Corneal Absorption Reinforcement of Certain Mydriatics

EUNICE S. N. WANG and E. ROY HAMMARLUND*

Abstract □ By using a prebuffer technique or by adding buffer and/or viscolizer to certain dilute mydriatic solutions, similar physiological effects were obtained as were given by unbuffered solutions that were 10 times more concentrated. Isotonic, sterile, 2.6% sodium borate solution was found to be an effective buffer for the following solutions: 0.1% homatropine HBr, and 1% phenylephrine HCl plus 0.1% cyclopentolate HCl. These concentrations provided adequate dilation for routine eye examinations. The addition of 0.5% hydroxypropyl methylcellulose, 4000 cps., to buffered mydriatic solutions did not show any further increase in pupil size of Caucasians compared to the same buffered solutions without viscolizer. However, when used similarly in the eyes of Orientals, the viscous solutions produced a more marked dilating effect. No eye irritation was reported by any of the human test subjects for any of the dilute experimental eye drops tested. The results of the animal experimentation indicated that the several biological buffers tested [tromethamine, 2-(*N*-morpholino)ethanesulfonic acid, and *N*,*N*-bis(2-hydroxyethyl)glycine] were not as effective as sodium borate for increasing the corneal absorption of the medicinal agents used. The pKa value of cyclopentolate HCl, determined by titration, was found to be 7.93.

Keyphrases □ Mydriatics—corneal absorption reinforcement □ Corneal absorption, mydriatics—reinforcement □ Buffer effect—corneal absorption □ pH effect—mydriatic activity □ Cyclopentolate HCl—pKa determination

Good therapy involves the efficient use of a drug so that the body encounters minimal chemical challenge to achieve the desired effect. In the case of ophthalmic solutions, modification of the vehicle must be continuously studied since it has the potential for improving therapy.

Because the cornea of the eye is more permeable to lipid-soluble than to water-soluble substances, the nonionized form of a drug, which is more lipid soluble, will be absorbed through the cornea more rapidly than the lipid-insoluble ionized form. The degree of dissociation of a drug in tears (normal pH 7.4), predictable from its pKa, and the capacity of tear buffers to overcome the buffer effect of the drug play vital roles in corneal drug absorption. The short-term alteration of pH also may affect the rate of corneal uptake.

Since the physiological effect of alkaloidal eye drops results from an absorption of the free base portion of the alkaloid, greater response should be obtained if the drugs are administered in alkaline media. However, due to the instability of many alkaloids in alkaline solution, this procedure is not usually possible. Previous studies by Boberg-Ans *et al.* (1) have shown that pretreatment of the eye with a drop of sterile, isotonic, 2.6% sodium borate solution (pH 9.2) can markedly reduce the dose required for an alkaloidal drug to produce a mydriatic or miotic response in the eye.

In 1966, Good *et al.* (2) described a series of organic buffers, called "biological buffers," for use in biological systems. No report has appeared on the effect of these biological buffers on the corneal absorption of ophthalmic drugs.

The objective of this study was to investigate some related aspects of prebuffering the eye or of buffering certain mydriatic eye drops with various alkaline buffers along with the utilization of some viscolizers in order to obtain increased physiological response in both rabbit and human eyes.

Since Howard and Lee (3) and others have shown that there is a difference in mydriatic response between persons of different races, both Oriental and Caucasian subjects were included in this study. In addition, the previously unreported dissociation constant for cyclopentolate HCl was determined experimentally.

EXPERIMENTAL AND RESULTS

Subjects and Materials—The experimental subjects employed were Caucasian and Oriental humans, both male and female, and New Zealand white rabbits, female, weight 2–2.5 kg. The mydriatics used were cyclopentolate HCl,[1] homatropine HBr USP,[2] and phenylephrine HCl USP.[3] The buffers utilized were sodium borate decahydrate,[4] *N*,*N*-bis(2-hydroxyethyl)glycine,[5] 2-(*N*-morpholino)ethanesulfonic acid,[6] and tromethamine.[7] The viscolizers employed were hydroxypropyl methylcellulose 4000 cps.[8] and polyvinyl alcohol.[9]

