Method accuracy was checked by analyzing some samples with the GLC method and with a previously described UV method (17). The results were plotted against each other and correlation analysis revealed a correlation coefficient, r, of 0.9960 (regression line: $y = 1.028x + 0.203$).

Plasma samples collected at different time intervals from six cancer patients to whom 1 g of fluorouracil had been administered intravenously were analyzed. Control runs with plasma blanks, collected before the drug administration, showed no major interfering peaks eluting in the regions corresponding to the drug or to the internal standard.

Figure 2 shows data obtained from three representative patients. In all cases, the fluorouracil concentration leveled off rapidly, with no measurable plasma levels occurring after 60 min and for three patients after 90 min. After an extremely short distribution phase, the drug appears to be eliminated by a logarithmic linear phase. The applicability of the method to these patients indicates that the procedure is suitable for clinical pharmacological studies on fluorouracil.

REFERENCES

(1) F. J. Ansfield, *J. Am. Med. Assoc.*, **190**, 686 (1964).

(2) E. M. Jacobs, W. J. Reeves, D. A. Wood, R. Pugh, J. Braunwald, and J. R. Bateman, *Cancer*, **27**, 1302 (1971).

(3) S. R. Lahiri, G. Boileau, and T. C. Hall, *ibid.*, **28**, 902 (1971).

(4) F. J. Ansfield, J. Klotz, T. Nealon, G. Ramirez, J. Minton, G. Hill, W. Wilson, H. Davis, and G. Cornell, *ibid.*, **39**, 34 (1977).

(5) B. Clarkson, A. O'Connor, L. Winston, and D. Hutchison, *Clin. Pharmacol. Ther.*, **5**, 581 (1964).

(6) E. R. Garrett, G. H. Hurst, and J. R. Green, *J. Pharm. Sci.*, **66**, 1422 (1977).

(7) J. J. Windheuser, J. L. Sutter, and E. Auen, *ibid.*, **61**, 301 (1972).

(8) J. L. Cohen and P. B. Brenan, *ibid.*, **62**, 572 (1973).

(9) H. W. Van Den Berg, R. F. Murphy, R. Hunter, and D. T. Elmore, *J. Chromatogr.*, **145**, 311 (1978).

(10) C. Finn and W. Sadée, *Cancer Chemother. Rep.*, **59**, 279 (1975).

(11) B. L. Hillcoat, M. Kawai, P. B. McCulloch, J. Rosenfeld, and C. K. O. Williams, *Br. J. Clin. Pharmacol.*, **3**, 135 (1976).

(12) A. P. De Leenheer, R. R. Roncucci, and C. Van Peteghem, "Quantitative Mass Spectrometry in Life Sciences II, Proceedings of the Second International Symposium on Quantitative Mass Spectrometry in Life Sciences" (Gent, June 13–16, 1978), Elsevier, New York, N.Y., 1978, p. 399.

(13) T. J. Giovanniello and J. Pecci, *Clin. Chim. Acta*, **67**, 7 (1976).

(14) A. P. De Leenheer and C. F. Gelijkens, *J. Pharm. Sci.*, **67**, 417 (1978).

(15) A. P. De Leenheer and C. F. Gelijkens, *J. Chromatogr. Sci.*, **16**, 552 (1978).

(16) A. W. Hofmann, *Ber. Dsch. Chem. Ges.*, **14**, 494 (1881).

(17) A. P. De Leenheer, M. C. Cosyns-Duyck, and P. M. Van Vaerenbergh, *J. Pharm. Sci.*, **66**, 1190 (1977).

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Influence of Cetylpyridinium Chloride on Corneal Permeability to Penicillin

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Received January 12, 1979, from the **Department of Ophthalmology and the* ‡*Department of Physiology, Medical College of Georgia, Augusta, GA 30901.* Accepted for publication March 14, 1979.

