Nonlinear Tissue Disposition: Salicylic Acid in Rat Brain

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Abstract □ A model was developed to detect nonlinear disposition of a drug in a tissue. The model was experimentally tested relating to salicylic acid disposition in the brain. Experimental data obtained in rats are reported for doses of 25 and 400 mg/kg ip. The parameters measured for each dose were the ratio of the area under the brain concentration–time curve to the area under the plasma concentration–time curve and the ratio of the maximum brain concentration of salicylic acid to the plasma concentration at that point in time. The ratios increased with dose; furthermore, ratios calculated using plasma concentrations corrected for plasma protein binding were dose dependent. Calculations performed on literature data for salicylic acid disposition in mouse brain corroborated the results of this study. The existence of a saturable transport system for the elimination of salicylic acid from the brain is supported by the data presented. The rationale necessary to apply the model to any tissue is discussed.

Keyphrases □ Transport, drug—nonlinear tissue disposition, salicylic acid in rat brain, model proposed, equations □ Drug disposition—nonlinear tissue disposition, salicylic acid in rat brain, model proposed, equations □ Tissue disposition, nonlinear—model proposed, equations derived, salicylic acid in rat brain as an example □ Salicylic acid—nonlinear tissue disposition in rat brain, model proposed, equations

A number of saturable processes may produce disproportionate changes in the tissue distribution of a drug when the dose of the drug is increased. Facilitated transport is one such process; in its presence the result is nonlinear tissue distribution. The existence of transport systems for the elimination of organic acids from the brain and cerebrospinal fluid has been documented (1–5). Steinwall (2, 3) postulated that such transport systems could be contributing to the hindrance of blood-to-brain transfer; that is, these systems could be a functional component of the blood–brain barrier.

Confirmation of an acid transport system in the brain requires knowledge of factors governing the distribution and disposition of drugs in the brain such as: (*a*) binding to blood proteins and tissue components, (*b*) metabolism of the drug, (*c*) diffusion in and out of the tissue, and (*d*) bulk flow of the cerebrospinal fluid. Similar processes are functional in other tissues as well as the brain.

This paper proposes a model to detect the presence of saturable processes that would influence the disposition of a drug in a tissue. The model is experimentally tested as it relates to the transport of salicylic acid from the brain; however, the model should be applicable to drug disposition in other tissues.

THEORETICAL

Consider the simplest model for the distribution of drugs into tissues, the case in which a drug enters and leaves a tissue only by passive diffusion. For such a system the change in the amount of drug in the tissue with time, dT/dt, may be expressed by an equation based on Fick's first law of diffusion and the assumption that only unbound drug is diffusible:

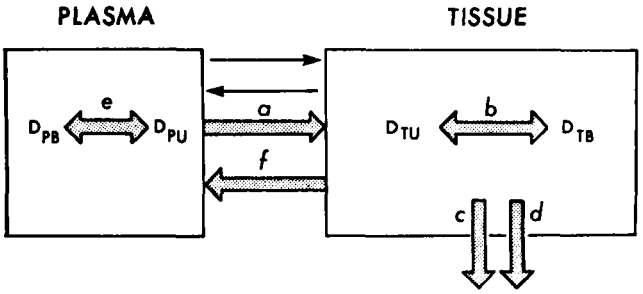

Figure 1—*Schematic diagram of the processes of disposition of a drug in a tissue. Key: thin arrows, passive diffusion; heavy arrows, saturable processes;* a, f, *unidirectional transport to and from tissue;* c, *metabolism;* d, *excretion; and* b, e, *binding to tissue components and plasma proteins. Symbols* D_{PB}, D_{PU}, D_{TB}, *and* D_{TU} *are the amounts of bound and unbound drug in plasma and tissue, respectively.*

$$\frac{dT}{dt} = k(C_1 - C_2) \qquad \text{(Eq. 1)}$$

where C_1 is the concentration of drug in the plasma free from protein binding, C_2 is the unbound concentration of drug in the tissue, and k represents a hybrid constant (units of clearance) for passage of the drug between plasma and tissue.

