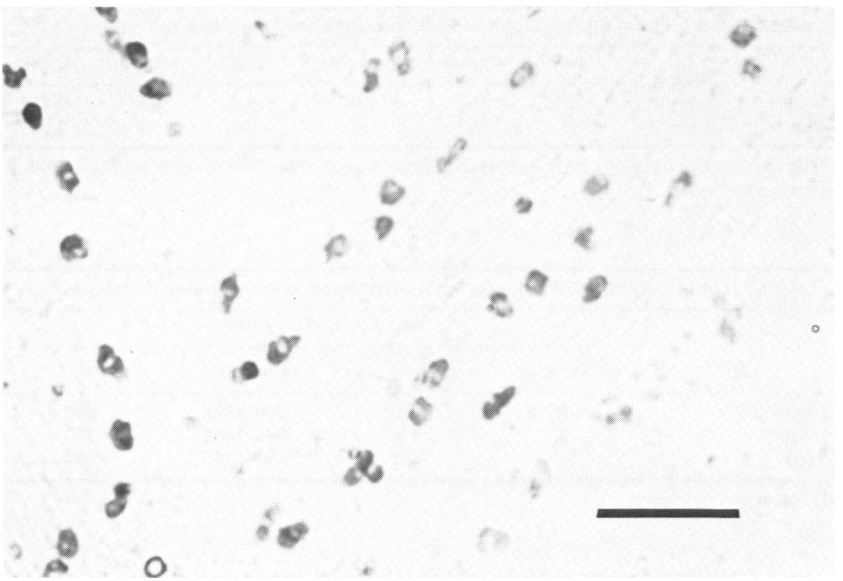

FIGURE 4
Flat choroidal mount with multiple mast cells aligned along branching arterial vessel (toluidine blue). Vessel wall is faintly visible (bar = 40 μm).

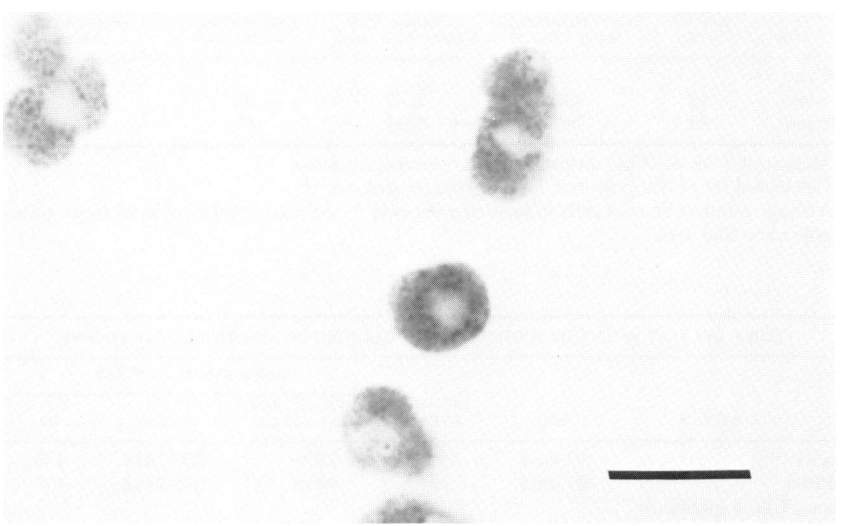

FIGURE 5
Flat choroidal mount with multiple mast cells along vessel (toluidine blue). Vessel walls are not visible with bright illumination but run vertically between single mast cell on left and cells lining other side of vessel (bar = 15 μm).

TABLE I: UVEAL MAST CELL COUNTS IN CROSS SECTIONS OF MALE RAT EYES*

	IRIS	RANGE	CILIARY BODY	RANGE	CHOROID	RANGE
Young eyes (2½ mo)	0	0-1	0	0-1	5	0-10
Older eyes (10 mo)	0	0-1	0	0-1	3	0-6

*Average count per ocular cross-section. 0 indicates more than 70% of cross sections did not show a mast cell.

TABLE II: FLAT MOUNT OF POSTERIOR POLE CHOROID OF SPRAGUE-DAWLEY MALE RATS

	TOLUIDINE BLUE STAIN			
AGE	NO. OF EYES	AVERAGE NO. MAST CELLS	RANGE	SD
43 days	6	960	594-1294	242
71 days	6	886	342-1451	347
10 mo	24	767	80-1370	367

SD, standard deviation.

TABLE III: MAST CELLS/mm^2 IN POSTERIOR CHOROID OF SPRAGUE-DAWLEY MALE RATS

		TOLUIDINE BLUE STAIN			
AGE	NO. OF EYES	AVERAGE NO. POSTCHOROIDAL MAST CELLS	AVERAGE EYE DIAMETER* (mm)	AVERAGTE POSTERIOR POLE CHOROIDAL AREA† (mm^2)	AVERAGE MAST CELL/mm^2 IN POSTERIOR POLE‡
43 and 71 days	12	923	4.75	35	26
10 mo	24	767	6.00	57	14

*Measured from multiple anteroposterior coronal sections.
†Calculated by ½ (for posterior half) of sphere area $4 \pi r^2$.
‡Average number of mast cells in posterior choroid ÷ average number of mm^2 in posterior pole choroidal area.

TABLE IV: FLAT MOUNT OF POSTERIOR POLE CHOROID OF ADDITIONAL RAT STRAINS

			TOLUIDINE BLUE STAIN		
STRAIN	AGE	NO. OF EYES	AVERAGE NO. MAST CELLS	RANGE	SD
Lewis	30 days	4	1236	760-1924	432
Wistar	90 days	12	1449	671-2142	456
Long-Evans (pigmented)	90 days	4	0*	. . .	. . .

*Mast cells not visible with flat preparations of choroid because of pigment interfernece. Bleaching with potassium permanganate solution appeared to alter mast cell and invalidated counts.

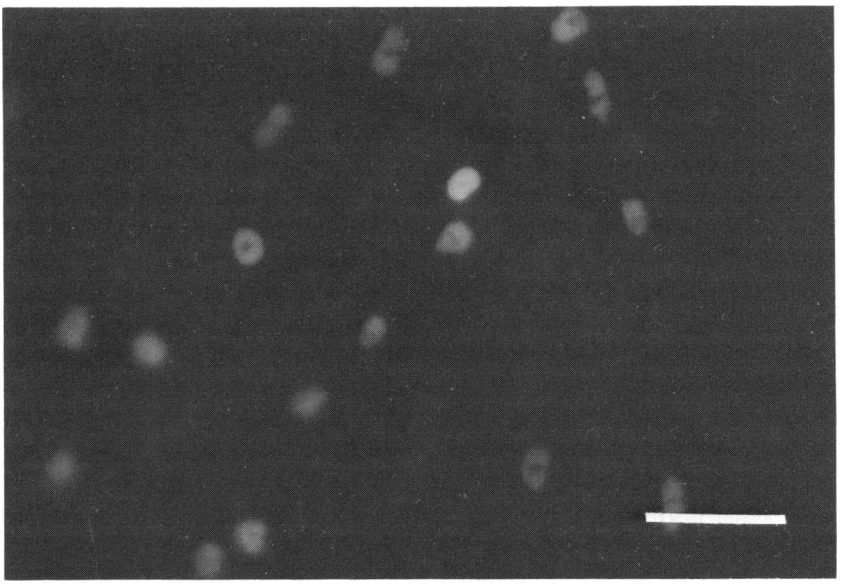

FIGURE 6
Flat choroidal mount with multiple rat choroidal mast cells along branching artery (berberine sulfate). Fluorescence of dense concentrations of heparin in cytoplasmic granules is seen under fluorescent microscopy. Mast cells are aligned along barely visible vessel wall and are more numerous at this branching location (bar = 40 μm).

fields in each quadrant of posterior choroid tissue using light microscopy, as described by Price.[60] Pigmented choroids were also prepared, but counts were not possible because of pigment interference. Bleaching with potassium permanganate solution seemed to alter mast cells in tissue.

