Table II—Second-Order Rate Constants for the Acid- and Base-Catalyzed Decomposition of III [a]

Temperature	$k_{H^+} \times 10^5$, M^{-1} sec^{-1}	k_{HO^-}, M^{-1} sec^{-1}
80°	394	2.16
60°	81.0	0.761
30°	6.72	0.118

[a] Calculated from the data in Table I.

indicates no other catalytic terms are necessary to explain the observed data. This also implies that it is likely that no significant buffer catalysis occurred in these solutions. It is also apparent from Fig. 3 that the presence of Cu^{2+} and Fe^{3+} had no effect on the rate of degradation of III.

The rate equation describing the pH dependence for the degradation of III (Eq. 1) is likely to hold at low temperatures as well as at 80°. The second-order rate constants k_{HO^-} and k_{H^+} at 60 and 30° were thus obtained by determining k_{obs} at high pH (9.3) and low pH (1.1) and calculating k_{HO^-} and k_{H^+} from Eqs. 2 and 3, respectively:

$$k_{HO^-} = \frac{k_{obs}[H^+]}{K_w} \quad \text{(Eq. 2)}$$

$$k_{H^+} = \frac{k_{obs}}{[H^+]} \quad \text{(Eq. 3)}$$

The second-order rate constants at 80, 60, and 30° are shown in Table II and were used to calculate the activation energies for the specific acid- (17 kcal/mole) and base-catalyzed (12 kcal/mole) hydrolysis of III[9].

The degradation kinetics of IV were also investigated at 80° from pH 1 to 7. The degradation reaction followed pseudo-first-order kinetics, and the observed rate constants obtained are shown in Table I. From the plot in Fig. 3 one can see that the degradation rate of IV is acid catalyzed and is significantly slower than that of its methyl ester (III) in the acidic pH region. The degradation rate for IV at pH 7.0 was too slow to measure. If the assumption is made that the degradation reaction of IV does not involve the C-1 carboxyl group[10], then this acid-catalyzed pathway is probably available to fenprostalene (III) itself. Hydrolysis of the C-1 methyl ester, however, is preferred by ~10-fold over this alternate degradation pathway for III at 80° in the acidic pH region.

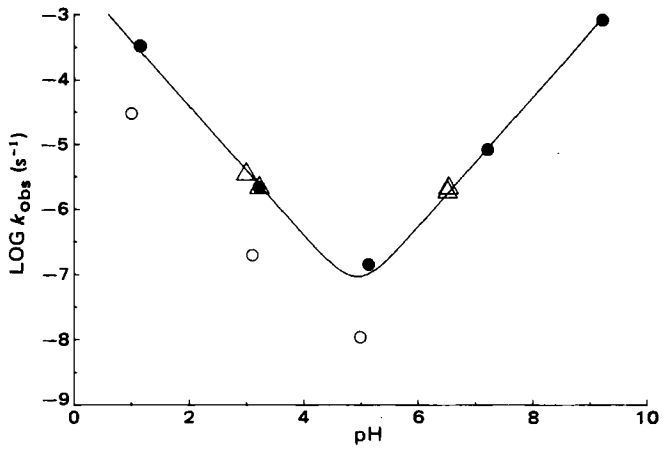

Figure 3—*Log* k_{obs} *versus pH profile for the hydrolysis of III at 80°. Key: solid circles represent simple buffer solutions, open triangles represent buffer solutions containing* Cu^{2+} *and* Fe^{3+} *ions, and the reaction of IV in aqueous solution at 80° is shown by the open circles.*

[9] These results are nearly identical to those reported for the hydrolysis of ethyl acetate (7).

[10] Jones *et al.* (3) studied the degradation of II in acidic solution under aerobic conditions. The products were a complex mixture of compounds, and no major products were identified. We have not identified the degradation products of IV in acidic media but have observed on HPLC that several products are formed.

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Determination of Hydrazine in Pharmaceuticals III: Hydralazine and Isoniazid Using GLC

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Abstract □ A GLC procedure has been developed for the determination of hydrazine in hydralazine and isoniazid drug raw materials, single and multicomponent tablets, injectables, and syrups. The method is based on the derivatization of hydrazine with benzaldehyde to form benzalazine. The minimum detectable amount of hydrazine in hydralazine and isoniazid raw materials and formulations is ~0.0003%. No hydrazine was found in the hydralazine raw material specimens examined. Traces of hydrazine (~0.0003%) were found in some tablet lots and ~0.02% was found in an injectable product. A trace of hydrazine was found in one lot of isoniazid raw material and low levels (0.0012 and 0.0029%) were found in isoniazid tablet products. An isoniazid syrup contained ~0.2% hydrazine.

