

Retinal detachment from hyperosmotic intravitreal injection

Michael F. Marmor

Hyperosmotic solutions were injected into the rabbit vitreous to study their effects upon the retina. Injection of 0.05 ml of a 1000 mOsm solution caused rapid whitening of the posterior retina followed by the development of a large detachment and permanent retinal degeneration. The weakest solutions which produced ophthalmoscopically visible changes in the retina (after an injection of 0.05 ml) were near 500 mOsm. Sodium chloride, sodium aspartate, EDTA, mannitol, sucrose, and penicillin were effective at similar osmolarities. An osmotic load in the vitreous caused immediate loss of the c-wave of the electroretinogram (ERG), and a slower decline of the a- and b-waves. The reported intravitreal toxicity of some drugs may relate to osmotic rather than pharmacologic effects. Osmolarity should be accounted for in planning the amount and location of any vitreous injection.

Key words: intravitreal injection, hyperosmotic solution, osmotic pressure, retina, retinal detachment, ERG, c-wave, drug toxicity, penicillin

The injection of a drug into the vitreous brings a high concentration near the retina. For this reason a number of therapeutic agents have been tried intravitreally in experimental and clinical situations,¹ and for most there is a critical dosage above which retinal degeneration occurs. Retinal detachment has occasionally been reported as a complication of such injections.²⁻⁴

Retinal detachment from an intravitreal drug was observed in a different context by Cunha-Vaz and Maurice.⁵ They found that penicillin competitively blocks the active

transport of fluorescein out of the eye and that high doses of intravitreal penicillin cause a detachment. This raised the possibility that a blockade of organic anion transport across the pigment epithelium might be responsible for the fluid accumulation in the subretinal space. On the other hand, Sanders and Peyman⁶ showed that detachments occur rapidly after intravitreal injection of strong hyperosmolar solutions of either ammonium chloride or sodium chloride.

The present series of experiments sought initially to produce experimental detachments in order to investigate the mechanisms of retinal adhesion.⁷ To determine whether the penicillin effect was specific to the pharmacologic properties of the drug, other agents of similar osmotic strength were tried for comparison—but they turned out to cause detachments with equal facility. The phenomenon of osmotically induced detachment of the retina and its implications for intravitreal therapy are the subject of this paper.

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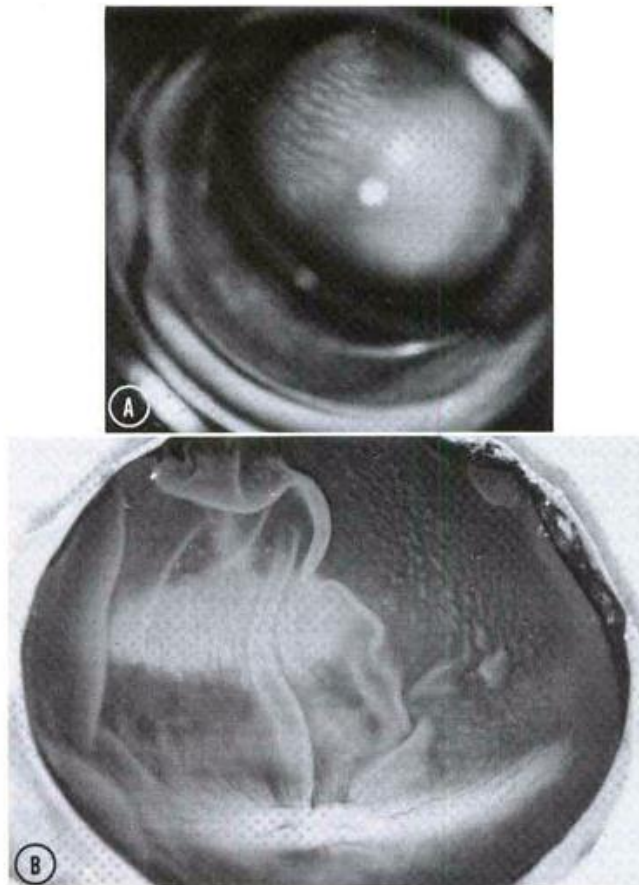


Fig. 1. Effects of midvitreal injection of a hyperosmolar solution. **A.** Photograph of the rabbit fundus (through a contact lens) 1 to 2 min after injection of 0.05 ml of 3M NaCl (approx. 5600 mOsm). Retina near the visual streak is edematous and grey, and detachment is beginning. **B.** Eyecup from the same experiment, enucleated and sectioned 24 hr after the injection. The detachment now involves the entire retina.

