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Corneal Absorption of Ophthalmic Drugs

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Abstract □ Corneal absorption of 0.0075% homatropine hydrobromide solution and 0.0025% tropicamide solution in rabbit eyes was reinforced by prebuffering the eyes with specific alkaline buffers or by adding viscolizers and a surfactant or by a combination of these measures. For 0.0075% homatropine hydrobromide solution, prebuffering with an isotonic sodium borate solution (2.6%) and two "biological" buffer solutions, 0.2 *M* cyclohexylaminopropanesulfonic acid and 0.2 *M* tris(hydroxymethyl)methylaminopropanesulfonic acid, was found to increase significantly the amount of corneal absorption of the mydriatic. Cyclohexylaminopropanesulfonic acid provided the greatest amount of increase. Two viscolizers, 0.7% hydroxypropyl methylcellulose (45 cps) and 0.375% guar gum (41 cps), increased the effect of tropicamide more than that of homatropine hydrobromide. Hydroxypropyl methylcellulose, providing slightly greater viscosity than guar gum, resulted in a slightly increased mydriatic effect with each drug. Dilute polysorbate 80 in the two mydriatic solutions gave a greater enhancement to the effect of tropicamide than to that of homatropine hydrobromide for the first 30 min, but the enhanced mydriatic effect of homatropine hydrobromide was longer lasting. The increased absorption of the mydriatics obtained by employing both prebuffer and surfactant was greater than when using either alone.

Keyphrases □ Ophthalmic (corneal) absorption of homatropine hydrobromide and tropicamide—effect of prebuffering and/or adding viscolizers and surfactant, rabbits □ Absorption, ophthalmic (corneal)—effect of prebuffering and/or adding viscolizers and surfactant, homatropine hydrobromide and tropicamide, rabbits □ Homatropine hydrobromide corneal absorption—effect of prebuffering and/or adding viscolizers and surfactant, rabbits □ Tropicamide corneal absorption—effect of prebuffering and/or adding viscolizers and surfactant, rabbits

Pretreatment of the eye with a drop of sterile, isotonic, 2.6% sodium borate solution (pH 9.2) was shown to reduce markedly the amount of an alkaloidal drug required to produce a mydriatic or miotic response in the eye (1). This technique did not affect the stability of ophthalmic solutions, since it was the eye and not the solution that was temporarily buffered.

Good *et al.* (2) introduced 12 "biological" buffers which they claimed to be suitable for biological research. In the current study the buffering effect of available biological buffers, cyclohexylaminopropanesulfonic acid[1] and tris(hydroxymethyl)methylaminopropanesulfonic acid[2], was compared with that of 2.6% sodium borate solution whose buffer action was previously known to be effective. Homatropine hydrobromide and tropicamide were the mydriatics employed for the comparisons; rabbits were used as the test subjects because the biological buffers have not been cleared by the Food and Drug Administration for use on humans.

Another objective of this study was to compare the effect of a guar gum viscolizer[3] with the routinely used viscolizer, hydroxypropyl methylcellulose, as an adjuvant for increasing the physiological effect of mydriatic eyedrops in rabbits.

Since it was reported (3–5) that agents which reduce surface tension will generally increase the permeability of certain biological membranes, another objective was to compare the mydriatic effect of homatropine hydrobromide and tropicamide solutions both with and without polysorbate 80.

[1] CAPS, Calbiochem, Los Angeles, CA 90054
[2] TAPS, Calbiochem, Los Angeles, CA 90054
[3] Jaguar J2S-1, Stein, Hall & Co., New York, N.Y.

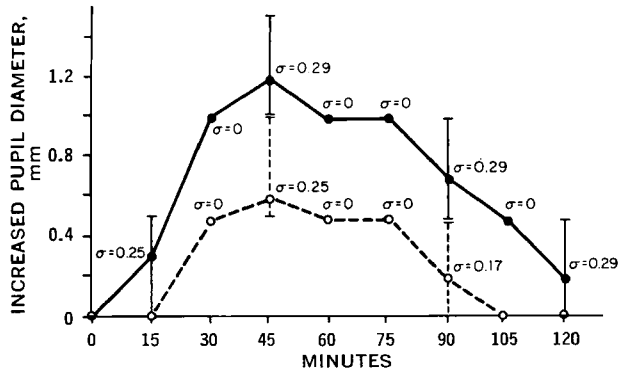

Figure 1—*Mydriatic effect of 0.0075% homatropine hydrobromide solution buffered* (●) *with 0.2* M *tris(hydroxymethyl)-methylaminopropanesulfonic acid or unbuffered* (○).