The rate of entry of the drug into the tissue is kC_1, while kC_2 is the rate of exit of the drug from the tissue. The integral of the rate of entry with time is the amount entering the tissue, $\int_0^\infty kC_1\,dt$, while the integral of the rate of exit with time equals the amount leaving the tissue, $\int_0^\infty kC_2\,dt$. The total amount that enters the tissue must equal the total amount that leaves the tissue; therefore:

$$\int_0^\infty C_1\,dt = \int_0^\infty C_2\,dt \qquad \text{(Eq. 2)}$$

Passive diffusion requires that the area under the unbound plasma concentration–time curve be equal to the area under the unbound tissue concentration–time curve.

In an experiment in which steady-state plasma and tissue levels are attained, that is $dT/dt = 0$, from Eq. 1:

$$C_1^{ss} = C_2^{ss} \qquad \text{(Eq. 3)}$$

where C_1^{ss} is the unbound plasma concentration at steady state, and C_2^{ss} is the unbound tissue concentration at steady state. The same relationship would be applicable for an experiment in which a single dose is administered. At the point in time, t_{max}, when the rates into and out of the tissue are equal, the unbound tissue level is maximal, C_2^{max}, and equal to the unbound plasma concentration, $C_1^{t\,max}$:

$$C_1^{t\,max} = C_2^{max} \qquad \text{(Eq. 4)}$$

Plasma and Tissue Binding—Unbound concentrations of a drug are not readily determined, particularly within a tissue. Usually, the total plasma concentration, C_P, and the total tissue concentration, C_T, are measured. The total plasma and tissue concentrations are complex functions of their respective unbound concentrations. The functions depend on the affinity of a drug for the plasma proteins and tissue components and on the total number of binding sites. The unbound concentration of the drug in the plasma is a fraction, α_1, of the total plasma concentration:

$$C_1 = \alpha_1 C_P \qquad \text{(Eq. 5)}$$

The fraction unbound varies with the total plasma concentration and, thus, with the dose. Consequently, the relationship between the area under the unbound concentration–time curve to the area under the total concentration–time curve becomes a function of the dose. This ratio can be thought of as the ratio of the mean value of C_1 with time, $\bar{C}_1$, to the mean value of C_P with time, $\bar{C}_P$, that is, the ratio of the time average values:

$$\frac{\int_0^\infty C_1\,dt}{\int_0^\infty C_P\,dt} = \frac{\int_0^\infty C_1\,dt \Big/ \int_0^\infty dt}{\int_0^\infty C_P\,dt \Big/ \int_0^\infty dt} = \frac{\bar{C}_1}{\bar{C}_P} \qquad \text{(Eq. 6)}$$

Similarly, for the tissue:

$$C_2 = \alpha_2 C_T \qquad \text{(Eq. 7)}$$

where α_2 is the fraction of drug in the tissue that is not bound to tissue components and:

$$\frac{\int_0^\infty C_2\,dt}{\int_0^\infty C_T\,dt} = \frac{\bar{C}_2}{\bar{C}_T} \qquad \text{(Eq. 8)}$$

Since the areas under the unbound plasma concentration–time curve and the unbound tissue concentration–time curve must be equal (Eq. 2), $\bar{C}_1$ equals $\bar{C}_2$. Dividing Eq. 6 by Eq. 8 yields:

$$R = \frac{\int_0^\infty C_T\,dt}{\int_0^\infty C_P\,dt} = \frac{\bar{C}_T}{\bar{C}_P} \qquad \text{(Eq. 9)}$$

The ratio, R, referred to as the area distribution ratio, is a measure of the concentration of drug in the tissue with time relative to that in the plasma with time. For both plasma and tissue, the fraction of the drug unbound normally increases with concentration; as the dose is increased, therefore, $\bar{C}_1/\bar{C}_P$ and $\bar{C}_2/\bar{C}_T$ increase. An increase or decrease of R with increasing dose depends on whether the plasma protein binding or tissue binding is the more readily saturable. The former results in an increase in R, the latter in a decrease.