Methods

All experiments were performed on Dutch rabbits weighing approximately 1.5 kg. Animals were sedated with a single intramuscular injection of chlorpromazine (approximately 10 mg/kg) and anesthetized with intravenous pentobarbital (approximately 20 mg/kg). The eyes were dilated with a 50/50 mixture of 1% cyclopentolate and 10% Neo-Synephrine. The fundi were observed with an indirect ophthalmoscope and with an operating microscope through a plano-concave contact lens.

The following stock solutions were diluted as needed: 1,000,000 U/ml sodium penicillin G (Up-

john); 3M NaCl; 25% mannitol (Invenex); 3M sucrose; 1.5M Na aspartate; 400 mM EDTA. Osmolarity was measured on a Fiske Model 130 osmometer.

Three types of experiments were performed:

1. *Injection into midvitreal.* A 0.05 ml amount of a solution was injected with a 27-gauge needle inserted about 3 mm behind the limbus. Direct observation ensured that the needle was neither in the lens nor adjacent to the retina.
2. *Local injection.* The conjunctiva was cleared from a portion of sclera, and a puncture was made about 3 mm behind the limbus with a

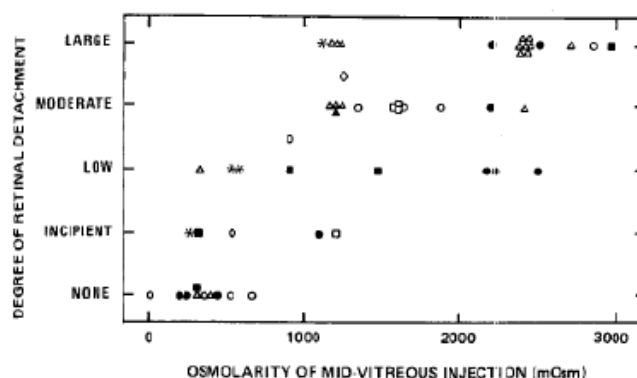


Fig. 2. Effects of midvitreal injection of 0.05 ml of the following solutions: o, NaCl; ●, Na aspartate; △, penicillin G; ▲, penicillin G with systemic benemidol; □, mannitol; ■, sucrose; ○, mixture of penicillin, NaCl and sucrose; *, EDTA. Clusters of points indicate multiple experiments at similar osmolarity. The effects were graded approximately 15 min after injection as follows: *incipient detachment*, if the vitreoretinal interface glistened but did not visibly separate; *low detachment*, if separation occurred without bullous elevation; *moderate detachment*, if the bullae were confined to the posterior pole; *large detachment*, if there was extension near or beyond the equator.

22-gauge needle. A blunted 30-gauge needle was gently inserted through the puncture site and moved under direct visual control to a location immediately adjacent to the retina. Approximately 0.005 ml of solution was ejected near the retinal surface. The needle was held in place for about 30 sec to ensure that viscous material would not retract with the needle. Several local injections could be made in different locations within each eye.

3. *Electrophysiologic recordings.* These were made from intact animals, as described previously.⁸ In brief, a contact lens containing a silver-silver chloride pellet was placed on the cornea, and similar nonpolarizable electrodes were inserted into the conjunctival cul-de-sac (as reference) and into the mouth (as ground). Direct current electroretinograms (D.C.-ERGs) were amplified by a Tektronix 5A22 amplifier and displayed on both an oscilloscope (for a- and b-waves) and a paper recorder (for c-waves).

Results

Injection into midvitreal. Midvitreal injection of 0.05 ml of a strongly hyperosmotic solution (≥ 1000 mOsm) typically produced the following sequence of events (Fig. 1):

1. *Within seconds to a minute.* Elevation

and glistening of the vitreoretinal interface in the posterior pole. The intraocular pressure (confirmed by Schiotz tonometry) was often increased for 5 to 10 min because of the fluid injected, but no pulsation or closure of vessels was ever observed.

2. *Within the next few minutes.* Grey-white opacification of the affected areas.