Eight eyes of Sprague-Dawley males were stained with berberine sulfate and counted, and then restained with toluidine blue and recounted to see if similar values would be obtained. The results are shown in Table V.

Conclusions—With the use of flat preparations of the posterior choroid, the problem of counting variation from one tissue section to the next is removed. Variability between animals, however, is considerable within comparable age-groups. A difference or variation in mast cell numbers between the strains utilized is also apparent. The numbers are lower than in a previous report,[48] and this may be due to the lower pH of the stain used, which is more specific for mature mast cells. Although numbers are variable even within the comparable groups, a significant trend is seen, suggesting a decrease in posterior pole choroidal mast cell numbers with

aging. This is especially notable if a mathematical adjustment for the increased size of the older eye is made. With this factor taken into account, the difference between mast cells/mm^2 of posterior choroid shows almost a 50% difference between the comparable Sprague-Dawley young and retired breeder animals (Table II). An additional factor is seen with immature mast cells that are present in the younger rat choroid that do not have typical granules present in the cytoplasm. Toluidine blue stains all mast cells and berberine sulfate is thought to be more specific for heparin.[69] The difference in cell counts shown in Table V suggests that not all mast cells in the choroid contain heparin.

Comparison of Number of Mast Cells in the Choroid and Mesentery

Methodology—Flat preparations of the posterior choroid and the mesentery of six Sprague-Dawley 2-month-old male rats were examined using the same grid. An ocular grid was used in the microscope and was calculated with a hemocytometer grid to determine the surface area of tissue in the ocular grid when using the 40× microscope lens. Counts of the mast

TABLE V: COMPARISON OF STAINING METHODS FOR CHOROIDS OF SPRAGUE-DAWLEY MALE RATS

NO. OF EYES	AGE	BERBERINE SULFATE		TOLUIDINE BLUE	
		NO. MAST CELLS*	RANGE	NO. MAST CELLS*	RANGE
4	4 mo	2268	2043-2698	3271	3063-3479
4	8 mo	540	385-641	750	712-789

*Average mast cell number per choroid.

TABLE VI: AVERAGE CHOROID AND MESENTERY VALUES IN SPRAGUE-DAWLEY MALE RATS

ANIMAL	CHOROID	CHOROIDAL MAST CELLS/ mm^2	MESENTERY MAST CELLS/ mm^2	RATIO CHOROID TO MESENTERY
1	Right	27	116	0.202
	Left	20		
2	Right	33	87	0.349
	Left	28		
3	Right	45	110	0.407
	Left	45		
4	Right	25	80	0.223
	Left	11		
5	Right	41	171	0.263
	Left	46		
6	Right	19	125	0.172
	Left	25		
Average		30	115	0.269

cells/mm^2 were then made of the mast cell-rich choroid and mesentery tissues of each animal. The number of mast cells/mm^2 was calculated for the choroid of both eyes and the mesentery. The average choroid and mesentery value for each animal is shown in Table VI.

Conclusions—With the grid counting method, a marked variation in the number of posterior pole choroid mast cells/mm^2 in different animals was noted. The mesentery counts also differed, and the ratio of choroidal mast cells to mesentery mast cells showed some variation.

Distribution of Mast Cells in Flat Mounts of the Posterior Choroid

Methodology—Flat mounts of all posterior choroids used in the previously described studies were reviewed to identify characteristics of the distribution of the mast cells in the posterior choroid. Verhoeff and Verhoeff–van Gieson stains were done on flat choroid mounts toshow the elastic components of the vessel walls.

Results—A preferential alignment of the mast cells along blood vessels was evident (Fig 7). Most of these vessels have thick walls and cross-striations seen only with arterial structures. These vessels have the branching

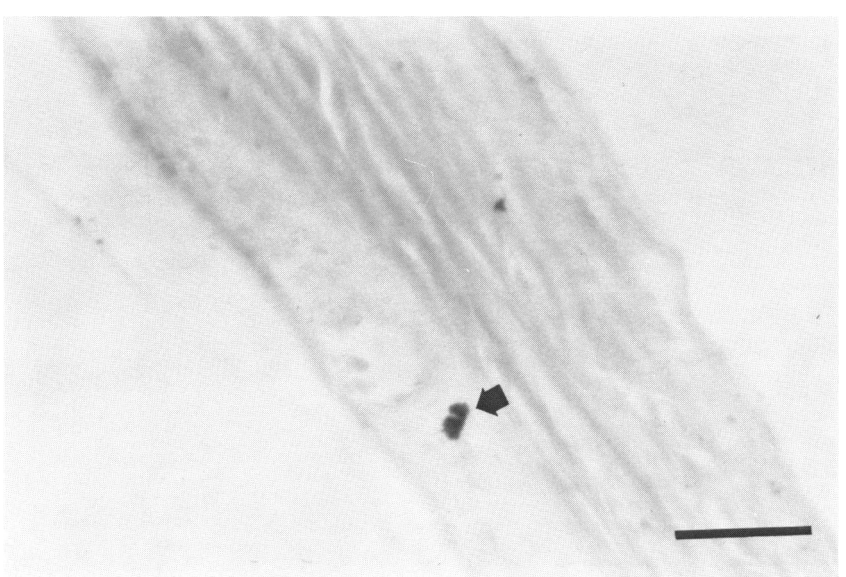

FIGURE 7
Tissue section of choroid to left of tissue and sclera to right (toluidine blue). Mast cell (*arrow*) is seen in adventitia surrounding vessel (bar = 40 μm).

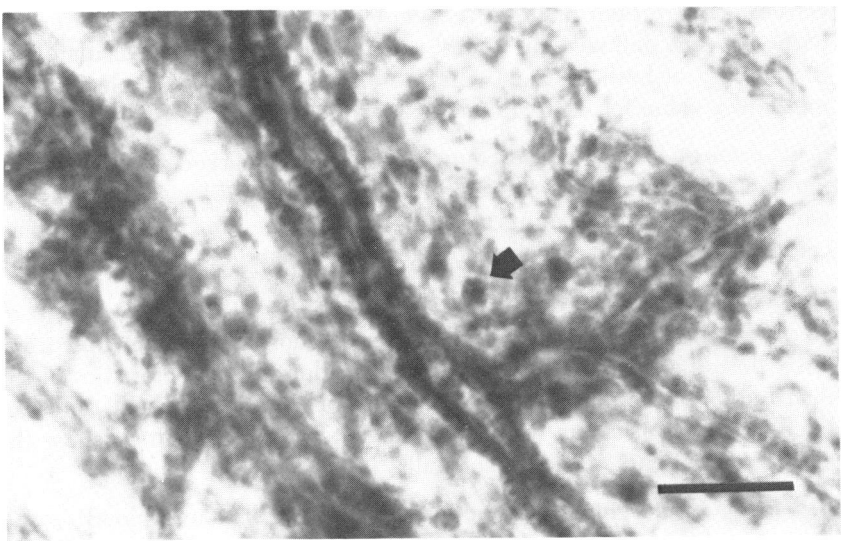

FIGURE 8
Flat choroidal mount of rat choroid (Verhoeff's stain). Elastic fibers are seen in deeply stained arterial wall structure. One mast cell of row of cells lininig wall is marked by *arrow* (bar = 40 μm).

