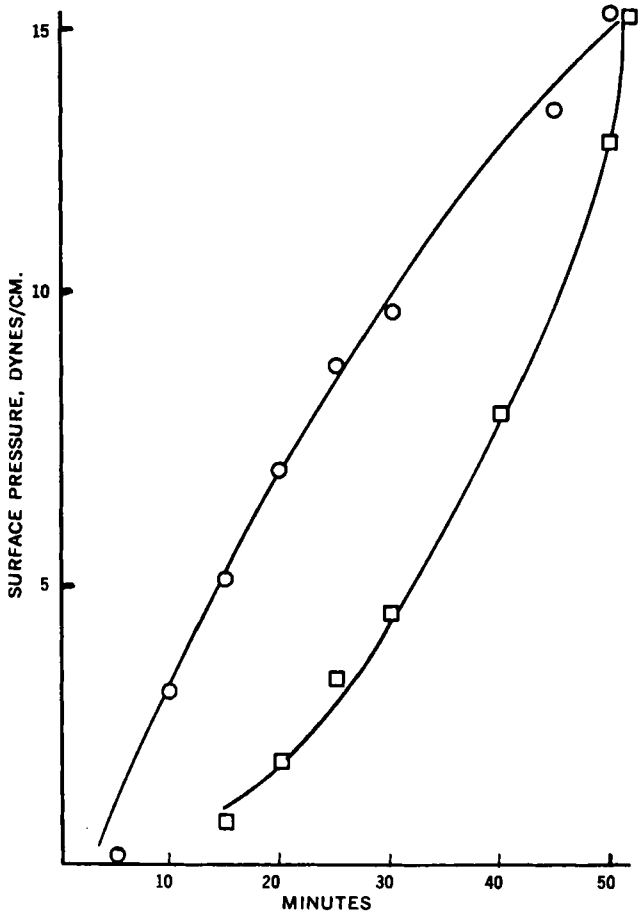

Figure 2—*Rate of surface pressure increase of a stearyl aldehyde film at pH 8.0 in the presence of 1 × 10^{-3} M gentamicin* (○) *and 1 × 10^{-3} M gentamicin with 0.05% sodium bisulfite* (□).

observed when gentamicin was studied in the absence of bisulfite ion.

In the case of a similar experiment conducted with 0.05% sodium bisulfite in the subphase, the previously observed lag period was increased to 14 min. The data clearly show that bisulfite significantly diminishes the interaction of gentamicin with stearyl aldehyde. This may be explained by the fact that bisulfite reacts with aldehydes to form α-hydroxysulfonic acid derivatives (12) which do not form Schiff bases.

The implications of the monolayer studies were extended by studying the effect of the bisulfite ion on the antibacterial activity of gentamicin. A disk-plate method (13), utilizing *Escherichia coli* as the test organism, was employed. Gentamicin-containing filter paper disks were placed on seeded nutrient agar plates containing various concentrations of sodium bisulfite. Following incubation at 37° for 18 hr., the plates were examined and the diameters of the observable zones of inhibition were measured (Table I).

The data indicate that bisulfite ion at 0.05% inhibits the antibacterial activity of gentamicin against *E. coli*. If the mechanisms of interaction of gentamicin at the monolayer and the bacterial membrane are the same, then it appears that bisulfite inhibits gentamicin activity by blocking membrane aldehyde sites which may be necessary for the transport of gentamicin into the bacterial cell.

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Received July 17, 1972.
Accepted for publication October 5, 1972.
The authors are indebted to Schering Corp., Bloomfield, N. J., for a generous supply of gentamicin and for awarding D. E. Auslander with a research fellowship. They also thank Miss Thelma Moss for her assistance in the biological studies.
▲ To whom inquiries should be directed.

Self-Association of Theophylline in Aqueous Solution

Keyphrases □ Theophylline, association in aqueous solution—determination, ultracentrifugation, molecular weights □ Association, theophylline—aqueous solution □ Ultracentrifugation—determination, theophylline self-association, molecular weights

Sir:

In 1957, Guttman and Higuchi (1) concluded that theophylline did not self-associate in water at concentrations from 2.3 × 10^{-3} to 28 × 10^{-3} *M*; partitioned with chloroform–isooctane (9:1) where the theophylline concentration was 1 × 10^{-4} to 12 × 10^{-4} *M*.

