Therapeutic and Toxic Drug Concentrations: A Correction

To the Editor:
The tabulation by Winek [Clin. Chem. 22, 832 (1976)] provides a concise review of the therapeutic, toxic, and lethal concentrations of drugs or chemicals in the blood. The therapeutic concentration of theophylline and aminophylline is listed as 20–100 mg/liter. Theophylline is a mainstay in the treatment of asthma and constrictive chronic obstructive pulmonary disease, and in fact clinical symptoms and pulmonary function tests begin to improve when serum theophylline concentrations are 5–10 mg/liter, whereas toxic symptoms appear at theophylline concentrations exceeding 15 mg/liter (1–7). Thus a more realistic therapeutic serum concentration of theophylline would be between 10–20 mg/liter. Also, a recent paper (8) reported eight patients who developed grand mal seizures after receiving intravenous theophylline; four of whom subsequently died. Serum theophylline concentrations measured within 1 h of the seizure ranged from 25–70 mg/liter (mean, 53 ± 4.8 mg/liter). Thus Winek’s indication of a therapeutic concentration of 20–100 mg/liter exceeds the therapeutic concentration and extends into the lethal range.

Winek also states that the therapeutic concentration of digitoxin is between 1.7–2.1 µg/liter. It has been demonstrated that mean serum digitoxin concentrations in patients without symptoms of toxicity are about 10-fold higher than those of digoxin, mainly from the substantially greater serum protein binding of digitoxin (9). Serum digitoxin concentrations between 4 and 50 µg/liter have been reported to be therapeutic (9–19). Digitals intoxication is a well-defined condition (20) and has been shown to correlate with serum concentrations. Beller et al. (10) evaluated the mean serum digitoxin concentrations in toxic and nontoxic patients receiving digitalis leaf and digitoxin. The concentration was 34 ± 18 µg/liter in the toxic group as compared to 20 ± 11 µg/liter in the nontoxic group. Bentley (13) found that digitoxin toxicity was associated with concentrations greater than 45 µg/liter. Butler (19) demonstrated that most nontoxic patients have serum digitoxin concentrations of less than 30 µg/liter while most intoxicated patients have concentrations in excess of 25 µg/liter. A therapeutic range of 20–35 µg/liter has been recommended for digitoxin, with toxicity observed with concentrations exceeding 45 µg/liter (21).

We agree with Mr. Winek that serum concentrations must be interpreted in relation to factors affecting the patient and drug as listed in his Table 1; however, we hope that our corrections will clarify the apparently erroneous therapeutic concentrations for digitoxin and theophylline.

References

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Ed. note: Mr. Winek is obliged to readers Dasta and Slaughter, and also to Thomas J. Persoon and Janice Quinn, (Univ. of Iowa), who also pointed out the error with respect to theophylline, which came from a 1968 reference. Reader Joseph S. Annino points out in addition that "an upper limit of normal for blood lead of 1.3 mg/liter appears high by any standard . . . especially when the range includes the author's stated toxic level of 9.7 mg/liter." Mr. Winek responds: "With regard to lead, this is an old problem. We have [seen concentrations] of [1 mg/liter] with no symptoms and have had the same level with symptoms. With lead, the total body burden is important. The expected or 'normal' range does overlap with the toxic. . . . The table is to be used as a guide and each case evaluated on its own basis. I suggest reading about Kehre's lifelong work on lead for enlightenment (In Essays in Toxicology, 1, 1969, chap. 4, 'Lead Poisoning. An Old Problem with a New Dimension,' by P. B. Hammond, pp 116–151)."

Inhibition of Alkaline Phosphatase Activity by Serum Albumin

To the Editor:
The diagnostic value of enzyme activity measurements in serum depends on the methodology used and the accuracy and reproducibility of the results. Of importance to these is the suitability of the substrate. For many enzymes, such as the aminotransferases, the choice is restricted to the natural substrate but for others, such as alkaline phosphatase.
In recent issues of Clinical Chemistry, a debate has developed over how best to adjust serum calcium values for changes in protein concentration, some authors advancing the use of a regression coefficient based on albumin (1), others advocating a regression coefficient based on total protein (2).

We do not wish to further this debate, but rather to question the need for a "corrected" total calcium.

"Total" calcium includes three different forms: (a) protein bound, approximately 45%; (b) Ca that is complexed with organic compounds, approximately 5%; and (c) Ca\(^{2+}\), approximately 50%.

