


Material and methods. Nineteen adult dogs, weighing 10 to 20 kg, were used in this study. The dogs were anesthetized with intravenous sterile sodium pentobarbital, 30 mg/kg body weight. The carotid artery was isolated through a vertical incision in the neck, and a 25-gauge needle was inserted. The needle was connected to a polyethylene tube. A bacterial or a control suspension was then injected slowly over 10 to 15 min.

The bacterial suspension was prepared as follows. The same strain of Streptococcus faecalis or American Type Culture Collection strain 25923 coagulase-positive Staphylococcus aureus was cultured in either 30 ml of the injected dog’s own plasma or 30 ml of trypticase soy broth at 37° C for either 5 or 16 hr. The resulting bacterial suspensions were diluted to 120 ml with sterile normal saline just prior to injection. The undiluted suspensions contained either 10^7-10^8 bacteria/ml (lower concentration, obtained from the 5 hr culture) or 10^9-10^10 bacteria/ml (higher concentration, obtained from the 16 hr culture). We used the urine colony count technique to determine the bacterial count. There was no difference in the bacterial count with trypticase soy broth or dog plasma used as the culture medium.

All 19 dogs were injected with bacterial suspensions, 14 with S. faecalis and five with S. aureus. Eleven of the 14 dogs injected with S. faecalis received the higher concentration (five cultured in trypticase soy broth, six cultured in the injected dog’s own plasma); three received the lower concentration (all three cultured in trypticase soy broth). Among the five dogs injected with S. aureus, four received the higher and one the lower concentration (all five cultured in trypticase soy broth).

Control suspensions consisted of 30 ml of either sterile trypticase soy broth or sterile plasma from the injected dog. A sterilized preparation of S. faecalis or S. aureus was not used as controls because of the unknown effect of toxins from dead bacteria. Control suspensions were incubated at 37° C for the same duration as the bacterial suspensions and diluted to 120 ml with sterile saline just prior to injection. Control suspensions were injected into the carotid artery opposite to the side of bacterial injection. Five of the 19 dogs received control injections 3 to 7 days prior to the bacterial injection (sterile trypticase soy broth in three dogs, sterile autogenous plasma in two dogs).

Eight of the 19 dogs received control injections at the same time as the bacterial injection (sterile trypticase soy broth in four dogs, sterile autogen-
ous plasma in four dogs). Six of the 19 dogs did not have control injections. Of the 13 control injections, 10 were done in dogs injected with *S. faecalis* and three in dogs with *S. aureus* injection.

The fundus was examined by indirect ophthalmoscopy, and color fundus photographs were taken. The pupils were dilated with 1% tropicamide (Mydriacyl) and 10% phenylephrine hydrochloride (Neo-Synephrine). Four to 48 hr after injection of the bacterial suspension, most of the animals were sacrificed. Their eyes were enucleated and fixed in either 10% formalin or 4% buffered glutaraldehyde and embedded in either paraffin or Epon-Araldite, respectively. Representative fundus lesions were sectioned serially either at 2 μm and stained with toluidine blue or at 8 μm and stained with hematoxylin-eosin or the Brown-Benn technique for light microscopic examination. Random sections of tapetal and nontapetal areas of control eyes were also studied by light microscopy. Two eyes with lesions produced by *S. faecalis* and their fellow control eyes were opened under sterile conditions at 24 hr, and swabs of the retina and choroid were cultured.

**Results.** Multifocal septic choroiditis with serous retinal detachments developed in dogs within 1 day following intracarotid artery injection of *S. faecalis* or *S. aureus*. Dogs injected with the former survived, whereas those injected with the latter died within the first day.

**Streptococcal experiments.** On clinical observation, *S. faecalis* produced small, occasionally hemorrhagic, serous retinal detachments, usually with dark centers. The lesions were located mainly in the tapetal area and were clearly visible 24 hr after injection (Fig. 1, top). All animals were examined 2 to 4 hr after bacterial injection, and there were no fundus lesions seen. The lesions developed consistently (in 11 out of 11 dogs) following the higher concentration (10^8-10^9 bacteria/ml) and inconsistently (in one out of three dogs) following the lower concentration (10^7-10^8 bacteria/ml). The fundus lesions, when present, always developed ipsilateral to the side of bacterial injection; however, in one of the 11 dogs injected with the higher concentration, fundus lesions were observed bilaterally. In this case, the contralateral eye showed only two very small lesions. In the dog with bilateral involvement, the control suspension had been given 3 days prior to the bacterial injection.

Two dogs with lesions from *S. faecalis* were followed for several weeks. In both cases, the fundus lesions faded after several days, leaving behind reflectile spots (in the tapetal area) or pigment irregularities (in the nontapetal area).