In steady-state experiments, the fractions unbound in plasma, α_1^{ss}, and in tissue, α_2^{ss}, are:

$$\alpha_1^{ss} = \frac{C_1^{ss}}{C_P^{ss}} \qquad \text{(Eq. 10)}$$

$$\alpha_2^{ss} = \frac{C_2^{ss}}{C_T^{ss}} \qquad \text{(Eq. 11)}$$

The total plasma and tissue concentrations at steady state are represented by C_P^{ss} and C_T^{ss}, respectively. As given in Eq. 3, the steady-state unbound concentrations in plasma and tissue are equal; therefore, dividing Eq. 10 by Eq. 11 yields a steady-state distribution ratio, R^{ss}:

$$R^{ss} = \frac{C_T^{ss}}{C_P^{ss}} = \frac{\alpha_1^{ss}}{\alpha_2^{ss}} \qquad \text{(Eq. 12)}$$

Similarly, in an experiment in which a single dose is given, there is a time, t_{max}, when the unbound tissue concentration equals the unbound plasma concentration as given in Eq. 4. The total concentration in the tissue, C_T^{max}, is at a maximum at the same point in time when C_2^{max} is achieved, assuming tissue binding is instantaneous. A t_{max} distribution ratio, R^{max}, may then be defined as:

$$R^{max} = \frac{C_T^{max}}{C_P^{t_{max}}} = \frac{\alpha_1^{max}}{\alpha_2^{max}} \qquad \text{(Eq. 13)}$$

The plasma concentration at the same time point when the tissue is at the maximum concentration is defined as $C_P^{t_{max}}$. The values of R^{ss} and R^{max} vary with dose in a similar fashion to R due to the decreased binding at higher concentrations.

Saturable Transport Systems—The presence of transport, metabolic, or excretory mechanisms also give rise to a change in R, R^{ss}, and R^{max} with dose, as determined by Eqs. 9, 12, and 13, respectively. As an example, consider the case in which the rate of entry into a tissue may be kinetically expressed as a combination of passive diffusion and a saturable transport. For such a case:

$$\text{rate in} = kC_1 + \frac{aC_1}{b + C_1} \qquad \text{(Eq. 14)}$$

where the maximum rate of transport is represented by a, b is the dissociation constant for the "drug–carrier" complex, and k is as defined in Eq. 1. The amount that enters the tissue is obtained by integration of Eq. 14:

$$\text{amount entering tissue} = k\int_0^\infty C_1\,dt + a\int_0^\infty \frac{C_1}{b + C_1}\,dt \qquad \text{(Eq. 15)}$$

When assuming that a drug leaves the tissue only by passive diffusion, the total amount leaving the tissue is $k\int_0^\infty C_2\,dt$, as shown in the derivation of Eq. 2. Furthermore, if the assumption is made that no protein binding occurs in the plasma or tissue, substituting C_P for C_1 and C_T for C_2 leads to the following:

$$k\int_0^\infty C_T\,dt = k\int_0^\infty C_P\,dt + a\int_0^\infty \frac{C_P}{b + C_P}\,dt \qquad \text{(Eq. 16)}$$

that is, the total amount leaving the tissue must equal that entering the tissue. The area distribution ratio as expressed in Eq. 9 becomes:

$$R = 1 + \frac{\frac{a}{k}\int_0^\infty \frac{C_P}{b + C_P}\,dt}{\int_0^\infty C_P\,dt} \qquad \text{(Eq. 17)}$$

The transport in gives rise to values of R greater than 1; however, as the dose is increased to the point that C_P exceeds b for prolonged periods, the value of R decreases toward a value of unity.

Conversely, transport out of the tissue with passive diffusion in and out of the tissue and no plasma or tissue binding results in the following:

$$\text{rate in} = kC_P \qquad \text{(Eq. 18)}$$

$$\text{rate out} = kC_T + \frac{a'C_T}{b' + C_T} \qquad \text{(Eq. 19)}$$

where a' is the maximum rate of transport out, and b' is the dissociation constant for the drug–carrier complex. Since the total amount entering the tissue equals the total amount leaving the tissue:

$$k\int_0^\infty C_P\,dt = k\int_0^\infty C_T\,dt + a'\int_0^\infty \frac{C_T}{b' + C_T}\,dt \qquad \text{(Eq. 20)}$$

the area distribution ratio then becomes:

$$R = 1 - \frac{\frac{a'}{k}\int_0^\infty \frac{C_T}{b' + C_T}\,dt}{\int_0^\infty C_P\,dt} \qquad \text{(Eq. 21)}$$

In this case, R is less than unity and an increase in dose gives rise to an increase in its value toward unity. The same argument holds for saturable metabolism or excretory mechanisms in the tissue.

