Pharmacokinetics of β-Methyldigoxin in Healthy Humans I: Intravenous Studies

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Abstract □ The pharmacokinetics of intravenously administered solutions of 0.30- and 0.60-mg bolus doses of ^{3}H-β-methyldigoxin, labeled in the 12α-position, were dose independent. Individual radioactivities assignable to the parent drug and specifically identified metabolites after TLC separation were followed in the plasma, urine, and feces. A sum of four exponentials described the plasma β-methyldigoxin data with apparent half-lives of 0.04, 0.33, 3.5, and 41 hr. β-Methyldigoxin was 10% plasma protein bound and had a red blood cell–plasma water partition coefficient of 0.9. The only significant metabolite observed in plasma was digoxin, although glucuronides and sulfates of β-methyldigoxin, digoxin, and digoxigenin also were observed in urine. As much as 92 ± 3% of the dose was excreted by all processes by 144 hr. Of this amount, renal excretion accounted for fractions that were 0.47 unchanged, 0.35 digoxin, and 0.058 water-soluble metabolites. The fraction in the feces was 0.13. The urine flow independent renal clearances of β-methyldigoxin and derived digoxin were 59 and 206 ml/min, respectively. The metabolism was a relatively fast process. The terminal pseudo-steady-state elimination of β-methyldigoxin with a half-life of 41 hr was reached 27 hr after drug administration and was primarily dependent on the slow release of sequestered or distributed drug from the tissues into the central compartment. The drug and metabolite levels in plasma and urine were consistent with analog computer fitting to the proposed pharmacokinetic multicompartmental model.

Keyphrases □ β-Methyldigoxin—intravenous, pharmacokinetics in humans, radiochemical–TLC study □ Pharmacokinetics—intravenous β-methyldigoxin, humans, radiochemical–TLC study □ Radiochemistry–TLC—study of pharmacokinetics of intravenous β-methyldigoxin in humans □ Cardiac glycosides—β-methyldigoxin, intravenous, pharmacokinetics in humans, radiochemical–TLC study

Frequently used methods for the assay of digoxin glycosides, radioimmunoassay (1) and liquid scintillation spectrophotometry (2–4) of labeled drug, are not specific for the parent compounds *per se* and measure drug plus metabolites. Their only valid use in pharmacokinetics or bioavailability studies is after proper separation of the drug and its metabolites or if the metabolic route of disposition is negligible. An argument could be made for those clinical circumstances where the sum of cardioactive drug and metabolites could be correlated with observed cardial or extracardial pharmacodynamic parameters.

Pharmacokinetic and bioavailability studies of glycosides with specific analyses of the parent drug and metabolites in plasma and urine as functions of the dose, formulation, and route of administration are needed. Apparently, no published studies demonstrate the linearity of the area under blood level–time curves (*AUC*) with the administered dose of digoxin-like drugs, a necessary prerequisite for the use of *AUC* as a criterion of bioavailability. The studies that did attempt to separate such drugs from their metabolites prior to assay by such nonspecific methods were limited. Column chromatographic (2, 3) and extraction (4) procedures were used to separate the labeled parent drug and metabolite. However, none of these papers clearly showed quantifications based on evaluated extraction efficiencies, nor did they demonstrate specific identifications of parent drug and metabolites in plasma or urine.

The present studies were conducted to determine the time course of radiolabeled ^{3}H-β-methyldigoxin (medigoxin) and its identified metabolites, separated and analyzed individually after both oral and intravenous administration at two dosage levels in the same individual, and to establish valid pharmacokinetic models and their parameters. This paper considers the pharmacokinetics of β-methyldigoxin after intravenous administration.

EXPERIMENTAL

Materials—The following were used: ^{3}H-β-methyldigoxin[1], digoxin[1], digoxigenin bisdigitoxoside[1], digoxigenin monodigitoxoside[1], and digoxigenin[1]. ^{3}H-β-Methyldigoxin was synthesized with the label in the 12α-position according to the method of von Wartburg *et al.* (5) and had a specific activity of 431 μCi/mg. β-Glucuronidase[2] and aryl sulfatase[3] were used for the enzymatic hydrolysis of conjugated metabolites. All organic solvents were analytical grade.