Histopathologic study of the acute lesions showed aggregations of polymorphonuclear leukocytes in the inner choroid and subretinal space surrounded by an exudative, occasionally hemorrhagic, retinal detachment (Fig. 2). The retinal pigment epithelium and photoreceptor outer segments were disrupted, but the inner retina was
Fig. 2. At 48 hr after intracarotid injection of *S. faecalis*, subretinal and choroidal aggregations of polymorphonuclear cells disrupt the outer retina but spare the inner retina. Top. Light micrograph of tapetal lesion seen clinically near long arrow of Fig. 1 and in inset of this figure (arrow). Bottom. Light micrograph of nontapetal lesion seen in inset by autopsy photo (arrow). (Toluidine blue; top ×235, bottom ×375.)
Fig. 3. At 5 hr after intracarotid injection of *S. aureus*, subretinal and choroidal aggregations of polymorphonuclear cells disrupt the outer retina but spare the inner retina. **Top.** Light micrograph of tapetal lesion seen in inset by autopsy photo (arrow). **Bottom.** Light micrograph of nontapetal lesion seen in upper inset by autopsy photo (arrow). Lower inset shows gram-positive cocci (arrow) beneath retinal pigment epithelial detachment of nontapetal retina. (Toluidine blue and Brown-Brenn (lower inset); top ×360, bottom ×380, lower inset of bottom ×300.)
not involved. When the retina and choroid of two eyes with lesions at 24 hr were cultured, pure colonies of S. faecalis grew, whereas cultures of the retina and choroid from the control eye of the same animals yielded no bacterial growth.

**Staphylococcal experiments.** S. aureus, on clinical examination, produced large areas of serous retinal detachment with multiple dark spots (Fig. 1, bottom). The lesions were located mainly in the tapetal area and appeared 2 to 4 hr after injection. These lesions, on indirect ophthalmoscopic examination, were more extensive and became visible earlier than those caused by S. faecalis. The fundus lesions were consistently (in four out of four dogs) present after injection of the higher concentration (10^8-10^9 bacteria/ml) and were absent in the one dog injected with the lower concentration (10^7-10^8 bacteria/ml). Involvement was ipsilateral in three dogs and bilateral in one, the contralateral eye again showing only minimal involvement. The dog with bilateral lesions did not have a control injection into the other carotid artery.

The acute lesions resulting from S. aureus resembled the lesions from S. faecalis on histopathologic study and exhibited gram-positive cocci with the Brown-Brenn stain (Fig. 3). However, the photoreceptor outer segments appeared either intact (Fig. 3, top) or mildly disrupted (Fig. 3, bottom) 5 hr after intracarotid injection of S. aureus, whereas there was more destruction of the photoreceptor outer segments 48 hr after S. faecalis injection (Fig. 2). This discrepancy may reflect the different age of the lesions.

Since the dogs injected with S. aureus always died during the first day, these animals were sacrificed for clinicopathologic correlation at 4 to 5 hr when the large fundus lesions were present. The small fundus lesions with S. faecalis were not seen until 24 hr after injection. These animals were sacrificed at 48 hr to allow for enlargement of the fundus lesions.

**Control experiments.** No fundus lesions were observed on clinical or histopathologic examination in the eyes on the side of the control injection in the five dogs receiving the control injections 3 to 7 days prior to the bacterial injection or in the eight dogs receiving the control injections at the same time as the bacterial injection.

**Discussion.** This study describes a model of multifocal septic choroiditis with serous retinal detachment in dogs after intracarotid injection of S. faecalis or S. aureus. The fundus lesions on ophthalmoscopic examination were much more extensive after S. aureus than after S. faecalis injection and were located mainly in the tapetal area of the fundus. These two aspects are of particular interest and will be discussed below.

The large retinal detachments observed on ophthalmoscopic examination with S. aureus may partially be due to its very potent toxins, notably alpha toxin which is known to cause vasoconstriction and necrosis of vascular smooth muscle cells. On the other hand, the small fundus lesions seen with S. faecalis may be due to its relatively low pathogenicity and lack of potent toxins.

The preferential location of lesions in the tapetal area of the fundus may be explained by the rapid slowing and turbulence of blood flow, predisposing this area to embolus formation. In the dog, the tapetal, in contrast to the nontapetal, area is likely to have such altered flow conditions because choroidal capillaries pass perpendicularly through the tapetum and then branch off at nearly right angles into the choriocapillaris. In our study, bacteria, each 0.5 to 1.0 μm in size, may have formed emboli of varying size in the choriocapillaris. Similarly, embolus formation may have been present after injection of H. capsulatum or latex microspheres. After intracarotid injections of H. capsulatum in rabbits, Smith et al. described foci of choroiditis with very minimal serous detachments of the retina. On histopathologic study, acute lesions showed a choroidal infiltration by polymorphonuclear cells, and chronic lesions exhibited a lymphocytic infiltration in the choroid. Multifocal choroidal microabscesses with large serous detachments of the retina, as seen in our model, were not described. After intra-arterial injection of microspheres, 7 to 28 μm in size, Gay et al. observed grayish white, nonelevated fundus lesions. These lesions developed 3 to 5 min after injection, were equally distributed throughout the fundus, and correlated with noninflammatory occlusions of choroidal and retinal vessels on histopathologic examination. In contrast, our bacteria-induced fundus lesions were delayed in appearance, occurred mainly in the tapetal area, and corresponded with microabscesses in the choroid and subretinal space without involving the inner retina and retinal vessels.