For the steady-state condition, if transport into the tissue is present as expressed in Eq. 14 and only passive diffusion accounts for the exit of the drug, then by assuming no binding effects the rate out of the tissue, kC_T, must equal $kC_P + [aC_P/(b + C_P)]$, the rate into the tissue. A steady-state distribution ratio is then defined by the following:

$$R^{ss} = 1 + \frac{a}{k(b + C_P^{ss})} \qquad \text{(Eq. 22)}$$

For transport out of the tissue:

$$R^{ss} = 1 - \frac{\frac{a'}{k}C_T^{ss}}{C_P^{ss}(b' + C_T^{ss})} \qquad \text{(Eq. 23)}$$

Following a bolus dose, the t_{max} distribution ratios are identical to Eqs. 22 and 23; C_T^{max} and $C_P^{t_{max}}$ replace C_T^{ss} and C_P^{ss}, respectively, in Eqs. 22 and 23.

A change in a distribution ratio with dose indicates the existence of nonlinear disposition of a drug in a tissue. As previously discussed, the direction of change in the distribution ratio with dose

Table I—Salicylic Acid Disposition in Rat Brain and Plasma

Hours after Treatment	Total Plasma Concentration[a], μg/ml	Assayed Brain Concentration[a], μg/ml
	25-mg/kg Dose	
0.5	105.8 ± 1.9	3.98 ± 0.45
1.0	96.5 ± 2.3	4.16 ± 0.30
5.0	65.2 ± 4.3	2.89 ± 0.16
10.0	15.2 ± 2.8	0.60 ± 0.08
15.0	8.5 ± 1.2	0.26 ± 0.03
20.0	4.2 ± 1.2	0.12 ± 0.04
	400-mg/kg Dose	
1.0	561.2 ± 41.7	119.7 ± 15.4
5.0	380.4 ± 14.4	71.9 ± 3.0
10.0	357.6 ± 22.7	62.4 ± 4.5
20.0	187.7 ± 14.7	21.3 ± 2.5
30.0	153.0 ± 5.1	12.1 ± 1.0
40.0	62.3 ± 12.8	3.4 ± 0.8
50.0	26.2 ± 7.2	1.2 ± 0.4

[a] The mean of five animals and standard error.

indicates which nonlinear process might exist. In reality, however, combinations of saturable processes are likely to be present.

Figure 1 schematically shows the mechanisms that would result in a dose-dependent distribution ratio. For combinations of saturable processes, the net change in the distribution ratio is a result of the dominant process if the effects are opposite. If the effects reinforce each other, the change is exaggerated. An increase or decrease in a distribution ratio with dose could thus help discern which saturable process is involved. For example, *if a distribution ratio decreases with dose*:

1. A unidirectional transport into the tissue may exist. Such a transport is described by process *a* in Fig. 1. As the dose increases, the transport system approaches saturation, resulting in tissue concentrations of the drug that are proportionately lower at the higher plasma concentrations.

2. Tissue binding of the drug may be occurring, process *b*. The possibility in this case is one of saturation of binding sites within the tissue as the dose increases. The apparent effect would be that the tissue loses the ability to accumulate the drug.

3. Metabolism of the drug in the tissue may be increasing, process *c*. This is an unlikely possibility unless the drug activates its own metabolism or the experiment involves chronic dosing with a drug which induces its own metabolizing enzymes.

If a distribution ratio increases with dose:

1. The degree of plasma protein binding may not be constant as the dose is increased, process *e*. This is to be expected, since the fraction of drug unbound normally increases with concentration.

2. A metabolic or an excretory system capable of being saturated may exist in the tissue, processes *c* and *d*, respectively. The administration of increasing doses results in a lower rate of elimination relative to the amount in the tissue. The net result would be an increase in tissue levels larger than the corresponding increase in plasma levels.

