An autoradiographic study of the penetration of subconjunctivally injected hydrocortisone into the normal and inflamed rabbit eye


The purpose of this study was to visualize the actual route of penetration of subconjunctivally injected hydrocortisone into the rabbit eye, and also to determine if lid movement or intraocular inflammation influenced this penetration. Albino rabbits, each with one eye inflamed by an intravitreal injection of 30 per cent bovine albumin and the other eye normal, were treated with subconjunctivally injected hydrocortisone to each eye in the superior temporal quadrant. Half of the animals were then anesthetized to prevent normal lid movement, and the remainder received no anesthesia, thus maintaining normal lid movement. The eyes of all the animals were then studied at specific time intervals with a dry-freezing technique and two different methods of autoradiography. This study indicated that subconjunctival hydrocortisone penetrates directly into the eye. Penetration was increased in the presence of inflammation but was unchanged as a result of lid movement. On the basis of these observations, it would appear that subconjunctival injections of hydrocortisone, to produce the maximum effect with the minimum dosage, should be immediately adjacent to the intraocular inflammation under treatment, rather than placing the injection haphazardly or always in the superior temporal quadrant, which is often the case.

Corticosteroids have become important therapeutic agents in ophthalmology for various inflammatory and allergic eye conditions in recent years.1-3 These drugs can be administered both locally and systemically. The mechanism of the local anti-inflammatory activity of the corticosteroids is not yet accurately defined, but they appear to hold the tissue response to cellular injury in abeyance, possibly by inhibiting glycolysis at the cellular level.4 These drugs do not appear actually to block the reaction between antigen and antibody.5

The local routes of application of corticosteroids, which include eye drops and subconjunctival injections, are of extreme interest to the ophthalmologist, since the therapeutic effect to the eye may be achieved but the systemic effects avoided. This feature is of utmost importance when it is necessary to treat the patient for a long period of time to control chronic ocular conditions.

The purpose of this study was to visualize the actual route of penetration of tritium-labeled hydrocortisone from a subconjunctival injection site into the eye, with an autoradiographic technique.

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Since hydrocortisone is known to be very soluble in organic solvents and slightly soluble in water, methods of autoradiography used by other investigators could not be used, since they require the use of water or organic solvents.\textsuperscript{6-19} Therefore, in this study, a modified method of autoradiography was devised to suit the purpose of the experiment.

**Methods and Materials**

Thirty-three albino rabbits, each weighing 2 to 3 kilograms, were anesthetized lightly with pentobarbital sodium, and a 0.2 ml. intravitreal injection of 30 per cent bovine albumin was given in the right eye of each animal to produce ocular inflammation. In 8 to 10 days' time, with a 26 gauge needle, 0.25 ml. of a 2.5 per cent suspension of tritium-labeled hydrocortisone\textsuperscript{*} was administered subconjunctivally to the inflamed right eye and the normal left eye of each animal in the superior temporal quadrant. Each injection contained 25 \( \mu \)c of labeled hydrocortisone. The suspension was a mixture of 0.2 mg. of tritium-labeled hydrocortisone containing 1.0 mc. and 250 mg. of inactive hydrocortisone\textsuperscript{†} suspended in 10 ml. of a sterile vehicle\textsuperscript{‡} which consisted of 0.9 per cent benzyl alcohol, 0.9 per cent sodium chloride, 0.4 per cent polyoxyethylene sorbitan monooleate, 0.5 per cent sodium carboxymethyl-cellulose, and distilled water.

The animals were then divided into two groups. Group I was anesthetized to prevent any lid movement, and Group II was not anesthetized, thus allowing normal lid movement. The normal and inflamed eyes of the two groups were then studied at specific time intervals, following the subconjunctival injection, of \( \frac{1}{2} \), 1, 2, and 4 hours. Additional unanesthetized rabbits were studied 12 and 24 hours after the subconjunctival injection.

Following the sacrifice of each animal, the injection site was marked by a black 6-0 silk suture, and the eyes were immediately enucleated and then "quenched" or rapidly cooled with the use of liquid air (Fig. 1).

The "quenching" was accomplished by immersing the whole eyeball in a metal container partially filled with isopentane, which acted as a conducting medium to transfer the extreme cold from the surrounding liquid air bath to the eye. The purpose of "quenching" was to freeze the tissue very rapidly, thereby keeping the size of ice crystals to a minimum while still maintaining the position of the soluble hydrocortisone in the tissue.

The frozen eyeball was then cut in quarters with a chilled razor blade and placed in an improvised heavy Pyrex dry-freezing vacuum chamber for a period of 4 days (Fig. 2). The whole apparatus was then placed within a cold chamber at \(-30^\circ\) C. The dry-freezing took place at a temperature of about \(-50^\circ\) C. and at a pressure of \(10^{-2}\) mm. Hg. Following the 4 day period of dry-freezing, the tissue was embedded in paraffin\textsuperscript{*} for 48 hours. The solubility of hydrocortisone in paraffin, which is very slight, was not considered to be a source of technical error. Five micron sections were then cut for autoradiographic study.

Two different techniques of autoradiography

\textsuperscript{†}British Drug Houses (Canada), Ltd., Toronto, Ontario.
\textsuperscript{‡}Supplied through the courtesy of Merck Sharp & Dohme of Canada, Ltd., Montreal, Quebec.

