organ clearance of the drug following systemic administration, and Q_b is the flow rate through the organ). In this case the fraction of the absorbed dose reaching the circulation intact (F) is given as:

$$F = 1 - Cl/Q_b \quad \text{(Eq. 11)}$$

Preabsorptive first-pass metabolism involves metabolism of a drug as it is absorbed into the circulation from the site of administration. In this case the fraction eliminated presystemically is not equal to the systemic extraction ratio.

The maximum pulmonary clearance of isoproterenol following systemic administration to the rabbit lung was ~2 ml/min, representing an extraction ratio of ~0.01. If only postabsorptive first-pass metabolism were occurring, this would predict that 99% of an intrabronchial or aerosol dose of isoproterenol should reach the circulation intact. If >1% first-pass metabolism occurs, as has been reported (7), preabsorptive first-pass metabolism must be involved. The preabsorptive metabolism would be observed experimentally as absorption of the metabolite into the circulation along with the parent drug. This was incorporated into the perfusion model as first-order absorption of both drug and metabolite, and various simulations with this model were compared to the experimental results following intrabronchial and aerosol administration of isoproterenol. These comparisons indicate little or no first-pass metabolism of isoproterenol following intrabronchial instillation of an aqueous solution of isoproterenol but suggest the possibility of a substantial first-pass effect when the drug was inhaled as an aerosol. The drug was also more rapidly absorbed following aerosol administration; it may be that this route of administration delivers the drug over a larger surface area in the airways than is achieved with intrabronchial administration. This may result in greater exposure of the drug to the epithelial cells of the bronchi and small airways, leading to more rapid absorption and avoiding local saturation of metabolizing capacity during absorption. This would hinder extrapolation of data following intrabronchial administration to situations involving aerosol inhalation of a drug and suggests that intrabronchial or endotracheal administration may be an inappropriate technique for studying drugs which are intended for aerosol administration.

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Isolated Perfused Rabbit Lung as a Model for Intravascular and Intrabronchial Administration of Bronchodilator Drugs II: Isoproterenol Prodrugs

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Abstract □ The pulmonary disposition of two diester prodrugs of isoproterenol (di-*p*-toluoylisoproterenol and dipivaloylisoproterenol) was studied in the isolated perfused rabbit lung preparation. High-pressure liquid chromatographic methods were developed to measure diester, monoester, isoproterenol, and 3-*O*-methylisoproterenol from a single 1-ml perfusate sample. The prodrugs were administered directly into the circulating perfusion medium and by endotracheal instillation. Perfusate concentrations of diester, monoester, isoproterenol, and 3-*O*-methylisoproterenol were measured for 180 min. The diesters were rapidly eliminated from the perfusate with a subsequent increase in monoester concentrations. Isoproterenol levels were observed within minutes of prodrug administration, peaked at 60–80 min, and declined slowly thereafter. The prodrugs were rapidly absorbed following endotracheal administration with 30–50% of the diester being metabolized during the first pass through the lung.

Keyphrases □ Isoproterenol prodrugs—isolated rabbit lung as model for intravascular and intrabronchial administration of bronchodilator drugs, high-pressure liquid chromatography □ Prodrugs—isoproterenol, isolated rabbit lung as model for intravascular and intrabronchial administration of bronchodilator drugs, perfusion, high-pressure liquid chromatography □ Perfusion—isolated rabbit lung as model for intravascular and intrabronchial administration of bronchodilator drugs, high-pressure liquid chromatography □ High-pressure liquid chromatography—isolated rabbit lung as model for intravascular and intrabronchial administration of bronchodilator drugs, prodrugs, perfusion, isoproterenol

Isoproterenol is a β-adrenergic agonist which is often employed in the treatment of bronchial asthma. The drug has limited oral activity because of the extensive first-pass metabolism which occurs during absorption of the drug from the GI tract (1, 2). Aerosol inhalation is the most commonly used route of administration and offers the advantages of a rapid onset of activity and delivery of the drug directly to the airways (3). The duration of action following inhalation is short, however, and frequent dosing is often required to maintain the desired effect. This can result in side effects such as cardiac stimulation, which can hinder effective therapy with the drug. Increasing the duration of action of isoproterenol would offer a significant improvement in the clinical use of the drug for the treatment of asthma.

Prodrugs have been used in the past to improve the delivery of pharmacological agents, and this approach seems feasible with isoproterenol. An inactive derivative of isoproterenol, which itself possesses more favorable physicochemical and pharmacokinetic properties than the

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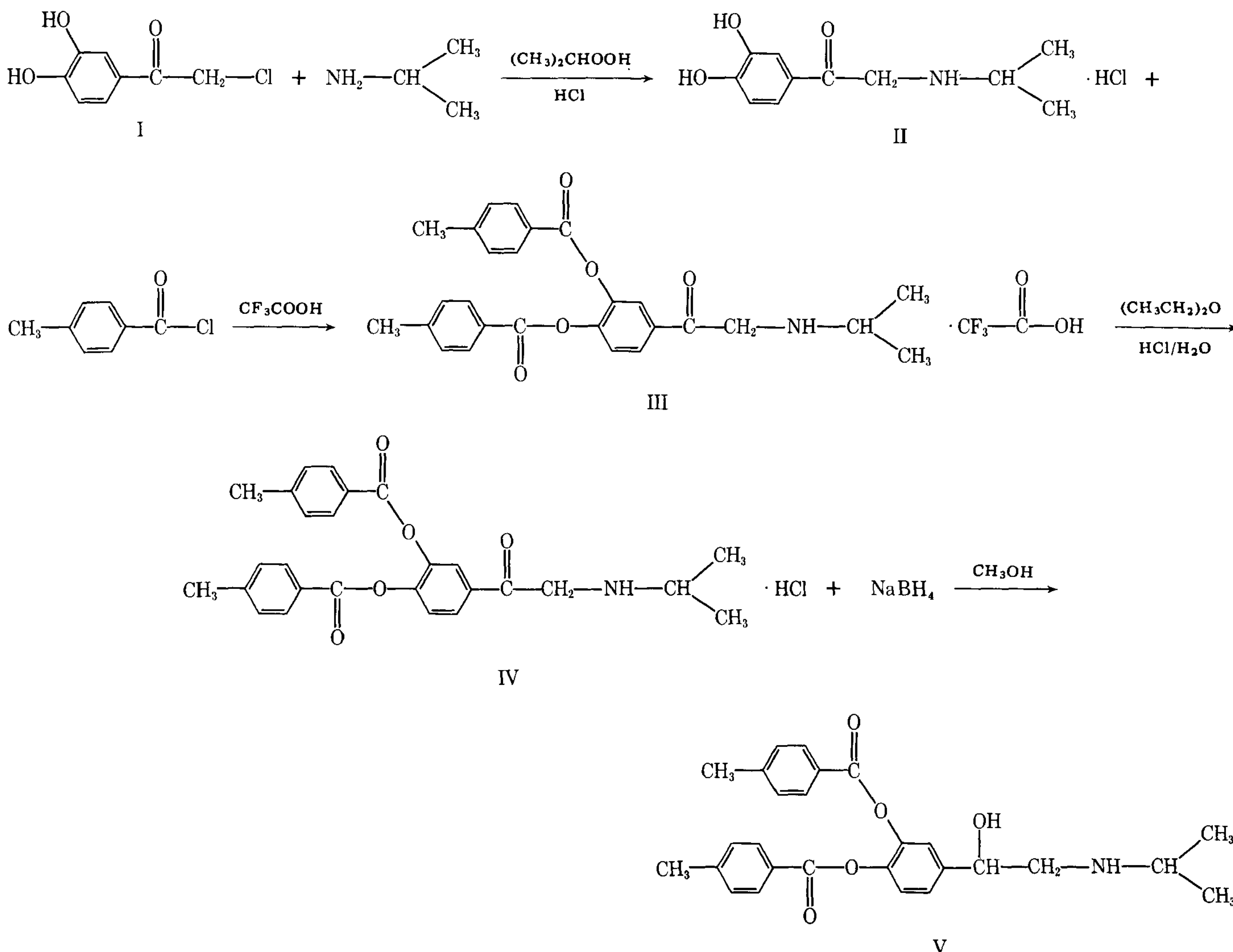