3. *Over 2 to 30 min.* Clearing of the opacification with formation of a bullous detachment; mild congestion of the visual streak vessels.

4. *Over the next several hours.* Widening detachment of thickened retina, often with multiple folds. The retinal vessels were now grossly congested and tortuous.

5. *Overnight.* Continuing detachment and permanent retinal degeneration. Small detachments (after weak osmotic injections) sometimes reannealed (showing pigmentary changes) but large detachments always persisted and progressed, sometimes leaving the retina fragmented and torn within the eye. The retinal vasculature remained abnormal.

This sequence of events was qualitatively similar whether the active agent was penicillin, sodium chloride, sodium aspartate, so-

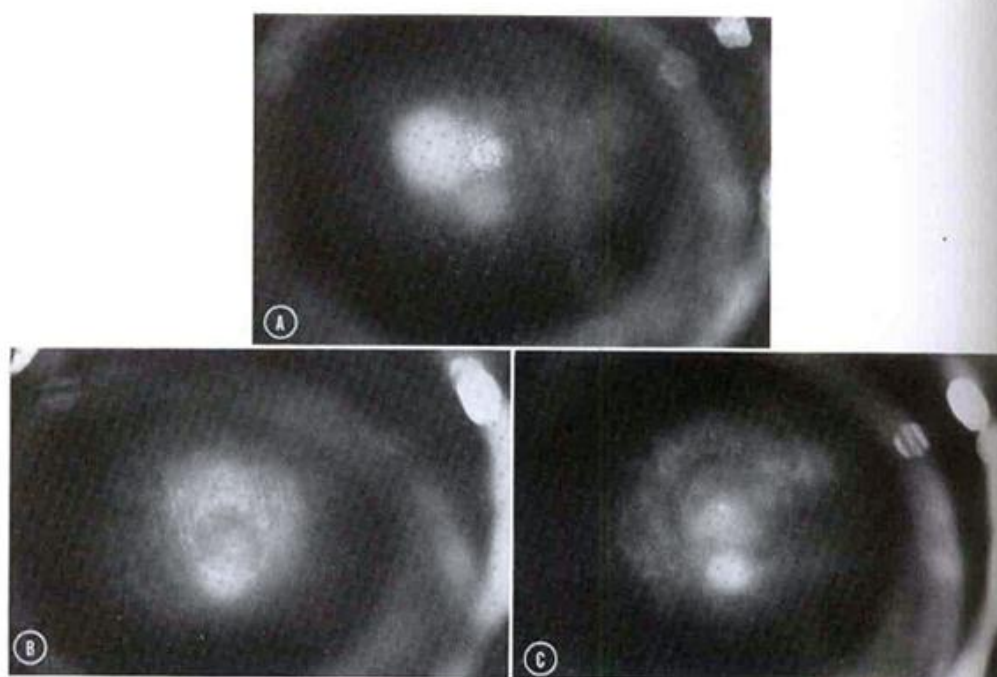


Fig. 3. Effects of injecting hyperosmolar solution adjacent to the retina. **A**, Rabbit eye a few seconds after local injection of penicillin G (1105 mOsm). Note the glistening interface near the region of retinal opacification. **B**, 1 to 2 min after injection. A definite bleb is forming at the site of the opacification. **C**, 5 min later. The local detachment has enlarged.

dium EDTA, mannitol, or sucrose. Preliminary histologic data confirmed that detachment occurred between the photoreceptors and pigment epithelium, and not by cleavage of cells; more detailed electron microscopic studies are in progress.

Fig. 2 shows the composite results after midvitreal injection of a variety of substances at different osmolarities. Control injections of normal saline, hydrochloric acid (pH 2) or distilled water had no visible effect on the retina. These data compare the action of penicillin with that of agents that have other pharmacologic actions (sodium aspartate, which blocks synaptic transmission; and sodium EDTA, which chelates calcium) and with agents that are largely inert (sodium chloride, sucrose, and mannitol). Regardless of the substance used, injections of less than 500 mOsm had little or no effect, whereas injections of more than 1000 mOsm had a strong effect.

