may be partly explained by the fact that deuterated *N*-demethyldiazepam is less effective than the hydrogenated analog as an inhibitor of the mortality induced by pentylenetetrazol in mice. The ED_{50} was 198 mcg./kg. for *N*-demethyldiazepam and 288 mcg./kg. for the deuterated analog.

However, there is also a decreased accumulation of the hydroxylated metabolite, oxazepam, in the brain of mice treated with the deuterated analog compared to the C_3-unlabeled *N*-demethyldiazepam. This finding is in agreement with previous studies indicating that the long duration of action of diazepam and *N*-demethyldiazepam in mice is related to the formation and persistence of brain oxazepam (9). The lack of accumulation of oxazepam from C_3-deuterated *N*-demethyldiazepam is due to a reduced C_3-hydroxylation of this benzodiazepine *in vitro* compared to the C_3-unlabeled compound, as shown by the experiments utilizing liver microsomal enzymes.

The deuterated *N*-demethyldiazepam may be less hydroxylated than the C_3-unlabeled analog for one of the following reasons: (*a*) slower rate of cleavage of the carbon–deuterium bond, (*b*) lower affinity for the active site of the enzymes involved in the hydroxylation, and (*c*) more stable complex with the enzyme. While these possibilities are open to experimental investigations, it remains established that the presence of oxazepam in the brain is the main factor accounting for the prolonged antipentylenetetrazol effect exerted by diazepam or *N*-demethyldiazepam in mice.

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COMMUNICATIONS

Effect of Hydroxy Group on Coacervate Formation by Sodium Hydroxybenzoates with Benzalkonium Chloride

Keyphrases ☐ Coacervate formation, sodium hydroxybenzoates and benzalkonium chloride—effect of position of hydroxy group ☐ Hydroxybenzoates—coacervate formation with benzalkonium chloride, phase transition diagrams, effect of hydroxy position

Sir:

Shah *et al.* (1) reported coacervate formation by sodium salicylate (sodium *o*-hydroxybenzoate) with benzalkonium chloride.

The phase transition diagram of this system (Fig. 1) illustrates two main regions: a biphasic coacervate system (I) and a monophasic equilibrium solution (II). The biphasic coacervate system shows two distinct regions. In one region (B) the coacervate phase has a higher density than the equilibrium liquid and it settles to the bottom of the container, and in the other region (T), the coacervate phase is lighter than the equilibrium liquid and floats on the top of the liquid.

This communication reports the effect of the presence and position of the hydroxy group on coacervate formation between hydroxybenzoates and benzalkonium chloride.

The coacervate systems are obtained by mixing various concentrations of sodium *m*-hydroxybenzoate, sodium *p*-hydroxybenzoate, and sodium benzoate with benzalkonium chloride in water. Figure 1 is the superimposed phase transition diagram of sodium *o*-, *m*-, and *p*-hydroxybenzoates. The phase regions for the hydroxybenzoates are equivalent, differing only in the positions of the boundary lines, and are as identified previously (1). Figure 2 shows a phase transition diagram of the sodium benzoate and benzalkonium chloride coacervate system.

The sodium benzoate system (Fig. 2) shows a lighter coacervate phase (T) throughout the biphasic coacervate region. The systems near the upper and lower transition lines showed the property of birefringence during flow. The coacervate phase in all three systems was observed to be isotropic under examination with a polarizing microscope.

From our previous (1) and present data, it is our belief that the density of the coacervate phase is a function of the molecular weight of the anionic electrolyte, the amount of this electrolyte associated with the micellar aggregation, and the size of the micelles.

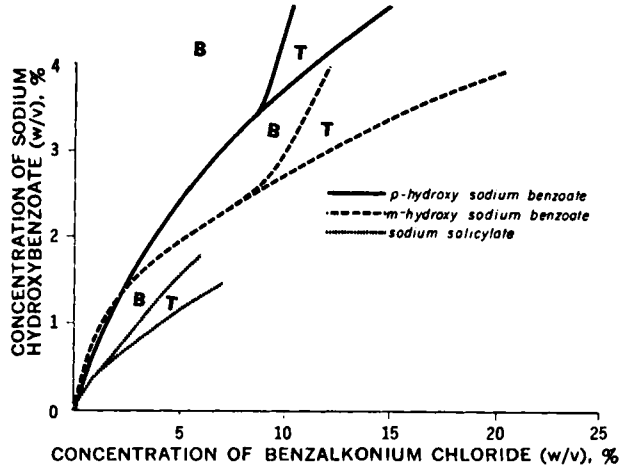

Figure 1—*Superimposed phase transition diagrams of sodium* o-, m-, *and* p-*hydroxybenzoates–benzalkonium chloride coacervate systems at 24°. (Data for sodium* o-*hydroxybenzoate from* Reference 1.)

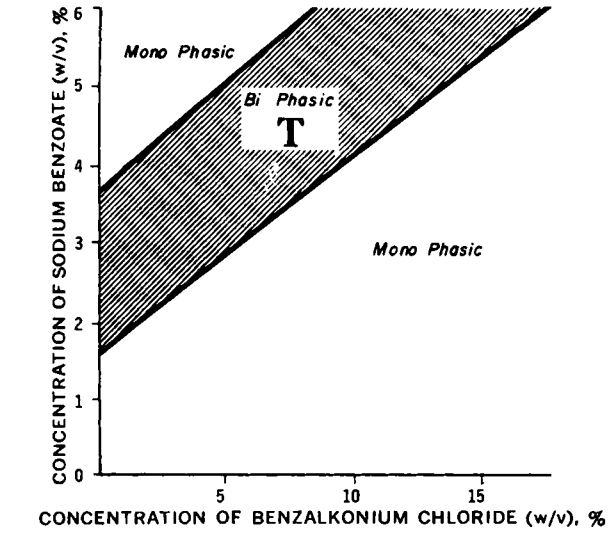

Figure 2—*Phase transition diagram of sodium benzoate–benzalkonium chloride coacervate system at 24°. Dark area [T] represents the region of coacervate formation at the top, and light area represents the region of no coacervate formation.*

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.

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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