*Scheme I—Synthesis of di-*p*-toluoylisoproterenol.*

parent drug, and which would be converted to the active compound in the body, could provide a mechanism for improving the efficacy of the drug while eliminating some of its problems. Esterifying the phenolic hydroxyl groups of isoproterenol is a practical approach as these functionalities are involved in the metabolism and chemical degradation of the drug. Esterases capable of hydrolyzing these esters are distributed throughout the body and have been demonstrated in the lung tissue itself (4–6). The rate of hydrolysis of the ester could be controlled somewhat by the choice of the ester moiety (7–9); a slowly hydrolyzed hydrophobic ester might be taken up extensively by the lung tissue with subsequent slow release of the parent compound, resulting in a prolonged bronchodilating effect.

The present report details the preliminary evaluation of two prodrugs of isoproterenol, di-*p*-toluoylisoproterenol and dipivaloylisoproterenol. The absorption, uptake, and metabolism of these two drugs by the isolated perfused rabbit lung system were evaluated following their administration into the circulation of the system and into the airways of the lung. Disposition of isoproterenol in the isolated perfused rabbit lung has been discussed previously (10). The drug was converted to its *O*-methyl metabolite in the lung by a dose-dependent process. Uptake of the drug by the lung tissue, however, was linear over a 100-fold concentration range. A physiological pharmacokinetic perfusion model was developed to describe the disposition of isoproterenol and 3-*O*-methylisoproterenol by the isolated lung system, and a similar model is used in the evaluation of the data from this investigation.

EXPERIMENTAL

Di-*p*-toluoylisoproterenol—Synthetic procedures were modeled after published methods for preparation of esters of isoproterenol (7) and *N-tert*-butylarterenol (9). The overall reaction scheme is shown in Scheme I.

3,4-Dihydroxy-α-(isopropylamino)acetophenone Hydrochloride—A mixture of 37.5 g (0.2 mole) α-chloro-3′,4′-dihydroxyacetophenone[1] (I), 125 ml of isopropyl alcohol, and 60 ml of isopropylamine was slowly heated to 75° and maintained at that temperature for 5 min. While cooling, the mixture was acidified with hydrochloric acid. The hydrochloride salt was precipitated by the addition of 400 ml of acetone and overnight refrigeration. The filtered product (II) was slurried with 100 ml of acetone, filtered, and dried to yield 21.2 g (43%) of II, mp 239–242°.

3,4-Di-*p*-toluoyl-α-(isopropylamino)acetophenone Hydrochloride—Twenty grams of II was suspended in 60 ml of trifluoroacetic acid, and 35 ml of *p*-toluoyl chloride was added slowly, with stirring, over a period of 20 min. The mixture was heated slowly to 80° and held at that

[1] Aldrich Chemical Co., Milwaukee, Wis.

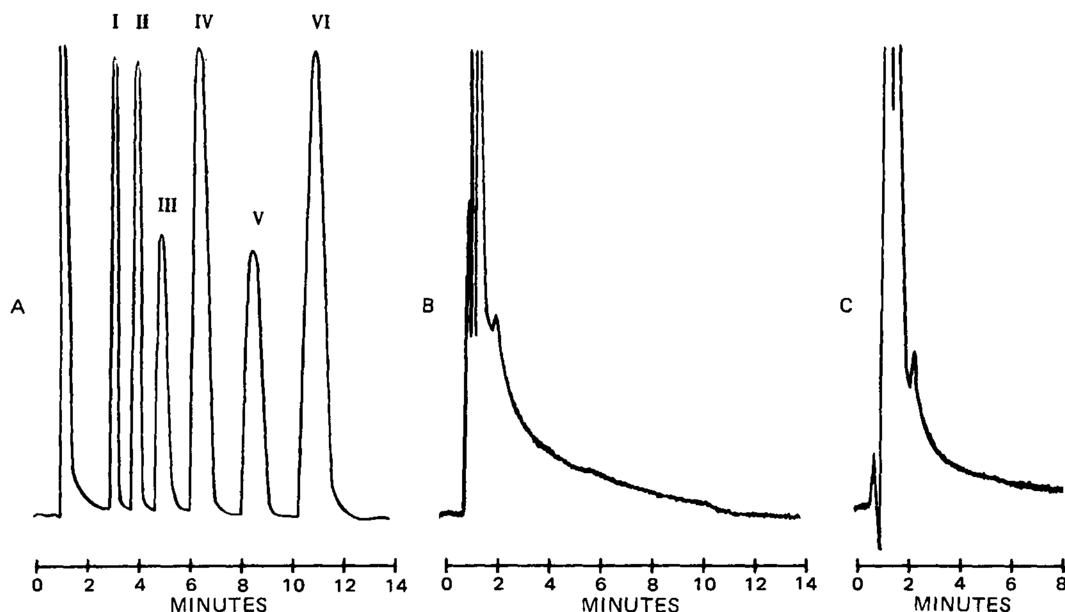

Figure 1—*(A) Separation of (I) isoproterenol, (II) 3-O-methylisoproterenol, (III) mono-*p*-toluoylisoproterenol, (IV) morphine, (V) di-*p*-toluoylisoproterenol, and (VI) codeine on a strong cation exchange column with 0.03* M *KH_2PO_4–25% acetonitrile (pH 3.0) flowing at 2.5 ml/min. (B) Blank methylene chloride perfusate extract. (C) Blank diethylhexyl phosphoric acid perfusate extract.*

temperature for 75 min. Most of the solvent was evaporated *in vacuo* and the residue stirred with 400 ml of ether and refrigerated. Filtration yielded 40 g of III. Compound III was partitioned between ether and water and made basic with sodium hydroxide. The ether layer was removed and 40 ml of 20% HCl was added slowly with constant stirring. The crystalline product that precipitated was filtered, dried, and recrystallized from isopropyl acetate–methanol (9:1) to yield 20 g of IV, mp 198–202°.