Local injection. To confirm the immediacy of the osmotic effects, injections of a small amount of hyperosmotic solution were made directly adjacent to the retina with a 30-gauge needle. Solutions of 1000 mOsm or more usually caused whitening of the retina within seconds, followed by the formation of a small bleb within minutes (Fig. 3). Solutions as weak as 700 mOsm were sometimes effective, and occasional injections of more than 1500 mOsm were ineffective. The effect was similar whether sodium chloride, penicillin, or mannitol was used. These local blebs persisted for several hours but were not studied over the long term. The injection of normal saline or hypo-osmotic solutions had no visible effect, and showed that injection itself did not cause detachment.

Electrophysiologic effects of osmotic detachment. Fig. 4 shows the effects on the conventional ERG of injecting 0.05 ml of either normal saline or penicillin into the

midvitreal of a rabbit eye. The injection of normal saline had minimal effect upon the ERG, confirming that neither the trauma of injection nor the transient rise in intraocular pressure was damaging to the retina. However, the injection of penicillin caused a steady decline in the a- and b-waves, which were reduced to 50% of control amplitude after 30 min and were nearly extinguished after 75 min. Hyperosmotic injections of sodium chloride and mannitol produced essentially the same effects.

In contrast to the a- and b-waves, the c-wave of the ERG was affected almost immediately, and much more severely, by hyperosmotic injections. Fig. 5 shows that the c-wave disappeared within 2 min after an injection of hyperosmotic sodium chloride, leaving a large negative response ("slow PIII") which was probably of retinal origin⁹ and was similar to the ERG after the systemic administration of sodium iodate.¹⁰ The a- and b-waves were also affected by this injection but not nearly to the same degree. After 9 min, further reductions of the a- and b-waves were accompanied by a reduction of the slow PIII. Intravitreal injection of penicillin produced a similar sequence of changes.

Discussion

Sanders and Peyman⁶ observed in 1974 that injection of strong salt solutions into the rabbit vitreous caused prompt detachment of the retina and the development, over several days, of vascular changes that simulated intravitreal neovascularization. Their primary interest was in the vascular model, but they inferred that the hyperosmolarity of the injections was responsible for detachment. The present study is concerned only with osmotic detachment and shows that intravitreal injection of a variety of agents, in addition to salts, will produce similar effects at similar osmolarity. The present results also indicate the ranges of drug osmolarity which are safe or potentially hazardous for intravitreal injection.

There are several mechanisms by which a hyperosmolar load in the vitreous may cause a detachment. The most obvious explanation

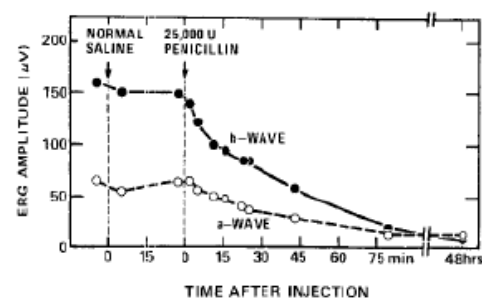


Fig. 4. ERG following midvitreal hyperosmolar injection. A control injection of normal saline had little effect, whereas injection of 25,000 U of penicillin G (approx. 1200 mOsm) caused a gradual decline in both the a- and b-waves.

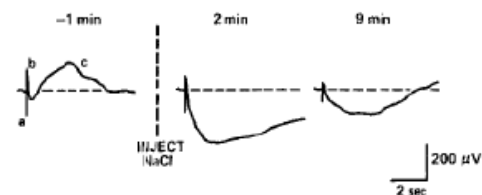


Fig. 5. D.C.-ERG after midvitreal hyperosmolar injection. A control response is shown on the left. Within 2 min after injection of 3M NaCl (approx. 5600 mOsm), the c-wave has disappeared (leaving a slow negative response) although the a- and b-waves are still relatively well-preserved. After 9 min, the a-wave is smaller and both the b-wave and slow negative response are proportionally reduced.