3. Elimination from the tissue may involve a saturable transport system, process *f*.

Correction for Plasma Protein Binding—An increase in a distribution ratio with dose due to the saturation of plasma protein binding can be readily ascertained. When assuming that the entry of a drug into a tissue depends upon the unbound drug in plasma, the ratio of the area under the tissue concentration–time curve to the area under the unbound plasma concentration–time curve would be independent of plasma protein binding. This ratio may be determined by dividing Eq. 9 by Eq. 6:

$$R_{cor} = \frac{\int_0^\infty C_T\,dt}{\int_0^\infty C_1\,dt} \qquad \text{(Eq. 24)}$$

The ratio, R_{cor}, is referred to as the area distribution ratio corrected for plasma protein binding.

A steady-state distribution ratio corrected for plasma protein binding, R_{cor}^{ss}, may be determined from steady-state tissue and unbound plasma concentrations:

$$R_{cor}^{ss} = \frac{C_T^{ss}}{C_1^{ss}} \qquad \text{(Eq. 25)}$$

Similarly, following a single dose, the t_{max} distribution ratio corrected for plasma protein binding is:

$$R_{cor}^{max} = \frac{C_T^{max}}{C_1^{t_{max}}} \qquad \text{(Eq. 26)}$$

The values of R_{cor}, R_{cor}^{ss}, and R_{cor}^{max} should be unity unless tissue binding or other saturable processes occur in the tissue. If the distribution ratios corrected for protein binding do not change over the dosage range tested, saturation of plasma protein binding is the mechanism by which R, R^{ss}, and R^{max} vary with dose. Any change in the corrected distribution ratios indicates the existence of other saturable processes. The direction of the change gives a further indication of the probable mechanisms.

EXPERIMENTAL

Materials—All chemicals used were of analytical grade; the sodium salicylate[1] was of certified grade.

Apparatus—A spectrophotofluorometer[2] was utilized in the salicylic acid assays. Protein binding studies were carried out with an equilibrium dialysis apparatus[3].

Animal Procedures—Male Sprague–Dawley rats[4], 190–210 g, were used. Two major studies were carried out: in one, the rats received a dose of sodium salicylate corresponding to 25 mg/kg; in the other, the dose was 400 mg/kg.

Several groups with five rats per group were randomly selected for each study. Except for the control group, each rat received an intraperitoneal injection of 1 ml of a sodium salicylate solution. After treatment the animals were sacrificed at different times by decapitation.

Upon decapitation the blood from each rat was collected in a 50-ml beaker containing 200 μl of a 0.05 *M* solution of edetic acid [(ethylenedinitrilo)tetraacetic acid disodium salt]. After centrifugation of blood, 1 ml of plasma was transferred to a centrifuge tube. The brain from each rat was removed after decapitation and quickly frozen in dry ice after blotting with a tissue to remove excess surface blood. Brain and plasma samples were stored frozen until the time of analysis.

Assay Procedures—Plasma samples were assayed using the procedure of Rowland and Riegelman (6). Standard curves were prepared by extracting control plasma samples containing known amounts of salicylic acid.

To assay the brain samples for salicylic acid, each brain was homogenized in a tissue grinder[5] using two volumes of distilled water for every gram of brain. Three milliliters of homogenate was transferred into a 20-ml culture tube containing approximately 2 g of sodium chloride and 0.3 ml of 6 *N* HCl. After mixing the samples, 5 ml of a solution of equal volumes of ether and *n*-heptane was added to the samples, and the samples were gently shaken mechanically for 30 min.

After centrifugation to separate the phases, an aliquot of the organic phase was extracted with 2 ml of a 0.5 *M* phosphate buffer, pH 7. The phosphate buffer extracts were read on the spectrophotofluorometer at the wavelengths for maximum activation and fluorescence, as determined using a standard sample of sodium salicylate in phosphate buffer. By using the described extraction procedure, known amounts of salicylic acid were added to homogenates of control brains to prepare the standard curves.

The value obtained for the concentration of salicylic acid in the brain homogenate had to be corrected for the contribution of drug from the residual blood. By using a value of 11 μl as the residual blood per gram of brain (7) and calculating the concentration of salicylic acid in the blood from the concentrations in plasma[6], it was possible to determine the amount of salicylic acid that had to be subtracted from the amount assayed in the brain.