Keyphrases □ Hydrazine—determination in hydralazine and isoniazid by GLC □ Hydralazine—GLC, determination of hydrazine □ Isoniazid—GLC, determination of hydrazine □ GLC—determination of hydrazine in pharmaceutics, hydralazine, isoniazid

Previous papers in this series described high-performance liquid chromatographic (HPLC) methods for the determination of hydrazine in isoniazid and phenelzine products and reported typical amounts found in commercial formulations (1, 2). Since hydrazine is a mutagen (3) and a carcinogen in laboratory animals (4), concern over its presence in drug products resulted in proposed regulatory action in the United States (5) and to the recall of

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an isoniazid product in Canada (6). Determination of hydrazine has now been extended to hydralazine, an antihypertensive drug.

Hydrazine contamination of hydralazine may occur during synthesis (7) or by degradation of the drug. Hydrazine is a metabolite of hydralazine in humans, and a GLC method for its identification and quantitation in urine has been described (8). Methods for the determination of hydrazine as a metabolite of isoniazid have also been reported (9, 10). Since attempts to determine hydrazine in hydralazine by the HPLC methods developed for isoniazid and phenelzine (1, 2) led to irreproducible results, a GLC method was developed. The sensitivity and stability of this method prompted its extension to isoniazid products as an alternative to the HPLC procedure (1).

EXPERIMENTAL

Materials—Hydrazine dihydrochloride[1], hydralazine hydrochloride[1], benzalazine[2], 5-chloro-2-methylaminobenzophenone[2], and benzaldehyde[3] were used as received. Solvents and reagents were commercial analytical reagent grade, except for *n*-heptane[4] which was HPLC grade.

Apparatus—The gas chromatograph[5] was equipped with a nitrogen–phosphorus alkali thermionic detector and a coiled-glass column (0.91-m × 4-mm i.d.) packed with 2% OV-101[6] on Chromosorb G-HP[7] (80/100 mesh). Instrument temperatures were: injector port, 275°; column, 250°; and detector, 300°. Gas flows were: nitrogen, 40 ml/min; air, 60 ml/min; and hydrogen, 1.75 ml/min.

Standard Solutions and Reagents—A stock solution of 5-chloro-2-methylaminobenzophenone in *n*-heptane (12 μg/ml) was diluted daily with *n*-heptane to obtain the working internal standard solution (1.2 μg/ml). The benzaldehyde reagent was prepared by dissolving benzaldehyde in a solution of equal parts (by volume) of methanol and water to yield a final concentration of 35 mg/ml.

Preparation of the Calibration Curve—An 80-μg/ml aqueous solution of hydrazine dihydrochloride (equivalent to 24.4 μg/ml of hydrazine) was diluted with distilled water to obtain five standard solutions ranging from 0.080 to 2 μg/ml of hydrazine dihydrochloride (representing 0.005–0.122 ng of hydrazine on-column). To a 2.0-ml aliquot of each solution, pipetted into separate 150 × 20-mm culture tubes fitted with polytef-lined screw caps, was added 1.0 ml of benzaldehyde reagent solution. The tubes were rotated for 10 min at 30 rpm, 10.0 ml of the working internal standard solution was added to each tube, and the tubes were rotated again for 30 min at 30 rpm. Duplicate 1-μl aliquots of the upper phase were chromatographed, and the slope was calculated from the ratio of the area counts of the benzalazine peaks to the area counts of the internal standard peaks *versus* the corresponding weight ratio.

Day-to-Day Calibration—Two hydrazine dihydrochloride standards, usually ~0.40 and 1.20 μg/ml, were prepared daily. Two-milliliter aliquots of these standards were derivatized as described above, and 1-μl aliquots were injected onto the chromatograph at regular intervals.