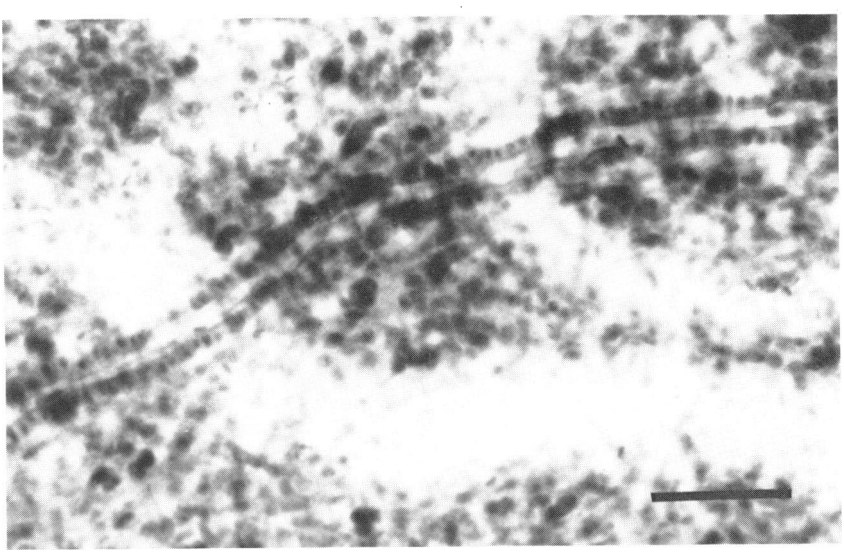

FIGURE 9
Flat choroidal mount of rat posterior choroid (Verhoeff–van Gieson). Elastic fibers are seen in artery vessel wall and mast cells in surrounding tissue. Smaller artery in lower part of field is not as clearly stained, but mast cells are visible along its course (bar = 40 μm).

characteristics of arteries. Verhoeff–van Gieson stain shows the elastic component of the vessel wall (Figs 8 and 9). The larger (40 to 60 μm) vessels have fewer mast cells along their course. Medium-sized (15 to 40 μm) vessels, however, are surrounded along their walls by many mast cells. A few mast cells are seen in the intervessel tissues, and it is difficult to be sure that they were not displaced during handling of the tissue. The mast cells along the arterial vessels seem to have moved circumferentially around the vessel so as to appear lined up along the vessel in the flat preparations, though they are probably scattered around the vessel in the pre-preparatory state (Figs 10 and 11). The thin-walled vessels that have characteristics of venous channels do not have significant numbers of mast cells; in fact, mast cells are seen in proximity to these vessels only occasionally (Figs 12 and 13). The younger choroids (2 months) (Fig 14) show immature cells that have faint granules, and the vessels are not as visible as in older choroids. These immature mast cells seem to be more evenly

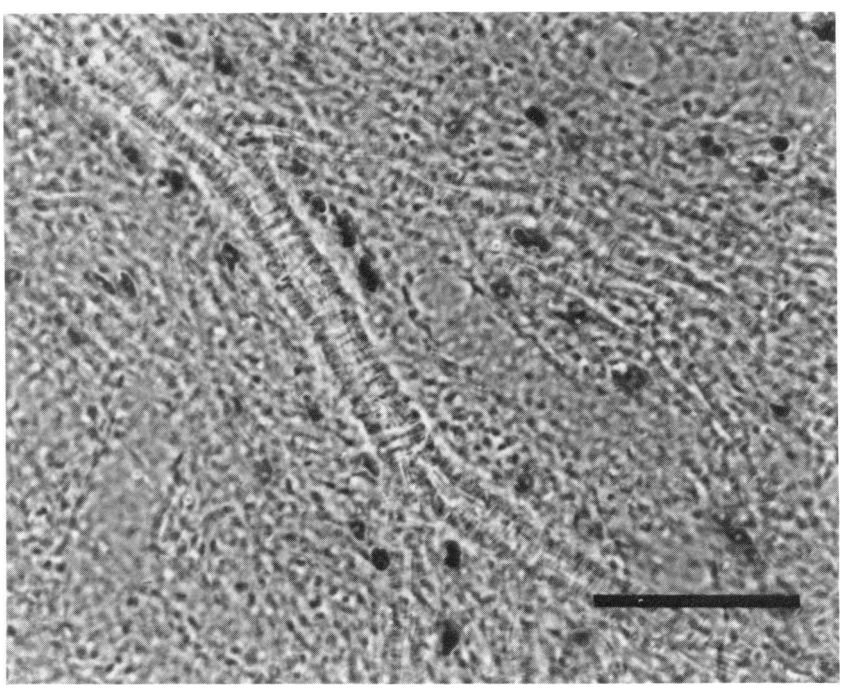

FIGURE 10
Flat choroidal mount of rat posterior choroid viewed with phase contrast microscopy. Thickened vessel wall and cross-striations are seen. Mast cells are visible along vessel wall (bar = 60 μm).

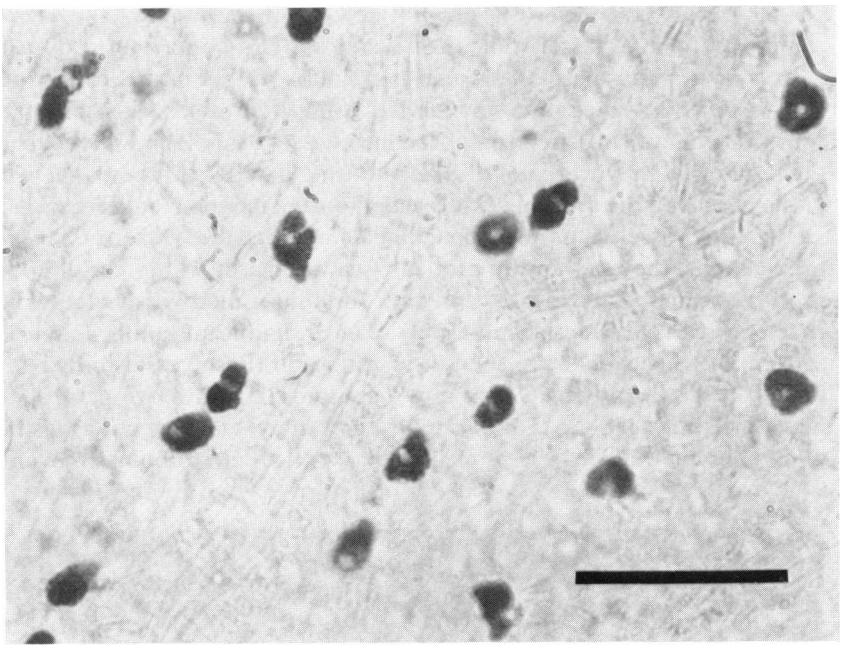

FIGURE 11
Flat choroidal mount of rat posterior choroid viewed at high power and bright illumination. Mast cell detail is clear, but vessel wall cross-striations and circular vessel detail are very faint (bar = 60 μm).

distributed throughout the choroidal tissue and appear to be in various stages of development. The younger choroids appear to be thicker than the older choroids. No dividing mast cells are seen in any of the tissues, which suggests that cells with any granules at all are already past the stage of mitotic activity. Multiple granules are seen in the tissue in some sites, suggesting that mast cell walls have been disrupted, perhaps by the handling of the tissue, the fixation, or the staining.

