or antisensitizing effects of the drug can be isolated from any sedative–anticonvulsant action it may have.

Physical impairment of hearing (*e.g.*, glycerin in the ear) during conditioning reduces the incidence of sensitization (and subsequent seizures); impairment at the time of testing reduces seizure severity. When hearing is impaired on both occasions, the two effects are combined (7). The latter should be the case when ototoxic drugs are tested with this screen. Transient and permanent effects can be partitioned by subjecting the mice to a third sound exposure 3 days after the test exposure.

A drug that blocks central perception of the sound stimulus should be detected by its ability to prevent sensitization when present during conditioning, but not when administered immediately afterward. Prototypes of many pharmacologic classes have been tested, but no drug has been found that effectively blocks audioconditioning at nontoxic doses. Latency changes and a decreased incidence of maximal seizures can be observed. However, because of the reduced metabolic and excretory potential of young mice, it is difficult to determine whether these are due to impairment of audioconditioning or to residual drug effects on seizure response. The latter remains a possibility even after the 2- or 3-day condition–test interval. Phenobarbital and diphenylhydantoin have been shown to be proconvulsant (rather than anticonvulsant) 2 days after their administration (8).

It is interesting to note that ether and pentobarbital anesthesias do not block central perception of the stimulus. Their presence during conditioning does not prevent sensitization but rather appears to enhance its development and to counteract the antisensitization effects of unilateral ear blockade (7).

In contrast to drugs that only inhibit or enhance sensitization, we recently have discovered that high doses of atropine sulfate (25 mg./kg., i.p.) completely block the development of sensitization. While this admittedly is an extremely high dosage, it is the first indication that audioconditioning can be prevented by drugs. Furthermore, since it is an ED_{100} dose, it is likely that a lower dose range can be found.

A drug that alters postconditioning development of sensitization would be detected by its ability to inhibit or enhance sensitization when administered after the conditioning stimulus. Several drugs of this type have been observed in our laboratory (8). Low doses of edrophonium (1–2 mg./kg., i.p.) inhibit sensitization when administered 30 min. after conditioning. When tested 2 days later, seizure incidence and severity are reduced and latencies are prolonged. The effect is similar but less striking when edrophonium is given before conditioning. This perhaps is explained by the fact that the postconditioning duration of drug action is shorter in this case. When edrophonium-treated mice are challenged at a 3-day condition–test interval, the usual seizures are elicited, indicating that this drug slows rather than blocks the development of sensitization.

A multitude of drugs promote or inhibit the seizure response when present at the test exposure to sound. In general, convulsants promote seizures, whereas anticonvulsants, sedatives, and tranquilizers inhibit their onset and severity (8). These drugs produce similar effects on audiogenic seizures in genetically susceptible strains of mice.

The advantages of audioconditioned seizures as a screening method lie in several areas. As a biomodel of stress-induced neurosis, it does not require the use of genetically susceptible strains, special diets, chemicals, or surgical manipulation. The test response is a quick quantal observation which can be easily assessed by a technician. Also, since it involves the use of immature animals, this screen may have special predictive value for drugs to treat neurologic diseases of children.

It is our conclusion that audioconditioning should serve as an important experimental approach to the study of neural hyperexcitability and as a useful screening method for drugs affecting the CNS.

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Binding of Salicylate to Crystalline Bovine Serum Albumin and to Fraction V Bovine Serum Albumin

Keyphrases ☐ Salicylate binding—bovine serum albumin ☐ Bovine serum albumin, crystalline, Fraction V—salicylate binding comparison ☐ Equilibrium dialysis—bovine serum albumin salicylate binding

Sir:

In the course of developing analytical methods for protein-binding studies, we have routinely used Fraction V bovine serum albumin (BSA) rather than the more costly crystalline BSA in preliminary work. For a drug such as sulfaethidole (SETD), there appears to be little difference in binding of the drug to Fraction V BSA and to crystalline BSA; a survey of references cited in a recent review (1) indicates that many workers have

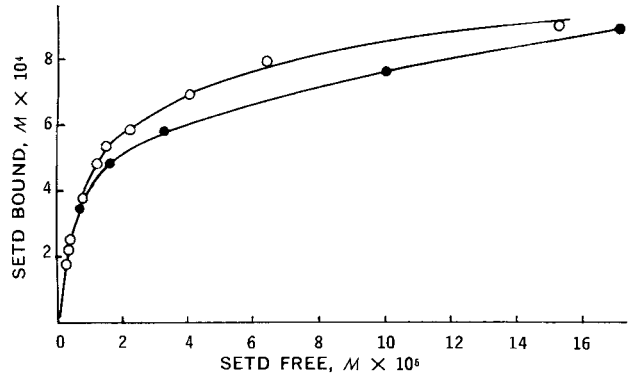

Figure 1—*Binding of SETD to 4.55% crystalline (●) and Fraction V (○) BSA, pH 7.4, 37°.*