It is of interest to comment on the differences and similarities between our dog model and the fundus lesions observed in human bacteremia. The quantity of bacteria we used exceeded the usual range of 10^3 to 10^6 organisms reported in human bacteremia. Retinal hemorrhages, with and without white centers, and cotton wool lesions have been seen in human bacteremia but were not observed in our dog model. On the other hand, in several patients with bacteremia Friedenwald and...
Bones\textsuperscript{1} and Dienst and Gartner\textsuperscript{2} described foci of septic choroiditis without involvement of the overlying retina. In chronic granulomatous disease of children, a disorder characterized by the inability of the patient's leukocytes to kill certain microorganisms after phagocytosis, Martyn et al.\textsuperscript{10} described numerous inactive chorioretinal scars on fundus examination. It is interesting to speculate that these scars may have resulted from foci of infection similar to those seen in our study.

Further investigation of this dog model may provide some insight into the pathogenetic mechanisms of septic inflammation in the choroid.

From the Department of Ophthalmology, University of Wisconsin Hospitals (Drs. Meyers, Wallow, Klein, and de Venecia), and the Department of Medicine (Dr. Wagnild), Division of Ophthalmology (Dr. Allen), and the Division of Laboratory Medicine (Dr. Lapinski), Veterans Administration Hospital, Madison, Wis. This research was supported in part by NIH grant 5-T01-EY-00039 from the National Eye Institute, by the Medical Research Service of the Veterans Administration, and by a grant from the Miller Foundation. Submitted for publication June 12, 1978. Reprint requests: Sanford M. Meyers, M.D., Department of Ophthalmology, University of Colorado Medical School, 4200 East Ninth Ave., Denver, Colo. 80262.

Key words: choroiditis, sepsis, retinal detachment, retina, choroid, bacteria

REFERENCES


Effects of epinephrine, indomethacin, acetysalicylic acid, dexamethasone, and cyclic AMP on the in vitro activity of lysosomal hyaluronidase from the rabbit iris.

Seiji Hayasaka and Marvin Sears.

The effects of epinephrine, indomethacin, acetysalicylic acid, dexamethasone, and cyclic AMP on lysosomal hyaluronidase activity in the rabbit iris were studied in vitro. Indomethacin and acetysalicylic acid inhibited the lysosomal hyaluronidase activity. Dexamethasone and cyclic AMP (adenosine-3',5'-cyclic monophosphate) had little effect on the enzyme activity. Epinephrine activated the enzyme activity. These drugs had no effect on the activities of either bovine testicular hyaluronidase or rabbit iridal acid phosphatase. The possible role of lysosomal hyaluronidase in the rabbit eye and the drug effects were considered.

We previously reported\textsuperscript{1-2} that the lysosomal hyaluronidase activity was high in the lysosomal extracts of rabbit iris. Very little is known about the activation-inhibition mechanism of the rabbit iridal lysosomal hyaluronidase. We therefore studied the activity of rabbit iridal lysosomal hyaluronidase under the influence of certain drugs.

Materials and methods

Chemicals. Indomethacin, acetysalicylic acid, dexamethasone acetate, epinephrine bitartrate, hyaluronic acid (grade I, human umbilical cord), bovine testicular hyaluronidase (415 NF units/mg of protein), and Coomassie brilliant blue C-250 were purchased from Sigma Chemical Co., St. Louis, Mo.; p-nitrophenyl phosphate from Dai-ichi Pharmaceutical Co., Tokyo, Japan; cyclic AMP (adenosine-3',5'-cyclic monophosphate) monosodium from Yamasa-Shoyu Co., Tokyo; carbocyanine dye (1-ethyl-2-[3-(1-ethyl-naphtho [1,2d]thiazol-2-ylidene) - 2 - methyl-propenyl] naphtho [1,2d] thiazolium bromide) from Eastman Organic Chemicals, Rochester, N. Y. All other reagents were of analytical grade or the best grade available. Drugs not soluble in 0.02M sodium acetate buffer (pH 3.8) were first dissolved in a small volume of dimethyl sulfoxide. The small amounts of dimethyl sulfoxide employed as a vehicle did not...