The insensitivity of the chicken eye to the inflammatory effects of x-rays in contrast to its sensitivity to other inflammatory agents

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The effects of x-rays and three chemical agents, known to cause intraocular inflammation in mammalian eyes, were studied on the chicken eye because this species was reported to be insensitive to the cataractogenic effects of x-rays. Intravitreal injection of Shigella endotoxin and topical and/or intravitreal administration of PGE₂, PGF₂α, or arachidonic acid caused a breakdown of the blood-aqueous barrier, as indicated by flare and increased protein concentration in the aqueous humor. Following endotoxin injection, there was also a large accumulation of cells in the anterior chamber. The ocular inflammatory effects of endotoxin and arachidonic acid were inhibited by indomethacin. Thus the chicken eye reacts to these inflammatory agents in a manner similar to that previously described for the rabbit. In contrast, the inflammatory response which was reported to occur in the rabbit eye 3 to 4 hr after exposure to 500 or 1000 rads of x-rays was not observed in the chicken eye even after exposure to 10,000 rads. Minimal flare and a small cellular infiltration were observed in some eyes only after extensive swelling of the surrounding tissues had developed. It is concluded that the insensitivity of the chicken eye to x-rays is due to some unique difference in the chain of events which mediates, or prevents, the effects of ionizing radiation rather than to a general insensitivity to inflammatory agents.

Key words: uveitis, eye, ocular inflammation, chicken, x-ray, bacterial endotoxin, prostaglandin, indomethacin, aqueous humor, cataract

Immediately after the administration of cataractogenic doses of x-rays, rabbit eyes develop typical signs of intraocular inflammation such as an initial rise followed by a fall of intraocular pressure (IOP), accumulation of protein in the aqueous humor, cellular invasion of the anterior chamber, decreased aqueous humor ascorbic acid concentration, and an inhibition of the transport capacity of the ciliary processes. This inflammatory response is shorter in duration but otherwise similar to that induced by the intravitreal injection of bacterial endotoxin or bovine serum albumin. Ocular inflammation and the consequent changes in intraocular fluid composition may be responsible for, or at least contribute to, the development of cataracts.

Since chickens have been reported to be insensitive to the cataractogenic effects of x-rays, the present experiments were undertaken to see whether this species shows a
similar insensitivity to the ocular inflammatory effects of x-rays in order to find a comparative approach to study the mechanism of x-ray-induced ocular inflammation and its possible relationship to cataractogenesis. For comparison, the effects of bacterial endotoxin, prostaglandins (PG's), and arachidonic acid were also studied on this avian eye.

Materials and methods

Adult bantam chickens of mixed breeds (0.5 to 1.5 kg) and of either sex were used. Since uveitis can occur naturally in domestic chickens primarily as a result of a viral infection, the animals were carefully screened before use. Birds that showed any signs of ocular damage, conjunctivitis, watery discharge, or cells or flare in the anterior chamber were rejected. In most cases the IOP was also measured with a floating tip pneumatic tonometer the day before and on the day of the experiment. During examination and tonometry the feet and wings were tied and each bird was wrapped in absorbent paper but was not anesthetized.

X-ray irradiation of the eyes. The chickens were lightly anesthetized with 0.4 ml of ketamine hydrochloride (100 mg/ml, intramuscularly; Parke, Davis & Co., Detroit, Mich.). In some experiments the head and neck were placed directly in the target area of a dual tube x-ray machine providing an equal rate of irradiation from above and below the target. In other experiments, the head and neck were shielded by two 7 mm lead plates having a 2.3 cm diameter circular opening to expose the eyes. In both cases, the rest of the body was covered with 4 mm lead sheets. The x-ray (210 kVp, 30 ma/tube filtered with 2 mm copper and 2 mm aluminum) was delivered at a rate of 308 rads/min, with the total exposure ranging from 500 to 10,000 rads.