Analysis of Drug Raw Materials and Tablet Formulations—An accurately weighed amount of hydralazine hydrochloride, isoniazid drug raw material, or a powdered tablet composite equivalent to 50 mg of drug substance was tumbled for 30 min in a screw-capped culture tube with 5.0 ml of distilled water. After centrifugation, a 2.0-ml aliquot of the aqueous extract was derivatized as described above, and 1-μl aliquots were injected onto the chromatograph.

Analysis of Liquid Formulations—An aliquot of hydralazine hydrochloride injectable or isoniazid syrup equivalent to 20 mg of drug was pipetted into a 150 × 20-mm culture tube with 1.0 ml of distilled water, if necessary, to bring the total volume to 2.0 ml. The solution was derivatized and chromatographed as described above.

Quantitation—Hydrazine was estimated by comparison of the benzalazine to internal standard peak area ratio of the sample to the corresponding ratio of the standard. When the area ratio of the sample was less than that of the daily calibration standard, a new standard was prepared to approximate the concentration of the sample in question. When the area ratio exceeded that of the daily calibration standard, the sample was diluted with the working internal standard solution to approximate the concentration of the standard.

[1] Sigma Chemical Co., St. Louis, Mo.
[2] K&K—ICN Pharmaceutical, Plainview, N.Y.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Burdick and Jackson Laboratories, Muskegon, Mich.
[5] Hewlett-Packard Model 5880A.
[6] Pierce Chemical Co., Rockford, Ill.
[7] Johns-Manville, Celite Division, Denver, Colo.

Table I—Hydrazine Levels in Hydralazine Hydrochloride Products

Products	Lot No.	Hydralazine Strength	Hydrazine, %[a]
Drug raw material	(4 lots)	—	ND
Tablet	A	10 mg/tab	Trace
Tablet	B	25 mg/tab	ND
Tablet	C	50 mg/tab	Trace
Injectable	D	20 mg/ml	0.0327; 0.0326
Tablet	E	50 mg/tab	Trace
Tablet (lot E, stressed[b])			0.0017; 0.0018; 0.0013
Tablet (with 0.2 mg of reserpine)	F	50 mg/tab	ND
Tablet (lot F, stressed[b])			0.0045[f]
Tablets (with 0.1 mg of reserpine and 25 mg of hydrochlorothiazide)	G	25 mg/tab	ND
Tablet (lot G, stressed[c])			ND
Injectable	H	20 mg/ml	0.0217; 0.0215; 0.0240
Injectable (lot H, stored[d])			0.0422; 0.0409; 0.0394; 0.0387
Injectable (lot H, stressed[e])			0.1083[g]

[a] Expressed as weight percent of the labeled amount of hydralazine hydrochloride. Key: (ND) none detected; (Trace) minimum amount detectable (~0.0003%). [b] Stressed at 37° and 75% relative humidity for 221 days. [c] Stressed at 22° (room temperature) and 75% relative humidity for 221 days. [d] Stored at room temperature for 221 days. [e] Stressed at 37° for 221 days. [f] Mean of five determinations; relative *SD* 7.0%. [g] Mean of four determinations; relative *SD* 0.83%.

RESULTS AND DISCUSSION

Hydrazine in hydralazine and isoniazid raw materials and formulations was determined as the benzaldehyde derivative by GC. Hydrazine dihydrochloride was chosen as the calibration standard because it is more stable in aqueous solution (50 days) than the sulfate (7 days) or the hydrate (1 day).

Concomitant with the formation of benzalazine in the derivatization step is the formation of a hydrazone from the condensation of hydralazine with benzaldehyde. This compound is not separated from benzalazine by extraction and appears at ~4.3 and 8.1 min, well after the appearance of benzalazine and the internal standard at 1.2 and 1.6 min, respectively. It is the presence of this compound which leads to the requirement for a GLC method: the compound accumulated on the HPLC column, only to elute after a time with a resultant baseline shift and other undesirable chromatographic features. Similar compounds form from phenelzine and isoniazid. In the former case the compound elutes from the HPLC column, while in the latter it does not enter the partitioning solvent.

