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SHORT COMMUNICATIONS

Aggregation of local anesthetics in solution

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SINCE the molecules of many drugs have both hydrophilic and hydrophobic portions, they may form charged aggregates in aqueous solutions according to well known models of surfactant interaction.[1,2] Such aggregates could affect the pharmacological activity of the drug directly at an active site, or indirectly through influence upon diffusion and permeability. Evidence for aggregation of certain medicinal amine salts has, in fact, been provided by the conductivity and osmolality data of Farhadieh *et al.*,[3] but these workers obtained little direct information on the size of the aggregates. In this investigation, we have used standard light-scattering techniques to study aggregates formed by the local anesthetics: dibucaine HCl, procaine HCl, and tetracaine HCl.

Dibucaine HCl, procaine HCl and tetracaine HCl were obtained from K & K Laboratories, Plainview, N.Y. Compounds were purified by recrystallization from acetone. Molar extinction coefficients obtained from ultraviolet spectra of the compounds in absolute methanol were slightly higher than those reported by Chatten *et al.*[4] None of the anesthetics showed any fluorescence in the light-scattering apparatus. Since dibucaine HCl was found to lose weight upon drying, concentrations of this compound were determined exactly by ultraviolet spectra of aqueous solutions. The molar extinction coefficient for dibucaine HCl in water was found to be $4{\cdot}36 \times 10.^3$ Spectral determinations of concentrations of procaine HCl and tetracaine HCl were found to correspond to dry weight concentrations. The pH values of the solutions were always low, indicating that the anesthetics were in the form of amine salts.

Light-scattering measurements were obtained with green ($\lambda = 5460$ Å) light in a Brice–Phoenix light-scattering photometer. Solutions were filtered through two Gelman VF filters to remove dust. Results were plotted as $\kappa c/R_{90}$ vs. c, where κ is the light-scattering constant, R_{90} is the reduced intensity of light scattered at 90°, and c is the concentration of anesthetic in grams per milliliter. None of the anesthetics showed any dyssymmetry, and molecular weights were calculated in the usual manner by extrapolation to $c = 0$.

Refractive index increments, dn/dc, were determined with green light in a Brice–Phoenix differential refractometer and were found to be independent of temperature and salt concentration over the range of anesthetic concentrations studied. Values of dn/dc for dibucaine HCl, procaine HCl and tetracaine HCl are, respectively, 0·2259, 0·2220 and 0·2351 ml/g.

The aggregates formed by the anesthetics which we have studied were found to be of two different types, the primary distinction being in their size. Dibucaine HCl formed large micellar aggregates resembling those formed by some soaps,[1,2] while procaine HCl and tetracaine HCl seemed to form small aggregates with very low aggregation numbers.

For dibucaine HCl, below a certain critical micellar concentration (c_o), scattering was as expected for a solution of monomers which did not interact appreciably with each other. Above c_o, scattering increased at a rate which would be predicted if all additional solute molecules dissolved in the form of aggregates of constant size. The value of c_o was estimated by plotting $(c\text{-}c_o)/R_{90}$ vs $c - c_o$ to produce the best-fitting straight line. For dibucaine HCl, c_o in water was thus found to be 0·0230 g per ml, and results obtained using this value are presented in Fig. 1. The molecular weight of dibucaine HCl micelles, calculated from this graph, is 5710, representing an aggregation number of 15. Local anesthetics are pharmacologically active in physiological solutions and, since it is generally known that micelle size increased in saline media, data were also obtained in NaCl. It was found that dibucaine HCl in 140 mM NaCl formed micelles with an aggregation number of 35 above a critical concentration, c_o, of 0·0133 g/ml.

The other type of aggregation, represented by procaine HCl and tetracaine HCl, is presented in Figs. 2 and 3. Scattering for these anesthetics was low and seemed to approach monomer scattering at

$c = 0$, with no sudden change in scattering which would define a critical concentration. The negative second virial coefficient evident as c approaches zero for these curves could indicate dissociation of aggregates formed by the anesthetics. The curve for procaine HCl in water closely approximates a theoretical curve calculated from an equilibrium of the type $2\,M \rightleftharpoons M_2$, where M represents a single monomer unit, and $K_{eq} = 10\ (g/ml)^{-1}$. Likewise, the data for tetracaine HCl in H_2O closely fall around a curve that would be expected for an equilibrium of the type $4\,M \rightleftharpoons M_4$ with $K_{eq} = 10^5$

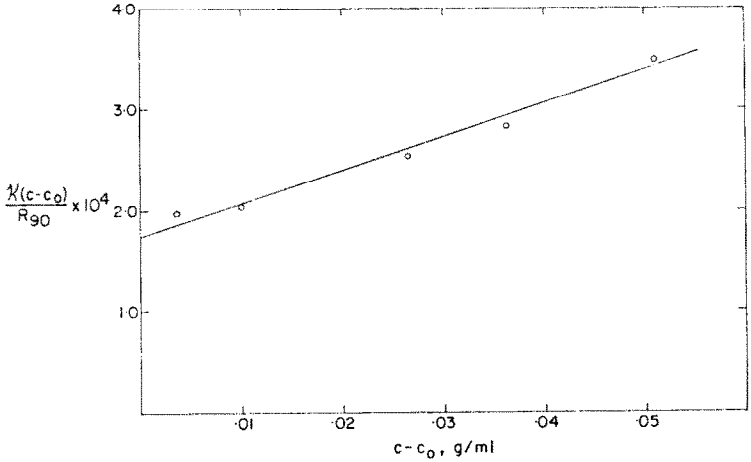

FIG. 1. Light scattering by dibucaine HCl in water. Extrapolation to $(c - c_o) = 0$ gives the reciprocal molecular weight of micelles formed above c_o.