In the choroids of the retired breeders, the mast cells are almost entirely along the thick-walled vessels, and granules are dense and sharply outlined, indicating primarily mature cells (Fig 15). Few mast cells are present in the tissue in many areas. The mast cells are in closer proximity to the smaller vessels and appear farther from the vessel wall as the vessel becomes larger. Some of the larger vessels (40 to 60 μm in diameter) in many sections have little branching, and only a rare mast cell is present near these vessels before they branch (Fig 13, arrow).

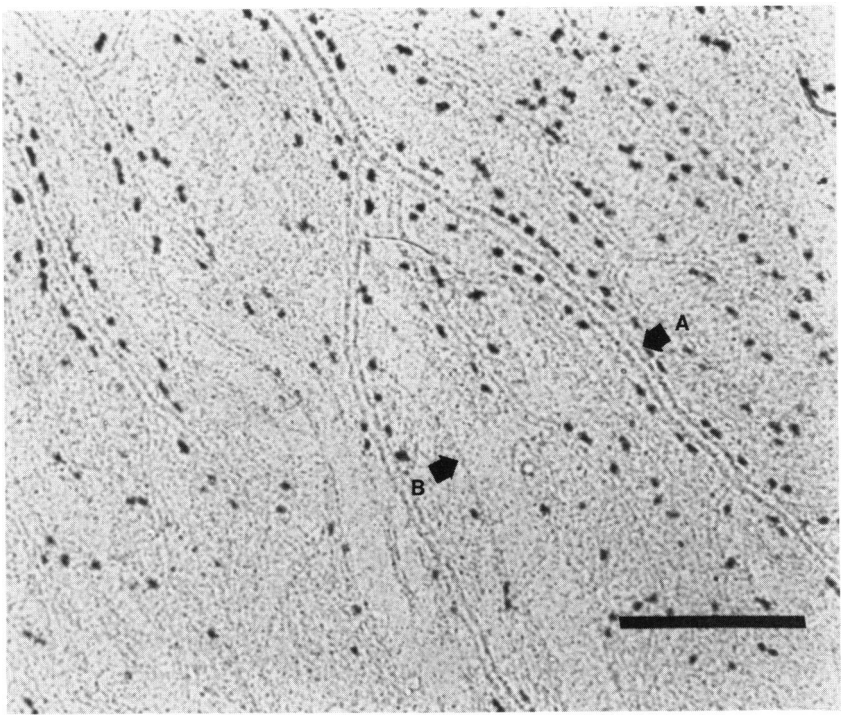

FIGURE 12
Flat mount of rat posterior pole. Low-power view shows larger field with alignment of mast cells along arterial (*arrow A*) structures. Less visible venous structures are not lined with mast cells, and occasional mast cell seen along venous channel (*arrow B*) may be related to artery that is less visible (bar = 130 μm).

Conclusions—The presence of many mast cells along the medium-sized arterial vessels of normal animals suggests that they have an effector role in blood flow and in the physiology of the choroid in this location. The preferential retention of mast cells in this location with aging points to a regulatory role and perhaps a role of the mast cell in age-related changes within the choroid. The potent effects of histamine, heparin, and other mast cell products are well known in other tissue. Similar potent effects are suggested for the choroid.

Distribution of Mast Cells in the Mesentery and Posterior Choroid

Methodology—Flat mounts of the mesentery and posterior choroid from each of six Sprague-Dawley male rats were studied. Three animals were 2 months of age and three were 10 months. The mounts were prepared and stained with toluidine blue and later with berberine sulfate as described

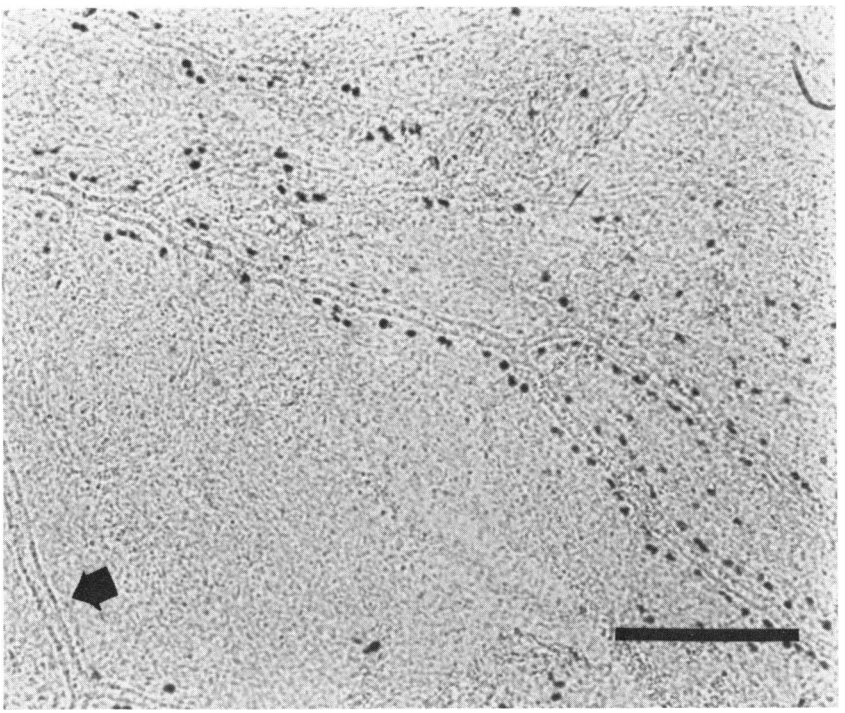

FIGURE 13
Flat mount of rat posterior pole. *Arrow* shows long area of vessel with no branching and no mast cells until branching occurs. This was seen numerous times in different preparations (bar = 130 μm).

previously under Materials and Methods, then examined by light and fluorescent microscopy.

Results—The numbers of mast cells in the mesentery of each animal was variable. The distribution of mast cells in the mesentery of young and old rats was similar and was regular throughout the tissues (Fig 16). The mesentery tissue vessel pattern has a predominance of venous channels, and the absence of arterial vessels appears to account for much of the difference in distribution when compared with the choroid (Fig 17).

Conclusions—The difference in number and distribution of mast cells within the mesentery and posterior choroidal tissues of the same animal suggests that these cells play different roles in the two tissues and that the function of the products differs as well.

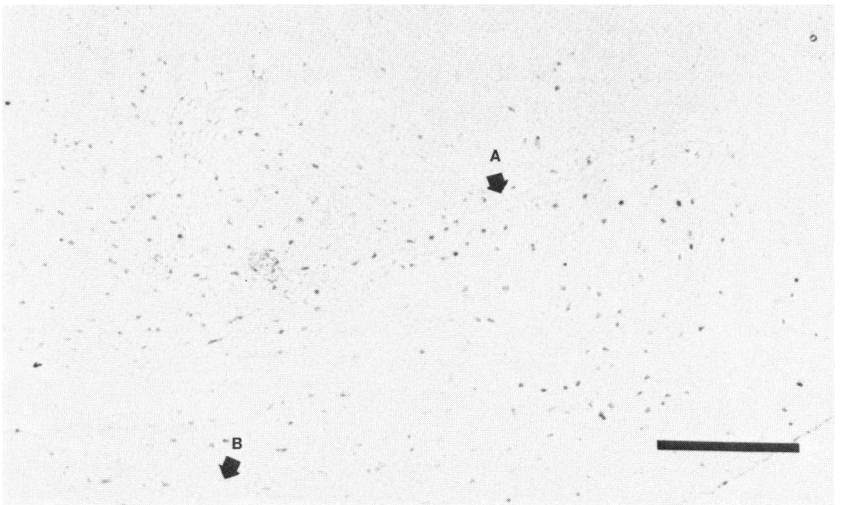

FIGURE 14
Flat mount of young (2-month-old) rat posterior pole choroid showing more diffuse arrangement of mast cells through choroid and less distinct staining of mast cells. Arterial vessel (*arrow A*) and venous channel (*arrow B*) are seen (bar = 100 μm).