EXPERIMENTAL

Subjects and Materials—Male New Zealand white rabbits, 2.0–2.5 kg, were used. At least four rabbits were used for each experiment. The mydriatics used were homatropine hydrobromide and tropicamide; the buffers used were sodium borate decahydrate, cyclohexylaminopropanesulfonic acid, and tris(hydroxymethyl)methylaminopropanesulfonic acid. The viscolizers were guar gum and hydroxypropyl methylcellulose (4000 cps). Polysorbate 80 was used as the surface-active agent.

Preparation of Aqueous Solutions of Mydriatics and Buffers—Aqueous solutions of 0.0075% homatropine hydrobromide, 0.0025% tropicamide, and 2.6% sodium borate were prepared and sterilized by filtration using a sterile filter adapter[4] and membrane (0.45 μm)[5]. Adjustment of the tonicity of the test solutions was not considered. Aqueous solutions of 0.2 *M* cyclohexylaminopropanesulfonic acid (pKa 10.4) and 0.2 *M* tris(hydroxymethyl)-methylaminopropanesulfonic acid (pKa 8.4) were prepared with the pH of each solution adjusted to the pKa value of each buffer with 1 *M* NaOH to obtain the maximum buffer capacity in each instance. Final solutions were sterilized by filtration.

Preparation of Mydriatics in Viscous Vehicles—Solutions of hydroxypropyl methylcellulose, 0.7%, and guar gum, 0.375%, were prepared and sterilized in an autoclave at 121° for 30 min. The required amount of homatropine hydrobromide powder was then added and dissolved in the viscolizer solution. Although this method did not guarantee perfect sterility, no bacterial infections were noted in the eyes in any test animal.

Preparation of Aqueous Mydriatic Solutions Containing Surfactant—Aqueous solutions of mydriatics containing polysorbate 80 were prepared by making aqueous solutions of 0.02% polysorbate 80, 0.015% homatropine hydrobromide, and 0.005% tropicamide. The solutions were sterilized by bacterial filtration,

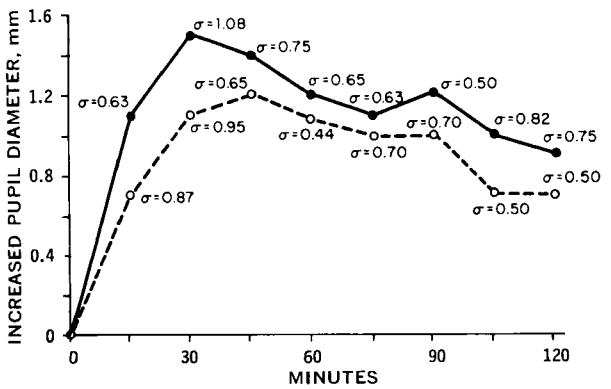

Figure 2—*Mydriatic effect of 0.0075% homatropine hydrobromide buffered with 0.2* M *cyclohexylaminopropanesulfonic acid* (●) *or 2.6% sodium borate* (○).

[4] Swinny.
[5] Type HA, Millipore Filter Corp., Bedford, Mass.

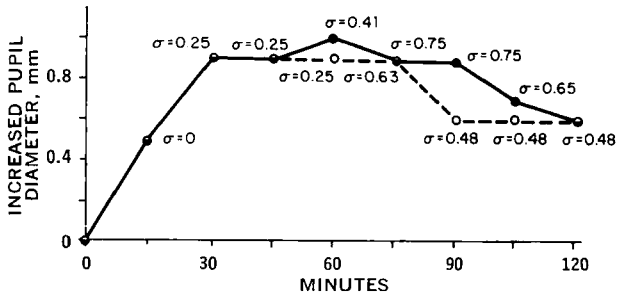

Figure 3—*Mydriatic effect of 0.0075% homatropine hydrobromide solution with 0.375% guar gum* (○) *or 0.7% hydroxypropyl methylcellulose* (●).

and equal volumes of each were mixed aseptically immediately prior to use.

Measurement of Pupil Size—The pupil sizes of the experimental rabbits were determined according to the method of Wang and Hammarlund (6). The pupil diameters were measured before the initial instillation of drops and at various intervals thereafter. The results are presented as millimeters of increased diameter of the average of at least four rabbits in each case.

