late the amine and gas chromatography with a nitrogen-specific detector to quantify it. Heating solutions of choline in 1 N sodium hydroxide at 60° for 14 days produced decomposition products in quantities of parts per million measured as trimethylamine. The rate and extent of decomposition appear to vary directly with the hydroxyl ion concentration of the medium.

Solutions of choline chloride, 2 mg/ml, were prepared in water with 0.01, 0.1, and 1.0 N sodium hydroxide. They were filled into 10-ml glass ampuls and sealed, then heated at steam bath temperature, ~100°, for 21 days. Intact choline was estimated by a modification of the Reinecke salt colorimetric method (2), the modification consisting of sparging the solutions with nitrogen gas to remove volatile amines. Three-milliliter portions of the ampule contents were acidified with sulfuric acid, 10 ml of 1% aqueous ammonium reineckate was added to each solution with stirring, and the mixtures were allowed to stand for 10 min. They were chilled in ice water and filtered through fine-frit sintered-glass crucibles. The precipitates were washed with ice water, then dissolved and made up to 10 ml with acetone. Absorbance of the solutions was determined in 1-cm cells at 523 nm, and the choline concentrations were calculated by reference to a standard curve constructed by carrying scalar amounts of choline chloride through the colorimetric procedure. The results support the hypothesis that degradation increases with increase in hydroxyl ion concentration; 96.0% of intact choline remained with water as the medium, 92.9% with 0.01 N, 89.8% with 0.1 N, and 80.4% in 1.0 N alkali.

Although drug decomposition at pH extremes generally is of little practical significance, it is important in this instance, for the pH of a saturated solution of oxtriphylline is >13. In a moist environment, oxtriphylline particles or granulation may be visualized as surrounded with a thin film of saturated solution. In support of this concept, it was found that adjustment of oxtriphylline granulations to a lower degree of alkalinity resulted in an odorless product. (Since the pKa of theophylline is ~8.8, a pH of 11 would afford all of the drug in the anionic form, yet the 100-fold reduction in hydroxyl ion concentration represented by the change from pH 13 to 11 minimizes the extent of decomposition.)

Solutions of choline chloride containing 50 mg/ml were prepared in water with 0.01, 0.1, and 1 N sodium hydroxide solutions. Aliquots of 2 ml were pipetted into the outer chamber of microdiffusion cells[1] and 2 ml of 1 M citric acid was pipetted into the inner chamber. The cells were sealed with a fine-ground flat glass plate using a minimum amount of stopcock grease and placed in an oven maintained at 60°. They were removed at intervals and allowed to cool to room temperature. A 1-ml portion of the citric acid solution from the inner chamber was pipetted into a 15-ml glass-stoppered centrifuge tube, then 3 ml of 30% chloroform in hexane and 5 ml of 1 N sodium hydroxide were added, the tube was stoppered and shaken for 5 min, and then centrifuged to obtain clear, immiscible phases. A 4-μl portion of the upper layer was injected into a gas chromatograph[2] fitted with a nitrogen-specific detector, using a 3.66-m × 2-mm i.d. glass column[3]. Temperatures were 110° for the column, 100° for the injection port, and 150° for the detector. Hydrogen pressure was 3 psi and attenuation × 8. The retention time for authentic trimethylamine was ~2 min and its detection limit ~5 ng on-column. Standard solutions of trimethylamine were prepared by serial dilutions of a 25% aqueous solution. Concentrations of 13.3–266 ng on-column, in 6 increments (3.3–66.5 μg/ml), provided a rectilinear plot of concentration *versus* peak area.

No time-dependent data were obtained for the solution in water or the lower alkali concentrations over a 24-day period; the amount of trimethylamine that diffused into the citric acid solution was ~6 μg (or 12 ng on-column), close to the limit of detection. Losses due to adsorption on glass surfaces or dissolution in the grease seal may be significant at this level. In 1 N NaOH, however, the trimethylamine determined was 43 μg after 2 days at 60° and 78 μg after 14 days, when the experiment was terminated. These data, with intermediate points obtained at 4, 7, and 10 days, gave a pseudo first-order rate constant of 7.14×10^{-5} day^{-1}. The amount of trimethylamine determined after 2 weeks at 60° represented <4 ppm of decomposed choline on a molar basis.

