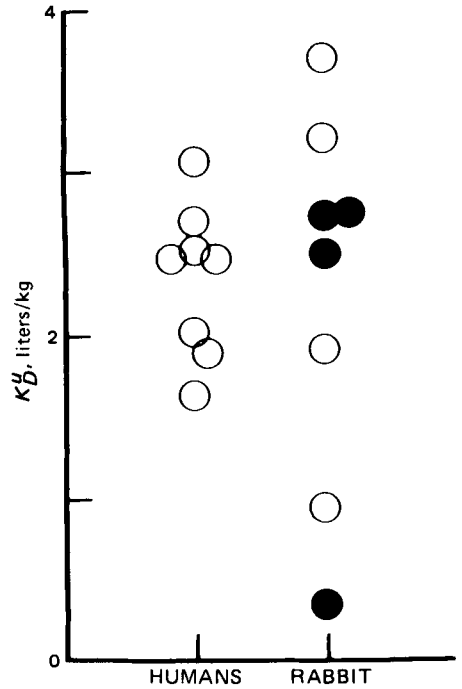

Figure 1—*Steady-state volume of distribution of unbound prednisolone calculated according to Eq. 7 for humans and rabbit.*

Table I—Protein Binding and Distribution Properties of Oxaprozin in Normal and Azotemic Subjects

Subjects	V_{ss}/TBW, liters/kg	f_{up}, %	K_D^u, liters/kg	f_{ut}, % Eq. 2[a]	f_{ub}, % Eq. 5
Normal	0.15 (0.01)	0.078 (0.003)	193 (11)	0.42 (0.03)	0.52 (0.03)
Renal impairment	0.18 (0.01)	0.177 (0.016)	103 (13)	0.79 (0.11)	1.03 (0.12)
Dialysis	0.21 (0.02)	0.283 (0.036)	79 (13)	1.05 (0.21)	1.43 (0.25)

[a] Assumes V_t = extravascular water space (554 ml/kg) and V_p = 46 ml/kg.

pharmacokinetics and binding were examined in subjects with normal and impaired renal function[2]. Direct plasma protein binding studies showed altered binding in plasma in the azotemic patients. The values of f_{ut} and f_{ub} were estimated by the two equations. The proposed equation did not obscure the apparent occurrence of impaired tissue binding in azotemic patients.

It is becoming common practice to calculate intrinsic clearance and the unbound volume of distribution directly from C_u *versus* time data. It is helpful to be able to interpret the resultant value in terms of overall drug binding. The necessary assumption that passive diffusion of unbound drug accounts for equilibration between tissues is retained. These arguments for simplified calculation of K_D^u, however, should not preclude the application of more specific tissue binding models when based on appropriate experimental data.

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[2] Unpublished data.

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Trace Decomposition of Choline

Keyphrases □ Choline—trace decomposition after exposure to high humidity or in unbuffered solutions □ Decomposition—trace, of choline after exposure to high humidity or in unbuffered solutions

To the Editor:

Decomposition of pharmaceuticals to the extent of parts per thousand, or less, usually is considered negligible. Such trace decomposition cannot be ignored where it affects the acceptability of drug products, *i.e.*, where it is manifested as insoluble matter in an injection, as discoloration, or by odor formation. The origin of a characteristic fishy odor in oxtriphylline (choline theophyllinate) exposed to high humidity for protracted periods or in unbuffered solutions led to the studies reported here. This odor, which is characteristic of many choline salts, can be attributed to trace decomposition; trace, because the presence of an odor has no measurable effect on physicochemical properties and decomposition, since quaternary ammonium salts are nonvolatile and would be expected to be odorless on that account.

A decomposition study of choline, measuring intact quaternary ammonium function, showed that it proceeded very slowly in solution at 100°. On heating choline has been reported (1) to yield 85–90% trimethylamine, 5% dimethylamine, 25% ethylene glycol, 4% acetaldehyde, and 10–15% acetylene on a molar basis with excess concentrated potassium hydroxide. The nature of the neutral reaction products would be expected to vary with reaction conditions, but it was assumed in this study that trimethylamine would constitute the bulk of the basic products. Because the amounts of trimethylamine formed were extremely low, a method was developed using microdiffusion to iso-

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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.

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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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