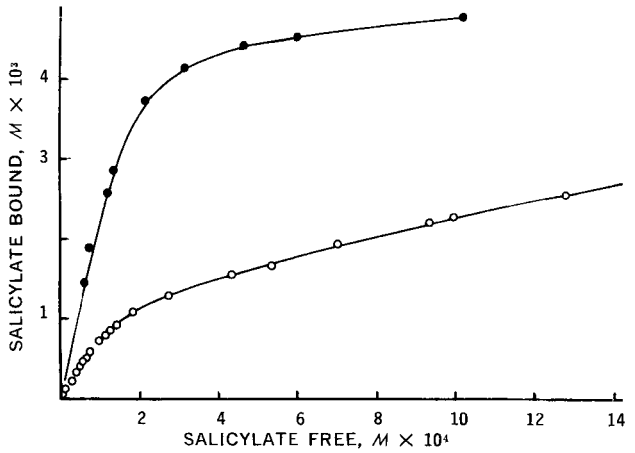

Figure 2—*Binding of salicylate to 4.55% crystalline (●) and Fraction V (○) BSA, pH 7.4, 37°.*

utilized Fraction V BSA to study drug binding. In studies with salicylate, however, we observed a marked difference in binding to these two BSA fractions. There have been reports of striking species differences in serum albumin binding of drugs (2–5), but the potential erroneous predictions which might arise from the assumption that if one drug exhibits similar binding to both crystalline and Fraction V BSA other drugs will also exhibit similar binding to both fractions have not been emphasized.

As shown in Fig. 1, SETD exhibits little difference in binding to Fraction V and crystalline BSA. Figure 2, however, illustrates considerably greater binding of salicylate to crystalline BSA than to Fraction V BSA. For example, at a total salicylate concentration of 47×10^{-4} *M*, 91% is bound in the presence of 4.55% crystalline BSA, while only 63% is bound by 4.55% Fraction V BSA.

A further and striking difference between the crystalline and Fraction V BSA is that 10 mg.% SETD, an agent that competes for binding sites with salicylate, and an agent that is effectively displaced from serum albumin by salicylate (6), greatly enhances the binding of salicylate to Fraction V BSA, while it exhibits the expected displacement of salicylate from binding to crystalline BSA and pooled human plasma (7). As illustrated in Fig. 3, at total salicylate concentrations less than about 6×10^{-4} *M* (9.6 mg.% sodium salicylate), there is displacement of salicylate upon addition of 10 mg.% SETD. But at higher salicylate concentrations, there is markedly enhanced binding of salicylate to the protein in the presence of 10 mg.% SETD.

It is apparent that Fraction V and crystalline BSA differ in such a manner that the binding of SETD is not

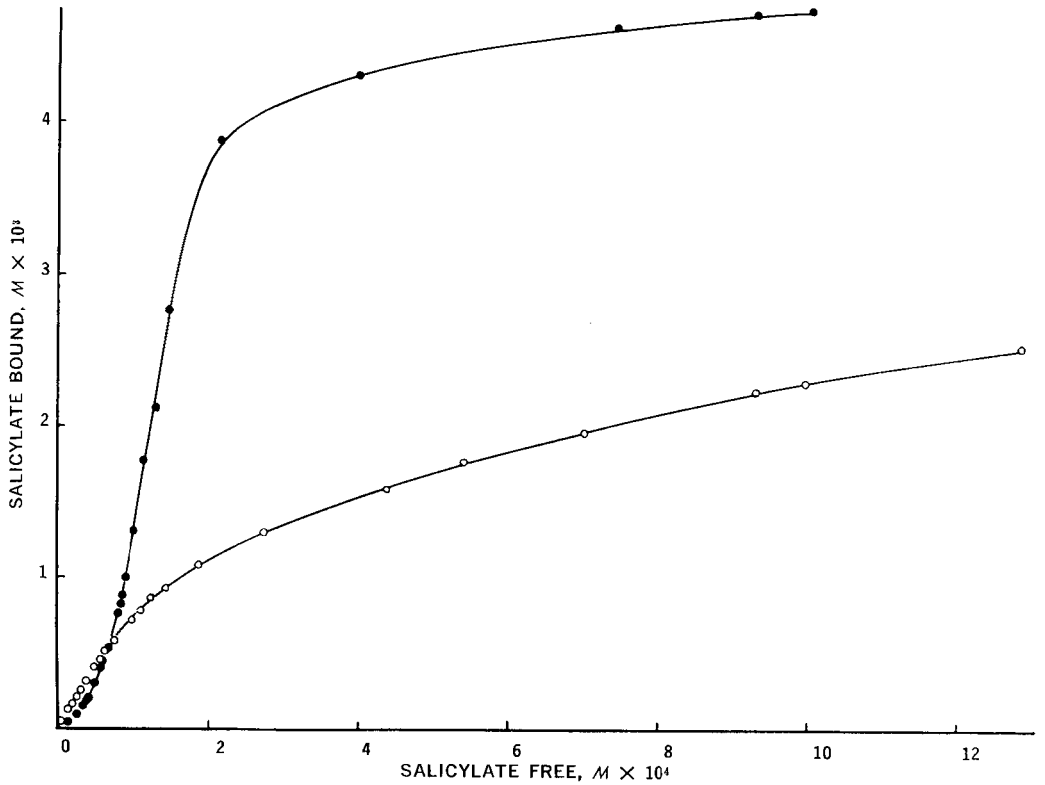

Figure 3—*Binding of salicylate to 4.55% Fraction V BSA (○) and the effect of 10 mg.% SETD on binding of salicylate (●), pH 7.4, 37°.*

