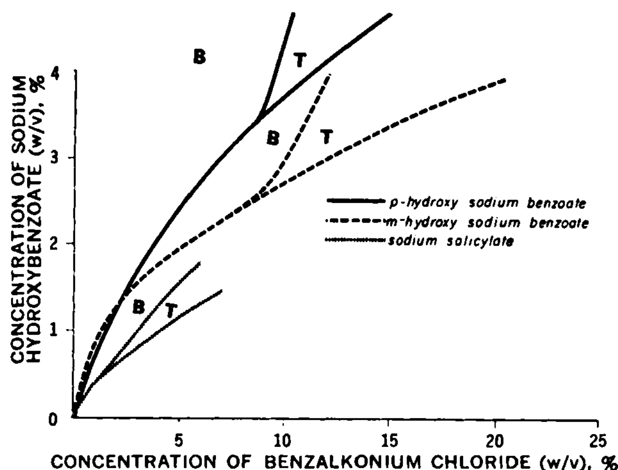

Figure 1—*Superimposed phase transition diagrams of sodium* o-, m-, *and* p-*hydroxybenzoates-benzalkonium chloride coacervate systems at 24°. (Data for sodium* o-*hydroxybenzoate from* Reference 1.)

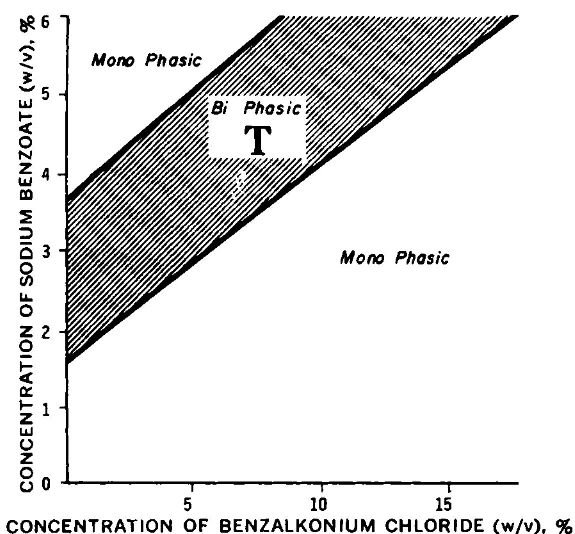

Figure 2—*Phase transition diagram of sodium benzoate-benzalkonium chloride coacervate system at 24°. Dark area [T] represents the region of coacervate formation at the top, and light area represents the region of no coacervate formation.*

In the case of the sodium hydroxybenzoate and benzalkonium chloride coacervate system, the denser coacervate phase, on heating, becomes lighter and moves to the top of the equilibrium liquid.

The increase in benzalkonium chloride concentration at constant salt concentration results in either a lighter coacervate phase or a monophasic solution (Fig. 1). Since benzalkonium chloride is lighter than water, an increase in micellar aggregation results in a lighter coacervate phase and hence the coacervate moves to the top of the equilibrium liquid. Figure 1 also shows the similarity in patterns of phase transition diagrams of all three salts of hydroxybenzoate, and they follow the same order as the degree of solubility (*o*-hydroxybenzoate being least soluble and *p*-hydroxybenzoate being most soluble). The sodium benzoate system did not form a denser coacervate phase; this is probably because of the structural changes in the micelle size and/or shape resulting from the absence of a hydroxyl group.

(1) D. Shah, B. Ecanow, M. Sadove, and F. Shieh, *J. Pharm. Sci.*, **62**, 1210(1973).

DEVENDRA SHAH
BERNARD ECANOW▲
REUBEN BALAGOT
College of Pharmacy
University of Illinois
Chicago, IL 60612
Surgical Service
Hines V.A. Hospital
Hines, IL 60141

Received May 25, 1973.
Accepted for publication August 16, 1973.
▲ To whom inquiries should be directed.