is that the normal flow of water from vitreous to sclera¹¹ is reversed because of the high vitreal osmotic pressure. Water flowing from the choroid towards the vitreous will tend to elevate the retina, because the retina exerts a measurable (although small) resistance to flow.¹² Under normal circumstances (in which fluid flows toward the choroid), this flow resistance may help to keep the retina in place, although it is certainly not the only mechanism for doing this. For example, asymptomatic retinal holes (which would defeat a hydrodynamic model) are not uncommon, and significant adhesive forces are measurable between the isolated retinal pigment epithelium and retina.^{7, 13} Bill¹¹ estimates that a hydrostatic and oncotic pres-

pressure gradient of 13 mm Hg is normally present from vitreous to choroid. Merely raising the vitreal osmotic pressure by 1 mOsm would shift this balance by approximately 18 mm Hg and create a 5 mm gradient toward the vitreous. Yet detachments did not occur in these experiments until the injection of at least 0.05 cc of a 500 mOsm solution, which, if it diffused evenly through the vitreous (about 1.2 ml in the rabbit), would produce approximately a 10 mOsm increase equivalent to 168 mm Hg. And this estimate is probably low, since detachment began focally beneath each bolus of hyperosmotic fluid. Osmotic detachment may indeed be a result of a vitreal flow of water, but the osmotic pressures which are required at the retinal surface are not easily predicted and must be quite high. This argument provides further evidence that adhesion is not maintained by hydrodynamic forces alone and that other adhesive mechanisms are remarkably strong in the normal eye.

A second mechanism by which an osmotic load may cause detachment is through damage to cells. The pigment epithelium and photoreceptors are closely interdigitated, and the pigment epithelium controls metabolism and transport across the subretinal space. Damage to these cells could weaken adhesion by blockage of metabolic systems which dehydrate the subretinal space, by destruction of the barrier properties of the pigment epithelium, or by physical disruption of the villous processes which ensheath the photoreceptors. Osmotic loads are unquestionably damaging to cells. Nonspecific shrinkage and disruption of normal cellular orientation probably account for the opacification which was observed acutely in many of these experiments, and permanent thickening and shredding of the retina became evident within hours after a highly osmotic injection. Small osmotic detachments were often observed after injections too weak to cause retinal opacification (in these cases, the retina glistened and then detached), but moderate to large detachments usually were associated with whitening of the retina under the injection site. These experiments sug-

gest, but certainly do not prove, that cellular damage may be an additional factor in causing the detachment.

The major conclusions of this paper depend upon the results of midvitreal injection (Fig. 2). These data exhibit considerable scatter, but this is not unexpected, since the injections could not be precisely controlled as to location, settling, or diffusion within the vitreous. This may be viewed as an experimental failing, but it is also a realistic model for the clinical situation in which injection sites are variable. Another source of scatter in the data is the subjective grading of detachments, but the trend of the results would not be altered by changing a few debatable points. Objections might be raised because the intraocular pressure was not kept at a normal level after injection. The transient elevations in pressure could not have caused detachments and were always far less than the osmotic pressures which caused detachment (see earlier discussion). Even with these reservations, Fig. 2 shows a clear trend of results from no detachment with isosmolar injections to invariable detachment with injections of more than 1000 mOsm. Moreover, within the accuracy of this figure, similar osmotic strengths of each agent (despite their physical and pharmacologic differences) produced similar effects. These data do not allow a precise judgment about the osmolality which is critically toxic, and they do not rule out the possibility that some of these substances may have toxic effects beyond osmolality. However, they do argue strongly that hyperosmolality alone is sufficient to produce both detachment and permanent damage to the retina.

The electrophysiologic experiments give indirect evidence that hyperosmotic solutions cause separation of the photoreceptors from the pigment epithelium (irrespective of possible toxicity to the neurosensory retina). Note that the c-wave of the ERG was much more seriously and rapidly affected by the hyperosmotic injections than were the a- and b-waves. The positive component of the c-wave is known to originate within the pigment epithelium,¹⁴ and recent work by Oak-

ley¹⁵ has shown that this response is a passive reaction by the apical membrane of the pigment epithelium to a fall of potassium concentration in the subretinal space. Thus even a slight separation of the retina from the pigment epithelium would cause a marked diminution in the c-wave by enlarging the diffusion space surrounding the photoreceptors. Loss of the c-wave can also be achieved pharmacologically by poisoning the pigment epithelium with sodium iodate,¹⁰ and the response remaining after such treatment is very similar to that shown in Fig. 5. The ERG data after hyperosmotic injection indicate that either pigment epithelial cells are immediately and diffusely damaged or that low separation of the retina has occurred over much of the fundus. These alternatives may not be separable, since compromise of the pigment epithelium probably affects hydration of the subretinal space.