Effect of Prebuffer Solution on Mydriatic Response—Aqueous 0.0075% homatropine hydrobromide was employed as the mydriatic test solution. Before any solution was instilled into the eyes, the normal pupil diameter was measured. Two drops of the buffer solution under test were dropped on the cornea of one eye, and the rabbit was allowed to blink normally several times. Any excess buffer solution on the eyelid was wiped off with soft tissue. No buffer was instilled into the other eye, which served as a control. Then 1 drop of the mydriatic solution was dropped on the cornea of each eye. The pupil diameters were measured every 15 min for 2 hr following instillation of homatropine hydrobromide. In succeeding tests comparing the relative effectiveness of two buffers on different eyes of the same rabbit at the same time, one eye of a series of four rabbits was prebuffered with 2.6% sodium borate and the other eye was prebuffered with 0.2 *M* cyclohexylaminopropanesulfonic acid prior to the instillation of homatropine hydrobromide solutions on the cornea of each eye.

Effect of Viscolizers in Mydriatic Solutions—The relative effectiveness of guar gum solution and hydroxypropyl methylcellulose solution on the corneal absorption of homatropine hydrobromide and tropicamide was tested in the eyes of four rabbits. Measurements of the pupil diameter were taken every 15 min for 2 hr following instillation of homatropine hydrobromide and every 10 min for 1 hr in the case of tropicamide.

Effect of Surfactant in Mydriatic Ophthalmic Solutions—A 0.01% aqueous solution of polysorbate 80, which is considerably greater than its critical micelle concentration (CMC), was used in this study. One rabbit's eye was treated with tropicamide in surfactant solution while the other eye was treated with a similar concentration of plain aqueous tropicamide solution. Another rabbit's eye was treated with homatropine hydrobromide in surfactant solution while the other eye was treated with plain homatropine hydrobromide aqueous solution. The pupil diameters were measured every 15 min for 2 hr for homatropine hydrobromide dilation and every 10 min for 2 hr in the case of tropicamide.

To find out if the surfactant would markedly increase the mydriatic effect given by the normal prebuffering effect of 2.6% sodium borate, each eye of a rabbit was first buffered with 2 drops of 2.6% sodium borate solution. One drop of 0.0075% homatropine hydrobromide solution was instilled into one of the two buffered eyes of a rabbit while 0.0075% homatropine hydrobromide in 0.01% polysorbate 80 solution was instilled into the other buffered eye. The pupil diameter was measured before the initial instillation and every 15 min thereafter over a 2-hr period for homatropine hydrobromide dilation.

RESULTS AND DISCUSSION

Before undertaking the mydriatic study in rabbits, it was necessary to ascertain the proper concentration of drug to give the desired response in rabbit eyes. The threshold concentration sought was the minimum concentration that would give a mea-

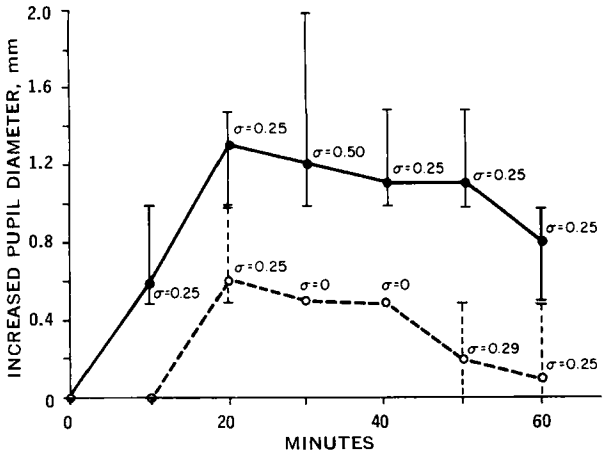

Figure 4—*Mydriatic effect of 0.0025% tropicamide solution in the presence* (●) *and absence* (○) *of 0.7% hydroxypropyl methylcellulose.*

surable mydriatic response but that was small enough for any increase in response brought about by an adjuvant to be apparent. The rabbit threshold concentration for tropicamide was found to be 0.0025%, and that for homatropine hydrobromide was reported to be 0.0075% (6).

It was observed in this investigation that the physiological effects of the ophthalmic solutions containing certain weakly basic drugs are increased by temporarily increasing the pH of the cornea with a prebuffering technique or by certain viscolizers or surfactants. The standard deviation was calculated for each set of results, and this value is indicated on each graph along with the plotted mean for four rabbits. The data for the four rabbits coincided exactly in those few cases where the standard deviation is listed as zero in the graphs. In some instances the ranges of the data from the experimental and control eyes overlapped slightly; in most instances they did not. For the experimental data in those ranges where the test and control values showed the greatest differences in the early stages of each determination, *t*-tests were performed.