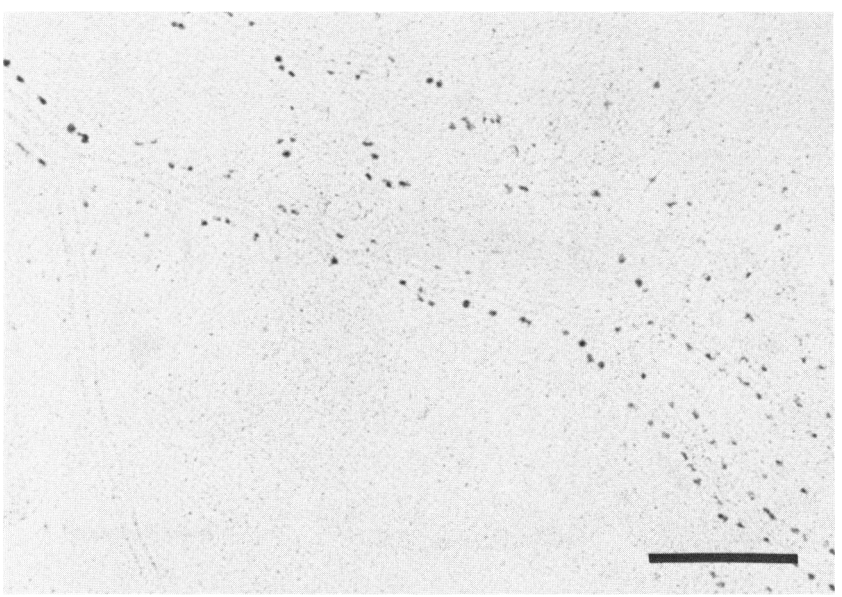

FIGURE 15
Flat mount of older (10-month-old) rat posterior pole choroid showing more dense staining of granules and more preferential alignment of mast cells along arterial vessels than seen in younger rat (Fig 14) (bar = 100 μm).

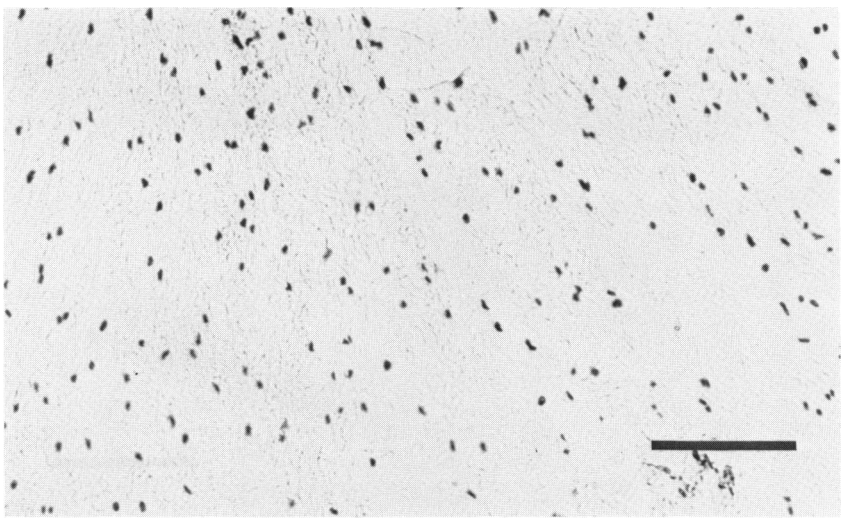

FIGURE 16
Flat mount of mesentery showing alignment of mast cells more related to venous channels and more diffuse tissue distribution (bar = 100 μm).

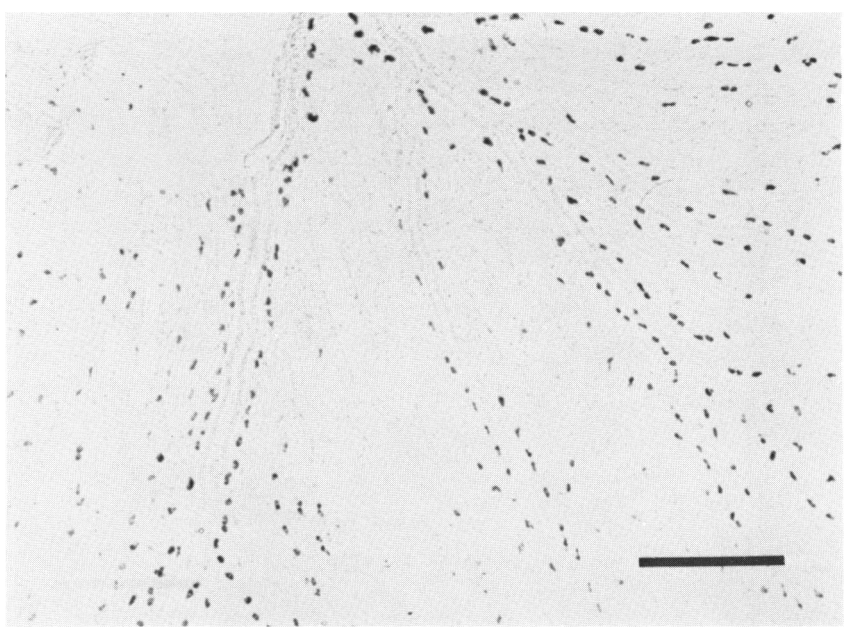

FIGURE 17
Flat mount of posterior choroid from animal in Fig 16. Preferential alignment is visible along arterial structures (bar = 100 μm).

DISCUSSION (EXPERIMENTAL STUDY I)

The variability of mast cell numbers among individual animals is striking and consistently encountered throughout all the groups examined. The relatively consistent agreement of mast cell numbers between the two eyes of the same animal is also noted and supports the validity of the observation of individual animal variations. The consistent presence of significant numbers of mast cells in all animals and their location along vascular arterial-like channels imply that these cells have important physiologic or pathologic roles. The trend of mast cells to decrease in number with aging is to be emphasized. The variability in their numbers from one individual to another is not inconsistent with the variation among individuals of unexplained susceptibility to certain disease states, particularly those related to senescence and involving the choroid and adjacent structures.

EXPERIMENTAL STUDY II: HISTAMINE AND HEPARIN CONTENT OF RAT EYES

Materials and Methods

Tissue was obtained from Sprague-Dawley rats of various ages. The eyes were removed after ether anesthesia and rapid exsanguination. The tissue was handled in one of two ways. One group of eyes was quick-frozen and stored at −70°C. Each eye was then held with a modified, watch-crystal lift vise and dissected over dry ice to separate the specific anatomic areas and to minimize histamine loss during the 20- to 30-minute dissection period. The anatomic areas were selected according to previous histologic studies, previously reported mast cell counts, and anatomic locations that correlate with the clinical division of ocular diseases. These areas were the (1) cornea and anterior sclera, (2) iris and ciliary body (anterior uvea), (3) equatorial choroid and retina (intermediate or peripheral), and (4) posterior pole choroid, retina (posterior), and posterior sclera (Fig 18). The retina was included along with the choroid because of the difficulty in separating choroid and retina in small frozen specimens and because no mast cells are visible in the retina on histologic examination. In the second group, the eyes were bisected just anterior to the equator, and the two halves pooled for histamine and heparin assays.