Preparation of Aqueous Solutions of Mydriatics and Buffers—All aqueous drug and buffer solutions (without viscolizer) were prepared and sterilized by filtration through sterile membrane filters[10] (0.45 μ) into sterile dropper bottles; subsequent transfers were made with sterile pipets. Adjustment of the tonicity of the test solutions was not considered. All eye drops prepared were stored in 0.5-oz., light-resistant, sterile, all-glass dropper bottles. To standardize the size of the drops instilled, only droppers that delivered 71–74 drops of water to give exactly 4 ml. when dropped vertically into a small cylindrical graduate were used.

Preparation of Ophthalmic Vehicles Containing Viscolizers—A double concentrated solution of each viscolizer, *i.e.*, 1% hydroxypropyl methylcellulose and 2.8% polyvinyl alcohol, was prepared

[1] Cyclogyl, Schieffelin and Co., New York, N. Y.
[2] Merck Co., Inc., Rahway, N. J.
[3] Neo-Synephrine HCl, Winthrop Laboratories, New York, N. Y.
[4] Reagent grade, J. T. Baker Chemical Co., Phillipsburg, N. J.
[5] Bicine, A grade, Calbiochem, Los Angeles, Calif.
[6] MES, A grade, Calbiochem, Los Angeles, Calif.
[7] Tris, buffer grade, Nutritional Biochemicals Corp., Cleveland, Ohio.
[8] Methocel, 90 HG, premium, Dow Chemical Co., Midland, Mich.
[9] 99% hydrolyzed; Matheson, Coleman and Bell, Cincinnati, Ohio.
[10] Swinnex, Millipore Filter Corp., Bedford, Mass.

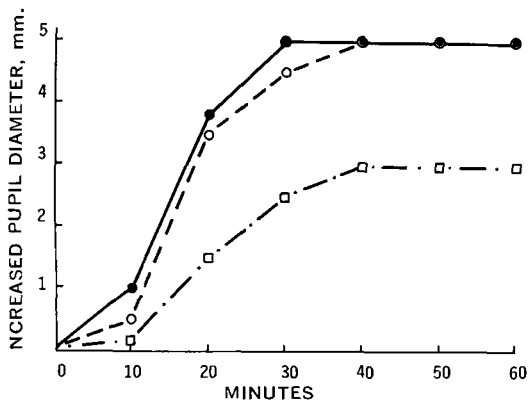

Figure 1—*Mydriatic effect of dilating solutions on humans (Caucasian), both with and without borate prebuffer. Key: ●, unbuffered concentrated dilating solution; ○, dilute dilating solution with borate prebuffer; and □, unbuffered dilute dilating solution.*

and autoclaved at 15 p.s.i. for 30 min. After cooling, a clear dispersion resulted. Equal volumes of the doubly concentrated sterile stock viscolizer solution and the doubly concentrated sterile aqueous mydriatic solution were mixed together before instillation, thereby giving the desired final experimental concentration. The final viscolizer concentrations of 0.5% hydroxypropyl methylcellulose and 1.4% polyvinyl alcohol were selected because those concentrations are often employed commercially.

Measurement of Pupil Size—Rabbits were placed on top of empty rabbit cages, the height of which subdued them sufficiently so that no further restraint was necessary; humans were seated in an evenly lighted room. A ruler with a millimeter scale was held firmly against the face, and the horizontal diameter of the pupil was estimated to the nearest 0.5 mm. in a similar manner in all cases. The pupil diameters were measured before the initial instillation of drops and at various time intervals thereafter. The results are presented as the average millimeter of increased diameter of at least two human or three rabbit subjects in each case.

Although statistical analysis could have been used in this study, it would contribute little. The range of values from which the averages were derived for the controls did not overlap those for the test subjects in any instance.