Commercially available scintillation fluids[4,5], solubilizer[6], dioxane, and a scintillation fluid prepared as described previously (6) were used for radioactivity counting. Heparin sodium[7] was used to prevent coagulation.

Heparinized blood and plasma with known protein fractions were obtained from healthy volunteers[8] who had no prior drug intake.

Criteria for Volunteer Selection—Seven healthy male Caucasian volunteers[9] between 21 and 25 years of age were selected. An individual was considered healthy when there was no history of problems with the cardiovascular system, kidneys, liver, blood, GI tract, and endocrine organs and no prostatic hypertrophy, drug addiction, and alcoholism. The volunteers had a normal status, ECG, blood pressure, and X-ray (chest).

The following initial laboratory checkup was performed on each volunteer: complete blood count (hematocrit included), sedimentation rate, serum electrophoresis, creatinine clearance, urinalysis, bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, and serum electrolytes (sodium, potassium, calcium, and inorganic phosphate). The following tests were repeated prior to the second, third, and fourth studies on each individual to determine toxic drug effects or clinical changes due to the experimental procedures: complete blood count (hematocrit included), sedimentation rate, serum electrophoresis, serum creatinine, and serum electrolytes.

Pharmacokinetic Procedures—Four diffferent studies were performed in each of the seven volunteers; 0.30- and 0.60-mg doses in solution were administered both orally and intravenously. A total of 28 studies was conducted with an incomplete Latin-square design. Intervals of 3 weeks were maintained between studies in a subject to exclude any possible enzyme induction due to repeated drug administration. The volunteers were supine during the first 24 hr after drug administration. Later they were ambulant, although no exercise was allowed.

The individuals were fasted 12 hr before drug administration in both the intravenous and oral experiments. After drug administration, the

[1] Boehringer & Co., Mannheim, West Germany.
[2] Ketodase, Warner-Chilcott Laboratories, Morris Plains, N.J.
[3] Calbiochem, San Diego, Calif.
[4] Scintillar, Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Spectrafluor, Amersham-Searle, Arlington Heights, Ill.
[6] Bio-Solv, Beckman Instruments, Fullerton, Calif.
[7] Heparin sodium injection USP, The Upjohn Co., Kalamazoo, Mich.
[8] Blood Bank, Shands Teaching Hospital and Clinics, Gainesville, Fla.
[9] Informed consent was obtained from all volunteers, and the experimental protocol was approved by both the Committee for Protection of Human Subjects and the Clinical Research Advisory Committee, Health Center, University of Florida.

volunteers fasted 8 hr. Within the first 24 hr after drug administration, food intake was restricted to fluids, *e.g.*, soups, milk shakes, and other beverages. The caloric intake was balanced in all studies to maintain constant weight. The daily fluid intake was fixed at 2500 ml/24 hr. Neither beverages nor food containing caffeine or other diuretically effective substances were allowed.

Three hours prior to drug administration, the volunteers were given water (10 ml/kg). One hour before the drug was given, a butterfly needle[10] was placed in a cubital vein. A constant drip of 0.45% sodium chloride[11], 1.7 ml/min in the oral experiments and 1.5 ml/min in the intravenous experiments, was started immediately through the butterfly needle. In the intravenous experiments, a catheter[12] was placed in the cubital vein on the other side and a constant drip of 0.9% sodium chloride (2.0 ml/min) was started immediately through the catheter. Both infusion solutions contained heparin[7], 1000 I.U./1000 ml, to prevent coagulation. Both the butterfly needle and the catheter were removed 11 hr after drug administration. Later blood samples were taken by venipuncture.

The freshly prepared solutions in 0.9% sodium chloride were assayed. They were intravenously administered at a constant rate for 1 min through the catheter, which was immediately flushed with 20 ml of 0.9% sodium chloride.

Blood (6 ml) was taken within 10 sec at 0, 1.5, 2, 2.5, 3.5, 7, 10, 15, 30, 45, 60, and 90 min and at 2, 3, 5, 7, 9, 11, 15, 20, 24, 36, 48, 60, 72, 84, 96, 120, and 144 hr after intravenous drug administration through the butterfly needle with syringes using a three-way stopcock[13]. An initial 2 ml of blood was discarded. The samples were immediately transferred to glass tubes[14] containing 12 mg of edetate disodium to prevent coagulation. After centrifugation at 1500 rpm for 20 min within 1 hr after sampling, the plasma was transferred to storage tubes and then frozen until assayed.

