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Keyphrases

Biopharmaceutics, drugs–parenteral
Drug administration—subcutaneous, intramuscular
Endothelial tissue—drug absorption
Diffusion—drug absorption
Phagocytosis—drug absorption
Biological factors—drug absorption
Drug formulations—absorption, biological factors

Research Articles

Procaine Interaction with the Corneal Surface and its Relation to Anesthesia

By VICTOR F. SMOLEN and FREDERICK P. SIEGEL*

The effects of pH and procaine on the apparent density of charged groups fixed to the corneal surface of guinea pigs were studied. The net density of fixed charge was found to be negative over the pH range of 5 to 9 and decreased in the presence of procaine. These results are interpreted in terms of procaine interaction with the surface and its implications with regard to the factors that may influence the characteristics of the anesthetic response. The electrometric method employed allowed the study to be performed under physiological conditions using live animals. The method is general and can be equally applied to study other drug–tissue interactions.

THE CORNEA possesses a moist surface primarily composed of amphoteric colloids; pH and the interaction of solutes determine the extent to which attached groups are ionized and the resulting net density of fixed charge on the surface. The present study is an effort to elucidate the role of these factors in the mechanism by which the effectiveness of procaine in the eye is increased in alkaline solution (1–4).

An electrometric method modified from Joseph *et al.* (5, 6) was used to determine the apparent fixed charge density of the corneal surface of the guinea pig in response to variations in the pH of applied buffer solutions in the presence and absence of procaine. The cornea is particularly suited to this approach due to its relative simplicity and accessibility. It has also been the subject of many investigations with local anesthetics. Reliable pharmacological data particularly with procaine were found to be readily available (1). The method is generally applicable to the study of other tissue surfaces and other drugs as well. It allows the results to be obtained under nearly physiological conditions without damage to the tissues involved. Thus, in the present study, the apparent density of fixed charge of the corneal surface was determined under the same conditions that are known to influence its anesthetic response to procaine.

THEORETICAL

The dissociation of counterions from ionogenic groups covalently bonded to a tissue surface or the absorption of ions onto neutral surface sites gives

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Hg	Hg_2Cl_2	Saturated KCl_1	Reference solution (0.15 *M* NaCl)	Reference surface (hind foot of guinea pig)	Ⓟ Experimental surface (guinea pig cornea)	Experimental solution or its 10-fold dilution	Saturated KCl	Hg_2Cl_2	Hg

Fig. 1—Circuit diagram corresponding to the experimental arrangement depicted in Fig. 2. Ⓟ represents the potentiometer.

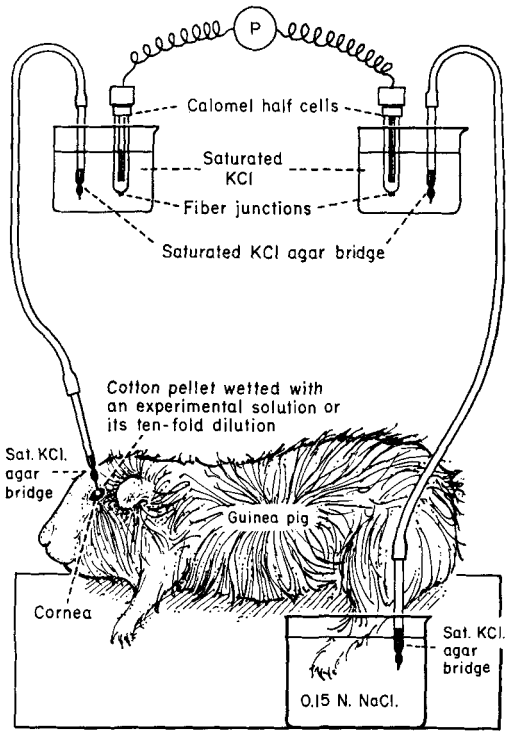

Fig. 2—Diagram of the experimental arrangement for the determination of the fixed charge density of the guinea pig cornea. Ⓟ represents the potentiometer.

