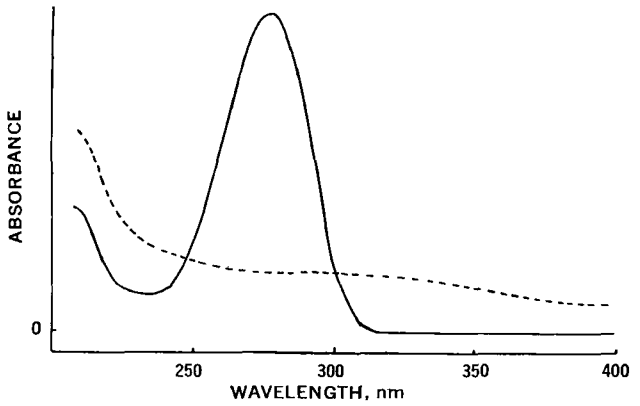

Figure 3—*Absorption spectrum of Ia* (—) *and brown precipitate obtained by heating 1% dextroamphetamine sulfate and 10% dextrose for 5 days at 60°* (- - - -). *Both spectra were determined in ethanol solution.*

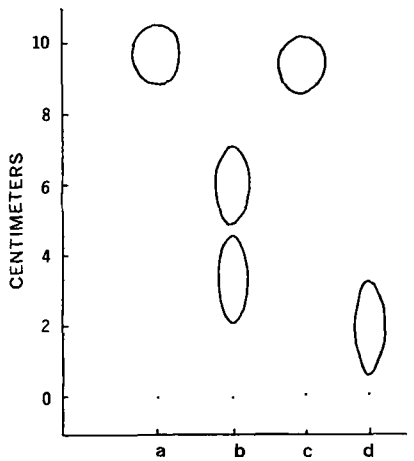

Figure 4—*Thin-layer chromatogram of dextroamphetamine sulfate–dextrose and Schiff-base solutions, developed in the lower phase of a mixture of ethyl acetate–pyridine–water (2:1:2) (see Ref. 1). Key: a, fresh 5-hydroxymethylfurfural; b, ethanol solution of precipitate from 1% dextroamphetamine sulfate and 10% dextrose solution heated at 60° for 5 days; c, Ia; and d, amphetamine base.*

would also expect Ia to absorb at longer wavelength than an amphetamine–sugar Schiff base (3). Consequently, it appears that neither the 298-nm band (1) nor the 320-nm band (2) is due to either Ia or the amphetamine–sugar Schiff base, although either of these may be *intermediates* involved in the browning reaction.

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Effect of Vehicles on Reduction of Brain Norepinephrine by α-Methyltyrosine

Keyphrases □ Methylcellulose—vehicle effect on α-methyl-*p*-tyrosine reduction of brain norepinephrine, compared to polysorbate 80 □ α-Methyl-*p*-tyrosine reduction of brain norepinephrine—vehicle effect, methylcellulose, polysorbate 80 □ Norepinephrine reduction by α-methyl-*p*-tyrosine—vehicle effect, methylcellulose, polysorbate 80

To the Editor:

Investigators seeking to elucidate the functional significance of brain norepinephrine have been aided by the development of the specific enzyme inhibitor, α-methyl-*p*-tyrosine (1). Spector *et al.* (2) demonstrated that α-methyl-*p*-tyrosine selectively inhibits tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of the catecholamines, and thereby depletes these amines from the brain. α-Methyl-*p*-tyrosine has been employed to estimate the turnover rate and the turnover time of the catecholamines (3) in various body tissues including brain.