[1] Fisher Scientific Co.

[2] Aminco-Bowman spectrophotofluorometer model 8210.

[3] Dianorm equilibrium dialysis system, supplied by Innovativ-Medizin AG., P.O. Box 31, CH-Esslingen, Switzerland.

[4] Horton Laboratories, Inc.

[5] Tenbroeck.

[6] M. A. Gonzalez and T. N. Tozer, unpublished data.

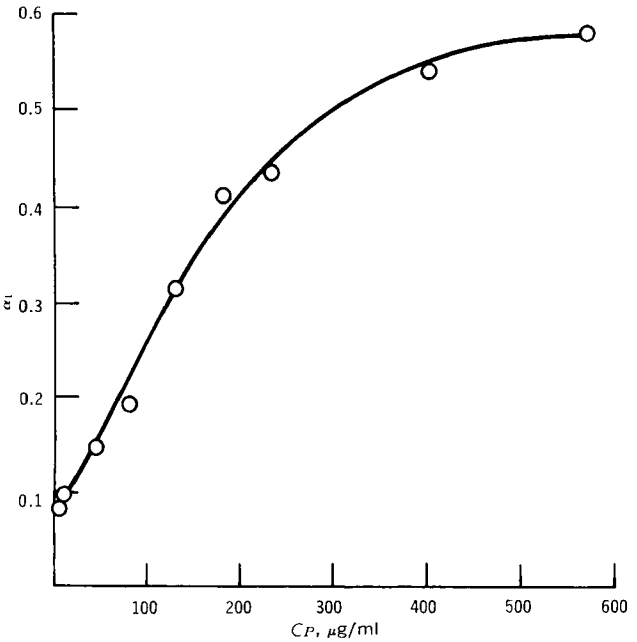

Figure 2—*Unbound fraction of total plasma concentration, α_1, as a function of total plasma concentration, C_P.*

Protein Binding Study—The degree of plasma protein binding for salicylic acid was determined using the equilibrium dialysis system. The apparatus consists of 20 cells (Teflon), each of which is divided into two compartments or half-cells with a 1-ml volume by a section of tubing[7] with a thickness of 0.025 mm. The cells are mounted on a rotor so that agitation is accomplished by rotation of the cells. The entire apparatus is watertight, so that it may be immersed in a constant-temperature water bath.

In the present study, 1 ml of a solution of sodium salicylate in Krebs–Ringer bicarbonate buffer was introduced with a tuberculin syringe into one half-cell, and 1 ml of fresh rat plasma was simultaneously injected into the other half-cell. Since the apparatus has 20 cells, it was possible to dialyze different concentrations of salicylic acid at one time. Preliminary experimentation showed equilibrium to be reached within 1–2 hr; therefore, the dialysis was carried out for 3 hr at 37°.

After equilibration, the solutions in the buffer and plasma half-cells were analyzed using the plasma assay previously mentioned. The concentration of drug in the buffer half-cell was taken to represent the concentration of the free ligand on both sides of the membrane. The total concentration of the bound and free ligand was obtained from the analysis of the plasma half-cell. The fraction of salicylate unbound was thus calculated by dividing the concentration in the buffer half-cell by the concentration in the plasma half-cell.

RESULTS AND DISCUSSION

The salicylate concentrations assayed in plasma and brain are summarized in Table I. The samples were analyzed separately; the values reported are the arithmetic means of five separate plasma or brain samples.

The results of the plasma protein binding study are depicted in Fig. 2. By using this graph, the values for total salicylate in plasma, C_P in Table I, were converted to the concentrations of unbound salicylic acid, C_1. The brain concentrations reported in Table I were corrected for the salicylic acid contributed by the residual blood as discussed previously. The corrected tissue concentrations, C_T, together with the unbound concentrations of salicylic acid in plasma are plotted in Fig. 3.