Abstract □ The epithelial surface or the deepithelialized anterior stromal surface of isolated rabbit corneas was perfused for 3 hr with ^{14}C-penicillin in 25 m*M* Ringer–bicarbonate solution with or without 1% albumin and with or without 0.02% cetylpyridinium chloride. The intact epithelium acted as a barrier to penicillin and impeded the flux rate by 66% when compared to the flux rate across the deepithelialized cornea. The presence of 0.02% cetylpyridinium chloride increased the penicillin flux rate across corneas with an intact epithelial layer to that of deepithelialized corneas. Cetylpyridinium chloride, 0.02%, had no effect on penicillin flux across deepithelialized corneas. The penicillin flux rate across corneas, with or without epithelium, was increased slightly following the inclusion of 1.0% albumin in the bathing solution. The flux rates across deepithelialized corneas in the presence of albumin, with or without cetylpyridinium chloride, were similar to fluxes found in the absence of albumin. Albumin–penicillin "binding" was not a significant factor in impeding penicillin flux, and this binding apparently was not altered by cetylpyridinium chloride. The surfactant appeared to alter epithelial permeability physiologically.

Keyphrases □ Cetylpyridinium chloride—effect on corneal permeability to penicillin, effect on epithelium □ Surfactants—cetylpyridinium chloride, effect on corneal permeability to penicillin, effect on epithelium □ Benzylpenicillin—corneal permeability, effect of cetylpyridinium chloride

The penetration efficiency of most topically applied drugs is very small, but penicillin penetration is especially poor (1, 2). Poor intraocular drug levels have been attributed (3) both to rapid drug removal by tears and to drug binding to the tear protein, thus rendering the drug biologically unavailable. Inclusion of 0.02% cetylpyridinium chloride with pilocarpine nitrate when applied topically to the rabbit eye caused 10 times the miotic effect of the same amount of pilocarpine without cetylpyridinium chloride. Previous investigators (4) hypothesized that cetylpyridinium chloride was a competitive inhibitor of pilocarpine protein binding and that the cetylpyridinium chloride allowed a higher percentage of drug to be unbound and bioavailable.

Cetylpyridinium chloride, when applied to the cornea, increased fluorescein penetration across the cornea; transmission electron microscopy revealed increased intercellular spaces in the superficial epithelial layer and lysis of the outermost cell membranes (5). Scanning electron microscopy revealed a loss of epithelial microvilli and microplicae, a process accompanied by surface pitting, which exposed deeper epithelial cells (6).

The purpose of this investigation was to delineate further the mechanism of cetylpyridinium chloride in increasing drug penetration into the eye by examining its effects on the corneal flux rate of a known albumin-bound drug.

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Table I—Penicillin Fluxes across Rabbit Cornea [a]

Group I	
Intact epithelium (n = 6, 36)	$0.043 \pm 0.001^{b,c}$
Deepithelialized (n = 6, 36)	$0.126 \pm 0.004^{b,c}$
Group II	
Intact epithelium (n = 5, 30)	$0.053 \pm 0.004^{b,c}$
Deepithelialized (n = 5, 30)	$0.179 \pm 0.016^{b,c}$
Group III	
Intact epithelium (n = 5, 30)	0.130 ± 0.005
Deepithelialized (n = 5, 30)	0.130 ± 0.005
Group IV	
Intact epithelium (n = 6, 36)	0.123 ± 0.004
Deepithelialized (n = 6, 36)	0.133 ± 0.004

[a] Values (10^{-9} mole/cm^2/hr) are the means ± *SEM*; n = number of corneas, number of experimental determinations. [b] $p < 0.001$, comparing flux rate of corneas with epithelium intact to flux rate of deepithelialized corneas within a given group. [c] $p < 0.05$, comparing flux rate of epithelialized corneas in the presence and absence of albumin (*cf.*, Groups I and II); also comparing flux rate of deepithelialized corneas in the presence and absence of albumin (*cf.*, Groups I and II).

EXPERIMENTAL

Forty-four adult albino rabbits of either sex, ~3 kg, were sacrificed with an overdose of pentobarbital sodium. Each eye was proptosed, and the epithelium was removed from one eye by scraping with a Gill corneal knife. The epithelium of the opposite eye was left intact and protected from abrasions during mounting. The corneas were removed and mounted in corneal flux chambers, using the specular microscope mounting system; this system permits the mounting of corneas without trauma to the corneal epithelium (7, 8).

