Quantitative IR Spectrophotometric Calculations

Keyphrases □ IR spectrophotometry—analysis, carbachol, absorbance calculation, error reported □ Absorbance calculations—IR spectrophotometric analysis of carbachol, error reported □ Carbachol—IR spectrophotometric analysis, absorbance calculation, error reported □ Cholinergics, ophthalmic—carbachol, IR spectrophotometric analysis, absorbance calculation, error reported

To the Editor:

In a recent paper (1), the authors made a common technical error in the calculation of absorbance from the observed values of percent transmittance as read off the recorder chart paper. According to the instructions by Frank and Chafetz (1), the length of the line read off the chart is a linear displacement of the absorption maximum along the chart ordinate from 100% transmission, in other words, a $1 - T$ absorption value.

It is fundamental that the concentration of an active absorbing species is not proportional to $1 - T$ but to log $1/T$. This is an error of a type, but a related error also was made by taking the log of $1/1 - T$ and equating this term to log $1/T$.

This point has been treated in the reference text literature (2). The correct procedure is to measure the log I_0/I values from the experimental values, using the experimental technique otherwise correctly described. This approach is well illustrated in the ASTM Recommended Practices (3).

As pointed out by Potts (2), log $1/T$ (correctly determined) is essentially directly proportional to $1 - T$ at high transmission values, and it is reasonable to assume from the excellent results reported by Frank and Chafetz (1) that this also holds for log $1/1 - T$ values. Since reflection and scatter effects are small and reproducible in transmission measurements of solutions and the baseline falls at high transmission values relative to the initial 100% T set-point, the practical effects in this work were small, with little detectable bias error in the final result.

However, since this paper is one of the few in dosage form analysis utilizing IR quantitation, clearly demonstrating the value of the specificity imparted by this technique, it is important to point out this academic procedural point.

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Tissue Binding of Drugs

Keyphrases □ Phenytoin—tissue binding, normal, nephrotic, and uremic humans compared □ Warfarin—tissue binding, rats with intrinsic high and low plasma binding ability compared □ Tissue binding—phenytoin compared in normal, nephrotic, and uremic humans, warfarin in rats with intrinsic high and low plasma binding ability □ Binding, tissue—phenytoin compared in normal, nephrotic, and uremic humans, warfarin in rats with intrinsic high and low plasma binding ability □ Anticonvulsants—phenytoin, tissue binding, normal, nephrotic, and uremic humans compared □ Anticoagulants—warfarin, tissue binding, rats with intrinsic high and low plasma binding ability compared

To the Editor:

Intersubject variability in plasma protein binding of drugs because of genetic or disease-related factors is widely recognized (1–5). The degree of variability in tissue binding of drugs, however, is essentially unknown. An important exception is the work of Jusko and Weintraub (6), who found a positive correlation between postmortem myocardial-to-serum concentration ratios and antemortem creatinine clearances in 15 patients. Based on these observations, they suggested that reduced tissue binding may explain the relatively small apparent volume of distribution of digoxin found in patients with impaired renal function (7).

A more general approach to gaining insight to variability in tissue binding was recently suggested (8, 9) based on the physiological approach to distribution developed by Gillette (10). It can be shown that:

$$V_{ss} = V_B + V_T \frac{f_B}{f_T} \qquad \text{(Eq. 1)}$$

where V_{ss} is the apparent volume of distribution at steady state, V_B is blood volume, V_T is the volume of other tissues in the body, and f_B and f_T are the fractions of unbound drug in blood and tissue, respectively. For most lipid-soluble drugs, the sum of V_B and V_T is equivalent to total body water; for drugs that do not penetrate cells, the sum of V_B and V_T is equivalent to the extracellular space. The term f_T may be viewed as the average fraction of unbound drug in the extravascular space weighted for tissue mass.