Determination of Threshold for Mydriatics in Rabbits—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 measurable mydriatic response but which was still small enough so that any increase in response brought about by any adjuvant would be apparent. The rabbit threshold concentrations were found to be 0.0075% for

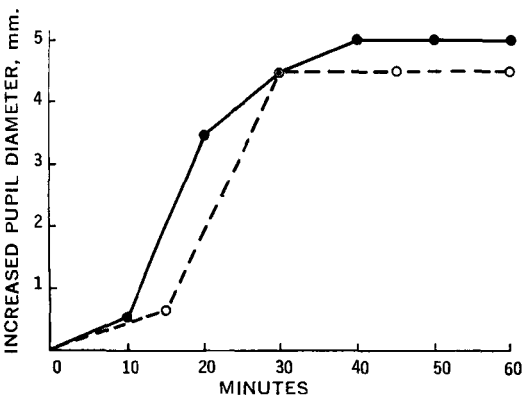

Figure 2—*Mydriatic effect of dilute dilating solution on humans (Caucasian) with borate prebuffer and in freshly prepared, 8-, and 24-hr.-old borate buffers. Key: ●, identical results from prebuffered eye, freshly prepared buffered dilute dilating solution, and 8-hr.-old buffered dilute dilating solution; and ○, 24-hr.-old buffered dilute dilating solution.*

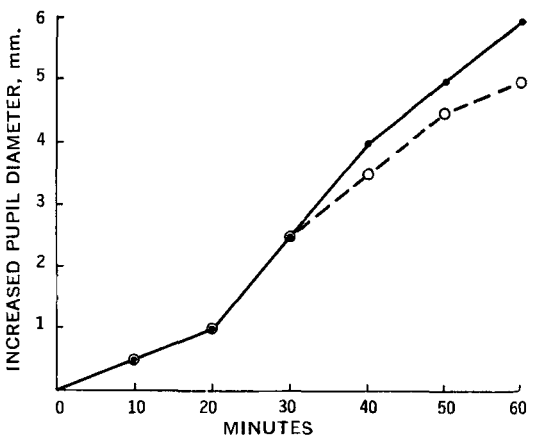

Figure 3—*Mydriatic effect on Orientals of buffered dilute dilating solution with and without viscolizer. Key: ●, borate-buffered dilute dilating solution with hydroxypropyl methylcellulose viscolizer; and ○, same solution without viscolizer.*

homatropine HBr and 0.02 and 0.002%, respectively, for the combination phenylephrine HCl and cyclopentolate HCl solution.

Comparison of Effects of Combination Solution of Phenylephrine HCl and Cyclopentolate HCl in Different Concentrations, Both with and without Sodium Borate Prebuffering—The combination solution of 10% phenylephrine HCl and 1% cyclopentolate HCl, which is used routinely in many eye clinics, is designated as "concentrated dilating solution," and a combination solution of exactly one-tenth this concentration is designated as "dilute dilating solution" throughout this study. The mydriatic effect and relative amount of eye irritation of both solutions were tested on human subjects. One drop of the concentrated dilating solution was instilled in one eye of the test subject, and one drop of the dilute dilating solution was instilled immediately in the other eye. Measurements of the pupil size were taken immediately before and every 10 min. after instillation for 1 hr. Figure 1 shows that the dilute dilating solution gave a weaker response than did the concentrated solution. The test subjects reported that the concentrated dilating solution was quite painful to the eye but that the dilute dilating solution was not.

In another series of experiments, one drop of sterile 2.6% sodium borate solution (pH 9.2) was first instilled in one eye of the test subject. After a few seconds, any excess buffer solution on the eye lid was wiped off with soft tissue, and this prebuffered eye received one drop of the dilute dilating solution while the unbuffered eye received one drop of the concentrated dilating solution. Figure 1 shows that there was a similar mydriatic response in both eyes, with both pupils reaching the same maximum diameter at about the same time. The degree of dilation of both eyes was confirmed by an ophthalmologist at the eye clinic as being adequate for any routine eye examination; the borate prebuffer solution gave no eye irritation.

The foregoing experiments were first carried out on rabbit eyes using the previously mentioned threshold concentration of the combination dilating solution. The results on rabbits corresponded to those reported in Fig. 1 for humans.