rise to an unequal distribution of free ions present at the surface as compared to the bulk of a solution with which it is in contact. An electrical potential difference also develops across the colloid-aqueous boundary. The magnitude of the distribution ratios (r) for the mobile ions, present at the colloid surface as compared to the bulk of the applied solution, as well as the sign and magnitude of the electrical potential, depend upon the net density of the charge fixed to the tissue surface. The measurement of a potential, E_1, includes this equilibrium phase boundary potential as well as all other potentials developed in the circuit (see Figs. 1 and 2 for a circuit diagram and the experimental arrangement, respectively). These extraneous potentials have been found to remain constant for the short period of time required to replace the initial solution in contact with the tissue surface with a dilution of this same solution followed by the immediate recording of a second potential, E_2. The difference of these two measured potentials ($E_2 - E_1$), termed the dilution potential E_d, is thus devoid of the extraneous potentials and includes only those potential differences developed between the aqueous medium at the tissue surface and the bulk of the applied solutions. This consideration allows the interpretation of E_d in terms of the familiar Donnan equilibrium (10, 11) and its treatment as a diffusion potential (5). Under the conditions of its measurement, E_d is characteristic only of the tissue surface rather than reflecting the properties of the bulk or interior of the tissue phase. If this were not the case, some question might be raised regarding the physical significance of the measurements (11).

Following an approach similar to that of Joseph *et al.* (5) the principal author has derived equations (Eqs. 1 and 2 below) for E_d and the fixed charge density (f) (12). These equations are presented in their general form.

Equation 1 expresses E_d in terms of a ratio (r) defined as the ratio of the concentration (m_{i+c} or m_{i-c}) of an i'th cation or anion at the charged colloid surface to its concentration in the bulk of the applied solution (m_{i+a} or m_{i-a}), raised to a power equal to the reciprocal of its valence (z_{i+}) for cations, and the negative of the reciprocal of its valence (z_{i-}) for anions. The value of r, obtained from the solution of Eq. 1, is substituted into Eq. 2 from which the fixed charge density (f) is obtained. In Eq. 1 the symbols λ^0_{i+} and λ^0_{i-} denote the limiting ionic conductances of the i'th cation and anion, respectively, while D is the factor by which the experimental solution is diluted for the measurement of E_2. In the present study its value is 10. The symbol F refers to the Faraday constant; R is the gas constant and T is the absolute temperature.

Equation 1 is derived on the basis of considering the Gibbs free energy changes accompanying the transfer of charged species between the colloid surface and the bulk of the solution and expressing the transport numbers for the ions in terms of Kohlrausch's law of independent ion migration (13). Equation 1 resembles equations describing liquid junction potentials (13) which in effect it does when applied to the measurements described in this study. Values of E_d calculated from Eq. 1 agree closely with values calculated from Henderson's equation (13), which describes a liquid junction potential developed across a continuous mixture boundary. Equation 2 is merely an expression of macroscopic electroneutrality at the tissue surface.

The described electrometric method is of general applicability. It has been verified quantitatively with wool (6); the results obtained were comparable

$$E_d = \left[\frac{\sum\left(r^{z}_{i+}\cdot m_{i+a}\cdot\lambda^0_{i+}\right) - \sum\left(\frac{m_{i-a}}{r^{z}_{i-}}\lambda^0_{i-}\right)}{\sum\left(z_{i+}\cdot r^{z}_{i+}\cdot m_{i-a}\cdot\lambda^0_{i+}\right) + \sum\left(\frac{z_{i-}\cdot m_{i-a}}{r^{z}_{i-}}\lambda^0_{i-}\right)}\right]\frac{2.303RT}{F}\log D \qquad \text{(Eq. 1)}$$

$$f = \sum\frac{z_{i-}m_{i-a}}{r^{z}_{i-}} - \sum z_{i+}\cdot r^{z}_{i+}\cdot m_{i-a} \qquad \text{(Eq. 2)}$$

to titration curves of wool obtained directly (22). When the method is used *in vivo*, it conveniently and rapidly yields results not readily attainable by other means.

MATERIALS AND METHODS

Materials—All reagents were of analytical grade, except procaine hydrochloride USP.[1] Male guinea pigs of approximately 3 months of age were used.

All electrical potentials were read from a Sargent model SR recorder. Saturated calomel electrodes[2] were inserted together with KCl-agar salt bridges into 100-ml. beakers containing saturated KCl. The salt bridges consisted of 3–4 ft. lengths of transparent tygon tubing filled with saturated KCl solution. The ends were blocked with 6-in. lengths of glass tubing drawn at one end into a capillary filled with hardened KCl-agar gel and constricted in the middle to prevent loosening of the gel and subsequent leakage. The transparency of the tubing allowed ready detection of air bubbles which can profoundly interfere with conductance through the bridges.