Di-*p*-toluoylisoproterenol (V)—A cold solution of 0.5 g of sodium borohydride in methanol was added to 5 g of IV in 25 ml of methanol over a 1-hr period in an ice bath. Most of the solvent was evaporated *in vacuo* and the residue was suspended in 20 ml of isopropyl acetate. This mixture was rapidly stirred and 25 ml of 10% sodium bicarbonate (pH 10.5) was slowly added. The organic layer was separated and dried over magnesium sulfate. The solution was partially concentrated *in vacuo* and refrigerated. The precipitated product was filtered and dried to yield 2 g (40%) of di-*p*-toluoyl isoproterenol (V), mp 110–111°.
cipitated product was filtered and dried to yield 2 g (40%) of di-*p*-toluoyl isoproterenol (V), mp 110–111°.

Anal.—Calc. for $C_{27}H_{29}NO_5$: C, 72.48; H, 6.48; N, 3.13; O, 17.89. Found: C, 72.49; H, 6.4; N, 3.12; O, 17.6. NMR (CD_3COCD_3): δ 6.9–8.3 (m, 11, aromatic); δ 4.5–4.9 (s, 1, —CHO*H*); δ 2.5–3.2 (m, 5, —C*H*$_2$—N*H*—CH$_2$—); δ 2.2–2.5 (s, 6, p-methyl); δ 0.9–1.2 [d, 6, —CH—(C*H*$_3$)$_2$]. The UV spectrum displayed a maximum of 245 nm with a molar absorptivity of 3.4×10^{-4} liters/mole/cm.

Isolated Perfused Rabbit Lung—Details of the perfusion technique have been described in detail in previous reports (10–12). Krebs-Ringer bicarbonate solution with 4.5% bovine serum albumin was used as the perfusion medium. Di-*p*-toluoylisoproterenol and dipivaloylisoproterenol were administered to the system by intravascular and intrabronchial administration. Doses of 10^{-5} and 5×10^{-5} moles of the two drugs were administered intravascularly as aqueous solutions added to the perfusion medium in the upper reservoir of the lung system. Intrabronchial administration of 5×10^{-6} and 10^{-5} moles of di-*p*-toluoylisoproterenol and 10^{-5} moles of dipivaloylisoproterenol was accomplished by delivering

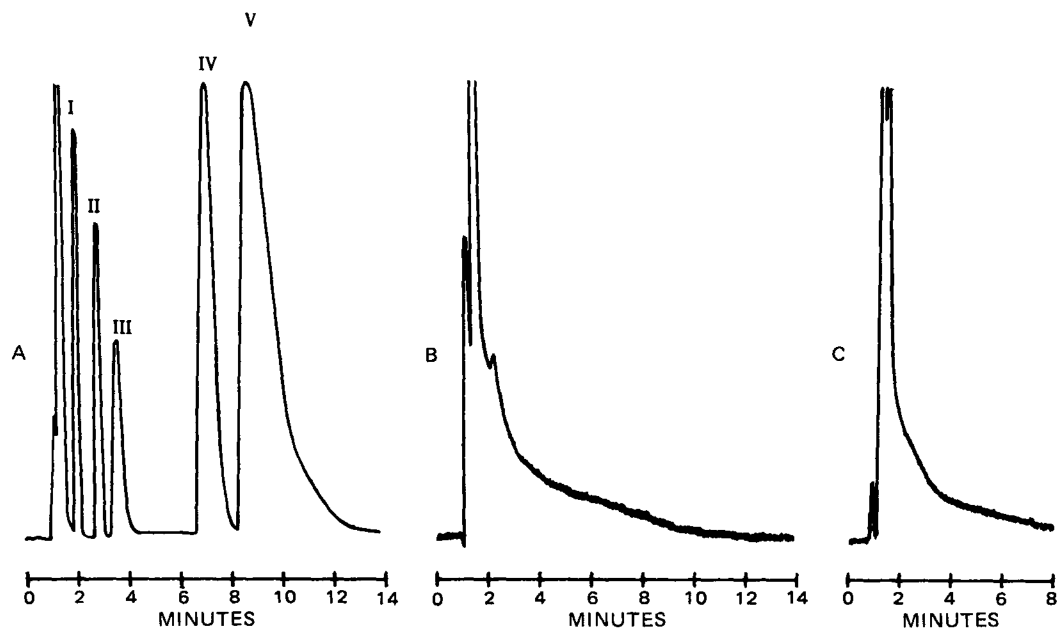

Figure 2—*(A) Separation of (I) isoproterenol, (II) 3-O-methylisoproterenol, (III) monopivaloylisoproterenol, (IV) dipivaloylisoproterenol, and (V) codeine on a strong cation exchange column with 0.01 KH_2PO_4–10% methanol (pH 3.0) flowing at 2.5 ml/min. (B) Blank methylene chloride perfusate extract. (C) Blank diethylhexyl phosphoric acid perfusate extract.*