It is the last that is most important physiologically and pathologically and that regulates and is regulated by parathyrin (parathyroid hormone). Moreover, nearly all the extravascular fluid calcium—e.g., that in cerebrospinal fluid—is ionized.

The importance of ionized calcium notwithstanding, the utility and practicality of directly measuring ionized calcium is still problematic. As an alternative to the direct measurement of ionized calcium, one can use the McLean-Hastings (3) nomogram, which enables one to determine ionized calcium indirectly from data on total calcium and total protein. Or one can calculate the ionized calcium by using the formula of Zeisler (4), which, like the McLean-Hastings nomogram, depends on information on calcium and total protein.

Although various studies have shown good correlation between values for ionized calcium in normal sera as obtained by direct measurement and values calculated by the Zeisler formula or the McLean–Hastings nomogram (5), there appears to be a lack of agreement in results for sera obtained from abnormal patients (6).

We have found that a modification of the Zeisler formula gives better correlation with direct measurement of ionized calcium in both normal and abnormal sera, and enables one readily to obtain an accurate result.

According to Zeisler (4), serum ionized calcium is calculated:

\[
\text{mg Ca}^{2+}/dl = \frac{6 \text{Ca} - (P/3)}{P + 6}
\]

where \(\text{Ca} = \) milligrams total calcium per deciliter, and \(P = \) grams of protein per deciliter. We modified this formula to reflect studies that show that protein-bound calcium is linearly related to albumin to the extent that about 81% of the protein-bound calcium is bound to albumin (5):

\[
\text{mg Ca}^{2+}/dl = \frac{6 \text{Ca} - (K/3)}{K + 6}
\]

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**Why Measure Total Serum Ca?**

To the Editor:

One contributing factor in the choice of substrate for alkaline phosphatase was revealed during investigations into assay conditions and the effect of serum components—especially serum protein, which, compared to enzyme concentration, is high—on the estimation of its activity.

Increasing the serum albumin concentration in the assay medium caused a decrease in the hydrolysis rate of \(p\)-nitrophenyl phosphate and \(\alpha\)-naphthyl phosphate. This is presumably due to binding of these substrates to albumin—as the protein concentration increases, the amount of substrate freely available to the enzyme diminishes, thereby causing the rate to decrease (1).

Figure 1 shows the influence of serum albumin on alkaline phosphatase catalysis in bicarbonate buffer, pH 9.5 and 30 °C. For both substrates in concentrations of less than 1 mmol/liter, enzymatic activity is inhibited, but this inhibition lessens as substrate concentrations are increased; that is, as proportionally more substrate becomes available to the enzyme. At concentrations exceeding 1 mmol/liter, the substrates inhibit alkaline phosphatase, but this inhibition is decreased by the presence of serum albumin, which complexes with the organophosphates.

Kinetic analysis of these results (1) indicates that there are at least two binding sites per albumin molecule, with association constants approximately 20 × 10\(^3\) mol\(^{-1}\), which, compared to a value of about 5 × 10\(^3\) mol\(^{-1}\) for their association with the enzyme active site, indicates that these substrates bind at least as strongly to the serum protein as to the alkaline phosphatase.

Although, at the concentrations of serum protein normally introduced via the specimen into the alkaline phosphatase assay medium containing these substrates, this association effect may not appreciably influence the rate, these observations do present two ramifications for clinical enzyme assays.

First, the substrates and cofactors used should have their association with other serum constituents characterized. Strong binding will decrease the concentration available to the enzyme and so yield underestimates of its activity, especially if suboptimal substrate concentrations cannot be avoided. In addition, formation of protein/ligand complexes is often accompanied by spectral changes; calibration curves derived from the influence of varying ratios of substrate plus product on the ultraviolet absorption maxima were used in the above rate calculations. It has been found that this effect is accentuated in those ultraviolet and fluorometric assays in which highly conjugated aromatic groups are present in the substrate molecule; for example, in the case of phenolphthalein, produced by alkaline phosphatase action on phenolphthalein phosphate, both of these compounds exhibit strong binding to serum albumin, coupled with considerable absorption changes.

Second, in agreement with Menaché (2), who found that both untreated and dialyzed serum decreased the activity of several routinely assayed enzymes, these results indicate that uniformity and standardization of serum dilution is a necessity for enzyme assays.

**References**


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