Histamine was measured with a sensitive enzymatic isotopic assay according to the method of Shaff and Beaven.[73] Methyltransferase was used to label standard amounts of histamine and unknowns with carbon 14 from S-adenosyl-L-methionine (C-14 methyl). The samples were then incubated at 37°C for 90 minutes. A carrier for the methyl histamine hydrochloric acid was added and extracted into chloroform. Each sample was then quantitated in a scintillation counter, and the amount of his-

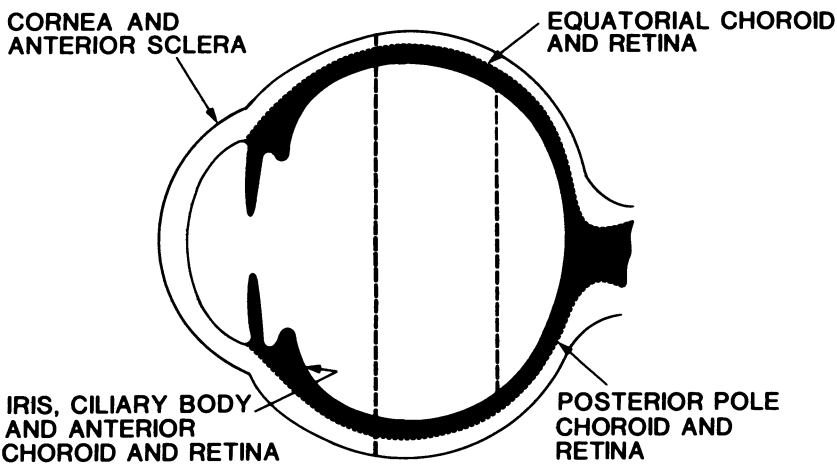

FIGURE 18
Ocular dissection used in experimental study IIa to test histamine distribution in eye tissues.

tamine was calculated according to the method described by Shaff and Beaven.[73] The sensitivity of this assay was 0.5 ng of histamine/ml.

Heparin was measured by the method described by Bitter and Muir.[74] The tissue was placed in sulfuric acid and maintained at 4°C. It was then heated in a boiling-water bath for 15 minutes. Carbazol reagent was added, and the tissue was heated again in a boiling-water bath for 15 minutes. The optical density was recorded at 530 nm. This was then compared with standards in order to calculate the heparin content. This is a sensitive test, detecting 4 μg of uronic acid/ml.

Experiment IIa: Distribution of Histamine in the Rat Uvea

Methodology—Initially 15 eyes from 250-gm Sprague-Dawley rats were dissected into anatomic segments as described previously. The tissue was pooled for each area (Fig 18). The anterior segment includes the iris and ciliary body. The equatorial contains the choroid and retina and sclera. The posterior pole includes the choroid, retina, and posterior sclera. Histamine was measured in these tissues as described, and the total wet weights were calculated. The histamine levels are expressed in nanograms per milligram of wet weight of tissue. These values are shown in Table VII.

Conclusion—The distribution of histamine is consistent with the distribution of mast cells. The retina contained no mast cells, and the posterior sclera did not show mast cells in significant number on previous studies.

TABLE VII: DISTRIBUTION OF HISTAMINE IN EYE TISSUE OF 250-gm MALE SPRAGUE-DAWLEY RATS

TISSUE	HISTAMINE*	
	WET WEIGHT (ng/mg)	TISSUE WET WEIGHT (mg)
Cornea and anterior sclera	2.38	13.64
Iris and ciliary body	0.23	6.32
Equatorial, choroid, and retina	1.02	9.04
Posterior pole, choroid, and retina	1.31	5.78

*Mean of 15 eyes.

The major source of intraocular histamine therefore appears to be the choroid. The equatorial tissue in this dissection extended posteriorly and contained more tissue than the posterior tissue pool. The posterior pool contained more histamine per milligram of tissue wet weight than the equatorial pool.

Experiment IIb: Histamine and Heparin Content Variations with Age

Methodology—As a consequence of the results of the previous experiment, the measurement of histamine and heparin in the uveal tissue was simplified into two samples, the anterior and posterior segments (Fig 19). The eyes were bisected with a razor blade anterior to the equator, and tissues were pooled with the iris, ciliary body, and anterior choroid in one group and the posterior choroid, retina, and sclera in the other. Previous indications that the mast cell number may decrease with age suggested that histamine and heparin be measured in young rat eyes and old retired breeder rat eyes. Twenty-four eyes of young rats (2 months) and 24 eyes of retired breeders (10 months) were tested. For comparison, the mesentery from each was also tested. The methodology was otherwise the same as in experiment IIa.

The results of experiment IIb are shown in Table VIII. Similar data were obtained in two additional repetitions of this experiment.

Conclusions—The overall values of histamine and heparin content in anterior and posterior choroidal tissues are variable, but the differences between posterior tissues in young rats and older rats consistently show that young rats tend to have higher posterior levels of histamine and older rats have slightly higher or similar posterior levels of heparin.

DISCUSSION (EXPERIMENTAL STUDY II)

The distribution of histamine seems consistent with mast cell distribu-

TABLE VIII: HISTAMINE AND HEPARIN LEVELS IN YOUNG AND OLD RAT EYES

AGE, ANATOMIC AREA	HISTAMINE	HEPARIN
Young, anterior	0.82 ng/ml wet wt	0.0400 μg/ml-mg
Young, posterior	8.65 ng/mg wet wt	0.0359 μg/ml-mg
Old, anterior	0.36 ng/mg wet wt	0.0436 μg/ml-mg
Old, posterior	1.14 ng/mg wet wt	0.0460 μg/ml-mg
Young peritoneal	23.4 pg/cell	9.5×10^{-6} μg/mast cell
Old peritoneal	32.6 pg/cell	19×16^{-6} μg/mast cell

tion. The levels of histamine in young rat posterior pole samples are higher than in older rat posterior pole samples in similar testing and handling situations.

Histamine and heparin were compared in the anterior and posterior choroidal tissue in two age-groups, young adult rats and retired breeders. A lower content of histamine was found in posterior pole in the older group. The number of mast cells present in the choroid was lower in the retired breeders. The histamine content per mast cell was greater in the retired breeders, which suggests that the mast cells undergo changes with aging and the mast cells of the choroid may differ from other tissues.

EXPERIMENTAL STUDY III: HISTAMINE AND HEPARIN RELEASE FROM UVEAL MAST CELLS

Materials and Methods

Spraque-Dawley rats were dissected by sectioning the unfrozen eyes slightly anterior to the equator (Fig 19) and removing the fresh anterior tissues and the choroid and retina from the posterior section and combining them into pools of tissue. These pools of tissue were suspended in cold Tyrode's buffer to study the heparin and histamine release by stimulation of the tissue with rabbit anti-rat IgE, compound 48/80, and ionophore A23187.

Experiment IIIa: Anti-IgE-Stimulated Release of Histamine from Uveal Mast Cells

Methodology—Posterior choroidal and retinal tissue pooled from 29 eyes from 250-gm Sprague-Dawley male rats was divided into aliquots in cold Tyrode's buffer and tested by stimulation with various concentrations of rabbit anti-IgE and with compound 48/80 as control. Net percent of histamine release was calculated as the percent of stimulated release minus spontaneous release. The results are shown in Table IX. Results were comparable to peritoneal mast cell release in the same experiment.