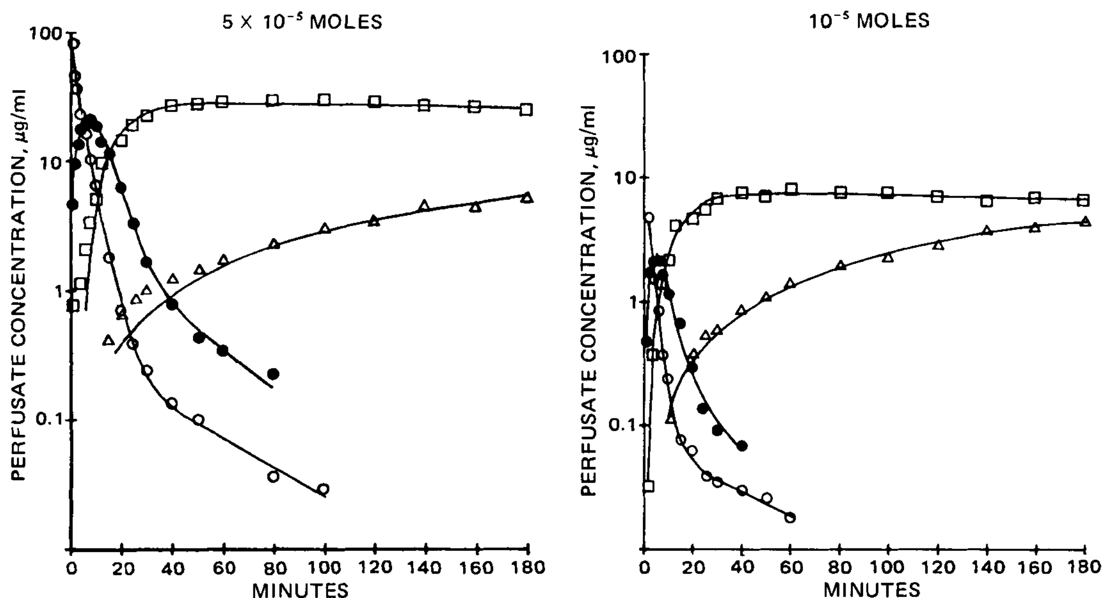

Figure 3—*Perfusate concentrations following the administration of 5 × 10^{-5} moles and 10^{-5} moles of di-*p*-toluoylisoproterenol into the circulation of the isolated perfused rabbit lung. Key: (○) di-*p*-toluoylisoproterenol; (●) mono-*p*-toluoylisoproterenol; (□) isoproterenol; (△) 3-O-methylisoproterenol.*

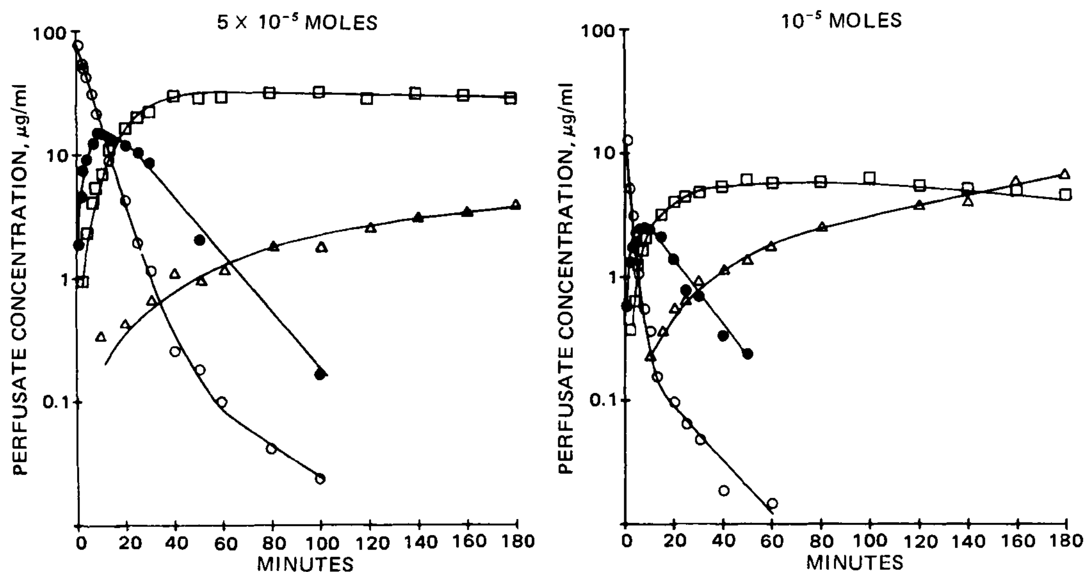

Figure 4—*Perfusate concentrations following 5 × 10^{-5} and 10^{-5} moles of dipivaloylisoproterenol administered to the circulation of the isolated perfused rabbit lung. Key: (○) dipivaloylisoproterenol; (■) monopivaloylisoproterenol; (□) isoproterenol; (△) 3-O-methylisoproterenol.*

250-μl aliquots of drug solution into the airways *via* a small polytef tube attached to a 1-ml syringe. Dipivaloylisoproterenol was dissolved in isotonic saline while di-*p*-toluoylisoproterenol was dissolved in saline which had been adjusted to pH 4.0 with hydrochloric acid.

Analysis—The perfusate concentrations of diester (di-*p*-toluoylisoproterenol or dipivaloylisoproterenol[2]), the corresponding monoester, isoproterenol, and 3-*O*-methylisoproterenol[3] were measured by high-pressure liquid chromatography (HPLC). The compounds were separated on a strong cation exchange column[4] and detected with a variable-wavelength UV detector[5]. Differences in physicochemical properties prevented the extraction of the four compounds with one procedure, and a double extraction technique was developed.

The same extraction procedure was used for both prodrugs. Samples were thawed to room temperature and 10 ml of methylene chloride containing codeine phosphate (internal standard) was added; this was vortexed for 1 min and centrifuged. The aqueous layer (750 μl) was transferred to a 15-ml culture tube and 100 μl of 1% ascorbic acid was added. This was stored at −20° and later analyzed for isoproterenol. The organic phase was evaporated under nitrogen, the residue reconstituted with 100 μl of methanol, and an aliquot was injected onto the HPLC system to measure the concentrations of the diester along with the monoester and 3-*O*-methylisoproterenol.

The 750-μl aqueous aliquot was thawed and 1 ml of 0.1 *N* acetate buffer (pH 4.0) containing morphine hydrochloride (internal standard) was added followed by 3 ml of 5% diethylhexyl phosphoric acid in ether. This was vortexed and centrifuged and the ether layer was transferred to a centrifuge tube; 100 μl of 1 *N* HCl containing 1% ascorbic acid was added, the mixture was vortexed and centrifuged, and a 10–50-μl aliquot was injected onto the HPLC system to measure isoproterenol.

Separation of di-*p*-toluoylisoproterenol, the monotoluoyl ester, isoproterenol, 3-*O*-methylisoproterenol, and codeine and morphine (internal standards) was accomplished with a strong cation exchange column and a mobile phase of 0.03 *M* KH_2PO_4 with 25% methanol and a final pH of 3.0. Flow rate was 2.5 ml/min and detection was at 220 nm. Aliquots

[2] Provided by the INTERx Corp., Lawrence, KS.
[3] Provided by C. H. Boehringer Sohn, Postfach 200, Germany.
[4] PXS 10/25 5CX, Whatman, Inc., Clifton, N.J.
[5] Vari-Chrom, Varian Associates, Palo Alto, Calif.