Use of Combination 1% Phenylephrine HCl and 0.1% Cyclopentolate HCl Solution in a Sodium Borate Buffered Vehicle, Both with and without Viscolizer—To lessen the number of drops that must be given a patient, an attempt was made to combine the borate buffer solution (both with and without viscolizer) and the dilute dilating solution so that the technique of prebuffering the eye was unnecessary. The buffered drops had a pH of 9.2. A brief study of the stability of the agents in aqueous, viscous and nonviscous, alkaline solutions was in order.

Comparison tests of the degree of mydriasis obtained were conducted first with rabbit eyes and then with a series of humans, using freshly prepared, dilute dilating solution in sodium borate buffer *versus* similar solutions which were 8 and 24 hr. old. All tests were made by instilling in a like manner one solution in one eye while the solution to which it was being compared was instilled in the other. The results for the average of three humans for each solution are presented in Fig. 2. The rabbit tests gave

similar responses. Clinically satisfactory and practically identical mydriatic and cycloplegic effects were obtained from the prebuffered solution and the freshly prepared and the 8-hr. old alkaline solution, but the 24-hr.-old alkaline solution produced slightly less mydriatic effect than did the others. No irritation to the eye was felt by any of the subjects receiving any of these alkaline, dilute dilating solutions. There was no change in physical appearance of the 24-hr.-old alkaline solution. However, after standing at room temperature for 3 days, the solution turned slightly yellow.

The addition of 0.5% hydroxypropyl methylcellulose to the freshly prepared sodium borate buffered, dilute dilating solution did not produce any increased mydriasis in the eyes of Caucasians because they were already at maximum dilation. However, when this viscous, dilute dilating solution was compared in Oriental eyes to the same solution without viscolizer, an increase in the degree of mydriasis in Oriental eyes was produced by the more viscous solution (Fig. 3). Since Oriental eyes are known to be more difficult to dilate than those of Caucasians, it was not surprising that one drop of the buffered, viscous, dilute dilating solution did not produce a completely satisfactory dilation, as shown in Fig. 3. It was necessary to instill a second drop 15 min. later into the eyes of Orientals to effect comparable dilation; these results are shown in Fig. 4 along with a one-drop dose of concentrated dilating solution. Comparison with Fig. 1 shows how much less effective one drop of concentrated dilating solution is for Orientals (Fig. 4) than for Caucasians (Fig. 1).

Effect of Various Prebuffers on Rabbit Corneal Absorption of Certain Mydriatics—In this study on rabbits, the enhanced mydriatic effect produced by using 2.6% sodium borate solution as a prebuffer was compared to the effect given by use of the following three biological buffers: 0.2 *M* *N,N*-bis(2-hydroxyethyl)glycine, 0.2 *M* 2-(*N*-morpholino)ethanesulfonic acid, and 0.2 *M* tromethamine for aqueous homatropine HBr solutions. The tests were all made in a similar manner, with sodium borate prebuffer solution being instilled in one eye of each rabbit and one of the biological buffer solutions dropped in the other eye prior to the instillation of the mydriatic solution. The diameter of the pupils was measured every 15 min. thereafter for 2 hr. and represented the average given by three or more rabbits in all cases. The results, shown in Fig. 5, indicate that the largest increase in mydriatic effect was given by the buffer with the higher pH and the smallest increase was given by the buffer with the lowest pH. A similar test on rabbits was made using sodium borate prebuffer solution in one eye and no buffer in the other eye to find out how much the mydriatic effect of a combination solution of phenylephrine HCl and cyclopentolate HCl is increased in rabbit eyes by prebuffering with sodium borate. The results, shown in Fig. 6, indicate that prebuffering markedly reinforces the mydriatic effect of phenylephrine and cyclopentolate solution in rabbit eyes.