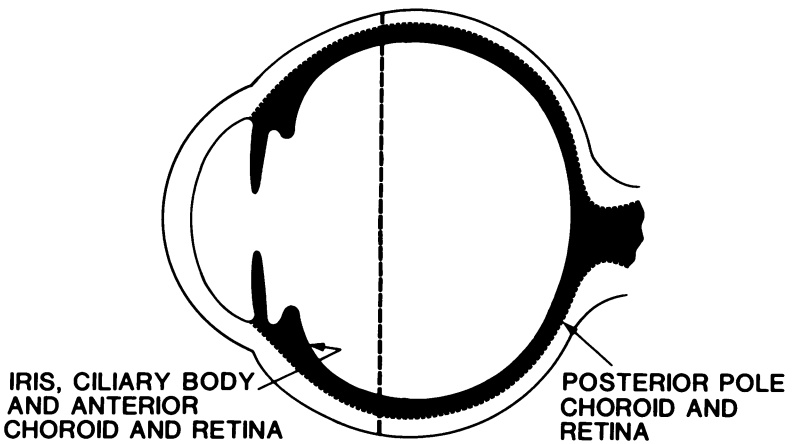

FIGURE 19
Ocular dissection used in experimental study IIb to test histamine and heparin concentrations in anterior and posterior uvea.

TABLE IX: IN VITRO HISTAMINE RELEASE FROM RAT EYES

ANTI-RAT IgE	NET % HISTAMINE RELEASE*
1:5	6
1:20	33
48/80 (0.1 μg/ml)	18

*Spontaneous release, 10%. Net % histamine release is percent stimulation-induced histamine release minus percent spontaneous release.

Conclusions—Mast cells from the posterior choroid showed a significant release of histamine with anti-IgE in a 1:20 dilution. Nearly twice as much histamine was released at this concentration than with compound 48/80.

Experiment IIIb: Histamine and Heparin Release from Choroidal Mast Cells Stimulated with Ionophore A23187

Methodology—Twenty-four eyes from 2½-month-old Sprague-Dawley male rats and 24 eyes from 10-month-old retired breeder Sprague-Dawley male rats were removed and sectioned as in experiment IIb, slightly anterior to the equator (Fig 19). The posterior pole intraocular contents, including the choroid and retina and anterior segment contents,

TABLE X: HISTAMINE RELEASE FROM CHOROIDAL TISSUE WITH IONOPHORE A23187 STIMULATION

AGE, ANATOMY	NET % HISTAMINE RELEASE WITH IONOPHORE A23187*	
	0.5 μg/ml	1.0 μg/ml
Young, anterior	22	22
Young, posterior	11	9
Older, anterior	22	28
Older, posterior	10	10

*Spontaneous release varied from 9% to 12% in different tissue. Net % histamine release is percent stimulation-induced histamine release minus percent spontaneous release. Heparin release was measured in same tissues, and no heparin release was detected.

were pooled from both sets of eyes. The tissues were blended and divided into aliquots for testing against various concentrations of calcium ionophore A23187, a nonphysiologic stimulator of cell activation. Measurements of histamine and heparin were made before and after stimulation of the tissue, with 0.5 and 1.0 μg/ml concentrations, using the techniques described in experimental study II. Net percent of histamine release was calculated as in experiment IIIa.

The results, expressed as net percent release, are shown in Table X.

Conclusions—Histamine release is more evident in the anterior segment tissues in all cases. The net percent release of posterior pole histamine with ionophore is similar in young and older posterior tissues. Heparin is not released by ionophore stimulation in either the anterior uvea or posterior choroid.

DISCUSSION

Although the choroidal mast cell seems to participate in immediate hypersensitivity reactions to a degree, a review of the literature and the investigative work done in these experimental studies infer a greater role for this cell in the modulation of inflammatory, immunologic, and physiologic processes. The choroidal mast cell appears to have some characteristics of the connective tissue mast cell elsewhere. However, there are indications that the significant numbers, the distributions along arterial structures, changes with age, and differences in release responses may represent properties of a group of mast cells with localized characteristics, differing from mast cells in other areas.

The attempts to learn more concerning the role of the choroidal mast cell in normal and diseased states have proven difficult because of limited numbers of cells present in the tissue and problems in obtaining enough cells to study in an unaltered, intact state. Studies using in vitro cultured cell lines have significantly altered not only the cells being studied but also the situations in which they are studied. Results from in vitro studies must be questioned, and additional clarification is needed.

Mast cells are difficult to identify in pigmented tissue and with hematoxylin-eosin stain and other stains frequently used for histologic study. Fixation methods alter mast cell staining characteristics and visibility. Any such study of tissue undertaken either by histochemical or histologic means represents a single time frame in a dynamic process.

Although the number of choroidal mast cells is high in comparison with many other areas, the amount of tissue available for study is small and the use of pooled samples is necessary. The variance among similar, individual animals presents additional obstacles. In spite of such difficulties, the importance of the mast cells and their roles in normal physiology and disease, as studied in other tissues, makes it clear that attempts must be made to study and better understand the role of mast cell in the posterior pole choroid of the eye. As a source of important chemical modulators of inflammatory and immunologic responses, the mast cell must significantly influence the health of the posterior uveal pole.

The rat is a useful model for studying choroidal mast cells. The albino rat, with unpigmented choroid, has mast cells that are easily visible with appropriate stains in flat choroidal-tissue mount preparation. A relatively high concentration of mast cells was observed in the posterior choroid of all animals in this study. Although mast cells are concentrated in the posterior pole, the numbers of mast cells in the posterior pole vary greatly from one animal to another and along blood vessels a short distance apart. Variation was seen even in branches of the same vessel. There was a close overall agreement between the numbers of mast cells in the two eyes of any individual.

The exact origin of the mast cell remains obscure. It has been speculated that a hematopoietic stem cell may be the precursor, and that this cell may also differentiate into granulocytes and may share a common bone marrow-derived precursor with basophils. Precursors have been noted in thymus and other lymphoid tissues. The mast cell apparently comes from a precursor cell that contains few or no granules. The granules become visible as the cell matures.[36] This theory would certainly be compatible with observations in the younger rat choroid, where cells of variable granule density were found. Since mast cells are not ordinarily

thought to circulate in the blood, it would seem consistent with these observations to state that the choroid becomes populated with mast cell precursors that are not visibly discernible by the usual methods of granular staining. The precursor cells may produce a local population of mast cells, which mature within the choroid and then share specific, regional characteristics. No cells showing mitotic changes with metachromatic granules were observed in any of the choroids studied.

In the younger rats, immature mast cells were visible in plentiful numbers and at many stages of maturity. The staining properties of the immature mast cell present problems in determining mast cell numbers in the young rat choroid. Also, immature cells seem more easily disrupted by handling than do mature mast cells. Cells observed ranged from clearly differentiated cells containing well-defined granules to those that were suggestive of being mast cells only because of their location along the blood vessels. Their faint staining characteristics differentiated them from adjacent cells and showed a similarity to cells slightly further along in development, containing immature but definite granules. The observations indicate that these faintly staining cells were earlier stages of the mast cell. In older rats the immature forms were absent, and the mast cell populations were more similar, with deeply staining granules and a monotonous appearance.