Shigella endotoxin (lipopolysaccharide B, S. flexneri; Difco Laboratories, Detroit, Mich.) was dissolved in sterile saline to yield concentrations of $10^{-7}$ to $10^{-13}$ gm/µl. Then 10 µl of one of these solutions were injected percutaneously into the vitreous with a 30-gauge needle connected to PE 190 tubing containing the endotoxin solution. After betadine was applied to the skin, the needle was passed through approximately 3 mm dorsal to the outer canthus and then guided between the edge of the orbit and the cartilaginous part of the anterior sclera. The needle was then tilted to enter the sclera and was passed into the vitreous chamber until it was observed through the pupil. The solution was delivered at a rate of 1 µl/sec with a syringe pump driven by a clutch motor. The needle was left in place for at least 10 sec after injection. The contralateral eye was injected in the same way with 10 µl of isotonic saline.

Topical and intravitreal administration of PG's and arachidonic acid. A 1 µg/µl solution of PGE$_2$ (free acid) in 10% ethyl alcohol, a 20 or 40 µg/µl solution of PGF$_2$a (tromethamine salt) in physiologic saline, or a stabilized solution of 5 mg/ml sodium arachidonate (courtesy Dr. Kenneth E. Eakins) was injected in a volume of 10 µl into the vitreous body of one eye, and the contralateral control eye was injected with the respective vehicle solution.

For topical application, PGE$_2$ was dissolved in 10% ethanol in physiologic saline and converted to its sodium salt by addition of sodium carbonate. The tromethamine salt of PGF$_2$a was dissolved in saline to yield a PGF$_2$a concentration of 10 µg/µl. The arachidonate solution described above was also used. In all cases, the contralateral eye was treated with an equal volume of the respective vehicle solution.

In vivo observations. Slit-lamp examinations of the eyes were made before x-ray irradiation or the administration of an inflammatory agent, and af-
Fig. 2. Relationship between the dose of intravitreally injected *Shigella* endotoxin and the peak inflammatory response of the chicken eye. The points represent the mean (±S.E.M.) of the highest values for cells (A) flare (B), and hyperemia (C) observed on each of six chickens during the first 6 hr after endotoxin injection.

Fig. 3. Effects of repeated systemic indomethacin treatment on the ocular inflammatory effects of $10^{-9}$ gm of *Shigella* endotoxin injected intravitreally. The points represent the mean (±S.E.M.) obtained on 6 normal and 6 indomethacin treated chickens.

Results

Intravitreal injection of 1 µg of *Shigella* endotoxin produced flare and an accumulation of cells in the anterior chamber, which became apparent in the first 2 hr and reached a maximum between 4 and 6 hr (Fig. 1, A and B).
Fig. 5. Induction of anterior chamber flare by topically applied arachidonic acid (100 μg applied five times at 5 min intervals). The points are means (±S.E.M.) obtained on six chickens.

Hyperemia of the iris was noted in all birds during the height of the cellular response (Fig. 1, C), but only a few irides showed microhemorrhages. These parameters returned toward normal during the second day. Significant differences between the IOP of the endotoxin-injected and the control eyes were not observed (Fig. 1, D).

A threshold cellular response was obtained following the intravitreal injection of $10^{-10}$ gm of *Shigella* endotoxin, whereas $10^{-9}$ to $10^{-8}$ gm caused a maximum response (Fig. 2, A). Pronounced anterior chamber flare was observed in all eyes that received $10^{-10}$ gm or more endotoxin (Fig. 2, B), but the extent of hyperemia increased more gradually in the dose range of $10^{-10}$ to $10^{-8}$ gm of endotoxin (Fig. 2, C).