Effect of Viscolizers on Rabbit Corneal Absorption of Homatropine HBr Solution—A series of rabbits received one drop of aqueous 0.0075% homatropine HBr solution in one eye and one drop of the same homatropine solution prepared in either 1.4% polyvinyl

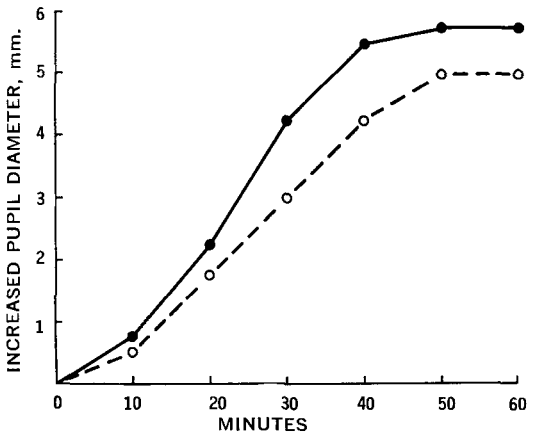

Figure 4—*Mydriatic effect on Orientals of concentrated dilating solution and two doses of buffered, viscous, dilute dilating solutions. Key: ●, borate-buffered, viscous, dilute dilating solution (two doses instilled); and ○, unbuffered concentrated dilating solution (one dose instilled).*

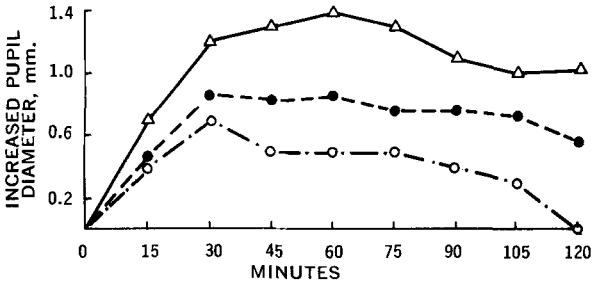

Figure 5—*Effect of various prebuffer solutions on rabbit corneal absorption of 0.0075% homatropine HBr solution. Key: △, with sodium borate prebuffer (pH 9.2); ●, with no buffer or with N,N-bis(2-hydroxyethyl)glycine prebuffer (pH 8.4) or with tromethamine prebuffer (pH 8.3); and ○, with 2-(N-morpholino)ethanesulfonic acid prebuffer (pH 6.2).*

alcohol or 0.5% hydroxypropyl methylcellulose in the other eye. Single-drop doses rather than measured volumes were used, because when the droppers were calibrated, the sizes of the drops were found to vary less than 6% whether or not they contained a viscolizer. The procedure was carried out in the same manner as described previously; the measurement of the pupil diameters was taken every 15 min. for 2 hr. The results are shown in Fig. 7 and indicate that both viscolizers reinforce the mydriatic effect of homatropine HBr solution but that the increase given by hydroxypropyl methylcellulose was much greater than that given by polyvinyl alcohol. This increased mydriatic effect produced by the drops containing 0.5% hydroxypropyl methylcellulose paralleled the somewhat greater viscosity that it had, *i.e.*, 13.2 cps. *versus* 2.3 cps. for the drops containing 1.4% polyvinyl alcohol.

Determination of pKa of Cyclopentolate HCl—An aqueous solution of known concentration of cyclopentolate HCl was neutralized with a stoichiometric quantity of standardized NaOH solution to liberate free cyclopentolate. A titration curve of the free cyclopentolate was obtained by titrating the neutralized solution with standardized dilute HCl, using a Beckman Zeromatic pH meter. The midpoint of the titration curve was ascertained, and the pKa value of cyclopentolate HCl was determined by finding the pH value that corresponded to this midpoint. In this way the pKa of cyclopentolate HCl was found to be 7.93, as shown in Fig. 8.

DISCUSSION AND CONCLUSIONS

The experimental data obtained from this investigation indicate that the physiological response of several mydriatic eye drops can be enhanced by proper utilization of certain buffers and viscolizers, with 2.6% sodium borate solution being the most effective buffer and 0.5% hydroxypropyl methylcellulose being the most effective viscolizer of those tested.