Derivatization Reaction—*Hydralazine*—Water was used as the medium for dissolution of the hydralazine and the derivatization reaction. The final pH values associated with dissolution of the drug were similar for all raw materials and formulated products (pH 2.9–3.2) and had no apparent effect on the derivatization reaction. The sodium acetate buffer (pH 6) used for sampling in the method for hydrazine in phenelzine (2) could not be used for hydralazine because it gave rise to an unidentified peak on the chromatogram that interfered with the internal standard peak. The time required for complete derivatization and the stability of the benzalazine formed were determined by measuring the GLC response due to benzalazine as a function of the time during which the derivatization was allowed to proceed. The derivatization of the hydrazine dihydrochloride standard was complete within 3–5 min, and the benzalazine formed was stable for at least 35 min. Hydralazine hydrochloride was stable under the derivatization conditions for 30 min after which time a peak due to a trace (0.0003%) of benzalazine appeared in the chromatogram. Neither hydralazine nor reserpine and/or hydrochlorothiazide (sometimes formulated with hydralazine) interfered with the derivatization reaction.

In contrast to the behavior of hydrazine dihydrochloride, the benza-

Table II—Hydrazine Levels in Isoniazid Products

Product	Lot No.	Isoniazid Strength	Hydrazine, %[a] Mean	Hydrazine, %[a] Range[b]
Drug raw material	(1 lot)	—	Trace	—
Tablet (stressed[c])	I	100 mg/tab	0.0012	0.0012–0.0013(2)
Tablet (with 50 mg pyridoxine hydrochloride[d])	J	300 mg/tab	0.0582	0.0571–0.0595(4)
Tablet (with 50 mg pyridoxine hydrochloride)	K	300 mg/tab	0.0029	0.0026–0.0034(5)
Tablet (lot K, stressed[e])			0.0199	0.0192–0.0210(4)
Tablet (lot K, stressed[f])			0.0052	0.0050–0.0055(3)
Tablet (lot K, stressed[g])			0.0671	0.0619–0.0757(3)
Syrup	L	10 mg/ml	0.235	0.234–0.236 (2)
Syrup	M	10 mg/ml	0.200	0.188–0.210 (3)
Syrup (lot M, stored[h])			0.349	0.326–0.378 (3)

[a] Expressed as weight percent of the labeled amount of isoniazid. Trace means the minimum detectable amount (~0.0003%). [b] Number of determinations is in parentheses. [c] Stressed at 45° and ambient humidity for 3 years. [d] Expired product. [e] Stressed at 22° (room temperature) and 75% relative humidity for 228 days. [f] Stressed at 37° and ambient humidity for 228 days. [g] Stressed at 37° and 75% relative humidity for 228 days. [h] Stored at room temperature for 228 days.

lazine derivative formed from hydrazine sulfate in water or acetate buffer was stable for only 10 or 20 min, respectively (2). There was no difference in the time required for the derivatization to go to completion.

Isoniazid—Either water or acetate buffer can be used for the sampling and derivatization of hydrazine from isoniazid products: the rate of derivatization of hydrazine dihydrochloride was the same in both solvents. Neither isoniazid nor pyridoxine interfered with the derivatization reaction. The mole ratio of benzaldehyde to hydralazine required to achieve quantitative derivatization of hydrazine in the presence of hydralazine, determined as previously described (1, 2), was 2.5:1. The method provides for a mole ratio of 3.0:1.

Neither hydralazine nor isoniazid decomposed to hydrazine during extraction and derivatization. This is demonstrated by the nondetectable and trace levels found in some drug raw materials and formulations (Tables I and II) and by the complete recovery of hydrazine from spiked samples (Table III).

Extraction and Partition—The extraction was done with *n*-heptane because it was free of interfering impurities and was a good solvent for the internal standard. To show that benzalazine was completely extracted from the derivatization medium, the latter was shaken with *n*-heptane in ratios of 1:20; 1:10; 2:10, and 3:10. The peak area ratios of derivative to internal standard divided by the corresponding weight ratios were 11.064, 11.028, 11.112, and 11.052, respectively, showing that partition was virtually complete. In addition, there was no significant change in the chromatographic response of internal standard solutions containing benzalazine after they had been extracted with the aqueous derivatization medium. The ratios of aqueous to the organic phase examined were 1:5, 2:5, and 4:5.

Tablet extraction conditions were verified by shaking portions of an isoniazid–pyridoxine tablet composite equivalent to 50 mg with 3.0, 5.0, 10.0, and 20.0 ml of water. After derivatization and extraction, hydrazine levels were found to be 0.0640, 0.0662, 0.0654, and 0.0602%, respectively. To check the time required for extraction, an isoniazid–pyridoxine tablet product was shaken with 5 ml of water for 10 and 30 min. Hydrazine levels, after derivatization and extraction, were 0.0060 and 0.0065%, respectively, identical within the experimental error.

