CONCLUSION

These findings are consistent with the hypotheses that these aliphatic amines act to antagonize adrenergic responses by competitive inhibition of the α-adrenergic receptors and that the active form is 1,18-diamino-6,13-diaza-9,10-dithiaoctadecane. If these hypotheses are correct, they suggest new ideas concerning the need for aromatic structural components for α-adrenergic receptor blocking drugs.

REFERENCES

(1) M. H. Heiffer, E. H. Herman, J. A. Vick, G. E. Demaree, R. L. Mundy, D. G. Reynolds, and D. P. Jacobus, *Fed. Proc.*, **28**, 611(1969).

(2) E. H. Herman, M. H. Heiffer, G. E. Demaree, and J. A. Vick, to be published.

(3) A. Cheung, A. Benites, and P. Lim, Stanford Research Institute Report No. 120, Apr. 8, 1969, U. S. Army Contract No. DA-49-193-MA-2753.

(4) R. F. Furchgott, in "Methods in Medical Research," A. D. Bruner, Ed., Yearbook Publishers, Chicago, Ill., 1959, p. 177.

(5) H. O. Schild, *Brit. J. Pharmacol. Chemother.*, **2**, 189(1942).

(6) J. A. Vick and M. H. Heiffer, *Pharmacologist*, **12**, 284(1970).

(7) J. A. Vick, M. H. Heiffer, W. H. Webster, and D. P. Jacobus, *Physiologist*, **12**, 383(1969).

(8) S. J. Phillips and J. A. Vick, *Pharmacologist*, **12**, 284(1970).

(9) G. E. Demaree, J. S. Frost, and M. H. Heiffer, *Fed. Proc.*, **29**, 420(1970).

(10) G. E. Demaree, J. S. Frost, M. H. Heiffer, and W. E. Rothe, *Proc. Soc. Exp. Biol. Med.*, **126**, 1332(1971).

(11) R. W. Caldwell and G. E. Demaree, *Fed. Proc.*, **30**, 508 (1971).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 7, 1970, from the *Division of Medicinal Chemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC 20012*

Accepted for publication July 12, 1971.

Hexachlorophene-Induced Changes in Electrical Response Specificity of Human Finger Epidermis for Sodium and Potassium Ions

VICTOR F. SMOLEN* and ROLLAND I. POUST†

Abstract □ Electrical potentials developed at a human epidermal surface–aqueous solution boundary in response to varying concentrations of sodium and potassium ions were measured *in vivo*, both in the presence and absence of hexachlorophene in the solutions. The results provide further evidence for a previously postulated mechanism of hexachlorophene interaction with epidermal colloids which implicated allosterically effected changes in the electron densities of ionogenic groups vicinally located to hexachlorophene interaction sites.

Keyphrases □ Hexachlorophene—role in changes in electrical response specificity for sodium and potassium ions, human finger epidermis, mechanism of action □ Epidermis, electrical response specificity—hexachlorophene-induced changes and its mechanism of action

In a recent article concerning the interaction of hexachlorophene with human finger epidermis (1), a molecular mechanism of interaction was postulated whereby unionized hexachlorophene molecules and/or anions hydrogen bonded to peptide linkages of the epidermal proteins to effect changes in the net fixed-charge density on the surface. An inductive effect, which allosterically altered the dissociation constants of ionogenic side groups located vicinally to hexachlorophene interaction sites, was implicated as operative in the observed phenomena. Ling (2) pointed out that such changes in proton-ionization constants of ionogenic groups can also induce an altered selectivity of the ionogenic groups for associating specific monovalent cations.

In accordance with Ling (2) and Eisenman (3), the electrical response of cation-selective electrodes to specific ions is dependent upon the anionic field strength of the groups fixed to the electrically responding surface; the field strength underlies the association affinity of the surface groups for specific cations and determines the rank order of ion selectivity manifested in the electrical response behavior of the surface. Therefore, provided the magnitude of the effect is sufficient for detection, the interaction of hexachlorophene with the epidermal surface colloids may be expected to alter inductively the rank order of their selective electrical response to cations in solution.

EXPERIMENTAL

Electrical potentials were measured, utilizing the previously described experimental arrangement (1), for solutions containing HCl, NaCl, and KCl ranging in concentration from 1.0 to 10^{-5} *M*, both in the absence and presence of saturated concentrations of hexachlorophene. The previously described (1) pretreatment of fingers was employed, with the exception that prehydration was carried out in double-distilled water to effect a removal of ions from the epidermal surface. The reference solution was 1.0 *M* KCl in all instances. Measurements were initiated at both high and low concentrations and were recorded only after the potentials remained constant for a minimum of 30 sec. All NaCl and KCl solutions had pH values above 5.75. Six replications were performed on a 25-year-old male volunteer.