(1) A. T. Babyan, L. K. Gamburyan, and E. O. Chukhadzhyan, *Dokl. Akad. Nauk Arm. SSR*, **44**, 29 (1967); through *Chem. Abstr.*, **67**, 43196u (1967).

(2) F. J. Bandelin, *J. Am. Pharm. Assoc., Sci. Ed.*, **37**, 10 (1948).

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[1] Conway microdiffusion cells, D3318, SGA Scientific, Bloomfield, N.J.
[2] Perkin-Elmer model 900.
[3] 28% Pennwalt 223 and 4% potassium hydroxide on 80/100 mesh Gas Chrom R.

Changes in Plasma Protein Binding of Drugs after Blood Collection from Pregnant Rats

Keyphrases □ Protein binding—plasma, changes after blood collection from pregnant rats □ *In vitro–in vivo* correlation—plasma protein binding changes after blood collection from pregnant rats

To the Editor:

Determinations of drug–protein binding in plasma are associated with a number of potential methodological and technical difficulties. It is, therefore, desirable to confirm the suitability of *in vitro* protein binding measurement procedures by *in vivo* studies, if possible. The steady-state concentration of phenytoin in cerebrospinal fluid is essentially identical to the concentration of free (unbound) phenytoin in plasma so that the ratio of phenytoin concentration in cerebrospinal fluid to the total (free plus bound) concentration of phenytoin in plasma gives an *in vivo* estimate of the free fraction of phenytoin in plasma (1). We have recently used this method in rats to demon-

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strate that the apparent decrease of phenytoin binding in plasma after heparin administration is an *in vitro* artifact (1) apparently due to increased nonesterified fatty acid concentrations caused by lipolysis in plasma after blood collection (2). On the other hand, there was excellent agreement between the *in vivo* free fraction of phenytoin in the serum of rats who received that drug alone or together with the competitive binding inhibitor salicylic acid, and the *in vitro* free fraction measurements by equilibrium dialysis (1).

More recent studies on pregnant rats yielded *in vivo* plasma free fraction estimates for phenytoin that were considerably lower than the free fraction values determined by *in vitro* equilibrium dialysis. Therefore, experiments were designed to assess these differences. Wistar-Lewis rats, 20 days pregnant and weighing ~320 g, received 14.7 mg/kg phenytoin through a jugular vein cannula by rapid injection followed immediately by an infusion of 195 μg/min/kg for 2 hr. The animals were then anesthetized with ether, and cerebrospinal fluid and 10 ml of blood from the aorta were obtained. The blood was collected in a plastic syringe containing 200 U of heparin. Plasma was separated immediately (10 min centrifugation at 1500×*g*). About 2 ml of plasma was placed in cellophane tubing which was mounted in stoppered plastic centrifuge tubes. They were centrifuged at 750×*g* for 30 min at about 37° in a controlled temperature centrifuge, whose head had been preheated to 37°, and about 0.1 ml ultrafiltrate (tested with trichloroacetic acid for absence of protein) was collected. The time interval between blood collection and the end of the ultrafiltration procedure was ~50 min. Another portion of plasma was placed in a closed glass vial which was rotated in a water-bath at 37° for 6 hr. That plasma was then subjected to ultrafiltration. A third portion of plasma was placed in a dialysis cell and dialyzed for 6 hr (sufficient to reach equilibrium in the case of plasma from normal animals) at 37° against an equal volume of pH 7.4 phosphate buffer (1). All samples were assayed for phenytoin by high-performance liquid chromatography (HPLC) as described previously (1).