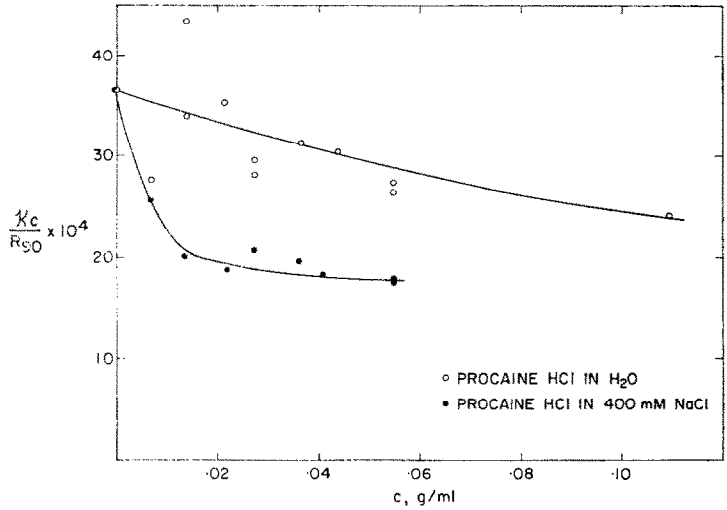

FIG. 2. Light scattering by procaine HCl in water and salt. No c_o is apparent for procaine HCl or tetracaine HCl, and extrapolation to $c = 0$ gives reciprocals of monomer molecular weights.

$(g/ml)^{-3}$. The data for procaine HCl and tetracaine HCl in salt show a marked increase in scattering over values obtained in water. These data are not easily characterized as simple equilibria and could represent more complex multiple equilibria between different aggregates.

It is of interest to note the comparison between procaine HCl and tetracaine HCl. Scattering by tetracaine HCl is considerably higher than that by procaine HCl, while differences in structure are small. Tetracaine HCl has alkyl substitutions of the paramino group at its hydrophobic end. Procaine

HCl, on the other hand, has ethyl rather than methyl substitutions of its hydrophilic amine. Evidently, the hydrophobic substitution on the tetracaine paramino group accounts for its greater tendency to aggregate. It is also interesting that the ability of the anesthetics to form large aggregates parallels the order of anesthetic potency usually given for these anesthetics.

Heretofore only two forms of a local anesthetic, the ionized molecule and the free base, have been considered as potentially active species. This research points out the presence of the aggregate as

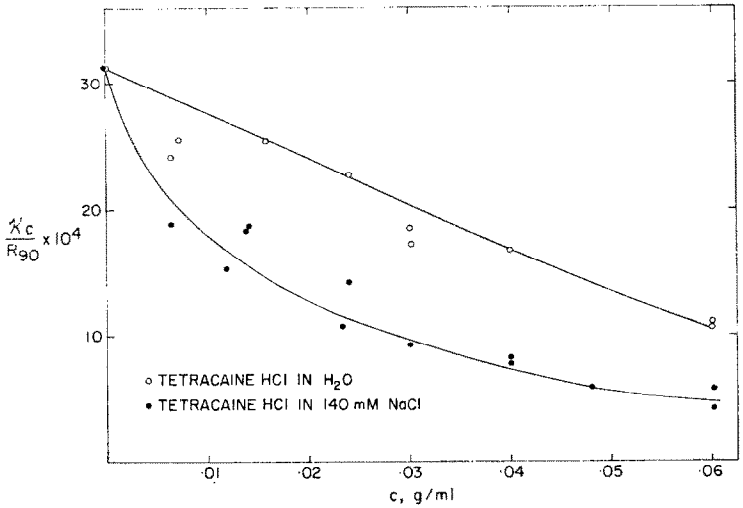

FIG. 3. Light scattering by tetracaine HCl in water and salt.

another form to be considered. It has been shown that local anesthetics undergo specific interactions with phospholipid micelles,[5,6] but the exact nature of the reactive form of anesthetic is still under investigation. In view of their ability to form charged aggregates, it is entirely possible that local anesthetics do not exist as either simple ions or free base in the complex environment of the cell wall. Additional experiments will be necessary to determine the physiological significance of these aggregated species.

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Department of Pharmacology,
Yale University School of Medicine,
New Haven, Conn. 06510, *U.S.A.*

E. M. JOHNSON
D. B. LUDLUM*

* Present address: Department of Cell Biology and Pharmacology, University of Maryland School of Medicine, Baltimore, Md.

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