Ringer–bicarbonate (2.5×10^{-2} *M*) solution (pH 7.35) containing reduced glutathione (0.092 g/liter) and adenosine (0.134 g/liter) with an osmolarity of 300 mOsm bathed both corneal surfaces (7–10). The volume of the endothelial (posterior) chamber was 0.45 ml, and that of the stromal or epithelial (anterior) chamber was 1.20 ml. The anterior chamber contained a polytef-coated magnetic stirring bar driven by an external magnet at 400 rpm.

Four epithelial surface perfusion solutions containing ^{14}C-penicillin G potassium[1] were prepared in Ringer solution to make a final concentration of 8×10^{-9} *M* ^{14}C-penicillin/ml. Group I corneas were perfused with penicillin in Ringer solution. Group II corneas were perfused with penicillin and 1% bovine serum albumin in Ringer solution; the mixture was stirred with a magnetic bar for 30 min prior to instillation into the flux chamber to allow maximal penicillin protein binding to occur (11).

Group III corneas were perfused with a Ringer solution containing penicillin, 1% bovine serum albumin, and 0.02% cetylpyridinium chloride (also stirred for 30 min prior to use). The penicillin and cetylpyridinium chloride were added simultaneously to allow any competitive inhibition of the protein binding sites by cetylpyridinium chloride to occur. Group IV corneas were perfused with penicillin and 0.02% cetylpyridinium chloride in Ringer solution.

All groups contained paired corneas, one epithelialized and the other deepithelialized. The endothelial surface was bathed with normal Ringer solution at all times.

The anterior flux chamber was filled with one of the radioactive penicillin solutions (Group I, II, III, or IV) immediately after mounting and allowed to equilibrate for 1 hr. Sample collection and quantitation of the penicillin flux by a liquid scintillation technique were performed as described previously (7) every 30 min over an additional 3-hr period, thus providing six sample periods for each cornea.

RESULTS

In Group I, the penicillin flux across deepithelialized corneas was three times greater than across corneas with an intact epithelium, indicating that the intact epithelial membrane provided a very significant barrier, $p < 0.001$ (Table I). This finding was confirmed by the data for Group II in the presence of 1% bovine serum albumin. In Group II, a small but significant increase in penicillin flux across both epithelialized and deepithelialized corneas also was noted in the presence of 1% bovine serum albumin when compared to the respective epithelialized and deepithelialized corneas in Group I that were perfused without albumin, $p < 0.05$ (Table I).

Penicillin penetration was the same for corneas in the presence or absence of epithelium when cetylpyridinium chloride was present (Groups III and IV). Cetylpyridinium chloride increased the penicillin flux across epithelialized corneas so that it equaled the flux across untreated deepithelialized corneas (Groups I and IV). This finding demonstrated that cetylpyridinium chloride converted the cornea with an intact epithelium into a physiologically deepithelialized cornea. The presence or absence of albumin made no difference in the cetylpyridinium chloride-evoked response (Table I, Groups I and IV *versus* Groups II and III).

Comparison of penicillin fluxes in Groups I and II reveals some puzzling trends. In both the epithelialized and deepithelialized corneas, penicillin penetration was increased by approximately one-fourth ($p < 0.05$) by the presence of 1% albumin. Such a difference was not noted between Groups III and IV, where again the only difference was the presence or absence of albumin.

DISCUSSION

The corneal epithelial barrier impeded the penicillin flux rate to one-third that of the flux rate across deepithelialized corneas. Cetylpyridinium chloride chemically converted the penetration characteristics of the epithelialized cornea into those of a deepithelialized cornea; *i.e.*, it increased its permeability so that both deepithelialized corneas and cetylpyridinium chloride-perfused epithelialized corneas showed equal penicillin fluxes.

An unexplained finding was that the penicillin flux significantly increased when 1% bovine serum albumin was present in the solutions perfusing the anterior corneal surface. Albumin apparently enhances the corneal permeability (across both the normal and deepithelialized corneas) in the absence of cetylpyridinium chloride, despite the known 60–65% binding of penicillin by albumin (11). Such a difference was not noted in the presence of cetylpyridinium chloride. The reason for this albumin-enhanced permeability is unknown.