Cholinergic Activity of Pilocarpine Methiodide: A Reinvestigation

Keyphrases ☐ Pilocarpine methiodide—cholinergic activity reinvestigated ☐ Cholinergic activity—pilocarpine methiodide ☐ Quaternary salts—cholinergic activity of pilocarpine methiodide

Sir:

While most compounds possessing cholinergic activity are quaternary amine derivatives, several tertiary amines are known to possess significant cholinergic activity. Pilocarpine is perhaps the most widely known example of a tertiary amine derivative possessing therapeutically useful cholinergic activity. The cholinergic activity, or lack thereof, of the quaternary methiodide salt of pilocarpine has been a point of interest for some time. Wojciechowski and Ecanow (1, 2) prepared a series of quaternary salts of pilocarpine and later reported (3) that pilocarpine methiodide lacks muscarinic activity. Hanin *et al.* (4) confirmed this report while presenting the correct chemical structure of the methiodide.

During studies designed to clarify the structural and conformational requirements for cholinergic activity of pilocarpine (5, 6), we examined the effects of pilocarpine methiodide on the ileum of the guinea pig. We confirmed the earlier reports that the quaternary salt lacks muscarinic activity but, more significantly, observed (5) that this salt possesses the ability to antagonize the muscarinic effects of acetylcholine. Subsequent to our observation, Ben Bassat *et al.* (7) reported the cholinergic antagonist activities of a large number of quaternary salts of pilocarpine but, curiously, neither reported any data regarding the methiodide salt nor mentioned previous studies on this compound. In view of these facts, we feel it of importance that we report our observations.

The methiodide of pilocarpine was prepared from pilocarpine in the standard manner, and the physical data (elemental analyses, IR and NMR data, and specific rotation) were consistent with the earlier reports. Antagonism of the muscarinic actions of acetylcholine

on the guinea pig ileum was measured by known procedures (8). A pA_2 value (9) of 2.2 was determined. While the degree of antagonism observed is much weaker than that observed for atropine [$pA_2 = 9.0$ (10)], it is significant that these effects have not been previously reported.

The effects of pilocarpine methiodide on eel acetylcholinesterase were studied[1], and weak, uncompetitive inhibition was observed ($K_i = 1.64 \times 10^{-4}$).

(1) N. J. Wojciechowski and B. Ecanow, *J. Pharm. Sci.*, **50**, 854(1961).
(2) *Ibid.*, **50**, 887(1961).
(3) N. J. Wojciechowski and B. Ecanow, *Pharmacologist*, **6**, 203(1964).
(4) I. Hanin, D. J. Jenden, and A. K. Cho, *Mol. Pharmacol.*, **2**, 325(1966).
(5) H. Y. Aboul-Enein, Ph.D. dissertation, University of Mississippi, University, Miss., July 1971.
(6) R. F. Borne, H. Y. Aboul-Enein, I. W. Waters, and J. Hicks, *J. Med. Chem.*, **16**, 245(1973).
(7) A. Ben Bassat, D. Lavie, H. Edery, and G. Porath, *ibid.*, **14**, 1066(1971).
(8) "Pharmacological Experiments on Isolated Preparations," Staff of Department of Pharmacology, University of Edinburgh, E. and S. Livingstone, Ltd., Edinburgh, Scotland, 1968, p. 25.
(9) J. H. Gaddum, *J. Physiol.*, **89**, 7P(1937).
(10) H. O. Schild, in "Quantitative Methods in Pharmacology," H. De Jonge, Ed., North Holland Publishing Co., Amsterdam, The Netherlands, 1961, p. 284.

I. W. WATERS▲
HASSAN Y. ABOUL-ENEIN
C. RANDALL CLARK
RONALD F. BORNE
School of Pharmacy
University of Mississippi
University, MS 38677

Received July 19, 1973.
Accepted for publication August 14, 1973.
Supported in part by the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.
▲ To whom inquiries should be directed.