greatly affected, but the binding of salicylate to Fraction V is greatly diminished. In Fraction V BSA, all of the salicylate binding sites may not be available or readily accessible to the drug. Upon addition of 10 mg.% SETD, however, there may be a subtle conformational change induced by the binding of SETD such that the Fraction V resembles the crystalline BSA in affinity for salicylate. Further studies in progress are directed toward exploration of the nature of the conformational change and its influence on the number of salicylate binding sites and the respective affinity constants.

Binding data were obtained by equilibrium dialysis for 12 hr. at 37°, in pH 7.4, 0.054 *M* phosphate buffer made isotonic with sodium chloride. Both inside and outside solutions were assayed for drug content, utilizing the Bratton-Marshall procedure for SETD (8), and both UV spectrophotometric analysis and ^{14}C were used for determination of salicylate.

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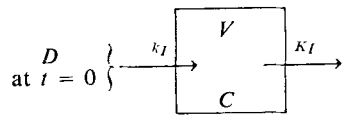
"Absorption Rate Constants" Calculated According to the One-Compartment Open Model with First-Order Absorption: Implications in *In Vivo–In Vitro* Correlations

Keyphrases □ "Absorption rate constants"—one-compartment open model □ *In vivo–in vitro* correlations, "absorption rate constants"—calculation method effect

Sir:

Plasma or serum concentrations of unchanged drug observed following oral administration of single doses of a drug frequently are readily fit by the one-compartment open model with first-order absorption (Model I). Wagner and Metzler (1) cited much of the literature on this fitting; such fitting leads to Eq. 1:



Model I

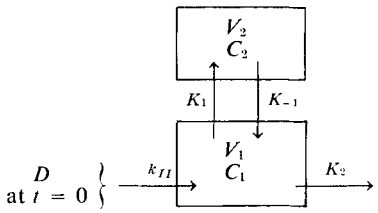
$$C = C^{\circ}\left(\frac{k_I}{k_I - K_I}\right)(e^{-K_I t} - e^{-k_I t}) \qquad \text{(Eq. 1)}$$

Even when simulated data are generated by application of Eqs. 2 and 2*a*,

$$C_1 = \frac{k_{II}D}{V_1}\left[\left\{\frac{K_{-1} - \alpha}{(k_{II} - \alpha)(\beta - \alpha)}\right\}e^{-\alpha t} + \left\{\frac{K_{-1} - \beta}{(k_{II} - \beta)(\alpha - \beta)}\right\}e^{-\beta t} + \left\{\frac{K_{-1} - k_{II}}{(\alpha - k_{II})(\beta - k_{II})}\right\}e^{-k_{II}t}\right] \qquad \text{(Eq. 2)}$$

$$\alpha,\beta = \tfrac{1}{2}\left[(K_1 + K_{-1} + K_2) \pm \sqrt{(K_1 + K_{-1} + K_2)^2 - 4K_{-1}K_2}\right] \qquad \text{(Eq. 2a)}$$

which are appropriate to the two-compartment open model with first-order absorption (Model II), most sets of C_1,t data can be fitted with two exponential terms (Eq. 1) rather than three exponential terms (Eq. 2). With real plasma or serum concentration data observed following oral administration, the same situation exists; however, in some cases, intravenous administration of the same drug tends to dictate the two-compartment open model.



Model II

When correlating "absorption rate constants" derived from plasma concentrations measured in man with *in vitro* rates of drug dissolution from dosage forms, one is most interested in relative values or ratios and not with absolute individual values.

The data in Table I are taken from Wagner and Metzler (1). When apparent "rate constants for absorptions," k_I, were estimated by nonlinear least-squares estimation, by applying Eq. 1 to data generated with Eqs. 2 and 2*a*, the absolute values of k_I deviated from the k_{II} values (either 0.5 or 2.0 hr.$^{-1}$), but the ratios of the k_I values were very close to the ratio of the true k_{II} values (namely, 4.0) when $8 \geq V_1/V_2 = K_{-1}/K_1 \geq 1$. It was previously shown (1) that where Model II was elaborated from actual plasma or serum level data, the ratio of parameters was within the limits shown in Table I.

This observation may help the dilemma of the biopharmaceutical and pharmacokinetic scientist who frequently can fit plasma or serum concentration data, obtained following oral administration, with Eq.