Results obtained from studies on nine animals are summarized in Table I. The free fraction values determined by immediate ultrafiltration were similar (~32% higher, on the average) to the *in vivo* estimates. These *in vitro* and *in vivo* free fraction values were strongly correlated ($r = 0.79$, $p < 0.02$). On the other hand, the free fraction values obtained by ultrafiltration after 6 hr were ~133% higher than the *in vivo* estimates and the free fraction values obtained by dialysis were ~63% higher. Clearly, a pronounced decrease in protein binding of phenytoin occurred in plasma from the pregnant rats during 6 hr after blood collection. Additional ultrafiltration studies with plasma incubated at 37° for 4 and 9 hr, respectively, showed that the maximum change in binding had occurred by 4 hr. This change was not prevented by the addition of antibiotics (mixture of penicillin G, streptomycin, and polymyxin B) to the plasma.

Table I—Protein Binding of Phenytoin in Plasma from Pregnant Rats [a]

Total phenytoin in plasma, μg/ml	12.9 ± 2.1
Cerebrospinal fluid/plasma concentration ratio	0.200 ± 0.029
Free fraction by immediate ultrafiltration	0.263 ± 0.027
Free fraction by ultrafiltration after 6 hr at 37°	0.461 ± 0.044
Free fraction by dialysis for 6 hr at 37°	0.322 ± 0.028

[a] Mean ± *SD*, $n = 9$.

Table II—Protein Binding of Salicylate in Plasma from Pregnant and Nonpregnant Rats [a]

	Pregnant Rats	Nonpregnant Rats
Total salicylate in plasma, μg/ml	234 ± 18	295 ± 33
Free fraction by immediate ultrafiltration	0.560 ± 0.081	0.435 ± 0.036
Free fraction by ultrafiltration after 4 hr at 37°	0.763 ± 0.118	0.468 ± 0.037
Free fraction by dialysis for 4 hr at 37°	0.733 ± 0.165	0.385 ± 0.029
Ratio of free fractions by ultrafiltration [b], 4 hr/immediate	1.36 ± 0.09	1.08 ± 0.04
Ratio of free fractions [b], dialysis/immediate ultrafiltration	1.30 ± 0.17	0.886 ± 0.040

[a] Mean ± *SD*, $n = 5$ per group. [b] These ratios are reported because one of the pregnant rats had an unusually low free fraction value in all determinations, thereby causing a relatively large coefficient of variation of the mean free fraction values.

The results of these studies do not mean that *in vivo* drug–protein binding alterations do not occur during pregnancy. The phenytoin concentration ratio, cerebrospinal fluid/plasma, in the pregnant rats is significantly larger than that ratio in nonpregnant rats from the same group of animals studied concurrently[1].

Additional protein binding studies were carried out with salicylic acid, but cerebrospinal fluid–plasma concentration ratios were not obtained because salicylate in cerebrospinal fluid, unlike phenytoin, does not equilibrate with free salicylate in plasma due to the active transport of salicylate from cerebrospinal fluid to blood (1). Twenty-days pregnant Wistar-Lewis rats and nonpregnant female rats (200–250 g) received a rapid intravenous injection of sodium salicylate, 100 mg/kg, and blood was collected 30 min later. Plasma was ultrafiltered immediately and after 4 hr of incubation at 37°, and another portion of plasma was subjected to equilibrium dialysis at 37° for 4 hr as described previously. All assays were carried out by HPLC (1). The results of these studies are summarized in Table II. There were only very small differences between free fraction values obtained by ultrafiltration immediately and 4 hr after blood collection, and between the 4 hr ultrafiltration and equilibrium dialysis free fraction values, in nonpregnant rats. On the other hand, there were considerably larger differences between free fraction values obtained immediately by ultrafiltration as compared with values obtained 4 hr later by ultrafiltration or equilibrium dialysis in plasma from pregnant rats. Results obtained by ultrafiltration at 4 hr and by equilibrium dialysis were essentially identical. These observations also indicate that drug–protein binding in plasma from pregnant animals decreases *in vitro* with time and that the magnitude of such changes in plasma from nonpregnant animals is much smaller.