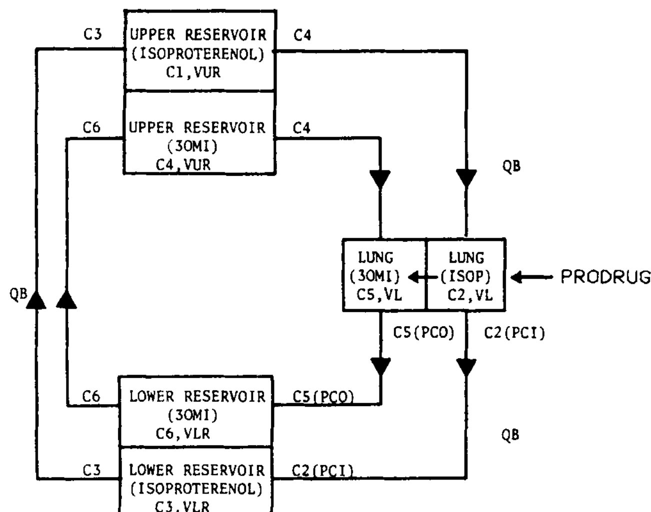

Figure 5—*Flow model used to describe the disposition of isoproterenol and 3-O-methylisoproterenol by the isolated perfused rabbit lung following administration of the diester prodrugs.*

(10–50 μl) from the methylene chloride extractions were injected onto this system to quantitate the diester, monoester, and 3-*O*-methylisoproterenol while aliquots (10–50 μl) from the diethylhexyl phosphoric acid extractions were injected to measure isoproterenol. This procedure provided adequate sensitivity for the monoester, isoproterenol, and 3-*O*-methylisoproterenol but was not capable of measuring diester concentrations <0.1 μg/ml. When diester concentrations below this limit were encountered, the methylene chloride extraction aliquots were also analyzed with an alternate chromatographic system utilizing a mobile phase of 0.04 *M* KH_2PO_4 with 20% acetonitrile and a final pH of 3.0 pumped through the cation exchange column at 3.0 ml/min with 250-nm detection. This provided sensitivity for the diester down to 0.01 μg/ml. Sample chromatograms are shown in Fig. 1.

A similar procedure was used for measuring dipivaloylisoproterenol. A cation exchange column was used; the mobile phase consisted of 0.01 *M* KH_2PO_4 with 10% methanol (pH 3.0) pumped at 2.5 ml/min with 220-nm detection. This system was used for measuring the monoester, isoproterenol, 3-*O*-methylisoproterenol, and concentrations of dipivaloylisoproterenol exceeding 0.5 μg/ml. The alternate system used to measure dipivalylisoproterenol concentrations <0.5 μg/ml also employed the cation exchange column with 0.045 *M* KH_2PO_4 with 20% methanol (pH 3.0) at a flow rate of 2.5 ml/min and 220-nm detection. Sample chromatograms are presented in Fig. 2.

For standard curves, the monoesters of di-*p*-toluoylisoproterenol and dipivaloylisoproterenol were produced by hydrolysis of the diesters in deoxygenated 1 *N* HCl containing 5% ascorbic acid as an antioxidant. Hydrolysis was relatively slow under these conditions and appreciable amounts of monoester could be produced while maintaining measurable amounts of the diester and isoproterenol by heating the solutions for 12–24 hr protected from light and air. These solutions were injected onto one of the previously described HPLC systems, and the concentrations of the diester and isoproterenol were determined by comparing their response to calibration curves prepared for the two compounds. Using the amounts of these two compounds and the diester initially added, the amount of monoester present in the solutions was determined using mass balance calculations.

Aliquots of these solutions were added to fresh blank perfusate samples along with equivalent volumes of 1 *N* NaOH such that the final perfusate sample volume was 1 ml. To each sample, 100 μl of 1 *M* phosphate buffer (pH 7.4) with 1% ascorbic acid was added. These samples were assayed in the manner described for the unknown samples. Standard curves for the diester, isoproterenol, and 3-*O*-methylisoproterenol were also generated. Peak heights were measured manually and the concentration *versus* peak height ratio of each compound to internal standard were fitted to a linear regression line. Correlation coefficients ≥0.98 were obtained using these methods. Standard curves for all compounds were produced on each day that samples were analyzed.

Care was taken in the storage, processing, and analysis of samples to minimize hydrolysis of the esters during this time. Small amounts of monoester and isoproterenol were observed in some diester standard perfusate samples processed in the same manner as the unknown samples, but these amounts never exceeded 5%.

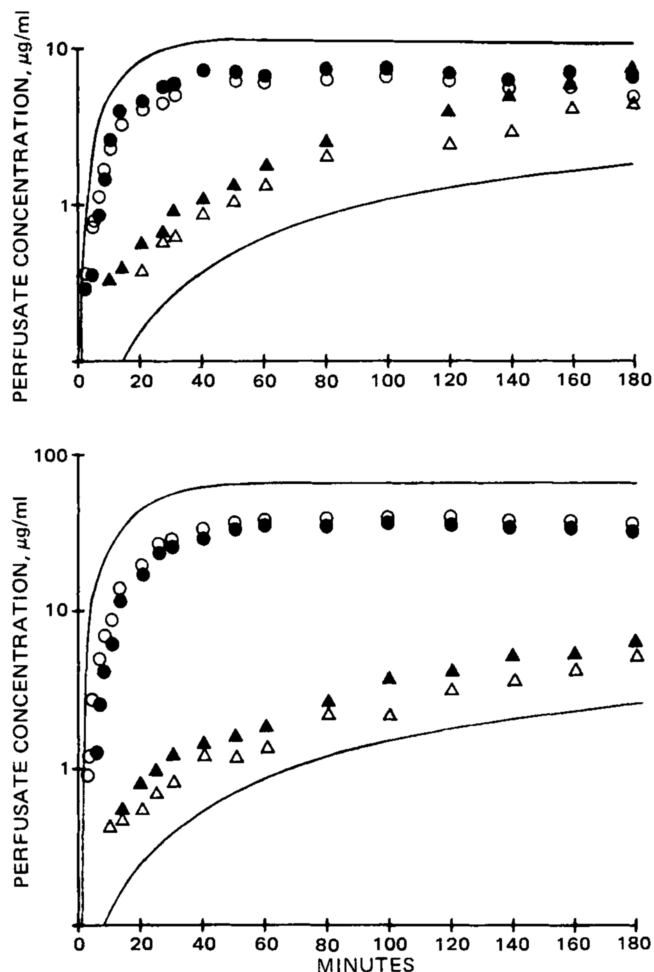

Figure 6—*Comparisons of observed concentrations to those predicted by the flow model for the intravascular administration of 10^{-5} moles of di-*p*-toluoylisoproterenol and dipivaloylisoproterenol (upper) and 5×10^{-5} moles of di-*p*-toluoylisoproterenol and dipivaloylisoproterenol (lower). Open symbols represent data from di-*p*-toluoylisoproterenol experiments, while closed symbols represent data from dipivalylisoproterenol experiments. Key: (○,●) isoproterenol; (△,▲) 3-O-methylisoproterenol.*

RESULTS AND DISCUSSION

Initial Studies—Preliminary studies involved administration of 10^{-5}-mole doses of dipivaloylisoproterenol and di-*p*-toluoylisoproterenol into the circulation of the isolated lung system. In each experiment, samples were withdrawn periodically from the upper reservoir and assayed for the prodrug, isoproterenol, and 3-*O*-methylisoproterenol. Both prodrugs were rapidly cleared from the perfusion medium, and in both cases isoproterenol appeared within 4–6 min. Following administration of each diester, an unidentified chromatographic peak was observed on the early sample chromatograms which eluted between 3-*O*-methylisoproterenol and the diester.