Bernard and Paolino (4), employing 80 mg/kg α-methyl-*p*-tyrosine suspended in 5% polysorbate 80, were able to lower the brain norepinephrine levels of adult male mice (C57BL/6J) by 52%, 4 hr following intraperitoneal injection. Similar results were obtained in wild rats (5) when using 250 mg/kg α-methyl-*p*-tyrosine. These depleted levels are similar to those obtained by other investigators employing other vehicles or acidification of aqueous solutions to increase the solubility of this enzyme inhibitor (6–9). The purpose of our experiment was to determine if a suspending agent such as 1% methylcellulose 400

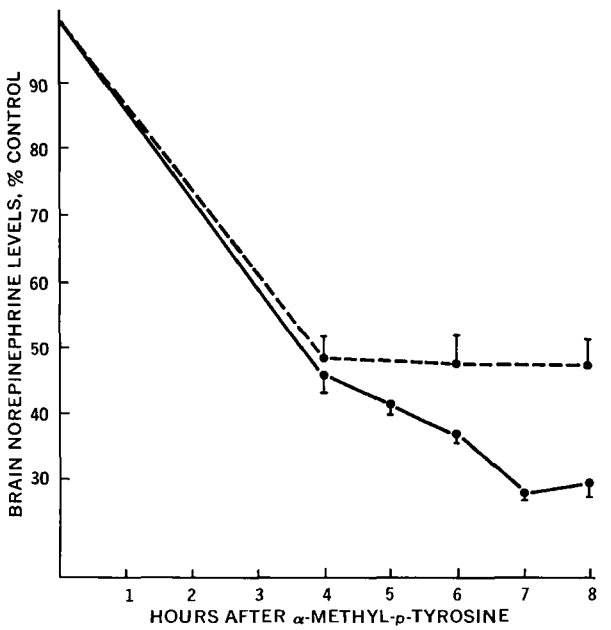

Figure 1—*Adult male mice were injected with α-methyl-p-tyrosine (80 mg/kg ip) in either 5% polysorbate 80* (- - - -) *or 1% methylcellulose 400* (—). *At various time intervals following injection, the animals were decapitated and their brains were removed and assayed for norepinephrine. Each data point represents the mean ± standard error* ($3 \leq n \leq 8$).

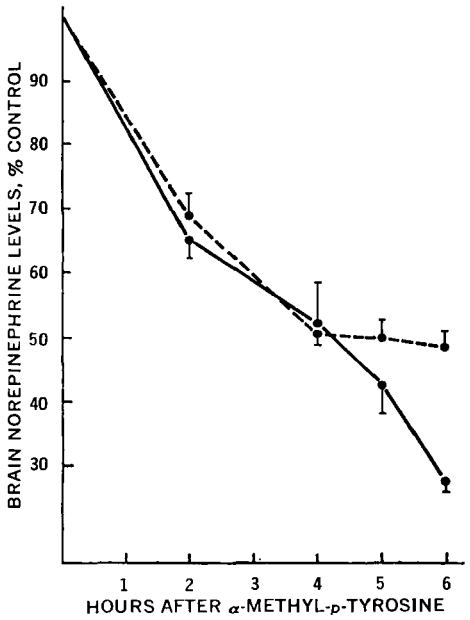

Figure 2—*Adult male rats were injected with α-methyl-p-tyrosine (250 mg/kg ip) in either 5% polysorbate 80 (- - - -) or 1% methylcellulose (—). At various time intervals following injection, the animals were decapitated and their brains were removed and assayed for norepinephrine. Each data point represents the mean ± standard error (3 ≤ n ≤ 8).*

would increase the effectiveness of α-methyl-*p*-tyrosine in reducing brain norepinephrine levels.

The time course for the decline of brain norepinephrine following an injection of α-methyl-*p*-tyrosine was determined in both male albino ICR mice and Wistar rats. Mice were injected with 80 mg/kg ip and rats with 250 mg/kg ip in either 5% polysorbate 80 or 1% methylcellulose 400. Animals were decapitated at various time intervals following injections, their brains were removed, and the norepinephrine content was analyzed by spectrophotofluorometry (4). We observed in rats and mice that 1% methylcellulose 400 significantly reduced brain norepinephrine levels after the 4-hr asymptote seen with polysorbate 80 (Figs. 1 and 2).