The results from use of the three prebuffering solutions, aqueous 2.6% sodium borate (pH 9.2), aqueous 0.2 *M* tris(hydroxymethyl)methylaminopropanesulfonic acid (pH 8.4), and aqueous 0.2 *M* cyclohexylaminopropanesulfonic acid (pH 10.4), on rabbit corneal absorption of homatropine hydrobromide are shown in Figs. 1 and 2. In Fig. 1 the prebuffered eyes showed a markedly increased mydriatic effect over the unbuffered eyes for the entire 2-hr test duration. A *t*-test confirmed that the mydriatic effect was greater at the 5% level of significance for the period between 15 and 30 min for the prebuffered eyes as compared to the nonbuffered control eyes.

Figure 2 shows that cyclohexylaminopropanesulfonic acid prebuffer enhanced the mydriatic effect of homatropine hydrobromide more than did the sodium borate prebuffer. But a strict paired *t*-test for the period between 15 and 30 min revealed that there was essentially no difference between the effects of these

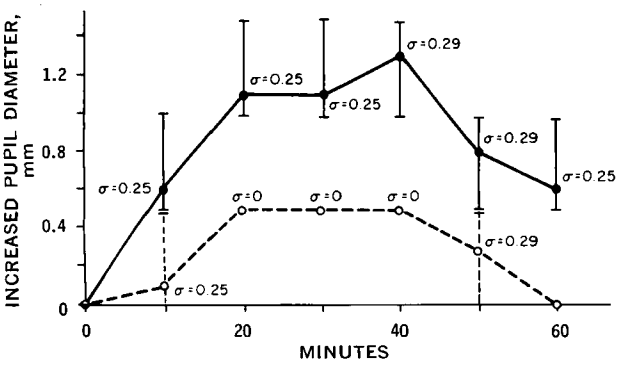

Figure 5—*Mydriatic effect of 0.0025% tropicamide solution in the presence* (●) *and absence* (○) *of 0.375% guar gum.*

two prebuffer solutions on the mydriatic action of homatropine hydrobromide. This might be because there was only a little difference in the pH values of the two prebuffers.

In comparing the enhanced mydriatic effects from the three prebuffers in Figs. 1 and 2, it is evident the amount of increase roughly parallels their differences in pH values, with the greatest increase produced by the buffer with the highest pH and the smallest increase produced by the one with the lowest pH. This is not to suggest that pH at the cornea is the only criterion affecting corneal absorption of ionizable substances; it was previously reported (7, 8) that other factors, such as buffer capacity, viscosity, surface tension, complexation, corneal contact time, and molecular size, also play a role. However, in this brief study where these other variables were fairly constant, the enhanced mydriatic activity did occur in the exact order of the increased pH of each prebuffer solution.

The amount of free base of homatropine hydrobromide available at the various pH's was calculated as follows from the pKa of each buffer:

$$\text{percentage free base} = 100\,\frac{\text{antilog (pH}-\text{pKa)}}{1 + \text{antilog (pH}-\text{pKa)}} \qquad \text{(Eq. 1)}$$

which gives an amount of free base of 0.2% at pH 7.4, 4.8% at pH 8.4, 24% at pH 9.2, and 83.3% at pH 10.4. However, the normal pH on the corneal surface is rarely as low as the theoretical value of 7.4 because some carbon dioxide is continually lost from the surface of the cornea to the air, thus increasing the actual pH of the eyes to about 8. This increase in pH varies considerably depending upon the amount of fresh tears, exposure of cornea to air, the frequency of eye blinking, *etc.*

Drop-size doses were used for the mydriatic solutions containing viscolizers as well as for the buffer solutions because the size of the drop was found not to vary enough to be significant. In fact, all of the solutions tested, whether they contained a viscolizer or not, required exactly 17 drops to measure 1 ml. The results on the effect of viscolizers on the corneal absorption of homatropine hydrobromide and tropicamide are shown in Figs. 3–5. When comparing reinforcement of corneal absorption given by two solutions containing viscolizers having quite similar viscosities, *i.e.*, 45 cps for 0.7% hydroxypropyl methylcellulose and 41 cps for 0.375% guar gum, one might expect that the presence of the viscolizers would provide a similar increase in response or that the slightly more viscous solution would have a slightly greater mydriatic effect. This was found to be the case, and Fig. 3 shows that hydroxypropyl methylcellulose produced only slightly greater mydriatic response than did guar gum for homatropine hydrobromide. But a *t*-test could not detect any difference at the 5% level of significance during the period between 60 and 105 min. Both viscolizers significantly increased ($p = 0.05$) the mydriatic effect of tropicamide about an equal amount (Figs. 4 and 5).