The values of osmotic injections which produced the detachment offer an interesting comparison with the osmolarity of drugs which have been shown to be retinotoxic in the rabbit. An injection of 0.05 ml of a 500 mOsm solution corresponds to 5 to 10 mg of a substance having a molecular weight of 400 (which is typical for antibiotics), depending upon the degree of dissociation of the substance in solution. Peyman and colleagues^{1, 16} found that a variety of antibiotics (including gentamicin, methicillin, cephaloridine, lincomycin, clindomycin, and chloraphenicol) caused histologic damage to the retina when 1 to 10 mg was injected into the vitreous. Retinal detachment was noted after an injection of 15 to 20 mg of carbenicillin,⁹ but most drugs have not been used in such high dosage. The present results raise the possibility that some of these diverse drugs may have appeared to be retinotoxic because of the osmotic dose which was delivered rather than specific pharmacologic toxicity. This conclusion has clear implications for human therapy. The estimation of retinotoxicity of a drug must include not only its pharmacologic effects upon the retina but also its osmotic effects which depend upon the molecular weight and dissociation of the drug molecule.

Translation of data from the rabbit to the human situation is unfortunately not a matter of simple mathematics. The rabbit vitreous volume is in the vicinity of 1.2 ml, in contrast to a human vitreous volume of 4 to 5 ml, but we have noted that osmotic toxicity is not a simple function of either drug dosage or vitreous volume. The effects of vitreous injection depend critically on factors such as the location of the injection, the pattern of diffusion within the eye, and possibly physiologic, vascular or hydrodynamic characteristics of the particular eye. The rabbit lens is much larger than the human lens, and in order to avoid hitting it during vitreous injection the needle must be placed in the posterior part of the vitreous cavity. Thus injected material is pushed towards the posterior pole and may have an osmotic effect beyond theoretical expectations.

In fact, most of the detachments in these experiments did begin in the posterior pole immediately adjacent to the optic disc. In the human, the smaller lens allows drug injection into more anterior parts of the vitreous, and the larger vitreous volume should allow greater osmotic loads to be tolerated without detachment. However, a significant risk will always remain from inadvertent injection of drugs deep into the vitreous or near to the retina. Furthermore, since cellular toxicity can probably occur at doses well below those which produce visible detachment or degeneration, a large safety margin should be incorporated in any calculations of intravitreal doses of drugs for human use.

Another difference between the rabbit and the human eye is the nature of the vascular system. An objection could be raised that the lack of retinal circulation in the rabbit does not allow the retina to protect itself against an osmotic load. However, preliminary experiments show that osmotic detachments also occur in the primate eye (Marmor, in preparation).

The present results have shown clearly that increased osmolarity of the vitreous is toxic to the retina, but they do *not* rule out the possibility that penicillin or other drugs may also have pharmacologic effects upon the

retina, or that metabolic and transport systems within the pigment epithelium may be involved with retinal adhesion. Cunha-Vaz and Maurice⁵ showed that fluorescein is rapidly transported out of the vitreous body by active transport across retinal vessels and the pigment epithelium. Penicillin, as well as other inhibitors such as iodopyracet and benemid, block this egress of fluorescein. Cunha-Vaz and Maurice⁵ postulated that since intravitreal penicillin causes detachment, the organic anion transport system may be involved in maintaining retinal adhesion. The high osmolarity of their injections accounts for their observations of retinal detachment, but the possibility that penicillin alters the transport of important materials across the pigment epithelium remains.

Retinal detachments have been noted with the injection of very small quantities (0.025 mg) of amphotericin B² and also after injections of 0.5 to 1 mg of prostaglandin.⁴ The amphotericin detachments were noted to occur near the intravitreal bolus of the drug, so that an osmotic effect may have been a factor, although the osmotic strength of the injected drug (even with solubilizing salts) should not have been very high. The prostaglandin detachments were noted only several days after the injections, and their relationship to osmotic detachments is unclear. There are, of course, a number of means other than osmotic pressure by which a drug could theoretically cause detachment of the retina. The results of this paper caution only that osmotic effects should be considered in evaluating the toxicity of intravitreal drugs and that effects which are presumed to be pharmacologic should be examined carefully to be sure of their drug specificity.

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