The results of this study of mast cell stimulation as well as review of the literature suggest that degranulation of the posterior choroidal mast cell with antigen anti-IgE and ionophore is not as complete as degranulation in other tissue unless the antigen challenge is excessive and rapid.[54,55,65,66] It appears that mediators are released in a slower, more complex manner than by degranulation observed in usual anaphylaxis. Anaphylactic changes in the choroid are not often observed clinically. This may be due to the life-threatening aspects of the anaphylactic reaction and to the possible rapid recovery of the choroid without long-term tissue effects. Experimental studies have shown anaphylactic changes in mast cells in certain areas of the eye, but the long-term effects on ocular tissues, including the choroid, have either not been remarkable or not been completely studied.[54,55,66] A release of mediators that occurs over a longer period, such as the "piecemeal" release of mediators,[36] may be of more importance. This type of mediator release does not occur by exocytosis of the entire secretory granule but seems to involve small vesicles of granule contents that flow to the surface of the cell and slowly discharge their contents from the cell surface. This has been described best in studies involving basophils and lectin Concanavalin-A stimulation.[36,75,76] This sort of active secretion of mediators would seem more likely to occur

in the choroid of the eye and could better play a role in many clinically observed, yet poorly understood, pathologic conditions involving the posterior pole of the eye.

Results of this study indicate that choroidal mast cells have IgE receptors and are able to release histamine when stimulated with small amounts of anti-IgE. A dose-related inverse response showed more release of histamine with a 1:20 dilution of anti-rat IgE than with a 1:5 dilution of anti-rat IgE. Choroidal mast cells also release histamine nonspecifically when stimulated with compound 48/80.

These mast cells are similar to the connective tissue mast cells of the mesentery in their responsiveness to anti-IgE and compound 48/80. The lung mast cells are less responsive to compound 48/80 by way of comparison and the mucosal mast cell is unresponsive to compound 48/80.[77,78]

The total amount of histamine present in the choroidal tissue varies significantly among individuals. This statement concurs with investigations involving other tissues. The relation to various conditions (eg, age, sex, genetic factors, pathologic states) needs to be more clearly defined.

The observations in this study and those reported by Mochizuki and associates[70] showing differences in the number of choroidal mast cells related to differences in rat strain deserve more investigation. These investigators also reported that experimental autoimmune uveitis was more likely to develop in strains with more choroidal mast cells. This supports not only a genetic predisposition for mast cell numbers to be increased or decreased but also presents evidence that those with more strain-associated choroidal mast cells are more likely to have an inflammation involving this area. Since there were no changes in the skin mast cell numbers, this also suggests a regional difference in the mast cells of choroid and a role in producing local inflammation stimulating activity.

The roles of prostaglandin D_2 and leukotrienes in ocular inflammation are unclear, but certainly their release during mast cell degranulation indicates that they very likely play important roles in inflammation of the eye.[28] The anatomic localization of mast cells and histamine in the posterior pole of the eye and their proximity to arterial vessels lend weight to speculation that the mast cell and its products could play an important part in such clinical conditions as macular edema, central serous retinopathy, and other poorly understood inflammatory and vascular-related diseases.

The more recently recognized roles of mast cell products regulating or altering immune responses may help suppress or prevent inflammatory disease states as well. Responses such as induction of suppressor T cell populations by histamine may promote tolerance and reduce tissue dam-

age from inflammation.[12,17,19,20] In the choroid, where inflammation is so destructive to the tissue and its subsequent function, a suppressed inflammation could be helpful. This would be especially significant when the stimulus for the inflammation itself was not destructive to the tissue or to the overall health of the whole organism. Teleologically speaking, the local suppression of ocular inflammatory reaction and protection of the choroid and retina from the destructive effects of the inflammatory reaction might have a survival value. By reducing tissue destruction and scarring caused by the inflammation, sight and function may be preserved when the etiologic agent or cause is not lethal and not as destructive to tissue as the inflammatory process effects.

There is a lack of clinical situations where local suppressor cell activity has been implicated. Demonstrating this, however, is technically difficult with current techniques. Systemic suppressor cell activity has been shown in systemic histoplasmosis,[79] and the possible existence of a mast cell-related local immunologic process, including a local suppressor cell population in the presumed ocular histoplasmosis syndrome, would offer reasonable mechanisms for further consideration in this poorly explained, recurring posterior pole disease. One example of ocular inflammation related to systemic suppressor cell activity was reported by Gardner and co-workers[80] in 1982 in a patient with an antigen-specific suppressor cell population that allowed a persistent systemic *Mycobacterium fortuitum* infection. Their study shows that a suppressor cell population can allow a systemic chronic infection to persist involving the uvea in a chronic inflammation, and that the patient may have relatively few symptoms of the infection.

Various other reactions may occur with effects on the microvasculature and inflammation. Local vasodilation and increased vascular permeability of the choroidal vessels, caused by the choroidal mast cell immunologically stimulated release of histamine and other mast cell products, could prolong local inflammation.[81] It would be important to have a local source of heparin for anticoagulant effect and to keep clotting in the choroid at a minimum when blood flow is reduced. Although heparin initially remains on the cell surface, and although this study was unable to show early heparin release with stimulation studies, the long-term fate of heparin is unknown.

Clinically, the rarity of posterior choroidal infarction is well recognized. Infarction of the peripheral retina and anterior choroid is clinically seen as an occurrence associated with aging in peripheral retinal degeneration, Elschnig's spots, and other conditions.[82,83] Macular degeneration and disturbances in the posterior pole circulation associated with aging might

also be related to mast cell changes and their regulatory role in the microcirculation. The lack of mast cells in the anterior choroid could contribute to increased difficulties in this area with blood flow regulation and might make the area more vulnerable to obstruction of the microvasculature in this region.

Another possible role of the mast cell in these pathologic conditions involves its participation in fibrosis and scar formation. This involvement of the mast cell is recognized systemically in scar tissue, keloids, scleroderma, and other scar-forming situations.[84] It would be attractive to involve the mast cell in some of the poorly understood posterior pole degenerative diseases that occur with long-term changes and involve posterior pole fibrosis and tissue scarring (such as occurs with macular degeneration).[85,86]

Mast cell interaction with eosinophils potentiates eosinophil activity. Histamine inhibits IgE-mediated histamine release and would tend to modulate parasitic disease reactions in the posterior pole.[12]

From the study presented and the literature reviewed, it seems justified to speculate that better understanding of the modulating effects of mast cell products may bring new insight into the management of posterior pole disease. Effector receptors such as H_1- and H_2-histamine receptors in the posterior pole must be clarified, and the effects of H_1 and H_2 agonists and antagonists need to be studied with the choroidal tissue. Knowledge of the time course of these reactions may lead to appropriate application of these drugs, which may be able to alter responses that are presently not treatable. The further characterizations of the numbers of mast cells present under various conditions, the sensitivity of release of histamine and the other mast cell products, and the conditions under which their release occurs are clearly worthwhile goals for future study.

SUMMARY

The experimental studies performed on nonpigmented rat choroids and the review of the important literature covered in this thesis seem to justify the following statements:

1. Mast cells are present in the choroid in significant numbers.
2. Mast cell numbers vary considerably from one individual to another and from one location in the choroid to another.
3. The major concentration of mast cells in the uvea is in the posterior choroid.
4. The mast cells of the choroid have a preferential location along arterial vessels.

5. Choroidal mast cell population density apparently decreases with senescence.

6. Mast cell products are present in sufficient quantity to exert substantial effects on physiologic, immunologic, and inflammatory responses in the choroid.

7. Choroidal mast cell products are released with appropriate stimulation and share some properties with the connective-tissue mast cell.

8. Choroidal mast cells demonstrate enough differences to suggest that a local differentiation may be present and may represent a locally controlled modulating effect for choroidal physiologic, immunologic, and inflammatory reactions.

ACKNOWLEDGMENT

I gratefully acknowledge the help provided by Daniel J. Stechschulte, MD, in determining tissue histamine and heparin content.

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