A comparison of the amount of the free base portion of an amine ophthalmic drug present at pH 9.2 (the pH of 2.6% sodium borate

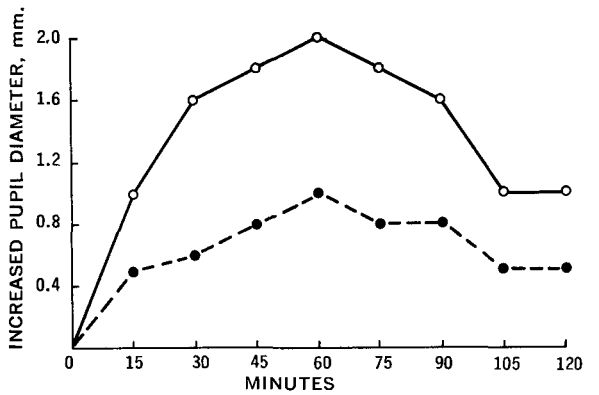

Figure 6—*Mydriatic effect on rabbits of combination solution of 0.02% phenylephrine HCl and 0.002% cyclopentolate HCl, both with and without borate prebuffer. Key: ○, with sodium borate prebuffer; and ●, without sodium borate prebuffer.*

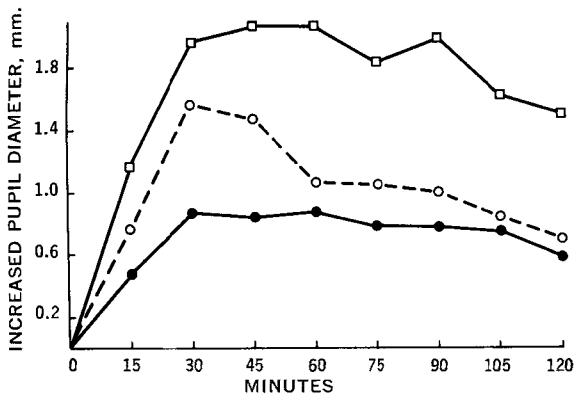

Figure 7—*Effect of viscolizers on rabbit corneal absorption of 0.0075% homatropine HBr solution. Key: ●, 0.0075% homatropine HBr solution; ○, same plus 1.4% polyvinyl alcohol; and □, same plus 0.5% hydroxypropyl methylcellulose.*

solution) and at pH 7.4 (the pH of normal tear fluid) by using the pKa of the particular drug can be used to predict whether or not buffering the eye to pH 9.2 would enhance the corneal absorption of this drug. For example, with phenylephrine HCl, which has a pKa of 8.86, the amount of free base present at pH 9.2 would at maximum be about 22 times greater than that present at pH 7.4; in a similar manner for homatropine HBr (pKa 9.7), the free base form would be about 48 times greater at pH 9.2. In the case of pilocarpine HCl with a pKa of only 6.85, the concentration of the free base portion at pH 9.2 is only about 1.3 times greater than that present at pH 7.4. This increase in quantity of free base is too small an amount to show any measurable increase in miotic effect. A study of pilocarpine HCl showed no measurable decrease in pupil diameter due to the prebuffer treatment with sodium borate.

Since the pH on the surface of the eye may fluctuate due to the loss of carbon dioxide and sometimes become as high as 8 (rather than 7.4), and since the pH of the mixed sodium borate solution and tear fluid which is continuously being diluted with more tears will decrease and be lower than 9.2 for much of the time after its initial instillation, the actual ratio of the free base molecules of instilled amine drops on buffered and unbuffered eyes will be considerably smaller than the theoretical calculated value. This study showed that the mydriatic effect of the combination solution of phenylephrine HCl and cyclopentolate HCl and of homatropine HBr each was increased about 10-fold when the sodium borate prebuffer method or borate-buffered drops were used. If the theoretical optimum buffering conditions could have prevailed, the mydriatic solutions should have given physiological responses of 2–4 times the experimental findings.

Although the most appropriate pH for eye drops is usually reported as being 7.4 for maximum eye comfort, Trolle-Lassen (4) and others have found that the eye can tolerate an isotonic solution up to about pH 9.7 as long as it has no other irritating properties. Therefore, the sterile, isotonic, 2.6% sodium borate solution is a suitable nonirritating buffer for ophthalmic use whenever the therapeutic drug has a sufficiently high pKa value for its effect to be appreciably enhanced.