The disposition of salicylic acid in rats is dose dependent, as evidenced by the difference in the apparent half-lives of elimination for the 25- and 400-mg/kg doses. A comparison of the half-lives calculated from the slopes of the plasma curves in Fig. 3 within the first 20–30 hr accentuates the dose dependency. The half-life of salicylic acid in the plasma after a 25-mg/kg dose was 2.8 hr, while a half-life of 11 hr resulted from a 400-mg/kg dose.

Dose-dependent elimination of salicylic acid in humans was reported previously by Levy *et al.* (8). These authors pointed out that monoexponential fits of plasma data are incorrect and that nonlinear kinetics best describe salicylic acid disposition; however, these conclusions are based on studies in individuals. Nonlinear kinetic parameters can be obtained by curve fitting the data from each individual. The present investigation involved a population study; therefore, calculations of nonlinear parameters are not as valid as when an individual is the test subject.

Dose dependency in the time course of the unbound plasma concentration will give rise to disproportionately larger areas under the C_1–time curve; since the amount entering a tissue must equal the amount leaving a tissue, the ratio of the areas under the C_T–time curve to the area under the C_1–time curve should not change. That is, the distribution ratio is independent of the time course of a drug in the body; saturable processes must be present to account for changes in this ratio with dose.

As presented in the *Theoretical* section, the calculation of the distribution ratios, R and R^{max}, after different doses of a drug serves as a test of the model described by Eqs. 9 and 13. To determine R, the areas under the cartesian plots of C_T–time and C_P–time were estimated using the trapezoidal rule. To determine R^{max}, C_T^{max} was approximated by selecting the highest mean value of C_T achieved. The plasma concentration obtained at the same time as C_T^{max} was used for C_P^{max}. The results are reported in Table II under the headings R and R^{max}. The large increase in R and R^{max} with increasing dose indicates nonlinear disposition of salicylic acid in the brain.

The increase in the distribution ratios could be attributed to a decrease in the fraction of salicylic acid bound in the plasma as the plasma concentration increases. To test this possibility, the distribution ratios corrected for plasma protein binding, R_{cor} and R_{cor}^{max}, were calculated from the data in Fig. 3. The corrected distribution ratios calculated for the two doses are listed in Table II. Inasmuch as these distribution ratios increase with dose, metabolism, excretion, or transport out of the brain must be involved in the salicylic acid elimination. Transport involvement in the entry of salicylic acid or tissue binding of the drug in the brain would cause a decrease in the distribution ratios if these processes were solely present.

As previously discussed, a combination of processes could be involved, but the change in the distribution ratios would be dependent on the dominant mechanisms. For example, entry and exit of salicylic acid in the brain could both be carrier mediated; however, if the transport out were more readily saturated than the transport in, the distribution ratios would increase with dose.

As further verification of the dose dependency of the distribution ratios for salicylic acid, R^{max} and R_{cor}^{max} were calculated using literature data for salicylic acid distribution in mice (9, 10). These studies included data for brain, unbound blood, and total blood concentration of salicylic acid at different times following administration of 50–800 mg/kg ip. The results of these calculations are

Table II—Experimentally Determined Distribution Ratios

Dose, mg/kg	R	R^{max}	R_{cor}	R_{cor}^{max}
25	0.035	0.035	0.180	0.146
400	0.139	0.205	0.302	0.353

Table III—Distribution Ratios Calculated from the Data of Sturman *et al.* (9) and McArthur *et al.* (10) in Mice

Dose, mg/kg	R^{max}	R_{cor}^{max}
50	0.153	0.305
100	0.213	0.386
200	0.278	0.487
400	0.294	0.506
800	0.459	0.766

[7] Visking.