Solutions—The composition of the solutions and the pH range selected for this study were chosen so that the results could be compared with those of Hiter (7) on the duration of corneal anesthesia. However, the pH 9.0 borate buffered solutions used by Hiter were found to give anomalous results in that observed and calculated diffusion potentials differed widely. As a consequence, 0.1 *M* phosphate buffers prepared from KH_2PO_4 and NaOH were used throughout the pH range of 5–9. The procaine solutions were always freshly prepared at the time of the experiment. Each buffered procaine solution below pH 9 contained 1% (36.7 m*M*) procaine hydrochloride. The pH 9.0 solution was saturated and the total concentration of procaine was calculated to be 4.35 m*M*. The calculation was based on the solubility of the free base given by Eisenbrand and Picher (8) as 0.13%, and from the degree of association, 44.2%, based on an acidic pK of 8.85 (9). An aliquot of each of the solutions was diluted 10-fold for use in the measurement of the potentials E_2 as described below.

The pH of the solutions was determined with a Beckman model 76 pH meter. The concentrations of the ionic species in each solution were calculated from the measured pH and the pK's of the dissociable substances (9).

Procedure—The position of the guinea pig in the experimental set-up is illustrated in Fig. 2. Figure 1 is a corresponding circuit diagram. A saturated KCl salt bridge was placed in a 0.15 *N* NaCl solution along with an immobilized shaved hind leg of the guinea pig which served as the reference surface. The animals were maintained immobile by the i.p. administration of 30–40 mg./Kg. pentobarbital.

The right eye of the guinea pig was used in the control study. The buffer solutions containing procaine were applied to the left eye. This order was reversed and alternated in subsequent experiments with the same animals which were allowed a minimum recovery time of 7 days. Animals observed to develop any abnormalities during this time were discontinued from further use.

The experimental procedure adopted for routine use involved a preliminary rinsing of the eye with isotonic NaCl solution to remove any secreted film on the cornea. This was followed by rinsing with the experimental solution to remove any solution remaining from a previous determination or treatment. Excess solution was removed by absorption into small strips of filter paper wick and gentle swabbing with dry cotton. A cotton dental pellet with a diameter less than the cornea was placed on the capillary end of a KCl-agar salt bridge and moistened with the experimental solution. The pellet was then carefully placed in contact with the cornea and the potential, E_1, was recorded for approximately 30 sec. The pellet was removed and the cornea swabbed lightly with dry cotton. A similar but smaller pellet was wetted with a 10-fold dilution of the previous solution and placed in the center of the area previously covered. A second potential, E_2, was recorded *immediately* upon contact. This procedure was repeated for all other pH values studied in a series. In developing this procedure to yield reproducible results, it was found necessary to carefully control the amount of solution applied to the cotton pellets and to prevent it from overflowing onto surrounding tissues by removing any excess onto filter paper wicks or cotton swabs. In more recent work the principal author has found that replacing the cotton pellets with solution wetted filter paper strips applied to the cornea with a micromanipulator, gave improved control and reproducibility.

The moist hydrated surface of the cornea allows diffusion and mixing of the solutions to readily occur at the surface causing initial changes in recorded potentials. The application of a procaine solution to a previously untreated cornea produces potentials (E_1) which approached relative constancy within approximately 15 sec. and remained at nearly the same value until the boundary was disturbed indicating the establishment of equilibrium at the surface. The application of diluted solutions may cause variations in recorded potential lasting up to several minutes, however in this case the initial value (E_2) is of interest. The effects of procaine were diminished or obliterated by rinsing the eye with isotonic NaCl or one of the control solutions indicating its effects are reversible.

RESULTS

Verification of Eq. 1—Equations for E_d and f specifically applicable to the solutions used in the present study were obtained by substituting appropriate values into Eqs. 1 and 2. The limiting ionic conductances of the various ions present were obtained from the literature (9, 13). The ionic conductance of procaine cation was experimentally determined by the measurement of liquid junction potentials (13). The accuracy of the resulting equations in describing the dilution potentials corresponding to each solution and its 10-fold dilution was experimentally checked by the measurement of diffusion potentials. Free diffusion-type boundaries were formed either within a capillary or by means of a filter paper bridge. The liquid junction potentials were found to be nearly inde-

[1] Supplied by Abbott Laboratories.
[2] Beckman fiber junction type calomel reference electrodes.