Total volumes of urine were obtained for the following collection periods: −3–0, 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–18, 18–24, 24–48, 48–72, 72–96, 96–120, and 120–144 hr. The pH was monitored, and aliquots were transferred to storage containers and frozen until assayed. Total feces were collected for the following collection periods: −12–0, 0–24, 24–48, 48–72, 72–96, 96–120, and 120–144 hr; the samples were frozen until assayed.

Analytical Procedures—The ^{3}H-β-methyldigoxin had a specific activity of 431 μCi/mg. Its purity was investigated in three different TLC systems: A, acetone–chloroform (4:6) on silica gel; B, ethyl acetate–formic acid (98:2) developed three times on basic alumina; and C, chloroform–ethanol–acetic acid–water (91.0:7.5:1.0:0.5) on basic alumina. Quantities and identities of the separable contaminants were determined by thin-layer scraping, elution of the material, and radioactivity counting in a liquid scintillation counter[15]. Corrections were made for background and efficiency using the channels ratio method (7).

When the ^{3}H-β-methyldigoxin used in the pharmacokinetic studies was chromatographed, the developed TLC spot assignable to the ^{3}H-β-methyldigoxin retained 97.6% (System A), 83.7% (System B), and 84.6% (System C) of the total radioactivity spotted in the three thin-layer systems used. It was concluded that 0.837 of the material represented ^{3}H-β-methyldigoxin, and this fraction of the total radioactivity was considered as the dose of β-methyldigoxin. The TLC separable contaminants assignable to digoxigenin monodigitoxoside (3.3%), digoxigenin (2.5%), digoxin (1.6%), and digitoxin and digoxigenin bisdigitoxoside (<1.0%) retained the percentages of the total radioactivity designated.

Since TLC System A was the least sensitive, only Systems B and C (with 250-μm basic alumina[16]-coated glass plates in activated chambers) were used in all other TLC studies on plasma and urine composition. The compounds were visualized by spraying with 50% aqueous sulfuric acid and heating the plates at 110° for 10 min. For the detection of the individual radioactive compounds, 4-mm bands of alumina were scraped from the plates using a zonal scraper[17]; the radioactivity in each zone was determined by liquid scintillation spectrophotometry using a prepared scintillation fluid (6). The radioactivity distribution pattern was then plotted by a computer plotter programmed to give the total radioactivity eluted from the plates, the number of radioactivity peaks, their R_f values, and the percentage of radioactivity in each peak. The efficiency of this process, 46.3 ± 1.3%, was determined by counting the scrapings of known spotted amounts. Corrections were made for the individual extractability of each compound.

For the determination of total radioactivity in plasma and urine, the samples were thawed and shaken, and 100- and 500-μl replicates were pipetted into vials containing 10 ml of scintillation fluid. In three of the seven investigated volunteers, total radioactivity was determined in the feces. Fecal samples were freeze dried and the dry powder was mixed thoroughly. Weighed aliquots were then combusted[18] to tritiated water, which was added to 10 ml of scintillation fluid and counted.

Aliquots of plasma (0.5–3.0 ml) were acidified to pH 4–5 with 10% HCl and extracted twice with three times the volume of ethyl acetate. The extracts were swirled over anhydrous sodium sulfate and decanted, and the solution was concentrated to dryness under nitrogen and then redissolved in 95% methanol (0.1 ml). Aliquots of the resultant solutions were then spotted on thin-layer plates, which were developed with Solvent Systems B and C. Sequential plasma samples with low radioactivity counts were pooled before extraction and TLC, and the weighted average of their sample times was taken as representative.

The extraction efficiencies into ethyl acetate of plasma samples spiked with various compounds were: β-methyldigoxin, 74.6 ± 0.7%; digoxin, 77.0 ± 1.6%; digoxigenin bisdigitoxoside, 74.3 ± 1.0%; digoxigenin monodigitoxoside, 85.5 ± 3.0%; and digoxigenin, 76.0 ± 1.0%.