When the mydriatic effect of 0.0075% homatropine hydrobromide and 0.0025% tropicamide solutions in 0.01% polysorbate 80

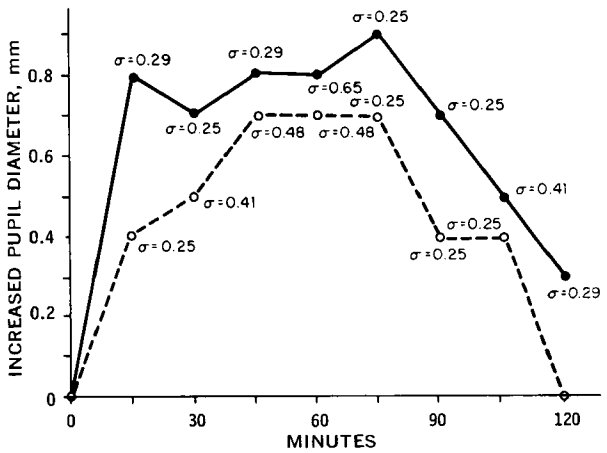

Figure 6—*Mydriatic effect of 0.0075% homatropine hydrobromide solution in the presence* (●) *and absence* (○) *of 0.01% polysorbate 80.*

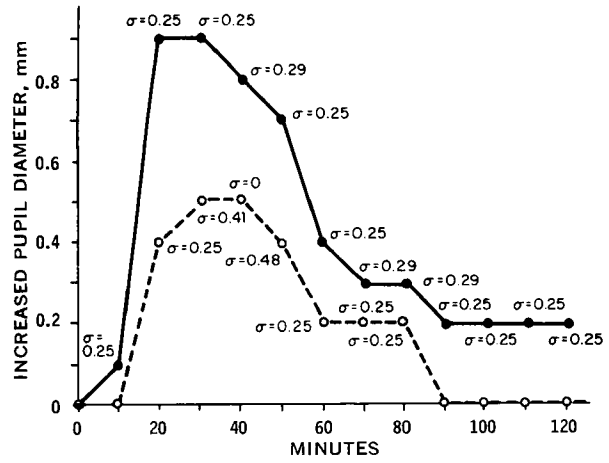

Figure 7—*Mydriatic effect of 0.0025% tropicamide solution in the presence* (●) *and absence* (○) *of 0.01% polysorbate 80.*

was compared to that given by identical but surfactant-free aqueous solutions, the mydriatic effect of both test solutions was found to have increased; *t*-tests were performed between 15 and 30 min on the data in Fig. 6 and between 20 and 40 min for Fig. 7. The increases were significant ($p = 0.05$) in both cases. The increase in mydriatic effect of both test solutions due to the presence of surfactant was much greater for tropicamide than for

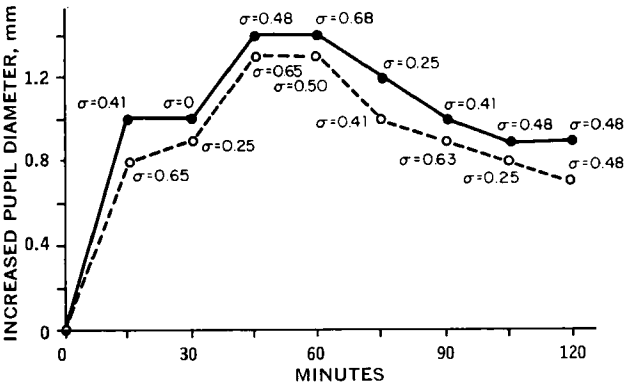

Figure 8—*Mydriatic effect of borate-prebuffered 0.0075% homatropine hydrobromide solution* (○) *and borate-prebuffered 0.0075% homatropine hydrobromide solution in 0.01% polysorbate 80* (●).

homatropine hydrobromide during the first 30 min. However, this greater effect was of shorter duration with tropicamide. To ascertain whether surfactant further enhanced the effect of borate prebuffer on the corneal absorption, 2.6% sodium borate was utilized in a similar series of experiments as a prebuffer solution. A further increase in mydriatic response was found at 15 min ($p = 0.05$) when the eye was prebuffered with sodium borate solution before instillation of the solution containing 0.0075% homatropine hydrobromide in 0.01% polysorbate 80. Therefore, it can be concluded that the effect of a mydriatic in rabbit eyes can be enhanced by employing a simple prebuffering technique for the administration of eyedrops or by adding certain adjuvants usually in addition to the prebuffer solution. This technique could make it possible to reduce the drug concentration in the drops or to give less frequent administration of drugs. It is reasonable to assume this dosage reduction would eliminate some undesirable side effects as in the case of atropine in which toxic effects have been reported to occur in patients with reduced renal function (9). The techniques studied in this investigation, if properly used, may likewise increase the efficiency of many other ophthalmic solutions normally employed in clinical practice if their pKa values are in the acceptable range.

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