TABLE I—COMPARISON OF EXPERIMENTALLY OBSERVED DIFFUSION POTENTIALS TO VALUES CALCULATED FROM EQ. 1

pH of Solution	Diffusion Potential Calculated, mv.	Diffusion Potential Obs., mv.	pH of Solution	Diffusion Potential Calculated, mv.	Diffusion Potential Obs., mv.
	Buffer Solutions			Buffer Solutions Plus 1.0% Procaine HCl	
5.18	20.56	20.5 ± 0.6	5.18	8.26	9.0 ± 1.0
6.06	20.35	20.2 ± 0.4	6.06	8.51	9.9 ± 1.1
6.70	19.09	19.0 ± 0.8	6.84	9.29	10.6 ± 0.8
8.18	16.95	17.6 ± 0.2	7.58	10.12	10.8 ± 0.9
9.00	16.82	17.0 ± 0.5	8.15	10.21	10.8 ± 0.5
			9.00	9.53	11.1 ± 1.5

pendent of the method which was employed to form the boundary. The electrical potentials measured in this manner are of the same magnitude as the corresponding dilution potentials at zero density of fixed charge, *i.e.*, when the ratio r equals one. The averages of 10 separate determinations performed with each experimental solution are listed in columns 3 and 6 of Table I along with maximum deviations. The agreement of these results with calculated values, listed in columns 2 and 5 of Table 1, was found in most cases to be within a mv.

Results of Applying Eqs. 1 and 2 to Measurements on the Cornea—Values of r corresponding to experimental values of E_d were obtained by graphical solution of Eq. 1. Eleven calibration curves, corresponding to the 11 buffer solutions used, were constructed by plotting calculated values of E_d as a function of r over a chosen range. Values of r corresponding to experimentally observed values of E_d were obtained from these curves and checked by calculation. An average value of E_d, observed from a minimum of six different determinations using six different animals, was used in each case. These values of E_d and corresponding values of r are listed in Table II for both the control buffer solutions and the buffered procaine solutions. The corresponding values of f are listed in columns 4 and 8 in Table II. They were calculated by substituting the values of r into Eq. 2. Figure 3 contains plots of f as a function of pH. Although the control and

TABLE II—RESULTS OF ELECTROMETRIC DETERMINATIONS ON GUINEA PIG CORNEAL SURFACE

pH of Buffer Solutions	Av. Obs. Dilution Potential[a] (E_d), mv.	Ion Distribution Ratio[b] (r)	Fixed Charge Density on Corneal Surface[c] (f), meq./L.
	Control Buffer Solutions		
5.18	21.8 ± 0.9	1.020	−4.10
6.06	22.2 ± 1.0	1.035	−7.40
6.70	22.0 ± 1.5	1.054	−15.1
8.18	23.0 ± 1.7	1.095	−51.3
9.00	33.0 ± 1.3	1.300	−140.
	Buffered Procaine Solutions (1%)		
5.18	9.0 ± 1.0	1.013	−3.60
6.06	9.9 ± 1.1	1.023	−6.80
6.84	10.6 ± 0.8	1.022	−8.50
7.58	10.8 ± 0.9	1.010	−5.50
8.15	10.8 ± 0.5	1.010	−6.20
9.00	11.1 ± 1.5	1.025	−17.1

[a] E_d is the difference of two measured potentials, $E_2 - E_1$, see text. The deviations are the maximum observed differences from the mean of a minimum of 6 determinations of E_d using different animals. [b] The values of r are obtained from E_d by graphical solution of Eq. 1. [c] The values of f in columns 4 and 8 are calculated by substituting the corresponding values of r into Eq. 2.

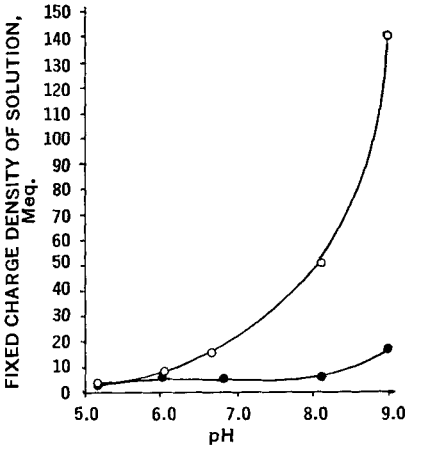

Fig. 3—Upper curve is the control titration curve of the guinea pig cornea; lower curve is the procaine displaced titration curve.

procaine displaced curves differ in shape, a negative net fixed charge density is common to each throughout the pH range studied. The isoelectric point (the pH below which the surface has a net positive charge) was found to be approximately 2.5. At normal physiological pH the anionic fixed charge density obtained from the control curve is 28 meq./L. of solution.