In summary, drug–protein binding in plasma obtained from pregnant rats can undergo rapid changes (decreases) *in vitro* after blood collection. Similar changes may occur in plasma of other species, including humans, and in plasma from animals with other pathophysiological conditions associated with alterations in drug–protein binding,

[1] Results to be published.

but the rates and magnitudes of these changes may be different. Careful attention will have to be given to the methodology of *in vitro* drug–protein binding determinations if artifactual results are to be avoided.

(1) R. C. Chou and G. Levy, *J. Pharmacol. Exp. Ther.*, in press.
(2) K. M. Giacomini, S. E. Swezey, J. C. Giacomini, and T. F. Blaschke, *Life Sci.*, **27**, 771 (1980).

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The Loss of Nitroglycerin from Intravenous Administration Sets during Infusion: A Theoretical Treatment

Keyphrases ☐ Nitroglycerin—loss from intravenous administration sets during infusion ☐ Administration sets—loss of nitroglycerin during infusion

To the Editor:

In a recent report (1), a model was proposed to explain the loss of nitroglycerin from solution into a plastic bag. Under static conditions, the kinetics describing the loss of drug was biexponential and expressed as:

$$A = \beta e^{-k_3 t} + (A_0 - \beta) e^{-k_1 t} \qquad \text{(Eq. 1)}$$

where A is the amount of drug in solution, k is the rate of drug absorption onto the surface of the plastic, k_3 is the diffusion of drug into the plastic, A_0 the initial amount of drug in solution, and β is a constant. With plastic bags, Eq. 1 showed an excellent fit to the experimental data.

During the infusion therapy, the above principles apply to large volume parenteral therapy. However, a dynamic situation exists in the infusion set due to the constant flow of solution through the plastic tubing. A model depicting the loss of nitroglycerin from solution when drug is diluted in a glass bottle (no absorption) and allowed to flow through the infusion set is shown in Scheme I.

In addition, because of the large amount of drug in the bottle, the tubing could become saturated. Thus, the amount of drug lost from solution is also dependent on the amount of drug present in the tubing. (It was assumed that the rate of absorption is not dependent on the amount remaining to be absorbed, $C_0 - C_T$, but on the amount present, C_T. The results appear to support this assumption.) The differential equations, therefore, describing the rate of change of C_0, C_I, C_S, and C_T are:

$$\frac{dC_T}{dt} = k_2 C_T \qquad \text{(Eq. 2)}$$

$$\frac{dC_S}{dt} = k_1 C_I - k_{-1} C_S - k_2 C_T \qquad \text{(Eq. 3)}$$

$$\frac{dC_I}{dt} = k_0 C_0 - k_1 C_I - k_3 C_I + k_{-1} C_S \qquad \text{(Eq. 4)}$$

$$\frac{dC_0}{dt} = 0 \qquad \text{(Eq. 5)}$$

To solve for this model, it must be assumed that the supply of drug in the bottle is infinite, and that the amount of drug and solution delivered through the infusion set is insignificant compared to what remains in the bottle. Solving for Eq. 2, then:

$$C_T = C_{T_0} e^{-k_2 t} \qquad \text{(Eq. 6)}$$

where C_{T_0} is the saturation concentration in the tubing. To solve Eq. 3, one needs to consider the total mass balance of drug (D) in that:

$$D_0 = D_I + D_B + D_S + D_T \qquad \text{(Eq. 7)}$$

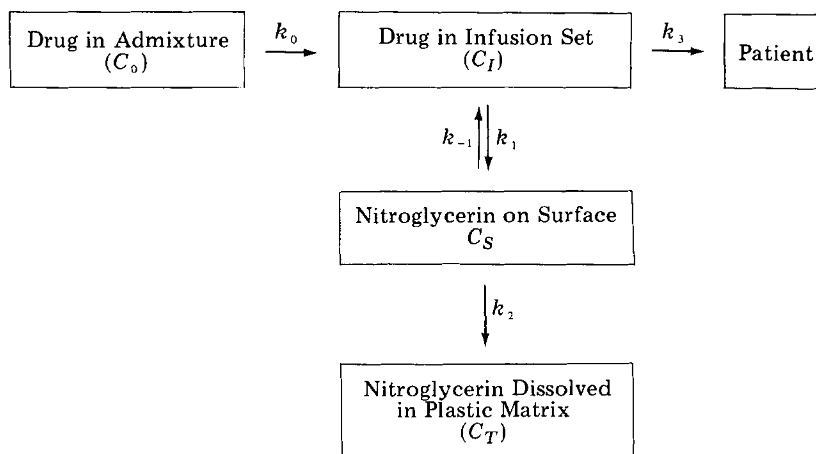
where D_0 is the total amount of drug, D_I is the drug in the infusion set (which includes the amount delivered), D_B is the drug in the bottle, D_S is the drug on the surface of the set, and D_T is the amount of drug in the plastic. Since the assumption was made that the amount of drug in the bottle is in infinite supply, then $D_0 \approx D_B$. Since D is the concentration (C) × volume (V), Eq. 7 can be approximated to be:

$$C_I = -\frac{V_S}{V_T} C_S - \frac{V_T}{V_I} C_T \qquad \text{(Eq. 8)}$$

Substituting Eqs. 8 and 6 into Eq. 3, and solving for C_S, one obtains the following solution for C_S:

$$C_S = Ae^{-at} - Ae^{-k_2 t} \qquad \text{(Eq. 9)}$$

where



Scheme I

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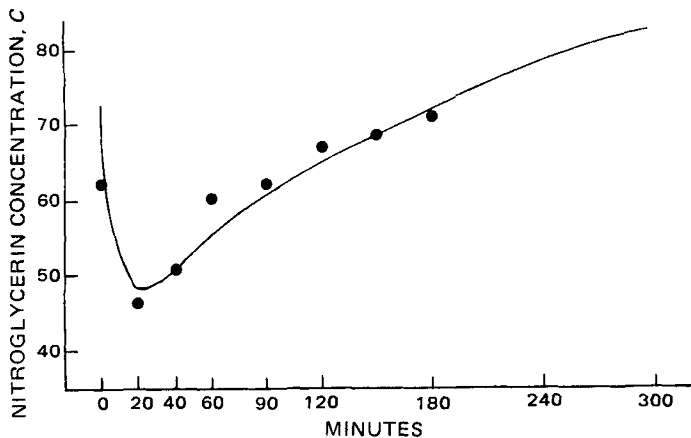

Figure 1—*Nitroglycerin loss: concentration in bottle = 100 μg/ml, flow rate = 1.0 ml/min. Equation describing loss:* $1 - (C/C_0) = 0.327e^{-0.0036t} - 0.270e^{-0.049t} + 1.08e^{-0.91t}$, *where* ● *is actual data and the smooth curve is computer-fitted data.*