[1] By G. Cocolas, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514

Binding of Sulfonylureas to Serum Albumin

Keyphrases ☐ Protein binding, human and bovine serum albumin—sulfonylureas, dynamic dialysis technique ☐ Sulfonylureas—binding to human and bovine serum albumin, dynamic dialysis technique ☐ Drug-protein binding—sulfonylureas ☐ Dialysis, dynamic—used to study sulfonylurea binding to serum albumin

Sir:

A recent paper (1) concerned the binding of the sulfonylureas, tolbutamide, chlorpropamide, and acetohexamide, to serum proteins. We have studied the binding of tolbutamide and chlorpropamide to serum albumin and find that there are substantial differences

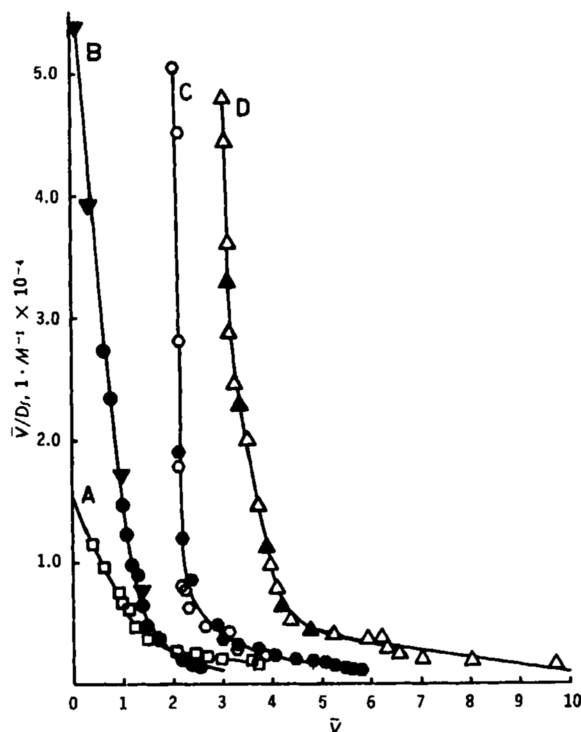

Figure 1—*Scatchard plots for the binding of tolbutamide to serum albumin in 0.067* M *buffer at pH 7.4 and 37°. Key: open symbols, 1% albumin; closed symbols, 2% albumin; A, 1% human serum albumin in tromethamine buffer; B, 2% human serum albumin in tromethamine buffer; ●, ▼, Judis' (1) results (40°); C, human serum albumin in phosphate buffer; and D, bovine serum albumin in phosphate buffer. All points are experimental while the solid lines were computed from the binding parameters.*

between our results and those of Judis (1). In this communication we present some results for comparison and attempt to reconcile the differences.

The drug-protein interaction was characterized using a dynamic dialysis technique. The method and apparatus were based on those described by Meyer and Guttman (2). A concentration of 1 or 2% serum albumin was used, buffered to pH 7.4 with 0.067 *M* phosphate buffer. The volume and frequency of sampling were such that the concentration of drug in the outer compartment never exceeded 5% of the free concentration in the protein compartment to ensure that dialysis proceeded under sink conditions.

Tolbutamide was estimated by two separate methods: (*a*) in buffer, by direct UV spectrophotometry at 228 nm., and (*b*) in solutions containing protein and/or other interfering materials, by a modification of the colorimetric procedure of Alessandro *et al.* (3). Chlorpropamide was estimated spectrophotometrically at 231 nm.

The decline in drug concentration, D_t, within the dialysis bag was followed as a function of time. From a knowledge of the intrinsic dialytic rate constant and the instantaneous dialysis rate at any time, *t*, the free concentration in the protein compartment was estimated. The instantaneous rate of dialysis was obtained by fitting the plot of D_t *versus* time to an equation of the form:

$$D_t = D_t^0 + at + bt^2 + ct^3 + dt^4 \ldots \quad \text{(Eq. 1)}$$