The peak height ratio *versus* time profile of this unknown component was suggestive of a monoester intermediate formed during the hydrolysis of the diester. The chromatographic behavior of this component under various conditions was identical to a similar component observed in partially hydrolyzed aqueous solutions of the corresponding diester. Earlier spectral kinetic studies with dipivaloylisoproterenol in diluted serum solutions had suggested the possibility of an intermediate in the hydrolysis of the diester[6].

These analytical comparisons help substantiate the presence of the monoester in the lung perfusate after administration of the prodrug, and chromatographic procedures were developed to separate and quantitate this compound in the perfusate samples.

Intravascular Administration—Dipivaloylisoproterenol and di-

[6] Unpublished data.

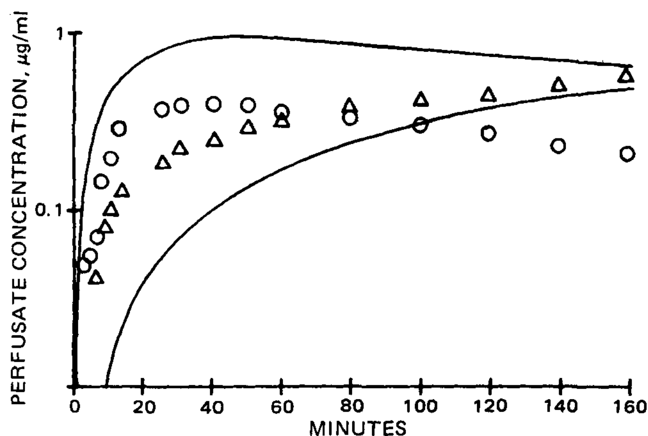

Figure 7—*Comparison of observed concentrations to those predicted by the flow model of the intravascular administration of 10^{-6} moles of di-*p*-toluoylisoproterenol to the isolated perfused rabbit lung. Key: (○) isoproterenol; (△) 3-O-methylisoproterenol.*

p-toluoylisoproterenol were administered to the circulation of the isolated lung system at 10^{-5}- and 5×10^{-5}-mole doses. Perfusate concentrations of diester, monoester, isoproterenol, and 3-*O*-methylisoproterenol were measured periodically for 180 min. A 10^{-6}-mole dose was also administered, but only isoproterenol and 3-*O*-methylisoproterenol were measured. The perfusate concentration–time profiles generated from these experiments are presented in Figs. 3 and 4.

Both diesters were rapidly cleared from the perfusion medium following their administration directly into the circulation. At the two 10^{-5}-mole doses, the clearance of di-*p*-toluoylisoproterenol was 92 and 106 ml/min, while that for dipivaloylisoproterenol was 59 and 89 ml/min. The diester perfusate concentration–time data were fitted best with a triexponential equation. The two initial rapid phases were barely distinguished from one another, but a slow terminal phase was observed for both diesters at very low concentrations. Detectable concentrations of the monoester intermediates were observed within 2–4 min of drug administration, indicating rapid uptake and hydrolysis of the diesters. Peak monoester concentrations occurred at 6–8 min. Isoproterenol appeared in the circulation within 6 min of prodrug administration but its elimination was very slow, as was the appearance of 3-*O*-methylisoproterenol. The perfusate concentration–time profiles of the four compounds of interest from the intravascular studies were initially fitted with complex compartmental pharmacokinetic models; the concentration–time profiles predicted from the curve fitting are represented by the solid lines in Figs. 3 and 4.

These models were successful in describing the data but provided little useful information about the disposition of these prodrugs. The limited number of experiments performed minimized the amount of pharmacokinetic information obtained, but the use of a physiologically based flow model provides some basic information concerning the disposition of isoproterenol following prodrug administration.

Perfusion Model Analysis—The perfusion model had been developed previously to describe the disposition of isoproterenol and 3-*O*-methylisoproterenol in the isolated perfused rabbit lung and was discussed previously (10). A similar model was used to evaluate the isoproterenol and 3-*O*-methylisoproterenol data resulting from the prodrug administrations (Fig. 5). Appearance of isoproterenol in the circulation of the lung system was modeled as a first-order release of the drug (from the prodrug) into the lung tissue with instantaneous partitioning into the perfusion medium. The parameters utilized to describe the model were, for the most part, identical to those described previously (10). An average lung volume of 11.7 ml and a rate constant for the appearance of isoproterenol of 0.05 min^{-1} were employed. The isoproterenol and 3-*O*-methylisoproterenol perfusate concentration–time profiles predicted by the perfusion model simulations are shown in Fig. 6 (5×10^{-5}- and 10^{-5}-mole doses) and Fig. 7 (10^{-6}-mole dose) along with the observed data.

These comparisons illustrate consistent disagreement between the observed and predicted data; the observed isoproterenol concentrations were lower than the predicted values, whereas the observed 3-*O*-methylisoproterenol concentrations were higher than those predicted by the model. This suggests that the disposition of isoproterenol is altered when the drug is delivered into the tissue *via* a prodrug. This might result from penetration of the more lipophilic prodrug into tissues to which isoproterenol does not penetrate.

Intrabronchial Administration—Solutions of the prodrugs in saline were instilled into the airways of the isolated lung; the upper reservoir was sampled periodically and perfusate concentrations of the diester, monoester, isoproterenol, and 3-*O*-methylisoproterenol were measured. Di-*p*-toluoylisoproterenol was administered at doses of 10^{-5} and 5×10^{-6} moles while dipivaloylisoproterenol was given at 10^{-5}-mole doses. In some studies, samples were initially collected at 15-sec intervals to characterize the absorption of the diester. The perfusate concentration–time profiles from these experiments are presented in Figs. 8 and 9.

Absorption of both prodrugs from the airways was quite rapid, with maximum concentrations of the intact diesters observed at ~2 min. Feathering the diester perfusate concentration–time curves yielded absorption rate constants of 1.4 min^{-1} and 0.9 min^{-1} for dipivaloylisoproterenol and di-*p*-toluoylisoproterenol, respectively, suggesting that absorption is virtually complete within 5 min. The subsequent metabolic profiles were quite similar to those following intravascular administration, except that the diester concentrations following intrabronchial administration were somewhat lower. Isoproterenol and 3-*O*-methylisoproterenol concentrations, however, were comparable to those following intravascular administration, and mass balance calculations suggest that most of the administered dose was absorbed.