Aliquots of urine (5 ml) were acidified with 10% HCl to pH 4–5 and extracted three times with equal volumes of chloroform. Each chloroform extract was swirled over anhydrous sodium sulfate, decanted, concentrated to dryness under nitrogen, and then redissolved in 95% methanol (0.1 ml). Aliquots of the resultant solutions were then spotted on thin-layer plates, which were developed with Solvent Systems B and C.

The aqueous phase was further analyzed in all urine samples in the 0.6-mg oral experiments of three volunteers. Aliquots of urine (5 ml) (Scheme I) were acidified with 10% HCl to pH 4–5 and extracted three times with equal volumes of chloroform to give chloroform extracts, C_1, which contained β-methyldigoxin and the less polar metabolites. The aqueous eluate, A_1 (pH 5.0), was incubated for at least 12 hr with 1.0 ml (5000 Fishmann units) of β-glucuronidase/ml of eluate. Extraction of A_1 with chloroform generated the chloroform eluate, C_2, which was chromatographed, and the generated aqueous eluate, A_2 (pH 5.0), was incubated with 0.1 ml of aryl sulfatase (5000 Whitehead units) for at least 12 hr.

After a third chloroform extraction of A_2, the newly generated aqueous eluate, A_3 (pH 5.0), was incubated with 1.0 ml of β-glucuronidase for at least 12 hr to assure complete enzymatic hydrolysis. The generated chloroform eluate, C_3, was subjected to TLC. After a final chloroform extraction of A_3, the generated aqueous eluate, A_4, was also chromatographed. The chloroform eluate, C_4, was not processed further, since it did not contain any radioactivity. Each chloroform extract was swirled over anhydrous sodium sulfate, decanted, concentrated to dryness under nitrogen, and assayed for percent recovery.

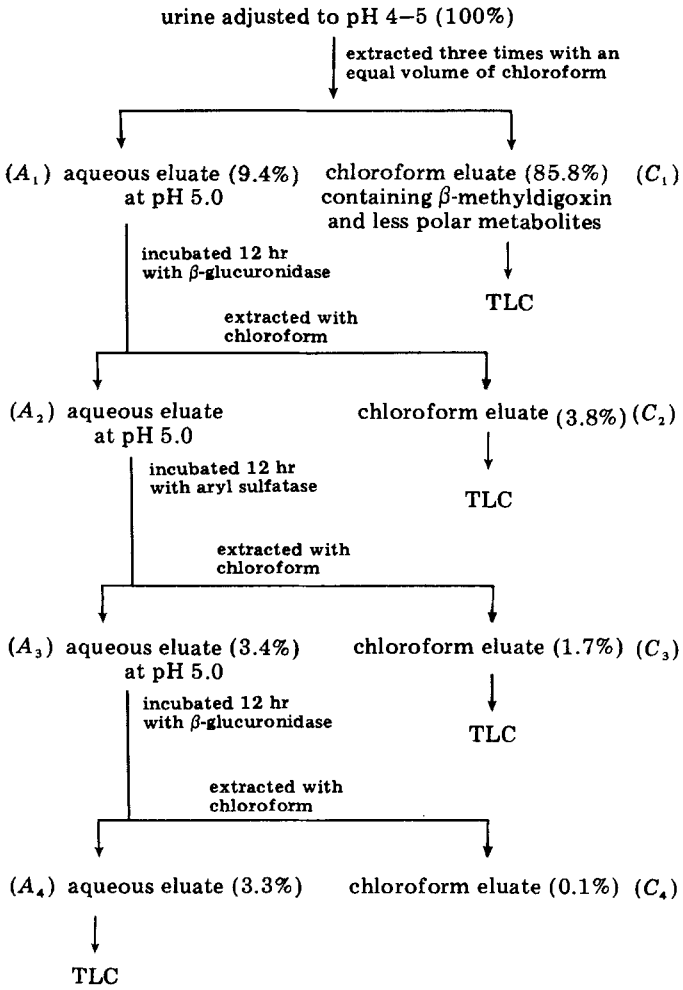
Recoveries of the compounds from urine spiked with specific radiolabeled compounds were, in the chloroform and residual water layer, respectively: β-methyldigoxin, 96.6 ± 1.5 + 2.1 ± 0.3 = 98.7%; digoxin, 100.9 ± 1.5 + 1.9 ± 0.3 = 102.8%; digoxigenin bisdigitoxoside, 104.2 ± 0.9 + 3.2 ± 0.8 = 107.4%; digoxigenin monodigitoxoside, 76.8 ± 2.7 + 18.9 ± 1.0 = 95.7%; and digoxigenin, 69.9 ± 1.8 + 31.6 ± 0.5 = 101.5%.