The penicillin fluxes across deepithelialized corneas (Groups I and II) and cetylpyridinium chloride-treated normal and deepithelialized corneas (Groups III and IV), with and without bovine serum albumin, were of the same general magnitude. These results illustrate that cetylpyridinium chloride increases corneal permeability to penicillin by its action on the epithelium and that albumin does not retard penicillin flux across the cornea. The permeability increase induced by cetylpyridinium chloride in the normal cornea is similar to that found in the deepithelialized cornea. There was no indication of either an increased or decreased penicillin protein binding caused by cetylpyridinium chloride.

These findings are in contrast to the findings of Mikkelson *et al.* (3, 4), especially to their hypothesis that competitive inhibition of protein binding of drugs by cetylpyridinium chloride makes more drug bioavailable in the unbound form. The current experiments confirm the concept that cetylpyridinium chloride increases drug penetration by reducing the epithelial barrier effect. In view of the present findings and those noted previously that indicated an effect of cetylpyridinium chloride on the epithelial ultrastructure (5, 6), it is not surprising that drugs placed on the surface of the eye in the presence of cetylpyridinium chloride would penetrate the cornea better and have a greater pharmacological effect.

In summary, cetylpyridinium chloride apparently enhances penicillin penetration across the cornea in a manner similar to that found for benzalkonium chloride both *in vitro* and *in vivo* (5, 12, 13); there is a direct effect on the epithelium, which causes disorganization of the normal structure and breakdown of the physiological barrier.

[1] Amersham Corp., Arlington Heights, Ill.

REFERENCES

(1) W. H. Havener, "Ocular Pharmacology," 3rd ed., Mosby, St. Louis, Mo., 1974.

(2) S. H. Witzel, I. Z. Fielding, and H. L. Ormsby, *Am. J. Ophthalmol.*, **42**, 89 (1956).

(3) T. J. Mikkelson, S. S. Chrai, and J. R. Robinson, *J. Pharm. Sci.*, **62**, 1648 (1973).

(4) *Ibid.*, **62**, 1942 (1973).

(5) K. Green and A. M. Tønjum, *Am. J. Ophthalmol.*, **72**, 897 (1971).

(6) K. Green, *Acta Ophthalmol.*, **54**, 145 (1976).

(7) D. S. Hull, K. Green, M. Boyd, and H. R. Wynn, *Invest. Ophthalmol.*, **16**, 883 (1977).

(8) S. Dikstein and D. M. Maurice, *J. Physiol.*, **221**, 29 (1972).

(9) E. I. Anderson, J. Fischbarg, and A. Spector, *Exp. Eye Res.*, **19**,

1 (1973).
(10) B. E. McCarey, H. F. Edelhauser, and D. L. Van Horn, *Invest. Ophthalmol.*, **12**, 410 (1973).
(11) "The Pharmacological Basis of Therapeutics," 5th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N.Y., 1975.
(12) K. Green and S. Downs, *Invest. Ophthalmol.*, **13**, 316 (1974).
(13) K. Green and A. M. Tønjum, *Acta Ophthalmol.*, **53**, 348 (1975).

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Renal Actions of Oxyphenbutazone

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Abstract □ Oxyphenbutazone decreased the renal excretion of sodium and water in anesthetized dogs. As these excretions decreased, the drug also produced a decrease in renal blood flow and in the glomerular filtration rate. Blood pressure increased slightly. These changes are consistent with an inhibition of renal prostaglandin synthesis and could explain why oxyphenbutazone is reported to produce weight gain and edema when used clinically.

Keyphrases □ Oxyphenbutazone—effect on renal sodium and water excretion, renal blood flow, glomerular filtration rate, blood pressure, prostaglandin synthesis ■ Anti-inflammatory agents—oxyphenbutazone, renal effects ■ Kidney—effect of oxyphenbutazone

Oxyphenbutazone[1] is an analog of phenylbutazone[2] and, like the latter compound, is employed as an anti-inflammatory agent. Both agents produce edema and weight gain as side effects (1). A possible renal mechanism has been suggested for phenylbutazone since this agent markedly reduced renal blood flow and decreased the glomerular filtration rate at the time that urinary volume and sodium excretion were reduced (2). In this study, the effect of oxyphenbutazone on several renal functions was determined.