A comparison of the apparent clearances of the diesters following in-

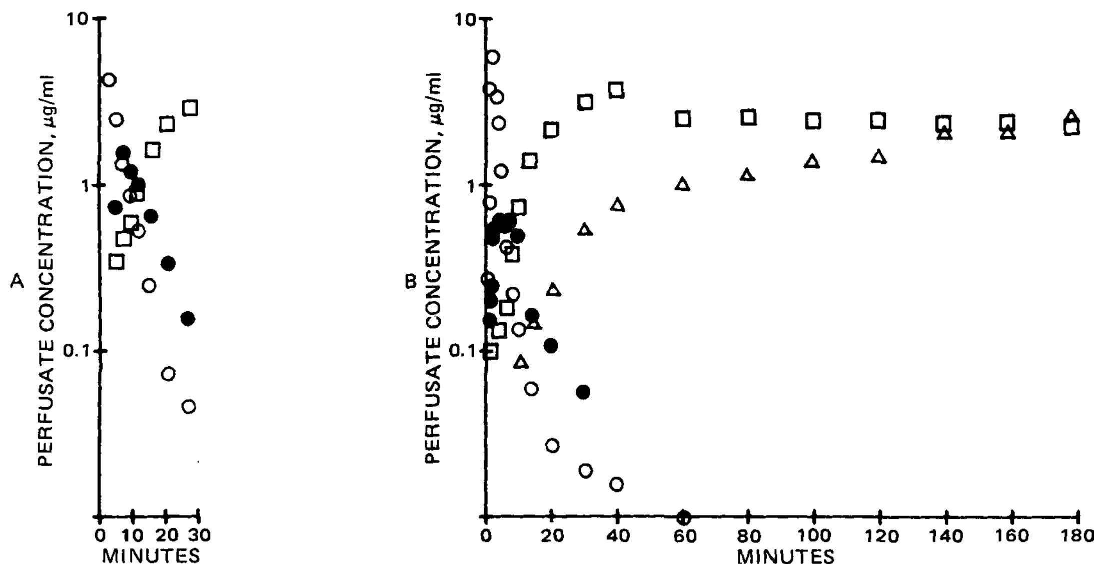

Figure 8—*Perfusate concentrations following the endotracheal instillation of 10^{-5} moles of di-*p*-toluoylisoproterenol (A) and 5×10^{-6} moles of di-*p*-toluoylisoproterenol (B). Key: (○) di-*p*-toluoylisoproterenol; (●) mono-*p*-toluoylisoproterenol; (□) isoproterenol; (△) 3-O-methylisoprotrenol.*

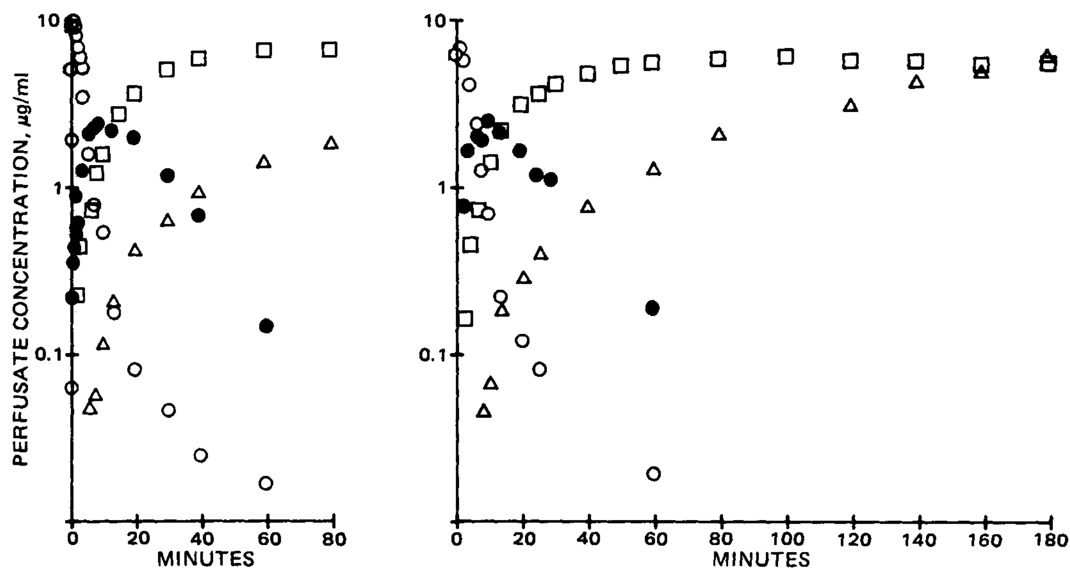

Figure 9—*Perfusate concentrations following the endotracheal instillation of two 10^{-5}-mole doses of dipivaloylisoproterenol. Key: (○) dipivaloylisoproterenol; (●) monopivalylisoproterenol; (□) isoproterenol; (△) 3-O-methylisoproterenol.*

trabronchial administration (172–203 ml/min for di-p-toluoylisoproterenol and 96–102 ml/min for dipivaloylisoproterenol) with those following intravascular administration (92–106 ml/min for di-p-toluoylisoproterenol and 59–89 ml/min for dipivaloylisoproterenol) suggests that a substantial portion of the intrabronchial dose does not reach the circulation as intact diester. From the data it appears that only ~50% of the di-p-toluoylisoproterenol and 75% of the dipivaloylisoproterenol reached the circulation intact after intrabronchial administration. This suggests that first-pass metabolism of the diesters following intrabronchial administration likely involves hydrolysis of the diester to the corresponding monoester (or isoproterenol) during the passage of the diester from the site of absorption to the systemic circulation. This possibility is consistent with the diester perfusate concentration data following intravascular administration to the lung from which an extraction ratio of 0.67 for di-p-toluoylisoproterenol and 0.5 for dipivaloylisoproterenol was calculated.

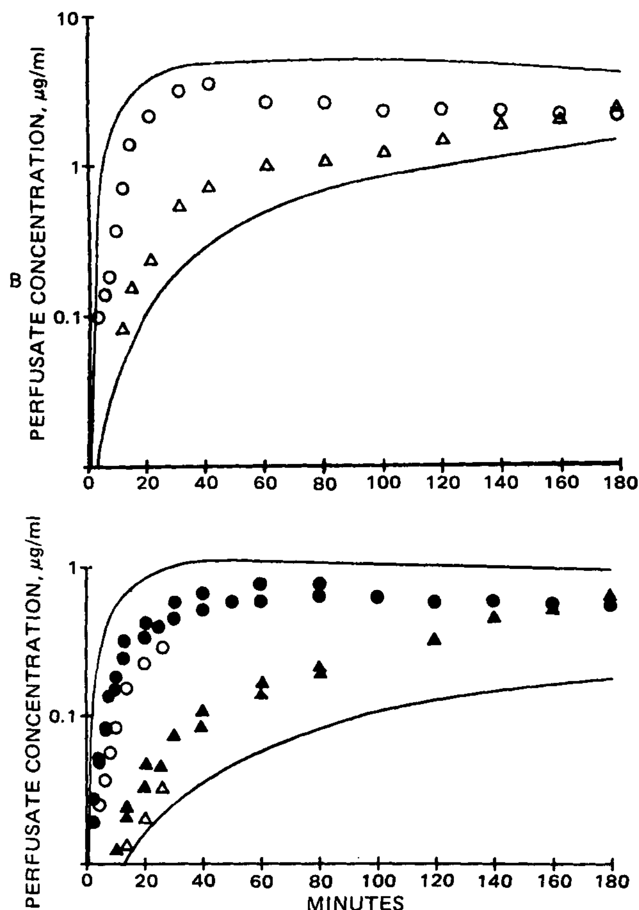

Figure 10—*Comparisons of observed concentrations to those predicted by the flow model for the intrabronchial administration of 10^{-5} moles of di-p-toluoylisoproterenol and dipivaloylisoproterenol (upper) and 5×10^{-6} moles of di-p-toluoylisoproterenol (lower). Key: (○,●) isoproterenol; (△,▲) 3-O-methylisoproterenol.*