One possible interpretation of these data is that the actual amount of α-methyl-*p*-tyrosine being injected into the intraperitoneal cavity was less with polysorbate 80 than with methylcellulose because of the poorer suspending characteristics of polysorbate 80. To check this hypothesis, suspensions of 1% methylcellulose and 5% polysorbate 80 containing either 4.8 mg/ml (80 mg/kg for mice) or 125 mg/ml (250 mg/kg for rats) of α-methyl-*p*-tyrosine were prepared. By using the typical injection procedure, aliquots were withdrawn and injected into previously weighed vials. The liquid was then evaporated, the appropriate vehicle controls were subtracted, and the final α-methyl-*p*-tyrosine weight was compared with the expected injected weight. The results indicated that at the lower concentration (4.8 mg/ml), both 1% methylcellulose and 5% polysorbate 80 delivered approximately the expected amount (2.4 mg). At the higher concentration (125 mg/ml), however, polysorbate 80 delivered significantly less α-methyl-*p*-tyrosine than did methylcellulose ($p < 0.001$; 71% of the expected dose of 125 mg)[1]. This discrepancy in injected α-methyl-*p*-tyrosine at the higher dose does not explain the results obtained in rats (Fig. 2) since polysorbate 80 still delivered 90% of the dose which completely inhibits tyrosine hydroxylase (3), and it fails to explain similar results obtained in mice (Fig. 1). However, the effectiveness of polysorbate 80 apparently decreases when employed as a vehicle for increasing amounts of α-methyl-*p*-tyrosine.

An alternative explanation for the differences observed between polysorbate 80 and methylcellulose in both the high and low doses is that even though the quantity delivered may be similar (as in mice), the amounts of α-methyl-*p*-tyrosine absorbed from the intraperitoneal cavity are less with polysorbate 80. It is well known that many physical properties of various surfactant solutions change at the critical micelle concentration (CMC). In fact, several investigators employing polysorbate 80 (10, 11) or polysorbate 20 (10–14) reported enhanced absorption of drugs across biological membranes when the CMC was not exceeded. Beyond this level, decreases in absorption could be explained as follows: *(a)* a micellar solution consists of two phases; *(b)* the partition ratio of drug between the micellar phase and the aqueous phase is constant, independent of drug concentration; and *(c)* absorption of the drug incorporated in the micelle is negligible. When the CMC was exceeded in the studies, there was a significant increase in the drug–micelle complexation, thereby decreasing the effective concentration of the drug available for absorption. This resulted in a decreased drug absorption rate (15).

Five percent polysorbate 80 exceeds the CMC. Therefore, if α-methyl-*p*-tyrosine formed micelles with polysorbate 80, a further increase in the concentration of the surfactant would probably not enhance absorption. This possible decrease in absorption of α-methyl-*p*-tyrosine would explain the results of Figs. 1 and 2, since the inhibition of tyrosine hydroxylase is known to be dose dependent (1). Although further experimentation will be required to confirm this α-methyl-*p*-tyrosine–micellar complexation theory, such a hypothesis does explain the pharmacological effects reported herein.

This paper presents evidence that 1% methylcellulose 400, when used as a vehicle for delivery of α-methyl-*p*-tyrosine, will result in a greater lowering of mouse and rat brain norepinephrine levels than either polysorbate 80 or some other presently used agents. This is accomplished without the necessity of acidifying the medium (and thus stressing the animal) to increase solubility. Since stress alters brain amine metabolism (16) and, therefore, might be expected to modify normal steady-state brain norepinephrine dynamics, this is an important factor for consideration.

[1] The higher concentration of 125 mg/ml in polysorbate 80 consistently delivered approximately 71% of the expected dose until the number of injections exceeded 75% of the vial volume. Thereafter, the injection concentration increased until it equaled and then exceeded the expected amount.