We used a modification of this equation to determine differences in the tissue binding of phenytoin between normal healthy volunteers and nephrotic (3) or uremic (4) patients and in the tissue binding of warfarin in rats who were intrinsically high or low plasma binders of the drug (5). This approach represents a new application of this equation. Assuming that V_β (11) is about equivalent to V_{ss} and incorporating the red blood cell volume into the "tissue" space give:

$$f_T = \frac{V_T(f_p)}{V_\beta - V_p} \qquad \text{(Eq. 2)}$$

where f_p is the fraction of drug unbound in plasma, V_p is plasma water, and V_T is the volume of total body water minus plasma volume. The term f_T incorporates binding to red blood cells. Apparent volumes of distribution and

Table I—Apparent Volumes of Distribution and Binding Parameters for Phenytoin in Healthy Volunteers, Nephrotic Patients, and Uremic Patients

Clinical Condition	V_β, liters/kg	f_p	f_T
Healthy volunteers (n = 6)[a]	0.3	0.10	0.22
Nephrotic patients (n = 6)[a]	0.6	0.19	0.19
Healthy volunteers (n = 4)[b]	0.64	0.12	0.11
Uremic patients (n = 4)[b]	1.40	0.26	0.11

[a] Apparent volumes of distribution and plasma binding data reported in Ref. 3. [b] Apparent volumes of distribution and plasma binding data reported in Ref. 4.

Table II—Apparent Volumes of Distribution and Binding Parameters for Warfarin in Rats

	V_β, liters/kg[a]	f_p[a]	f_T
High plasma binders (n = 6)	0.14	0.004	0.025
Low plasma binders (n = 7)	0.19	0.014	0.058

[a] Values were reported in Ref. 5.

plasma binding data were obtained from the literature (3–5). Total body water was assumed to be 599 ml/kg in humans and 660 ml/kg in rats. Plasma volume was assumed to be 46 ml/kg in humans and 42 ml/kg in rats.

The findings with phenytoin are summarized in Table I. Both types of renal disease resulted in a twofold increase in both the fraction of free drug in the plasma and the apparent volume of distribution compared to those observed in healthy volunteers. Consequently, in neither case was the apparent tissue binding affected. The reduced plasma binding observed in nephrotic patients was essentially secondary to hypoalbuminemia (3), whereas that observed in uremic patients was presumably due to changes in the molecular structure of albumin or to the accumulation of an avidly bound endogenous inhibitor that acts like a displacing agent (12).

In view of the unaltered tissue binding of phenytoin in nephrotic patients, either hypoalbuminemia in the extravascular space is less pronounced than that observed in the vascular space or molecules other than albumin are principally responsible for binding phenytoin in the tissues. In uremic patients, phenytoin binding sites in the tissue are less susceptible to disease-related changes than phenytoin binding sites in the plasma.

As shown in Table II, differences in plasma protein binding of warfarin in rats were paralleled by qualitatively similar differences in tissue binding. Rats that showed relatively high plasma binding of warfarin also showed relatively high tissue binding of the drug. Consistent with this finding is the observation that the fraction of warfarin unbound to liver homogenates correlated strongly with the fraction of warfarin unbound to serum in the same animals (5). Perhaps a similar factor is responsible for the intersubject variability of warfarin binding in both plasma and tissue of rats.

The examples presented illustrate the utility, at least in a conceptual sense, of considering drug distribution in a physiological framework (10). Estimations of apparent tissue binding can provide guidelines for more extensive distribution studies.

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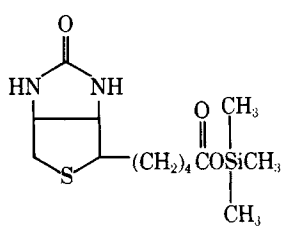
Characterization of Biotin Trimethylsilyl Derivative

Keyphrases □ Biotin trimethylsilyl derivative—structure determined □ Trimethylsilyl derivative of biotin—structure determined □ GLC–mass spectrometry—analysis, trimethylsilyl derivatives of biotin, structure determined □ Vitamins—biotin, structure of trimethylsilyl derivative determined

To the Editor:

Analytical methods are available for the determination of biotin (1, 2). In view of the need for a stability-indicating assay procedure, the GLC method (2) was investigated. The purpose of this communication is to report that the silyl derivative actually prepared according to this procedure is not the silyl ester reported (I) but the trisilyl de-



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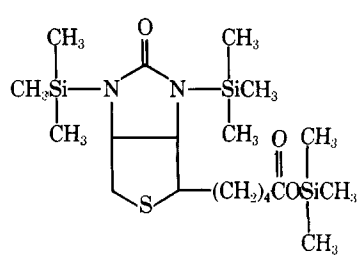

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