At 4 hr after the intravitreal injection of 1 μg of endotoxin, the protein concentration of the aqueous humor was elevated nine-fold (0.3 ± 0.1 mg/ml in the control eyes vs. 2.8 ± 0.7 in the endotoxin-injected eyes; n = 4, p < 0.05). The PGE and PGF$_{2\alpha}$ concentrations in two aqueous samples, each pooled from six control eyes, were 20 and $24 \times 10^{-12}$ gm/ml and 12 and $18 \times 10^{-12}$, respectively. Two aqueous samples, each pooled from three endotoxin-injected eyes, yielded 14.5 and $1.8 \times 10^{-9}$ gm of PGF$_{2\alpha}$ and 57 and $36 \times 10^{-9}$ gm of PGE per milliliter of aqueous. Thus the aqueous PG levels of the endotoxin-injected eyes at 4 hr were elevated by about 1500-fold over the control eyes. No cells were detected in individual aqueous samples from 11 control eyes, and aqueous samples taken at 24 hr after endotoxin injection showed 20,000 to 90,000 white cells and 1,000 to 2,000 red cells per cubic millimeter of aqueous.

Pretreatment of the chickens with indomethacin (80 mg/kg intramuscularly) 1 hr before and 2, 5, and 8 hr after injection of $10^{-9}$ gm of endotoxin delayed the onset of the cellular invasion of the anterior chamber by at least 3 hr and significantly reduced the height of the peak response (Fig. 3, A). Systemic indomethacin treatment resulted in an even more pronounced inhibition of flare and iridial hyperemia (Fig. 3, B and C).

Topical application of PGE$_2$ resulted in the development of anterior chamber flare which depended on both the amount and the concentration of PGE$_2$ applied (Fig. 4). Administration of 50 μg of PGE$_2$ in 50 μl resulted in a
Fig. 6. Development of flare in the anterior chamber of the chicken eye after intravitreal injection of PGE$_2$ (A) and PGF$_{2\alpha}$ (B and C). The points are means (±S.E.M.) obtained on six chickens.

Slight flare in some of the eyes, whereas the same dose administered in 5 μl volume caused a clearly visible flare in all eyes treated (Fig. 4, A vs. B). When 50 μg of PGE$_2$ was applied five times in 5 μl at 5 min intervals, the flare was much more pronounced (Fig. 4, C), reaching a peak which was comparable to that caused by an intravitreal injection of 10$^{-10}$ gm of endotoxin. At 3 hr after PG application, the protein concentration in the aqueous of a group of six animals was about 10-fold greater in the experimental (5.7 ± 0.7 mg/ml) than in the contralateral control eye (0.6 ± 0.06; p < 0.001).

Topical application of 500 μg of sodium arachidonate (given in five doses of 100 μg in 20 μl of saline 5 min apart) caused the development of significant flare (Fig. 5) which was completely prevented by the prior administration of indomethacin (80 mg/kg intramuscularly, 1 hr before). No cells were seen in the anterior chamber during 3 days of observation, and only minimal hyperemia was observed in some of the arachidonate-treated eyes.

Intravitreal injection of 10 μg of PGE$_2$ per eye caused an anterior chamber flare similar to that observed after the topical application of 250 μg of PGE$_2$ (Figs. 6, A vs. 4, C), whereas intravitreal injection of as much as 400 μg of PGF$_{2\alpha}$ caused less flare than 10 μg of PGE$_2$ (Fig. 6, A vs. B or C). Topical application of 200 μg of PGF$_{2\alpha}$ (4 doses of 50 μg in 5 μl) had no observable effect on the eye. No cells were observed in the anterior chamber, and hyperemia was minimal after the intravitreal injection of either of these PG's.

Exposure of the chicken eyes to 500 rads of x-ray did not have a noticeable effect on the eye or the surrounding tissues (Fig. 7). A marked effect on the skin and lids occurred with 1,000 to 3,000 rads. A purple circle of approximately 2.5 cm diameter, corresponding to the opening in the lead shield, was observed within the first 2 to 5 hr after irradiation, and a swelling of the region became apparent soon after. In spite of these extraocular effects, there were no significant intraocular reactions. Exposure to more than 3,000 rads had a much more severe effect on the skin and lids. By the end of the first 24 hr after exposure to 4,000 rads, the lids were so swollen that they had to be forced apart for the slit-lamp examination of the anterior segment. The IOP could not be measured on these eyes, since the probe could not be held on the cornea without touching swollen conjunctival tissue. All eight chickens that received 4000 rads died within 3 days. In spite of this extensive reaction of the surrounding skin and lids, the eyes of only two of these chickens showed a breakdown of the blood-aqueous barrier (anterior chamber flare), and in only two eyes of one chicken were cells noted in the anterior chamber during the first day after irradiation. These intraocular effects
were noted only six hr or more after irradiation, at a time when the inflammatory reaction of the skin and lids showed a pronounced worsening (Fig. 8).