The procaine displaced curve lies entirely below the control curve indicating the anionic fixed charge density was decreased at every pH. The number of titratable groups is noted to be decreased by 90% in the presence of procaine. The changes at the corneal surface were generally found to be reversible as indicated by the fact that the shapes of the curves in Fig. 3 are independent of the order in which the corresponding E_d values are experimentally observed.

DISCUSSION

Interpretation of Titration Curves—The curves in Fig. 3 are analogous to titration curves observed for wool (14–15) and other insoluble proteinaceous structures (5, 6, 16, and 17). The net negative charge of the corneal surface that is apparent even at relatively low pH may in part be attributed to the strongly acidic sulfate groups of mucopolysaccharides (16). The titratable groups in the pH range of 5–9 may be assumed to be phosphatic, imidazolyl, imino, and α-amino groups. Carboxyl and sulfate groups are likely dissociated below pH 5 while ϵ-amino and phenolic groups are generally titratable above pH 9. Generally the tendency of

any group to dissociate is influenced by the composition of its medium and the state of the colloids as a whole (18, 19).

Mechanism of Procaine Effect—There are several mechanisms that could operate alone or in combination to bring about the lowering of anionic fixed charge density observed in the presence of procaine. Among them are a possible increase in the acidic pK's of the dissociable groups, an increase in the specific affinities of fixed anionic sites for binding the metal ions (Na^+ and K^+), the rupture of salt linkages between fixed groups of opposite charge followed by binding of mobile cations to the freed anionic groups, the absorption of procaine or other cations to uncharged sites on the colloids, and direct salt binding of procaine to fixed anionic sites. The complex nature of the possible interactions and the probable heterogeneity of the binding sites allows only speculation as to the relative contribution of each mechanism. However, lacking evidence to the contrary and considering that the two curves were obtained with solutions differing only in that one series contained procaine, it may be reasonably assumed that the lowering of the anionic charge density results primarily from the binding of procaine cations. Presumably the neutral species of procaine which is in equilibrium with the cationic form could also interact with the corneal surface. Its binding would not alter the net charge of the surface except perhaps indirectly by an inductive effect (18). The binding of the cationic species would be reinforced by long-range electrostatic forces which render its approach to binding sites increasingly favorable with an increasing density of negative fixed charge. Procaine cations taken up by the surface in a particular pH range need not necessarily bind to the anionic groups dissociating in that range. In fact, binding to uncharged sites on the surface would also lead to a stoichiometric decrease in anionic fixed charge density. That amine binding can occur is demonstrated by the fact that basic amino dyes such as toluidine blue are good tissue stains. Sawinski and Rapp (20) have shown that two binding sites are available for procaine on human serum albumin. Glassman (21) has concluded that long chain organic cations form a stoichiometric complex with plasma albumin. Steinhart and Zaiser (22) have also demonstrated binding of organic cations to wool and egg albumin.

In their studies of colloidal surfaces, Catchpole *et al.* (16, 17) have attributed the effect of cations on the fixed charge density of colloidal surfaces to a direct neutralization of fixed anionic groups due to salt binding. They also assumed a stoichiometric relation between the equivalents of bound cation and the observed decrease in negative fixed charge density. Adopting a similar approach to the treatment of the present results, the outermost surface of the cornea can be described to behave essentially as a cation exchanger having a variable affinity for procaine cations which is dependent upon the pH of the environment. Variations in the apparent affinities for association of procaine cations with the surface may be expected since the binding sites are probably dissimilar and thier intrinsic association affinities may be affected by the state of the colloids as a whole.