The amount of *n*-heptane required to extract the hydrazine derivative from the aqueous phase was established by experiments with a hydralazine injectable and an isoniazid elixir. For the injectable, aqueous to organic phase ratios of 2:5, 2:10, 2:15, and 2:20 gave apparent hydrazine levels of 0.0257, 0.0240, 0.0256, and 0.0260%, respectively. For the isoniazid syrup formulation, the hydrazine levels were 0.374, 0.370, 0.363, and 0.389%, respectively, for the same solvent ratios. The method calls for a ratio of 2:10.

Linearity, Reproducibility, and Accuracy—A standard curve was prepared by derivatizing, extracting, and chromatographing known amounts of hydrazine dihydrochloride. Over the range from 0.005 to 0.122 ng of hydrazine injected (as benzalazine), the slope of the area count ratio of benzalazine to internal standard *versus* the corresponding weight ratio was 11.7121 and the intercept did not differ significantly from zero (7.2×10^{-3}). The standard deviation of the slope was 0.2131, and the correlation coefficient was 0.9987. This range represents hydrazine levels from 0.0003 to 0.0061% for 2000 ng of drug injected.

The chromatographic reproducibility was established by injecting seven aliquots of each of three solutions of derivatized and extracted hydrazine dihydrochloride. The amounts of hydrazine injected (as benzalazine) were 0.0264, 0.0486, and 0.0972 ng, and the relative standard deviations of the count/weight ratios were 1.60, 0.63, and 0.89%, respectively. The reproducibility of the procedure as applied to formulated drug products can be gauged from the data in Tables I and II. Relative standard deviations ranged from 4.0% for a hydralazine injectable (4 replicates, 0.0403% hydrazine) to 10.3% for isoniazid tablets (5 replicates, 0.0029% hydrazine).

The recovery of hydrazine in the presence of formulation matrices was determined by adding known amounts of hydrazine dihydrochloride at the extraction step and carrying the assay through to completion. Recoveries ranged between 93.4 and 103.2% (Table III).

The minimum detectable amount of hydrazine, as benzalazine on-column, is ~0.005 ng, with the minimum quantifiable level about three times this amount. This permits detection of hydrazine at the level of ~0.0003% in drugs. This method is 20 times as sensitive as the previously described HPLC methods (1, 2).

Hydrazine in Drug Raw Materials and Formulations—No hydrazine was found in raw material lots of hydralazine. Traces of hydrazine were found in some tablet lots and these amounts increased in tablets subjected to stress (Table I). Hydrazine levels in injectable hydralazine products were higher than in tablets and increased with time and temperature stress (Table I). It would appear that hydrazine is a degradation product of hydralazine.

Only a trace of hydrazine was found in the isoniazid raw material lot examined, but the formulated products examined contained hydrazine. The levels increased in products subjected to temperature and humidity stress (Table II). Again, it appears that hydrazine is a degradation product of the drug.

Table III—Hydrazine Recoveries

Product	Hydrazine, ng/injection Original	Added	Found	Recovery, %
Hydralazine hydrochloride drug raw material	0	1.305	1.285	98.5
	0	2.18	2.19	101.0
	0	3.44	3.45	100.1
	0	5.74	5.68	98.9
Hydralazine hydrochloride and reserpine tablet (lot F)	0	0.350	0.327	93.4
	0	0.606	0.606	100.3
Hydralazine hydrochloride, reserpine, an hydrochlorothiazide tablet (lot G)	0	0.0263	0.0263	100.0
	0	0.584	0.556	95.2
Isoniazid syrup	2.289	0.315	2.61	100.2
	2.289	0.315	2.65	102.0
Isoniazid and pyridoxine hydrochloride tablet (lot K)	0.253	0.715	0.949	98.0
	0.261	1.072	1.265	94.9
Isoniazid and pyridoxine hydrochloride tablet (lot J)	0.0243	0.0210	0.0443	97.8
	0.0493	0.0552	0.1078	103.2
	0.582	0.1071	0.696	101.0

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Theophylline Blood–Brain Barrier Transfer Kinetics in Dogs