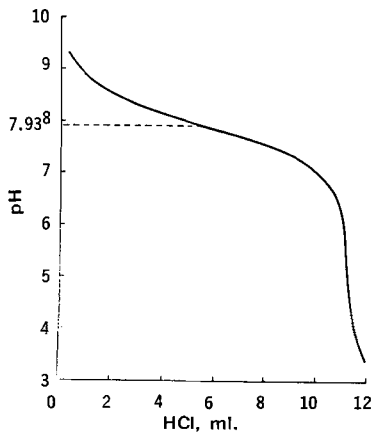

Figure 8—*Titration curve of cyclopentolate.*

Recommended Dilating Solutions for Routine Eye Examination—The following two rather dilute mydriatic preparations will give a physiological response equal to that of the frequently used more concentrated dilating solution composed of 10% phenylephrine HCl and 1% cyclopentolate HCl:

1. If the administration of a drop dose of each of two separate solutions presents no problem, it is recommended that one drop of sterile, 2.6% aqueous sodium borate solution be first instilled in the eye, followed immediately by one drop of a sterile, aqueous, combination solution of 1% phenylephrine HCl and 0.1% cyclopentolate HCl. This is the so-called prebuffering technique.

2. If it is desired to have the total medication in a single drop, as it might be for children, one drop of a special solution should be instilled which is prepared by mixing together equal volumes of the aqueous mydriatic and viscous buffer solutions not more than 8 hr. prior to their use. The buffer solution is composed of double concentrations of sodium borate, 5.2%, and hydroxypropyl methylcellulose, 1.0%, and has been sterilized in an autoclave. The mydriatic solution consists of double concentrations of phenylephrine HCl, 2%, and cyclopentolate HCl, 0.2%, and has been sterilized by filtration. Equal volumes of these two solutions, each in a sterile bottle, are mixed by pouring the viscolizer and buffer solution into the dropper bottle containing the mydriatic solution and shaking the contents well for a few seconds to effect complete mixing. The resulting solution will have the recommended concentration of 1.0% phenylephrine HCl, 0.1% cyclopentolate HCl, 0.5% hydroxypropyl methylcellulose, and 2.6% sodium borate, and it should not be used more than 8 hr. after being mixed.

While the use of the buffered solution has the advantage of requiring the instillation of only a single drop, it also has the obvious disadvantage of requiring that the two separate solutions must be mixed together daily since the mixture is only stable for 1 day. Also, when using the prebuffer technique, if there is any excess buffer solution on the cornea or lids following its instillation, it should be wiped off gently with tissue before instillation of the mydriatic drops. Otherwise, due to the occasional limiting capacity of the cul-de-sac of the eye, a portion of the medicinal drop will flow out from the eye and down the cheek. The partial loss of the drop will cause a deficient response. Use of the preparation with both medicament and buffer combined in one solution will avoid this disadvantage.

For use on races other than Caucasian, it is recommended for either buffering method that a repeat instillation be made about 15 min. after the first one to ensure adequate dilation.

Duration of Activity and Decreased Side Effects—All of the dilute mydriatic solutions used with prebuffer or with both buffer and viscolizer studied in this investigation gave mydriatic effects which were of considerably shorter duration than those given by the more concentrated dilating solutions used without buffer, although the duration of action of the various solutions is not being specifically reported in these results. Since it has been found that angle closure glaucoma can be precipitated by dilating the pupil (5), the shorter duration of mydriasis should be a decided advantage for using a dilute mydriatic solution with a buffer rather than a nonbuffered, more concentrated one.

Carpenter (6) states that cyclopentolate in its usual and more concentrated dosage has been found to produce disorientation, hallucinations, apprehension, and cerebral disfunction in some cases, lasting for as long as 11 months in one instance. Another report (7) concludes that cyclopentolate is a strong enough hallucinogen to produce symptoms similar to those of LSD in some persons. Since an appreciable amount of any instilled eye drops descends through the nasolacrimal duct into the nasopharynx and is eventually swallowed or absorbed through the pharyngeal mucosa, a 10-fold less concentration of any therapeutic agent instilled into the eye—if it produces an acceptable therapeutic response—should have the added advantage of producing considerably less side effects.

