Several reports of endogenous *Candida* endophthalmitis successfully treated with intravenous amphotericin B have appeared recently.\textsuperscript{1-8} There are, however, very few reports of successfully treated exogenous fungal endophthalmitis.

Veirs and Davis\textsuperscript{4} described the only cure of culturally or histologically proved exogenous intraocular fungal infection, in which there was a good visual result.

Although amphotericin B is effective against a wide range of fungal pathogens, its usefulness in treating fungal endophthalmitis is severely limited by poor ocular penetration. Green and associates\textsuperscript{5} found no amphotericin B inside the vitreous of normal rabbits after intravenous and subconjunctival administration. Even with experimentally induced uveitis, they found only occasional trace amounts in the vitreous. An additional drawback of systemic amphotericin B therapy is the variety of toxic side effects encountered, ranging from nausea, vomiting, and malaise to anemia and renal failure.\textsuperscript{6} The incidence of side effects has been reported to be as high as 80% after intravenous administration.\textsuperscript{6}

In light of this antibiotic's poor ocular penetration and toxic potential when administered systemically, this experiment was designed to investigate whether amphotericin B can be tolerated by the rabbit eye when injected by the intravitreal route.

**MATERIALS AND METHODS**

Both commercially available amphotericin B (Fungizone) and pure crystalline amphotericin B (both supplied by Squibb Institute for Medical Research) were used in this experiment. The commercial preparation comes in vials containing 50 mg amphotericin B, 41 mg sodium deoxycholate (used as a solubilizing agent), and sodium phosphate buffers. Because saline can cause precipitation of the antibiotic, dilutions were made with sterile water to achieve the desired concentrations of solubilized amphotericin B. These solutions were clear, and depending on concentration, amber to pale yellow in color. Pure crystalline amphotericin B was mixed with sterile water, and the mixture was agitated until the drug was well suspended. This suspension was hazy and yellow. (Aqueous solutions of sodium deoxycholate alone are clear and colorless.)

We used 28 albino and four pigmented rabbits weighing 2 to 3 kg. The pupils were dilated with 1% cyclopentolate hydrochloride and 2.5% phenylephrine hydrochloride, and the animals were anesthetized by intravenous administration of sodium pentobarbital. We performed all intravitreal injections using a 1.0-ml tuberculin syringe with a 25-gauge needle, and no matter what substance or concentration was injected, the volume injected was always 0.1 ml. We made only one intravitreal injection into each eye.

After topical application of 0.5% proparacaine hydrochloride, the superior and inferior rectus muscles were grasped with toothed forceps to stabilize the eye. We introduced the needle 2 mm posterior to the limbus and directed it toward the center of the vitreous. With the aid of an indirect ophthalmoscope (without hand lens), the needle could be well visualized inside the vitreous. Prior to injection—but with the needle already inside the vitreous—we tapped the anterior chamber with a 25-gauge needle to lower the intraocular pressure. Injection was
performed slowly, and after the plunger had been pushed down completely, the rectus muscles were released, and the needle was left inside for approximately 20 seconds before being slowly withdrawn. No apparent leakage of fluid from the injection site occurred during any of the injections.

The animals were divided into eight groups of four animals each. Pigmented rabbits were used in group 8; albino rabbits were used in all the other groups.

Groups 1 through 6—The objectives in these groups were (1) to determine the amount of amphotericin B that could be injected into the vitreous without causing damage and (2) to characterize the nature of any toxic effects of the drug on the ocular tissues. The right eye of each animal received solubilized amphotericin B. The amounts injected were as follows: group 1, 500 μg; group 2, 250 μg; group 3, 100 μg; group 4, 25 μg; group 5, 10 μg; and group 6, 5 μg. The left eyes were all used as controls, each left eye receiving 0.1 ml sterile water.

The animals in groups 1 through 4 were examined daily by direct and indirect ophthalmoscopy. After ten days, these animals were killed with intravenous sodium pentobarbital. Groups 5 and 6 were observed for four weeks before being killed. We performed electroretinograms four weeks after injection on both eyes of two of the animals in group 5.

Group 7—This group was used to evaluate the separate effects of amphotericin B and sodium deoxycholate. The right eyes of four rabbits received 100 μg sodium deoxycholate (equal to the sodium deoxycholate in 100 μg solubilized amphotericin B). The animals were observed for ten days and then killed.

Group 8—These animals were used to study the early effects of amphotericin B on the retina. We used pigmented rabbits because early retinal changes are somewhat easier to detect clinically in pigmented rabbits than in albinos. The injection technique differed from all the other groups in that injections were performed with the needle tip very close to the retina. We used the indirect ophthalmoscope (with hand lens) to direct the needle close to, but not touching, the retina. The right eyes received 25 μg solubilized amphotericin B; the left eyes received sterile water. Two rabbits were killed two hours after injection, and two were killed after 36 hours.