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Influence of Solute Properties on Release of *p*-Aminobenzoic Acid Esters from Silicone Rubber: Theoretical Considerations

Keyphrases □ Silicone matrix—chain-length effect on *in vitro* release of *p*-aminobenzoates, theory, equations □ *p*-Aminobenzoic acid esters—*in vitro* release from silicone matrix, effect of solute properties, chain length, theory, equations □ Chain-length effect—*in vitro* release of *p*-aminobenzoates from silicone matrix, theory, equations

To the Editor:

The use of silicone rubber as a carrier for therapeutic agents is well documented (1–5). The release of drug from such an inert matrix is dependent upon certain solute properties, *i.e.*, solubility and diffusivity, along with other parameters independent of the particular drug in question. Examples of the latter are the geometry of the matrix, the diffusion layer thickness, the amount of drug per unit volume in the matrix, the particle size, and the filler content.

A mathematical model describing drug release from homogeneous and heterogeneous systems was originally presented by Higuchi (6). Within the assumptions of the model, equations were derived that predict a linear dependence of the amount released, Q, upon the square root of time, $t^{1/2}$. Subsequently, the model was extended and equations were derived that consider the solvent boundary diffusion layer as an additional diffusional pathway (7). According to this extended model, plots of Q *versus* $t^{1/2}$ may not be linear during the early stages of the release process. This has been experimentally verified (8) with a series of progestins. Nonlinear behavior was also observed for chlormadinone acetate (9) and for ethynodiol diacetate (10). However, a systematic evaluation of the influence of the properties of a homologous series on the release rate has not been reported.

This communication utilizes these derived mathematical relationships to describe the effect of chain length of esters of *p*-aminobenzoic acid on the *in vitro* release of drug from a silicone matrix. The assumptions and conditions of the model are: *(a)* pseudo-steady state exists; *(b)* diffusion is rate controlling rather than dissolution of drug particles; *(c)* total concentration of drug within the matrix, A, is substantially greater than its solubility, C_s, in the matrix phase, *i.e.*, $A \gg C_s$; *(d)* transport of the drug species occurs through the matrix phase; and *(e)* ideal sink conditions exist in the dissolution media. The amount released per unit area, Q, from a planar surface as a function of time, t, is given by the following expression (7):

$$Q = \frac{-D_s h_a K A \epsilon}{D_a \tau} + \left[\left(\frac{D_s h_a K A \epsilon}{D_a \tau}\right)^2 + \frac{2AD_s C_s \epsilon t}{\tau}\right]^{1/2} \quad \text{(Eq. 1)}$$

where:

A = total concentration of drug in matrix (milligrams per square centimeter)
D_a = diffusion coefficient in aqueous phase (square centimeter per minute)
D_s = diffusion coefficient in matrix phase (square centimeter per minute)
K = partition coefficient (C_s/C_a)
C_a = solubility (milligrams per milliliter) in dissolution media
C_s = solubility in matrix phase (milligrams per milliliter)
h_a = boundary diffusion layer (centimeters)
ϵ = volume fraction
τ = tortuosity

Differentiating Q with respect to time yields the rate equation:

$$\frac{dQ}{dt} = \text{rate} = \frac{\alpha C_s}{2(\beta^2 K^2 + \alpha C_s t)^{1/2}} \quad \text{(Eq. 2a)}$$

where:

$$\alpha = \frac{2AD_s\epsilon}{\tau} \quad \text{(Eq. 2b)}$$

$$\beta = \frac{D_s h_a A \epsilon}{D_a \tau} \quad \text{(Eq. 2c)}$$

For a homologous series of moderate chain length, D_s and D_a can be considered to be relatively constant (11); thus, α and β are constant for a given set of experimental conditions. For this situation, the release rate (Eq. 2*a*) is dependent only upon C_s and C_a (or, alternatively, C_s and K). As the carbon chain length

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