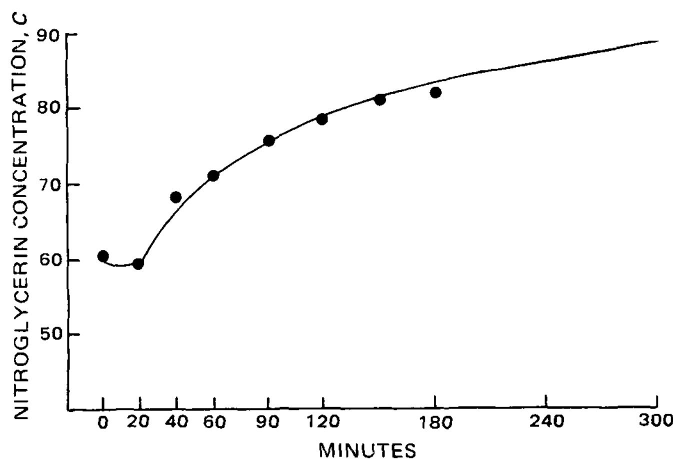

Figure 2—*Nitroglycerin loss: concentration in bottle = 100 μg/ml, flow rate = 0.5 ml/min. Equation describing loss:* $1 - (C/C_0) = 0.201e^{-0.068t} - 0.57e^{-0.004t} + 0.59e^{-0.118t}$, *where* ● *is actual data and smooth line is computer-fitted data.*

$$A = \frac{k_1 \frac{V_T}{V_I} C_{T_0} + k_2 C_{T_0}}{k_2 - k_{-1} - k_1 \frac{V_S}{V_I}} \quad \text{(Eq. 10)}$$

and

$$a = k_1 \frac{V_S}{V_I} + k_{-1} \quad \text{(Eq. 11)}$$

Finally, substituting Eq. 9 into Eq. 4 and solving for C_I:

$$C_I = \frac{k_0 C_0}{k_1 + k_3} - \left(\frac{k_0 C_0}{k_1 + k_3} - \alpha + \beta\right) e^{-(k_1+k_3)t} + \alpha e^{-at} - \beta e^{-k_2 t} \quad \text{(Eq. 12)}$$

where

$$\alpha = \frac{k_1 A}{a - k_1 - k_3} \quad \text{(Eq. 13)}$$

and

$$\beta = \frac{k_1 A}{k_2 - k_1 - k_3} \quad \text{(Eq. 14)}$$

To further simplify Eq. 12 at the initial phase, prior to adsorption of nitroglycerin, $k_1 = 0$, $k_3 = k_0$, and rearranging Eq. 12:

$$1 - \frac{C_I}{C_0} = \left(\frac{k_0}{k_1 + k_3} - \frac{\alpha + \beta}{C_0}\right) e^{-(k_1+k_3)t} - \frac{\alpha}{C_0} e^{-at} + \frac{\beta}{C_0} e^{-k_2 t} \quad \text{(Eq. 15)}$$

The results of the treatment of the infusion data using Eq. 15 is shown in Figs. 1 and 2, using different flow rate conditions. The data were listed using back projection (stripping) technique (2). As predicted by the model, a triexponential loss of nitroglycerin is seen. The model shows that the initial loss is due to adsorption and loss due to infusion, followed by equilibration on the inside surface of the infusion set and, consequently, the rate-limiting adsorption of nitroglycerin by the plastic. The model also shows that when no adsorption/absorption occurs, the concentration of drug delivered is the same as the concentration in the bottle.

All factors affecting nitroglycerin loss have been documented previously for static conditions (1). All these factors apply here, in addition to the loss also being dependent on the flow rate.

(1) A. W. Malick, A. H. Amann, D. M. Baaske, and R. G. Stoll, *J. Pharm. Sci.*, **70,** 798 (1981).
(2) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence, Hamilton, Ill., 1975.

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General Derivation of the Equation for Time to Reach a Certain Fraction of Steady State

Keyphrases □ Pharmacokinetics—derivation of the equation for time to reach a certain fraction of steady state □ Equations—for time to reach a certain fraction of steady state, derivation

To the Editor:

The time to reach a certain fraction of a given steady-state plasma concentration for a drug which exhibits multiexponential characteristics is not a simple function of the terminal disposition rate constant or half-life. Rather, it is a complex function of all coefficients and disposition rate constants in the equation describing the concentration–time curve. A given fraction of steady state is reached sooner with a drug that demonstrates multiexponential behavior than one that demonstrates monoexponential behavior. Recently, Chiou (1, 2) developed a general equation that permits the estimation of fraction of steady state from area ratios. The derivations were based on the superposition principle, or assume constant rate input of drug into the body. The following appears to be a more general approach for the derivation of the area equation.

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