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\textsuperscript{*}Tissuemat M.P. 52 to 54° C., Fisher Scientific Co., U. S. A.
were used: (a) the "wet" technique and (b) the "dry" technique. In the "wet" technique the paraffin sections were fixed to warm 1 by 3 inch glass slides and then dipped in Kodak NTB2 liquid photographic emulsion. The "dry" technique consisted of transferring the paraffin sections by means of a warm (50° C.) mercury surface, or a surface of Teflon
to 1 by 3 inch glass slides previously coated with the Kodak NTB2 photographic emulsion approximately 4 to 5 μ in thickness.

Exposure of the photographic plates to the radioisotope was carried out by storing the plates in an upright position in sealed, light-free, black slide boxes with a small amount of Drierite1 in the refrigerator for a period of 3 weeks. Following this period of exposure of the photographic emulsion to the energy released by the isotope, the position of the hydrocortisone in the tissue was now marked clearly by small areas of the exposed photographic emulsion and all fluids so carefully avoided previously because of the serious solubility problem could now be used.

Processing of the exposed photographic plates of the two techniques was carried out in the darkroom in the following way.

"Wet" technique.
1. The plates were developed with the Kodak D19 developer for 2 minutes at 20° C., followed by fixer for 4 minutes, and then were washed for 15 minutes in tap water.
2. Deparaffinization was then carried out in a routine manner with a xylol series followed by an alcohol series.
3. The plates were then lightly stained with hematoxylin and eosin and a cover slip applied.

"Dry" technique.
1. The plates were dipped in a solution of cellulose acetate (0.5 Gm. cellulose acetate, 100 ml. 2-butanone, 10 ml. acetone) to prevent the tissue sections from moving or falling during deparaffinization or development.
2. The plates were then placed in a closed container for a period of 4 to 6 hours to allow slow evaporation of the cellulose acetate solvents. (This step was found to be extremely important to prevent the movement of the tissue.)
3. Deparaffinization was then carried out as in the above technique.
4. Development.
5. Staining.

The slides of the two techniques were then examined to ascertain the position of the tritium label (as shown by tiny black dots of exposed photographic emulsion) and to relate the position of the radiisotopic label to the ocular tissues under the variable experimental conditions.

Control slides, with nontreated tissue, were also studied with both the "wet" and "dry" technique to rule out exposure to light or defective technique in each experimental condition.

Results
The "wet" and "dry" techniques of autoradiography gave comparable results, indicating that the liquid photographic emulsion had not altered the position of the soluble drug in the tissue sections. Lid movement, which was present in the unanesthetized animals in Group II, did not appear to increase the penetration of the hydrocortisone into the eye by any appreciable amount. The sclera-choroid layer

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*Eastman Kodak Co., Rochester, N. Y.
†Produced by E. I. DuPont de Nemours and Co., Wilmington, Del.
‡Anyhydrous CaSO₄, manufactured by W. A. Hammond Drierite Co., Xenia, Ohio.

Fig. 3. Autoradiograph showing the penetration of the tritium-labeled hydrocortisone (R) into the underlying sclera (S) and choroid (C) of a normal rabbit eye. (×480.)
directly under the subconjunctival injection site showed the greatest amount of radioactivity, and this level decreased directly as the distance from the injection site. This appeared to be the case for all time intervals in both the normal and the inflamed eyes (Fig. 3).

The penetration of the hydrocortisone into the inflamed eye appeared to be much greater than the penetration into the normal eye. The maximum penetration in both the normal and the inflamed eye occurred between 2 and 4 hours after the subconjunctival injection, both with and without lid movement (Fig. 4). There was also evidence of the hydrocortisone reaching the vitreous and the retina of the inflamed eye 4 hours after the subconjunctival injection. However, this was not a high concentration.

The anterior segment of the eye, including the corneal stroma, iris, and lens, contained only negligible amounts of hydrocortisone both in the normal and inflamed eyes with and without lid movement. The corneal epithelium appeared to prevent the penetration of the drug into the eye by the corneal route (Fig. 5).

**Discussion**

From this study, it appears that the major route of penetration of tritium-labeled hydrocortisone from a subconjunctival injection site is by direct penetration through the underlying tissues. The percentage of the total dose which penetrates into the eye appears to be quite small. Although this technique is not quantitative, there is fairly good correlation with the results of others, who estimate that the total penetration into the eye is in the range of 1 per cent to 2 per cent with the subconjunctival route. Our results do not agree with those of Azuma and co-workers, who showed much greater penetration of hydrocortisone in the corneal stroma and in the lens. However, these workers used a different technique of causing intraocular inflammation, which included the use of 10 per cent NaOH to cause corneal damage.

It would seem advisable, in the light of the present observations, if this subconjunctival route is to be used clinically, that the injection be given as close as possible to the intraocular inflammation under treatment rather than haphazardly or always in the superior temporal quadrant as is commonly the case.

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Penetration of hydrocortisone into rabbit eye

Fig. 5. Autoradiograph showing the relatively poor penetration of a small amount of tritium-labeled hydrocortisone (R) that has regurgitated from the subconjunctival depot, probably through the needle puncture site, over the corneal epithelium (E). (x480.)

REFERENCES