In all groups the eyes were enucleated immediately after the animals were killed. After formaldehyde fixation, the eyes were opened in the vertical plane and examined with a dissecting microscope. The tissue was embedded in paraffin, and sections were cut at 8 μ and stained with hematoxylin and eosin.

**Results**

Groups 1 through 6—All the doses of amphotericin B used in groups 1 through 4 (25 to 500 μg) produced toxic effects. The 500 and 250 μg doses caused immediate clouding of the vitreous. By the third day, there were retinal detachments and vitreous opacities in all of these eyes. Immediately after injection of the 100 and 25 μg doses, the vitreous remained clear but had a uniformly pale yellow color. Within two days after injection of 100 and 25 μg, each fundus had a white area of approximately 3 to 4 disk diameters through which no choroidal vessels could be seen. By the fourth or fifth day, these whitish areas appeared to be torn through, and the surrounding retina was detached. The vitreous became hazy at about five days after injection, but remained clear enough to permit examination. The vitreous changes were more pronounced in group 3 (100 μg) than in group 4 (25 μg).

Gross examination of the enucleated right eyes in groups 1 through 4 confirmed the presence of retinal detachments. In two of the four 500-μg eyes, we found an amorphous, yellow jelly-like mass resembling a clot in the vitreous. The yellow material probably represented precipitated amphotericin B.

Microscopic examination of the eyes in groups 1 through 3 revealed a totally de-
Fig. 1 (Axelrod, Peyman, and Apple). Group 3 (100 μg solubilized amphotericin B). There is total retinal detachment ten days after intravitreal injection. The lens deformity is artifact. S indicates subretinal exudate (hematoxylin and eosin, X5).

Fig. 2 (Axelrod, Peyman, and Apple). Deeper section taken from same eye as in Figure 1. Note extensive retinal degeneration, most marked in the photoreceptor layer (arrows). Inflammatory cells, primarily mononuclear, are seen in the vitreous (V) and subretinal space (hematoxylin and eosin, X175). was to determine whether the toxic changes observed in groups 1 through 4 might be due to the sodium deoxycholate in the commercial preparation. None of the eyes injected with pure sodium deoxycholate suffered any toxic effects that could be observed clinically or histologically. In contrast, the eyes injected with 100 μg pure crystalline amphotericin B paralleled the course of those eyes in group 3 injected with 100 μg solubilized amphotericin B. Because the amphotericin B in this group was injected as a suspension, we observed numerous small yellow flecks of insoluble amphotericin in the vitreous clinically.

Group 8—This group was used to investigate the nature of the early changes in the retina caused by amphotericin B. Immediately after injection of 25 μg close to the retina, a white area approximately 2 disk di-

Group 7—The purpose of this group

tached retina overlying a proteinaceous exudate (Fig. 1). Inflammatory cells, mainly mononuclear, were present in the vitreous (Fig. 2) and to a lesser extent in the subretinal exudate. The lens was not affected by the inflammatory reaction. The findings were similar in group 4 (25 μg), but the inflammation was much less marked.

Except for mild conjunctival irritation caused by the injection procedure, there were no clinical changes observed in any of the control eyes or in the eyes receiving 5 or 10 μg solubilized amphotericin B (groups 5 and 6). Nor were there microscopic changes found in any of these eyes (Fig. 3, left), even in sections cut through the site of injection (Fig. 3, right). To rule out the possibility of damage without morphologic change, electroretinograms were performed on two animals in group 5 (10 μg). We noted no significant differences between the treated and control eyes.

Group 7—The purpose of this group

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amphotericin B. Group 6 (5 μg solubilized amphotericin B). Left, No significant changes are evident four weeks after injection (hematoxylin and eosin, ×250). Right, Injection site of a globe injected with 5 μg of soluble amphotericin B, two months following injection. Note normal healing of the wound tract (arrow) and the normal appearance of the adjacent retina (hematoxylin and eosin, ×80).

Fig. 3 (Axelrod, Peyman, and Apple). Group 6 (5 μg solubilized amphotericin B). Left, No significant changes are evident four weeks after injection (hematoxylin and eosin, ×250). Right, Injection site of a globe injected with 5 μg of soluble amphotericin B, two months following injection. Note normal healing of the wound tract (arrow) and the normal appearance of the adjacent retina (hematoxylin and eosin, ×80).