RESULTS AND DISCUSSION

The mean values of electrical potentials observed when the measurements were initiated at high and low ion concentrations are plotted as a function of the negative logarithm of the cation concentration in Figs. 1 and 2. The initial linear portions of the curves represent a least-squares regression analysis fit to the data. The relative specificities of the epidermal proteins for interaction with the ions were computed using the following equation as presented

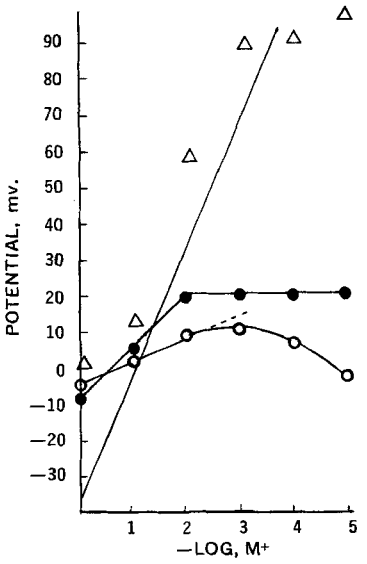

Figure 1—*Electrical response of human finger epidermal surface to hydrogen* (△), *sodium* (●), *and potassium* (○) *ions in the absence of hexachlorophene.*

by Eisenman (3) for glass ion-selective electrodes and electrically responding surfaces in general (4):

$$V_I - V_H = \frac{RT}{F} \ln K_{HI} \qquad \text{(Eq. 1)}$$

where V_I and V_H are the observed potentials (least-squares intercepts) for the experimental and reference (H^+) ions, respectively; R is the gas constant; T is the absolute temperature; and F is the Faraday constant. The symbol K_{HI} is an empirical constant describing the relative selectivity of the protein to the experimental cation *versus* the reference hydrogen cation. The value of K_{HI} in the presence and absence of hexachlorophene was calculated for each cation. The results of these calculations are summarized in Table I; it appears that in the absence of hexachlorophene, potassium ion is more selectively interacted while sodium ion is preferred when hexachlorophene is present. A paired *t*-test comparison of the two curves revealed the reversal between sodium and potassium specificities as statistically different at $p < 0.05$.

These results indicate a hexachlorophene-induced change in the specificity of the anionic surface groups interacting with the cations. As mentioned earlier, Ling (2) stated that shifts in dissociation constants of ionogenic groups may give rise to alterations in ion specificity. These shifts, described in terms of changes in *c*-value, can change an anionic site's preference for certain cations and can be brought about by the inductive effect. The *c*-value is a parameter

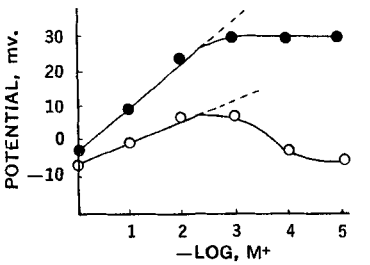

Figure 2—*Electrical response of human finger epidermal surface to sodium* (●) *and potassium* (○) *ions in the presence of hexachlorophene.*

Table I—Summary of the Relative Electrical Response Selectivity of the Human Epidermal Surface to Sodium and Potassium Ions in the Absence and Presence of Hexachlorophene

Cation	K_{HI}[a] Hexachlorophene Absent	K_{HI}[a] Hexachlorophene Present
Na^+	2.78	3.74
K^+	3.36	3.33

[a] The differences are statistically significant at the 95% confidence level.

expressing the electron density of the anionic oxyacid group and underlies the differences in pK values of ionogenic groups. Thus, the inductive effect, which was previously postulated for hexachlorophene interaction, is capable of altering the ion specificity of the epidermal proteins.

Exchange resins (5) having weakly acidic carboxyl groups (high pK) selectively adsorb sodium ion in preference to potassium ion, whereas strongly acidic sulfonic groups (low pK) selectively adsorb potassium ion over sodium ion. Therefore, if hexachlorophene, through an inductive effect, does raise the pK values of the titrable groups in the region above 5.6 as was postulated (1), the observed reversal in the affinity of the epidermal protein to sodium and potassium ions could be expected.

SUMMARY AND CONCLUSIONS

The selectivity of the proteins comprising the epidermal surface to sodium and potassium ions was studied, both in the presence and absence of hexachlorophene. It was found that a reversal in the affinity of ionogenic groups to sodium and potassium ion occurred; it is attributable to a shift in the pK values of the cation-interacting ionogenic groups as a result of hexachlorophene interaction producing an inductive change in their electron densities. These results constitute predictive evidence and lend additional credence to the previously postulated (1) mechanism of hexachlorophene interaction with epidermal protein. In addition, the presently described experiment exemplifies the manner in which the electrometric study of specific ion–tissue surface interactions may be utilized to provide mechanistic insight into the nature of the processes involved in drug–tissue binding.

REFERENCES

(1) R. I. Poust and V. F. Smolen, *J. Pharm. Sci.*, **59**, 1461(1970).

(2) G. N. Ling, *Texas Rep. Biol. Med.*, **22**, 244(1964).

(3) G. Eisenman, "The Glass Electrode," Interscience, New York, N. Y., 1969.

(4) G. Eisenman, in "Membrane Transport and Metabolism," A. Kleinzeller and Kotyk, Eds., Academic, London, England, 1960, pp. 163–179.

(5) J. I. Bregman, *Ann. N. Y. Acad. Sci.*, **57**, 125(1953).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 1, 1971, from the *Biophysical Pharmaceutics Area of the Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication August 2, 1971.

Abstracted in part from a thesis submitted by R. I. Poust in partial fulfillment of the Ph.D. degree requirements at Purdue University.

* To whom communications should be addressed.

† Present address: Department of Pharmaceutics, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15213