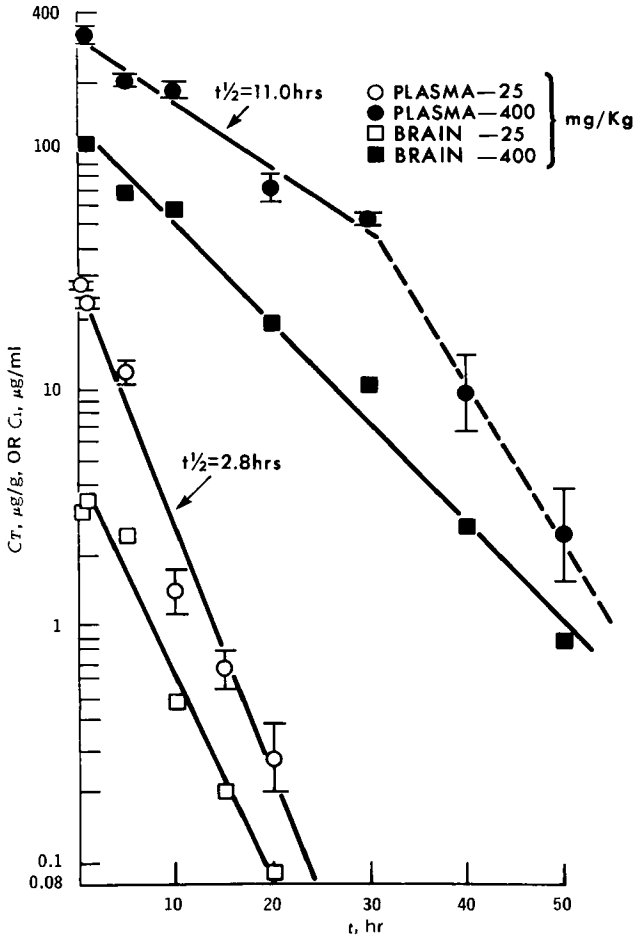

Figure 3—*Semilogarithmic plots of salicylic acid concentrations in rat brain and plasma* versus *time after administration of 25 and 400 mg/kg ip.* C_1 *is the unbound concentration of salicylic acid in the plasma;* C_T *is the concentration of salicylic acid in the brain corrected for residual blood.*

summarized in Table III; the results agree with those of the present study; that is, the distribution ratios increase with dose.

One interpretation of the changes in the values of the distribution ratios could be the existence of a saturable metabolic pathway in the brain, as discussed in the *Theoretical* section. This explanation is unlikely since Wolff and Austen (11) reported no salicylate metabolites in rat brains. Sturman *et al* (9) reached the same conclusion in their studies with mice.

An alternative explanation is the existence of a saturable transport system which functions to rid the brain of unwanted acidic compounds, in this case salicylic acid. As previously discussed, if the rate of elimination relative to the amount in the tissue becomes lower due to saturation of the transport system, the tissue levels increase more than the corresponding increase in plasma levels. This would result in a distribution ratio that increases with dose. The hypothesis that a transport system is responsible for the dose dependency of the distribution ratios presupposes that there are no major physiological changes produced by the drug under study. Especially critical is that the cerebral blood flow remain unchanged throughout the study.

The fact that the values for the distribution ratios corrected for plasma protein binding are always less than one also points to a transport system that prevents salicylic acid in the brain from achieving equilibrium with the plasma. Referring to Eqs. 24 and 26, if there were tissue binding, R_{cor} and R_{max} should be greater than one. McArthur *et al.* (10) reported that binding does not occur in brain tissue. The values of R_{cor} and R_{cor}^{max}, therefore, should be equal to one if there is no metabolism in the tissue, no transport system, and no other means of drug elimination. Cerebrospinal fluid bulk flow could be invoked as responsible for the elimination of acids from the brain, but this process is not saturable and does not explain the dose dependency of the corrected distribution ratios. Since the metabolism is considered unlikely, the existence of a saturable transport system is postulated.

If it were possible to saturate completely a transport system in the brain, the corrected distribution ratios should approach unity. This is observed with the R_{cor}^{max} values in Table III.

The dose dependency of the distribution ratios does not conclusively prove the existence of a transport system for the elimination of salicylic acid from the brain. Additional proof could be obtained by the administration of known inhibitors of acid transport systems if the distribution values should increase in the presence of the inhibitor.

The model and techniques presented should be applicable to any tissue. Further studies are planned to test the general usefulness of the model as a means of screening drugs for saturable metabolism, binding, and transport within specific tissues.

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ACKNOWLEDGMENTS AND ADDRESSES

Received January 18, 1974, from the *Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143*

Accepted for publication July 22, 1974.

Supported by a grant from Eli Lilly and Co. M. Gonzalez also gratefully acknowledges receipt of a U.S. Public Health Service Traineeship (5T01 GM006728-07).

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