The overall equilibria pertinent to this model are shown below, where fHP, PH^+, and P refer to bound procaine, free procaine cations, and base, respectively, while f^- refers to the sites and fH to sites occupied by protons, H^+.

$$fHP \rightleftharpoons f^- + PH^+ \rightleftharpoons P + H^+ \quad \text{(Eq. 3)}$$

$$fH \rightleftharpoons f^- + H^+ \quad \text{(Eq. 4)}$$

In accordance with this scheme the extent to which procaine is bound and the accompanying decrease in fixed negative charge density depends upon the availability of anionic binding sites and procaine cations. Both factors depend upon pH. The increasing rate of lowering of fixed anionic charged density—evidenced by the widening vertical gap between the two curves in Fig. 3—with increasing pH, may in part be ascribed to the greater rate of availability of binding sites relative to the decrease in procaine cations occurring with pH.

The concentration of procaine bound to the corneal surface at each pH can be estimated from the assumed stoichiometric decrease in the negative charge density, Δf, which corresponds to the vertical difference between the two curves presented in Fig. 3. These values are listed in column 2 of Table III. The concentration of free procaine cations present at the corneal surface, $[P^+]_c$, can be calculated using the appropriate value of r and the concentration present in the applied solutions, $[P^+]_s$, *i.e.*, $[P^+]_c = r[P^+]_s$. Using these values apparent association constants, K_B, have been calculated from Eq. 5 where f' denotes the negative charge density taken from the lower, procaine, curve in Fig. 3. The values of K_B are listed in column 3 of Table III. As expected these values are not constant but increase with pH.

$$K_B = \frac{\Delta f}{f'[P^+]_s \cdot r} \quad \text{(Eq. 5)}$$

The volumes of the applied procaine solutions placed in contact with the cornea can be assumed to be relatively large in comparison to the volume of the corneal surface with which they equilibrate; the concentrations of the various components of the solutions can also be assumed to remain constant throughout the equilibration process. The total concentration of procaine occurring at the corneal surface, $[P]_t$, at each pH studied (taken as the sum of the concentrations of free procaine cation, free base, and bound procaine), can be estimated using Eq. 6.

$$[P]_r = r[P^+]_s + [P]_s \quad \text{(Eq. 6)}$$

TABLE III—CALCULATED RESULTS RELATING TO THE PROCAINE–CORNEAL SURFACE INTERACTION

pH of Solutions	Bound Procaine[a] (Δf), mmole/L.	Association Constant (K_b), L./mmole	Total Procaine Concentration at Corneal Surface, mmole/L.
5.18	0.4	3.0	37.6
6.06	0.6	3.0	38.1
6.84	9.5	30	46.9
7.58	27	140	64.0
8.15	44	231	81.0
9.00	123	461	133.

[a] Values of Δf corresponding to each pH listed were obtained from the vertical differences in the curves presented in Fig. 3.

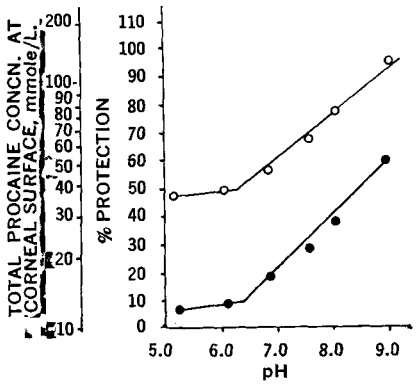

Fig. 4—Curve A represents the relationship of the logarithm of the total procaine concentration at the corneal surface to the pH of the applied solutions. Curve B represents the relationship of the duration of procaine-induced corneal anesthesia (plotted as percent protection) to the pH of the applied solutions.

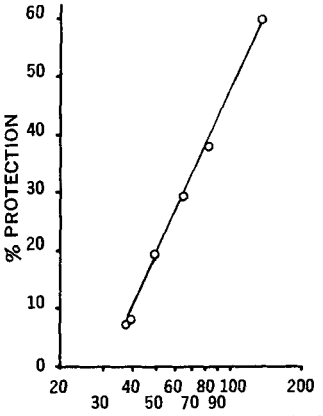

Fig. 5—Relationship of the logarithm of total procaine concentration at the corneal surface to duration of procaine-induced corneal anesthesia plotted as percent protection.

Since we are concerned with only the outermost surface of the cornea which resides in the same aqueous medium, the concentration of procaine free base at the surface can be assumed as equal to that in the bulk of the applied solutions $[P]_s$.

Values of $[P]_t$, listed in column 4 of Table III, show nearly a fourfold increase in the pH range of 5.18–9.0 arising primarily from increasing concentrations of bound procaine.