EXPERIMENTAL

Mongrel dogs of either sex, 13–16 kg, were anesthetized with pentobarbital sodium (30 mg/kg iv), and the trachea was intubated to maintain an open airway. Mean arterial blood pressure was monitored with a pressure transducer[3] *via* a femoral artery catheter. A femoral vein was catheterized for administration of a 0.9% NaCl infusion (4 ml/min) during preparation of the animals and throughout the experiments. Inulin was added to the infusion to estimate the glomerular filtration rate.

The left kidney was exposed *via* a retroperitoneal flank incision, and a flow probe was placed around the renal artery and connected to a square wave electromagnetic flowmeter[4] to monitor renal blood flow. Blood pressure and renal blood flow were recorded[5] continuously. Urine was collected at timed intervals by a cannula placed in the left ureter. Arterial blood samples were obtained from the femoral artery catheter. After stabilization of the preparation, control urine collections (10-min periods) and blood samples at the midpoint of alternate urine collection periods were obtained. Oxyphenbutazone was administered intravenously over 3 min, and urine collections were continued for several periods.

[1] Tanderil, Ciba-Geigy Corp., Summit, N.J.
[2] Butazolidin, Ciba-Geigy Corp., Summit, N.J.
[3] Model P23AA, Statham, Hato Rey, Puerto Rico.
[4] Carolina Medical Electronics, King, N.C.
[5] Dynograph (type R), Beckman Instruments, Fullerton, Calif.

Table I—Effect of Oxyphenbutazone (5 mg/kg iv) on Renal Function

Parameter	Control Period[a]	Oxyphenbutazone Period[b]	Difference
Blood pressure, mm Hg	115	128	+13 ± 5[c]
Renal blood flow, ml/min	262	220	−42 ± 5[c]
Urine volume, ml/min	1.1	0.4	−0.7 ± 0.2[c]
Glomerular filtration rate, ml/min	33	23	−10 ± 4[c]
Sodium excretion, μEq/min	136	77	−59 ± 1.9[c]

[a] Values are from the clearance period preceding oxyphenbutazone administration. [b] Values are from the clearance period 10–20 min after oxyphenbutazone administration. [c] Significant difference ($p < 0.05$); Student paired t test; $n = 7$.

Urinary and plasma inulin levels were determined by a reported method (3). Urinary sodium concentrations were measured with a flame photometer[6]. Data were analyzed using the Student paired t test (4); a $p < 0.05$ was the significance criterion.

RESULTS AND DISCUSSION

The effect of oxyphenbutazone on renal blood flow and blood pressure is shown in Fig. 1. Following oxyphenbutazone administration, 5 mg/kg iv, blood pressure increased slightly while renal blood flow decreased gradually. These effects, as well as effects on other renal functions, are summarized in Table I. At 20 min after oxyphenbutazone administration, blood pressure was increased while renal blood flow, the glomerular filtration rate, urinary volume, and sodium excretion were decreased. All changes were statistically significant. These changes also were present at 60 min.

Since similar changes were reported with phenylbutazone (2) and since both agents are similar structurally, it seems reasonable that these two agents act by the same mechanism. Both oxyphenbutazone and phenylbutazone have been reported to be inhibitors of prostaglandin syn-

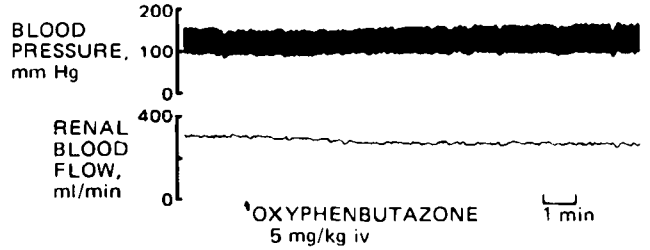

Figure 1—*Effect of oxyphenbutazone, 5 mg/kg iv, on blood pressure and renal blood flow.*

[6] Instrumentation Laboratories, Lexington, Mass.

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