The isoproterenol and 3-O-methylisoproterenol perfusate concentration data from the intrabronchial experiments were compared with perfusion model predictions in the same manner as the intravascular data. These comparisons are presented in Fig. 10 and exhibit a pattern very similar to that seen with intravascular data. Isoproterenol concentrations were lower than predicted, whereas 3-O-methylisoproterenol concentrations were higher, again suggesting that the disposition of isoproterenol by the lung was altered when the drug was administered as a prodrug.

Diester prodrugs of sympathomimetic amines have been suggested to increase the potency and duration of action of these drugs. Bitolterol, the di-p-toluate ester of N-*tert*-butylarterenol, has been studied rather extensively (13–15). This compound is very similar in structure to di-p-toluoylisoproterenol, and the disposition of the two might be expected to be similar. When administered to humans by aerosol inhalation, bitolterol displayed a 5-hr duration of activity compared with 1 hr for the parent compound (15). This has been attributed to uptake of the intact diester by the lung followed by slow release and subsequent hydrolysis to N-*tert*-butylarterenol, an active β_2 adrenoceptor agonist (14). Studies with dipivaloylisoproterenol and di-p-toluoylisoproterenol were designed to determine if these prodrugs exhibited a similar pattern.

These results suggest that the isoproterenol prodrugs do not accumulate to a great extent in the lung tissue and do not support the assumption that the prolonged activity of similar prodrugs is related to accumulation in the lung. Both dipivalylisoproterenol and di-p-toluoylisoproterenol were rapidly taken up by the lung but were quickly hydrolyzed, first to monoesters and then to active drug. The monoesters appeared in the circulation in appreciable concentrations and under normal conditions, *i.e.*, in the whole body, most of this intermediate would be swept away into the general circulation. These results do not explain the prolonged activity displayed by bitolterol and suggest the need for further studies in this area.

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NOTES

An Improved High-Pressure Liquid Chromatographic Assay for Secobarbital in Serum

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Abstract □ A high-pressure liquid chromatographic method for the analysis of secobarbital in serum was developed. Secobarbital was extracted from buffered serum (pH 5.5) with a solvent mix of hexane–ether–*n*-propanol. 5-(4-Methylphenyl)-5-phenylhydantoin was added as an internal standard. Separation of secobarbital and internal standard from serum constituents and other drugs was achieved on a 5-μm C-18 reversed-phase column using an acetonitrile–phosphate buffer (pH 4.4) mobile phase. The eluent was monitored at 195 nm. The sensitivity limit of the assay was ~0.02 μg/ml with 0.5 ml of serum sample. The application of this method to pharmacokinetic studies in pediatric patients was demonstrated.

Keyphrases □ Secobarbital—high-pressure liquid chromatographic assay in human serum, anticonvulsants, pharmacokinetics □ High-pressure liquid chromatography—secobarbital, anticonvulsant, pharmacokinetics, human serum □ Anticonvulsants—secobarbital, high-pressure liquid chromatographic assay in human serum, pharmacokinetics □ Pharmacokinetics—secobarbital, high-pressure liquid chromatographic assay in human serum, anticonvulsant

Secobarbital is used primarily as a hypnotic agent. Like many other barbiturates, when administered in anesthetic doses it is an effective anticonvulsant (1). Rectal secobarbital is prescribed for the emergency home treatment of prolonged seizures in poorly controlled epileptic children (2). In an attempt to study the bioavailability of rectally administered secobarbital in pediatric patients, a sensitive method for quantitating the drug in serum was required.

A number of analytical procedures for determining secobarbital in serum have been described in the literature. These include spectrophotometry (3), gas-liquid chromatography (GLC) (4–8), and high-pressure liquid chromatography (HPLC) (9, 10). However, many of these existing procedures are aimed at detecting high concentrations (>5 μg/ml) of secobarbital for screening purposes related to drug abuse. The more sensitive methods such as GLC with electron capture detection require tedious sample clean up and derivatization procedure prior to analysis. Also, with many of these methods, the problem of interference from other drugs and their metabolites in serum has not been evaluated. This report describes an improved HPLC method that permits the determination of submicrogram quantities of secobarbital in small volumes of serum in the presence of various antiepileptic drugs.

EXPERIMENTAL

Reagents and Chemicals—Secobarbital[1] and 5-(4-methylphenyl)-5-phenylhydantoin[2] (internal standard) were used as supplied. The solvents used for extraction and chromatography were all of HPLC grade[3,4]. All chemicals were of analytical reagent grade.

A stock solution of sodium secobarbital (120 μg/ml) and the internal standard (7 μg/ml) were prepared in water. The phosphate buffer component of the mobile phase was prepared by adding 300 μl of 1.0 *M* KH_2PO_4 and 50 μl of 0.9 *M* H_3PO_4 to 1800 ml of water (pH 4.4). For the extraction procedure, a pH 5.5 acetate buffer consisting of 0.01 *M* sodium acetate–0.01 *M* acetic acid (88.5:11.5, v/v) was prepared.

Apparatus and Operating Conditions—The liquid chromatograph consisted of a constant flow pump[5], a variable volume sampling valve[6], and a variable wavelength detector[7]. A 4.6-mm × 25-cm column packed with 5 μm of microporous silica chemically bonded with octadecylsilane was obtained from a commercial source[8]. The mobile phase consisting of 28% acetonitrile and 72% pH 4.4 phosphate buffer (v/v) was filtered[9]

[1] Eli Lilly and Co., Indianapolis, Ind.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] Fisher Scientific Co., Rochester, NY.
[4] J. T. Baker Chemical Co., Rochester, NY.
[5] Model M6000A, Waters Associates, Milford, Mass.
[6] Model U6K, Waters Associates, Milford, Mass.
[7] Model SF770, Kratos Inc., Schoeffel Instrument Div., Westwood, N.J.
[8] Partisil 5 ODS-3, Whatman Inc., Clifton, NJ 07014.
[9] 0.5 μm FH type filter, Millipore, Bedford, Mass.

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