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Interactions of Drugs with Proteins II: Experimental Methods, Treatment of Experimental Data, and Thermodynamics of Binding Reactions of Thymoleptic Drugs and Model Dyes

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Abstract □ The binding to bovine albumin of the model dyes and drugs—bromocresol green, eosin, imipramine, and desipramine—has been studied using equilibrium dialysis, ultracentrifuge sedimentation, and difference spectrophotometry. An improved apparatus for equilibrium dialysis has been developed. Bromocresol green interacts with two types of binding sites: four ligands are bound by H-bonds, electron-donator-acceptor (and possibly hydrophobic) forces stabilized by electrostatic forces, and three to four ligands are bound by electrostatic forces only. Eosin is bound by van der Waals' forces and electron-donator-acceptor forces to three binding sites and by electrostatic forces to six binding sites. Imipramine interacts with only one type of binding site by van der Waals' and possibly hydrophobic forces, stabilized by dipole-dipole forces and involving tyrosyl residues. There are $n = 6$ binding sites, and the intrinsic association constant $k = 5 \times 10^3$ M^{-1}. Desipramine binding exhibits a more complicated mechanism, probably involving exposure of additional binding sites upon a drug-induced conformational change. Given concentrations of drug and protein yield the free-drug concentration and degree of binding as experimental values. From these the following parameters for each type of binding site have been determined by computer: n, k, free-enthalpy change, enthalpy change, and entropy change. From these parameters, as well as from spectral shifts and dependence on pH, ionic strength, and temperature, the modes and forces of interaction have been deduced according to previously discussed binding models and methods. Identical results are obtained by dialysis and ultracentrifugation if pH, ionic strength, and temperature are kept constant.

Keyphrases □ Plasma protein-drugs—interactions □ Thymoleptics, dyes—bovine albumin binding □ Dyes, thymoleptics—thermodynamics, bovine serum binding □ Thermodynamics—drugs-albumin binding □ Dialysis, equilibrium—albumin binding □ UV spectrophotometry—analysis

It is generally assumed that a small molecule interacting with a biopolymer induces a conformational change which is responsible for the action of the small molecule (drug). Similar interactions or binding of drugs also occur with biopolymers not involved in pharmacological action, *i.e.*, with unspecific receptors such as plasma proteins.

The binding of a small molecule can influence the chemical reactivity at different sites of the macromolecule. These phenomena are mainly due to long-range electrostatic forces, to shorter range specific interactions such as hydrogen and hydrophobic bonds (1), and finally to proton dispersion forces; the latter obey the same laws as London dispersion forces (2). In addition, primary drug–protein complexes are often stabilized by charge transfer forces. These forces, however, should not be used to estimate the overall complex stability, since the interactions are mainly due to van der Waals–London forces. The stability of a drug–protein complex is expressed by its association constant, which is also important for the pharmacokinetic behavior of the drug.

Numerous methods are currently used for the study of drug–protein interactions (3). Thermodynamic methods and optical rotatory dispersion (ORD) or circular dichroism measurements are tools for the detection of drug-induced conformational changes of a biopolymer. Equilibrium dialysis and ultracentrifugation, as well as spectroscopy in the visible and UV range, allow the determination of association constants. The resulting energy and entropy effects are useful parameters for the interpretation of the mechanism of interaction. Difference spectra in the 220–310-mμ range yield information on conformational changes in the environment of phenylalanyl, tyrosyl, and tryptophyl residues. High resolution NMR spectroscopy is a powerful tool for the study of primary binding sites and of drug atoms interacting with the macromolecule. Information on the mobility of the interacting groups can, in this way, be obtained by the determination of relaxation times.

In this paper, the authors present the results of experiments with triphenylmethane dyes and bovine albumin designed to test methods used in the study of drug–protein interactions. Some of these methods were used in the earlier study of tricyclic thymoleptic drugs (4). This paper also contains additional data on the thermodynamics and binding mechanism of the drugs mentioned.