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Abstract □ A simple diffusion-based pharmacokinetic model is proposed relating blood–brain barrier transfer kinetics of theophylline to the difference in the free concentrations of the drug in serum and cerebrospinal fluid (CSF). The model predicts that the CSF drug level is proportional to the serum drug level convoluted by $\exp(-kt)$, where k is the blood–brain barrier diffusion rate constant. An excellent agreement was found by nonlinear regression analysis between serum and CSF theophylline data in eight dogs and the proposed model for the blood–brain barrier transfer kinetics of theophylline. The ratio of the free fractions of theophylline in serum and CSF predicted from the model also agrees with the value determined experimentally.

Keyphrases □ Theophylline—blood–brain barrier transfer in dogs, kinetics □ Kinetics—of theophylline, blood–brain barrier transfer in dogs □ Blood–brain barrier—theophylline transfer in dogs, kinetics

The narrow therapeutic range of theophylline and the substantial intersubject variability in its disposition have resulted in extensive studies of the pharmacokinetics and clinical dosage management of the drug (1–8). Several investigations have related the bronchodilator effect of theophylline to its serum concentration level (5, 6, 9–13). However, it is the adverse effects rather than the therapeutic effect that dictates the dose administered and limits the therapeutic efficacy. The main adverse effects appear to be of CNS origin. It may be misleading, therefore, to use serum levels as a guide for the clinical management of the drug without any *a priori* knowledge of the kinetics of the blood–brain barrier transfer of theophylline. The theophylline concentration in the cerebrospinal fluid (CSF), should provide a better correlation to the CNS effects. The object of this study is to investigate the serum–CSF disposition of theophylline and the blood–brain barrier transfer kinetics. By establishing the kinetic relationship between the serum and CSF drug levels, a more rational approach to the usage of serum theophylline determinations can be established.

Although it was recognized early that theophylline enters the cerebrospinal fluid, neither the rate of equilibration with serum nor the serum–CSF concentration ratio have been defined adequately. In fact, little is known about the kinetics of transfer of drugs across the blood–brain barrier. The few CSF samples that have been correlated with serum samples in humans provide only a very limited insight into the kinetics (14–17). The use of dogs in the present study allowed comprehensive CSF sampling enabling a proper pharmacokinetic analysis of the serum–CSF theophylline disposition. Animals are often a poor predictor of human pharmacokinetics mainly due to substantial differences in the elimination processes. However, the present manner of analysis of the serum–CSF transfer kinetics is not influenced by absorption or elimination or other disposition processes. Furthermore, the tissues that constitute the blood–brain barrier apparently do not differ significantly between dogs and humans (18). The results from this study should therefore be of clinical interest.

EXPERIMENTAL

Study Design—After an 18-hr fast, eight dogs were anesthetized with 30 mg/kg iv of sodium pentobarbital; supplemental doses were given as needed during the remainder of the experiment. A polyethylene catheter in the left lateral saphenous vein was used for the infusion of aminophylline. Aminophylline[1] for intravenous use was utilized containing 25 mg of aminophylline (20.63 mg of anhydrous theophylline)/ml of solution. Aminophylline, 9 mg/kg (7.43 mg/kg theophylline) was diluted with saline to 19.4 ml and infused with a constant-infusion pump over a total of 20 min.

Blood samples for theophylline level determination were taken from a catheter in the left external jugular vein. An 18-gauge needle was percutaneously placed in the cisterna magna for obtaining the CSF samples. Cerebrospinal fluid and blood for theophylline levels were obtained at time zero (start of the infusion) and 20 (end of infusion), 50, 80, 140, 200, 260, 320, 350, and 380 min. The dogs were ventilated through a cuffed endotracheal tube using a constant-volume ventilator[2] with periodic hyperinflation to prevent atelectasis.

Theophylline Assay—The serum from 2–3 ml of blood and 0.5 ml of CSF were frozen and later assayed for theophylline, usually within 1–3 days. Theophylline concentrations were determined by the GC method of Least and coworkers (19) using 100-μl samples and substituting iodobutane for iodopentane in the derivation procedure. Theophylline concentrations were calculated using peak height ratios of theophylline to internal standards.

Protein Binding—The protein binding of theophylline was deter-

[1] Searle Laboratories.
[2] Harvard model 607.

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