Relation of Procaine Binding to Duration of Anesthetic Effect—The procaine solutions used in the present study are similar to those used by Hiter in his study of the duration of procaine anesthesia induced upon the guinea pig cornea as a function of pH. His experimental approach was based upon a modified Chance and Lobestein technique (23). The procaine solutions were instilled into the eye and allowed to remain in contact with the cornea for 15 sec., a time found to be sufficient for the establishment of equilibrium between the procaine solution and the corneal surface. Excess solution was then removed from the cornea by the initiation of a blink reflex. The anesthetic effect was noted with a loss of the blink reflex when the cornea was stimulated at 1-min. intervals. The duration of the anesthetic effect was recorded as percent protection.[3]

The durations of anesthetic responses Hiter observed with 1% procaine solutions in the pH range of 5–9 are plotted in the lower curve in Fig. 4. The upper curve in Fig. 4 is a semilogarithmic plot of $[P]_t$ *versus* pH. The marked similarity in the two curves may be explained if the fact is considered that under conditions of constant pH, the duration of surface anesthesia in the eye is approximately a linear logarithmic function of the concentration of the local anesthestic in solution (1, 2). It may be expected that the duration of the anesthestic effect is also an approximately linear function of the logarithm of the concentration of the local anesthetic at the corneal surface itself. That this prediction is realized is evidenced by the curve shown in Fig. 5 where this relationship is demonstrated.

[3] The actual duration of the anesthetic effect was recorded in minutes, divided by 60 and multiplied by 100.

The treatment of the data which have been presented has in effect converted the pH dependency of the duration of anesthesia into a concentration dependency. The significance of this result lies in that it indicates that the duration of surface anesthesia can be related to and is dependent upon the total concentration of procaine estimated to be present at the corneal surface. Since the reversibly bound procaine is primarily responsible for the increasing total concentrations and is in equilibrium with free procaine, it may be assumed to act as a reservoir for the drug allowing it to be slowly released from its binding sites. By this means the bound procaine could serve in a manner analogous to continuous release dosage forms in prolonging the anesthetic response by maintaining threshold or suprathreshold levels of procaine at the biological sites of action for extended periods of time.

The role of lachrymal fluid in the onset and duration of the anesthetic response in relation to the effects of pH and buffer capacity (2) will be discussed in a future paper.

CONCLUSIONS

Although much of this treatment has been qualitative, it provides an example of the role that tissue structures may have in modulating their response to drugs. It also points out factors, in addition to the liberation of free base, which may be operating to increase the effectiveness of procaine in alkaline solution.

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Keyphrases

Local anesthesia–guinea pig cornea
Procaine–interaction with corneal surface
Density, ionic–corneal amphoteric colloid
pH effect–corneal ionic density
Anesthesia, corneal–relation to charge density

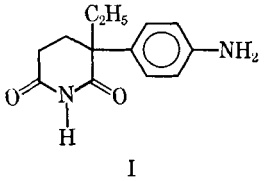
Synthesis of Derivatives of *N*-Methyl-2-phenylsuccinimide Involving a Lithium Salt Condensation and a Novel Application of the Mannich Reaction

Potential Anticonvulsant Agents

By HARRY CARL CLEMSON*, EDWARD O. MAGARIAN†, GEORGE C. FULLER, and RONALD O. LANGNER

The preparation of several Mannich bases of *N*-methyl-2-phenylsuccinimide was accomplished under a variety of conditions. There is no report in the literature of the application of the Mannich reaction to *N*-substituted-2-arylimides. The next higher homologs of these Mannich bases were prepared by a base displacement reaction in toluene between the lithium salt of *N*-methyl-2-phenylsuccinimide and the appropriate β-chloroethylamine hydrochlorides. Preliminary pharmacological data are reported.

GLUTETHIMIDE, a well-known sedative and hypnotic agent, has been reported as having an unreliable anticonvulsant effect at normal hypnotic doses (1). Introduction of an amino group into the *para* position of the benzene ring produces amino-glutethimide (I) which was used, until recently, in the clinical control of epileptic seizures. In their investigation of the effect of the presence of an amino group on the anticonvulsant properties of mephobarbital, Craig and Shideman (2) demonstrated that II has a higher



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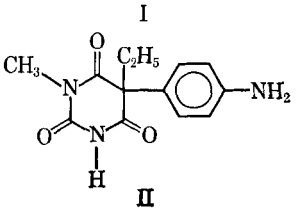

II

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