During the first 6 hr following exposure of six chickens to 10,000 rads of total head irradiation, slit-lamp examination revealed no iridial hyperemia and no flare or cells in the anterior chamber. At 6 hr after irradiation, these chickens were killed, and the aqueous humor was collected and assayed for protein. The mean protein concentration of 0.57 ± 0.03 mg/ml (n = 8) was not significantly different from that of the aqueous humor of normal, unirradiated chickens (0.52 ± 0.03 mg/ml; n = 17). No attempt was made to keep these birds for more than 6 hr because of the extensive damage to extraocular tissues.

Four of the chickens that received 1000 rads and five that received 2000 rads irradiation of the eyes were examined periodically for 9 months. At the end of this period, all eyes appeared normal, either grossly or by slit-lamp examination. The lenses were clear, and there was no indication of cataracts. One of the chickens that received 2000 rads had a wartlike growth on the lower lid of one eye, but in all other birds, the lids and the exposed area of the skin appeared normal.

Discussion

The present experiments clearly show that the chicken eye reacts to the intravitreal injection of bacterial endotoxin and to the topical or intravitreal administration of PG's and arachidonic acid in a manner very similar to that observed in the rabbit eye. Yet it is essentially refractory to the intraocular inflammatory effects of x-rays.

In a recent study, clear signs of intraocular inflammation were observed within 3 hr after a single exposure of rabbit eyes to 500 rads of x-rays, and 1000 rads produced an inflammatory reaction comparable in extent to, but shorter in time course than, endotoxin-induced uveitis. In contrast, the present studies show that even 3000 rads of x-rays produced virtually no intraocular inflammatory effect on the chicken eye. The small number of cells and low-level flare which was observed in some eyes after exposure to 4000 rads may well be secondary to an inflammation of adjacent extraocular tissues, since they were observed only after the development of pronounced serous discharge and swelling of the lids and surrounding skin. In none of the cases were flare, cells, or iridial hyperemia observed during the first 6 hr after irradiation, which is the period when the x-ray–induced intraocular inflammatory reaction reaches a peak in the rabbit eye.

In contrast to a resistance to x-rays, the chicken eye develops an inflammatory reaction to intravitreal injection of bacterial endotoxin which is similar in severity but more rapid in time course than that previously reported for the rabbit eye. The more rapid onset of the inflammatory reaction may simply be due to the fact that much of the...
chicken vitreous is fluid; thus intravitreally injected endotoxin may reach the surrounding tissues more rapidly than in the rabbit. The chicken eye appears, in fact, to be slightly more sensitive to intravitreally injected endotoxin (submaximal dose about $10^{-10}$ gm) than the rabbit eye\(^9\) (submaximal dose $10^{-8}$ gm).

Topical application of PG's, especially PGE\(_1\) or PGE\(_2\), to the rabbit eye causes a transient rise in IOP\(^{10}\) which normally peaks within 30 min and is associated with or followed by large increases in aqueous humor protein concentration. In the chicken eye, no significant changes in IOP were observed, yet PGE\(_2\) clearly caused a breakdown of the blood-aqueous barrier as evidenced by the development of pronounced flare 1 to 2 hr after topical application. PGF\(_2\alpha\) is less inflammatory than PGE\(_2\) in the rabbit\(^6, 10, 11\); this difference in potency is even more pronounced in the chicken, since flare and iridial hyperemia were observed only after the intravitreal injection of 200 or 400 μg of PGF\(_2\alpha\) but not after the topical applications up to a total of 200 μg of PGF\(_2\alpha\).