Fig. 3 (Axelrod, Peyman, and Apple). Group 6 (5 μg solubilized amphotericin B). Left, No significant changes are evident four weeks after injection (hematoxylin and eosin, ×250). Right, Injection site of a globe injected with 5 μg of soluble amphotericin B, two months following injection. Note normal healing of the wound tract (arrow) and the normal appearance of the adjacent retina (hematoxylin and eosin, ×80).

ameters in size formed on the retina directly below the tip of the needle. This area enlarged to about 4 to 5 disk diameters over a period of 36 hours. There was a very sharp border separating the white area from the surrounding fundus (Fig. 4).

Microscopic examination showed these lesions to be focal areas of retinal necrosis (Figs. 5 and 6). The retina appeared normal in all areas peripheral to the lesion. In the

Fig. 4 (Axelrod, Peyman, and Apple). Fundus photograph taken two hours after injection of 25 μg amphotericin B close to the retina. The arrows delineate the advancing border of the white area referred to in the text.

Fig. 4 (Axelrod, Peyman, and Apple). Fundus photograph taken two hours after injection of 25 μg amphotericin B close to the retina. The arrows delineate the advancing border of the white area referred to in the text.

Fig. 5 (Axelrod, Peyman, and Apple). Microscopic section of the eye shown in Figure 4. The zone of transition (arrow) from normal retina (left of arrow) to the necrotic area corresponds to the border of the white area in Figure 4 (hematoxylin and eosin, ×100).

Fig. 5 (Axelrod, Peyman, and Apple). Microscopic section of the eye shown in Figure 4. The zone of transition (arrow) from normal retina (left of arrow) to the necrotic area corresponds to the border of the white area in Figure 4 (hematoxylin and eosin, ×100).
Fig. 6 (Axelrod, Peyman, and Apple). The center of the white lesion seen in Figure 4. There is total loss of normal retinal architecture (compare with normal appearance of the retina shown in Figure 3). The retinal neural elements are dispersed throughout the entire thickness of the retina in random fashion. No recognizable photoreceptor elements are observed (hematoxylin and eosin, ×250).

two eyes enucleated after two hours, the retina was not detached. However, in the eyes enucleated after 36 hours, the focus of necrotic retina, along with some normal adjacent retina, was separated from the pigment epithelium.

DISCUSSION

Doses of 5 and 10 μg amphotericin B were injected into the vitreous of normal rabbit eyes without causing any changes that could be detected clinically, microscopically, or by ERG. Intravitreal doses of 25 μg or higher proved to be extremely toxic to the retina. From the results in group 7 (pure crystalline amphotericin B versus pure sodium deoxycholate), we must conclude that the toxicity of the commercial preparation is due to the amphotericin B itself and not to the sodium deoxycholate in the amount used as a solubilizing agent.

Since even a 5-μg dose is potentially toxic if injected forcefully very close to the retina, emphasis must be placed on the importance of injecting slowly into the center of the vitreous. The uniform yellow coloration of the vitreous after injection of the higher doses would seem to indicate that solutions injected slowly into the center of the vitreous are dispersed fairly evenly throughout the ocular cavity. In groups 5 and 6, after injection of 5 or 10 μg, no one area of the retina was exposed to a high enough level of the drug to suffer any adverse effects.

Although the vitreous volume in humans is greater than in rabbits (4 ml compared to 1.5 ml), it is not necessarily valid to multiply the safe dose in rabbits by 4.0/1.5 to arrive at the human dosage. Furthermore, the greater the dose employed, even in the theoretically safe range, the greater is the chance of inadvertently subjecting one area of retina to a toxic level. For these reasons and because of the low margin of safety with amphotericin B, we would not advise injection of more than 10 μg into the vitreous of a human eye. A 10-μg dose would theoretically provide a concentration of 2.5 μg/ml, which is effective against most fungal pathogens.

The mechanism of the retinal detachments observed with the higher doses may be explained by the direct toxic effects of amphotericin B on the retina. Amphotericin B alters membrane permeability by combining with sterol groups in the cell membrane. Therefore, the detachments may partially result from alterations in permeability causing transudation of fluid into the subretinal space.

Foster and associates injected amphotericin B into the anterior chamber of rabbits, and found that iritis, anterior chamber exudate, and lens opacities occurred after doses of 25 μg or higher. In our experiment there were no lens changes or anterior chamber reaction in response to intravitreal injection of 25 to 500 μg.
SUMMARY

Intravitreal injections of 5 to 10 µg amphotericin B in normal rabbits do not cause any toxic changes that can be detected clinically, microscopically, or by electroretinography. Injection must be performed slowly into the center of the vitreous.

Injection of 25 µg or higher causes retinal necrosis and detachment. These changes occur whether pure amphotericin B or the commercial preparation of amphotericin B (which contains sodium deoxycholate as a solubilizing agent) are used. Sterile water, 0.1 ml, and 0.1 ml pure sodium deoxycholate in the amount used as solubilizing agent do not cause toxic changes when injected into the vitreous.

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