In 1971, Ng (2) presented IR evidence for the self-association of theophylline by hydrogen bonding in nonaqueous (deuterochloroform) solutions. Thakkar *et al.* (3) showed, from NMR spectra of aqueous solutions (5 × 10^{-3} to 42 × 10^{-3} *M*), that theophylline self-associates by hydrophobic interactions in water. The present communication presents data, obtained

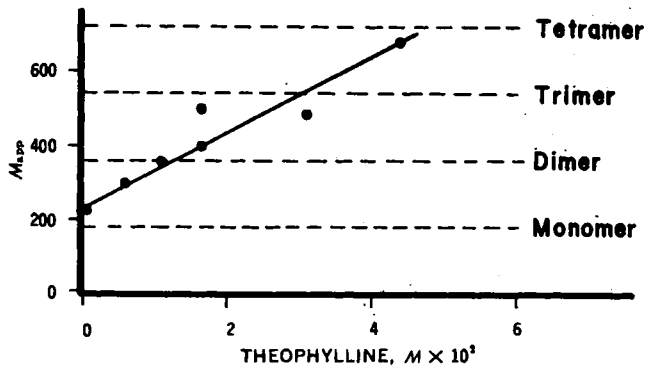

Figure 1—*Plot of apparent molecular weight* (M_{app}) *against concentration of theophylline (mol. wt. 180). Hatched horizontal lines indicate the calculated molecular weight of the monomer, dimer, trimer, and tetramer.*

with the aid of an analytical ultracentrifuge, that confirm the formation in aqueous solution of species of theophylline with molecular weights higher than the formula molecular weight of 180.

Theophylline[1] was authenticated by its IR spectrum using a mull and a spectrophotometer[2]. The molecular weight was determined in an analytical ultracentrifuge[3] by the Archibald approach-to-sedimentation equilibrium method, using the schlieren optical system and solutions with concentrations from 1 mg. (5.5×10^{-3} *M*) to 8 mg. (4.4×10^{-2} *M*) solute/ml. solvent. The solvent was water or 0.075 *M* NaCl or 0.2 *M* NaCl–0.02 *M* sodium phosphate buffer, pH 6.95. Attempts also were made to determine the molecular weight of 1 mg. (5.5×10^{-3} *M*) and 0.1 mg. (0.55×10^{-3} *M*)/ml. theophylline solutions using the UV scanner attachment to the ultracentrifuge.

Apparent molecular weights (M_{app}) were determined using Eq. 1:

$$M_{app} = \frac{2RT}{(1 - \bar{v}\rho)\omega^2 Cr} \times \frac{dc}{dr} \qquad \text{(Eq. 1)}$$

where R is the gas constant, $\bar{v}$ is the partial specific volume, T is the absolute temperature, ρ is the density of the solvent, ω is the angular speed of rotation, dc/dr is the concentration gradient at either the meniscus or cell bottom, C is the total solute concentration at either the meniscus or cell bottom, and r is the distance from the center of rotation at either the meniscus or cell bottom.

The concentration gradients at the meniscus and cell bottom were measured from schlieren patterns recorded on metallographic plates[4] and enlarged 10-fold on a magnifier[5]. Total concentration was measured by use of a capillary centerpiece in an interference cell. The integral of the concentration gradient was evaluated by the procedure of Engelberg (5). The partial specific volume, $\bar{v}$, of theophylline was calculated to be 0.72 cm.^{3}/g. from density data, using methodology described by Schachman (6).

[1] Calbiochem.
[2] Perkin-Elmer model 237.
[3] Spinco model E.
[4] Kodak.
[5] Nikon.

Figure 1 summarizes the apparent molecular weights (M_{app}) obtained at various theophylline concentrations. The data indicate the existence of monomers, dimers, trimers, and tetramers, a property similar to some other xanthines (1). The average deviation of 11% between the experimentally determined multimer molecular weight and the nearest integral multimer molecular weight can be explained best by the presence of at least two self-associating species at most concentrations. Indeed, at a concentration of 3 mg./ml. (1.65×10^{-2} *M*), the M_{app} at the meniscus was 400 daltons and the M_{app} at the bottom of the ultracentrifuge cell was 500 daltons, indicating some separation by the ultracentrifuge of dimer from trimer. Another possible source of error, especially at the lower concentrations where small gradients had to be measured, could have been caused by difficulty in measuring the concentration gradients. This latter possibility is less likely than the first explanation since two different ultracentrifugal techniques yielded M_{app} between monomer and dimer at the lower concentrations.

The conclusion by Thakkar *et al.* (3) of self-association of theophylline at concentrations between 5×10^{-3} and 44×10^{-3} *M* was verified by our direct evidence, although their NMR techniques apparently could not differentiate dimers from the higher molecular weight species existing under their experimental conditions[6]. Our data also demonstrate the well-known dissociation of a self-associating system with decreasing solute concentration (11, 12), since monomer predominated at a concentration of 0.55×10^{-3} *M*, dimer predominated at a concentration of 1.1×10^{-2} *M*, and higher molecular weights were found at higher concentrations.

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Received January 31, 1972.
Accepted for publication November 13, 1972.
The author thanks Dr. David Guttman of Smith Kline and French and Dr. Arvind Thakkar and Dr. Lowell Tensmeyer of Eli Lilly for helpful discussions of their results, Mrs. B. Toeplitz for her help with the interpretation of the IR data, Dr. Klaus Florey for a critical review of the manuscript, and Dr. C. H. Chervenka of Beckman Instruments for the molecular weight determinations performed with the UV scanner attachment to the analytical ultracentrifuge.

[6] See also the discussion by Guttman and Higuchi (10).