Podos et al.\(^12\) have shown that the ocular response to topically applied arachidonic acid is dependent upon ocular PG synthetase activity, since it is inhibited by nonsteroidal anti-inflammatory agents. Thus the arachidonate-induced flare and its inhibition by systemic indomethacin pretreatment indicate that the chicken eye has a cyclo-oxygenase system. The release of PG's and related autacoids seems to play a role in the inflammatory reaction of the chicken eye to Shigella endotoxin, since indomethacin delayed and reduced the development of flare and the entry of cells into the anterior chamber. Indomethacin has also been shown to reduce the ocular inflammatory signs induced by the intravitreal injection of endotoxin in the rabbit.\(^12\) However, even the repeated systemic administration of a high dose of indomethacin could not completely block the endotoxin-induced appearance of cells or flare in the anterior chamber of the chicken eye. Thus other pathways or chemical mediators of inflammation seem likely to operate in the avian eye during the defensive response.

It was considered in these experiments that the drainage of topically applied solutions from the corneal surface and conjunctival cul-de-sac of the chicken eye must be extremely rapid, since a saline solution could be delivered to the surface of the cornea of unanesthesized chickens at a rate of up to 300 μl/min without overflow of the fluid (unpublished observations). The effectiveness of topically applied PG's and arachidonic acid may therefore be greatly attenuated in the chicken by a rapid drainage and possibly by a high normal rate of tear flow or by stimulation of tearing. Direct comparison between the sensitivity of the chicken and the rabbit eye to topically applied PG's seem therefore unwarranted because of the possibility of greatly different contact times.

The lack of significant changes in the IOP of the chicken eyes during any of these experiments need not indicate that the mechanism of ocular inflammation is basically different in the avian and mammalian eye. Simultaneous changes in outflow facility and secretion, together with a breakdown of the blood-aqueous barrier and vascular changes, could occur without a net effect on IOP. Unfortunately, very little is known about the aqueous humor dynamics of birds, and the few available reports are somewhat contradictory. The rate of aqueous flow in white Leghorn hens was found to be 1.7% to 2%/min by the para-aminobipyrurate technique\(^14\) and nearly 20% (10 to 15 μl/min) in the White Rock chicken with a fluorometric technique.\(^15\) The latter study reported a mean IOP of 11.1 mm Hg by direct manometry of cannulated eyes, whereas Sears\(^16\) found in Rhode Island hens an IOP of 15.6 mm Hg. In contrast, Smith et al.,\(^17\) using noninvasive tonometry, found an IOP in a New Hampshire strain of 20.8 mm Hg. Sears failed to observe a significant decrease in the IOP after the systemic administration of acetazolamide, but Smith et al.\(^17\) reported a significant reduction in IOP. Clearly, further studies will have to be done to explain the apparent lack of IOP change in this species.

In summary, our results clearly indicate that bacterial endotoxin, PG's, and arachidonic acid have inflammatory effects on the
chicken eye which are basically similar to those in the mammalian eye. Furthermore, the chicken eye has a PG synthetase system which plays a role in endotoxin-induced ocular inflammation. Thus failure of the chicken eye to show an inflammatory response to x-ray irradiation cannot be explained on the basis of a general insensitivity to inflammatory agents or to the lack of a PG system. This, of course, does not rule out the possibility that there are basic differences between the avian and mammalian eye in the triggering mechanism for PG synthesis. Such differences in the control of autacoid release or differences in free radical formation and/or elimination may account for the insensitivity of the chicken eye to doses of x-rays up to 20 times higher than the x-ray dose that causes pronounced uveitis in rabbits.

Previous evidence for the resistance of the chicken eye to the cataractogenic effects of x-rays4 and present findings concerning the lack of an anterior uveal inflammatory response to this ionizing radiation support the suggestion that uveitis and the consequent long-term alteration in secretory processes and intraocular fluid composition may cause, or at least contribute to, the development of cataracts.5 Comparative studies on the effects of x-rays on mammalian and avian eyes and on the intraocular fluid composition of these eyes may help to elucidate the mechanism of x-ray–induced ocular inflammation and cataractogenesis and may reveal the relationship between these two phenomena.

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