The β-methyldigoxin and metabolites in the extracts of plasma and urine were identified by admixing known standards with the extracts and separating in Systems B and C. The scrapings that corresponded to the R_f values were eluted with 95% methanol and definitely identified by three consecutive recrystallizations to constant specific activity (8) with standard deviations of less than ±4%. The metabolites in plasma and urine whose extraction efficiencies and recoveries were studied were checked by this procedure.

The extent of tritiated water formation was checked in urine by distillation (9). Equal aliquots of five total volumes of urine collected between 0 and 10 hr after 0.3 mg po was administered to one volunteer were combined and distilled. Distillate aliquots were assayed for tritiated water and compared with aliquots of water serving as controls. Triplicates (0.1 ml) of each were added to 10 ml of scintillation fluid and counted. Only 0.035% of the total radioactivity excreted was tritiated water. This amount was considered as a measure of negligible tritium exchange of labeled β-methyldigoxin with body water.

Protein Binding of β-Methyldigoxin—Plasma was equilibrated with chromatographically pure β-methyldigoxin (TLC System B) for 0.5 hr

[10] Abbott Laboratories, North Chicago, Ill.
[11] Baxter Laboratories, Division of Travenol Laboratories, Morton Grove, Ill.
[12] Intracath, Deseret Pharmaceutical Co., Sandy, Utah.
[13] Tomac, American Hospital Supply, Evanston, Ill.
[14] Vacutainer, Becton-Dickinson, Rutherford, N.J.
[15] Mark II, Searle Analytic Inc., Des Plaines, Ill.
[16] Woelm, Waters Associates, Framingham, Mass.
[17] Snyder-Kimble, Analabs Inc., North Haven, Conn.
[18] Tri-Carb oxidizer, Packard Instruments Co., Downers Grove, Ill.



Scheme I—Isolation scheme for separation of metabolites of ^{3}H-β-methyldigoxin in urine. Typical percentages of total radioactivity in each fraction are given in parentheses.

and filtered at 1200 rpm for 18–20 min in a centrifuge[19] through two to four high flux cone membranes[20]. Half-filtration was preferred, since complete filtration of plasma might trap and inhibit the free passage of other soluble plasma components (10–12). Total drug concentration (bound and unbound) in the plasma prior to filtration and the free drug concentration in the plasma water of the filtrate were determined by liquid scintillation spectrophotometry.

The protein binding of β-methyldigoxin was investigated at five pharmacokinetically observed concentrations between 0.49 and 76.0 ng/ml at 25–29°, pH 7.32–7.34, in the fresh human plasma of two healthy fasted volunteers with no prior drug intake. The total protein content and the protein fractions of the two plasmas were determined (13).

The concentration-dependent membrane binding of β-methyldigoxin was compensated for by presaturating the cones. Several prior half-filtrations of 3–4 min of plasma water spiked with drug of the same concentration as the plasma were half-filtered through the cone. Only those membranes that yielded greater than 85% recovery of plasma water concentration and less than 5% variability in two consecutive filtrations were used.

Red Blood Cell–Plasma Water Partition Coefficient of β-Methyldigoxin—Centrifugally separated red blood cells were washed twice with sterile isotonic sodium chloride solution, recentrifuged, and suspended in plasma water obtained by ultrafiltration. Chromatographically pure β-methyldigoxin (TLC System B) was added to prepare 45.2-, 5.53-, and 2.90-ng/ml concentrations in the presence and absence of unlabeled digoxin at the pharmacokinetically anticipated 31 and 42% of these concentrations. Aliquots were centrifuged, and their plasma water concentrations were assayed at 6, 10, and 16 min. The reproducibility of the hematocrits[21] after each sampling demonstrated thorough mixing of the red blood cell–plasma water suspensions.

The partition coefficient was also determined in whole blood with 8.3–47.8 ng/ml of radiolabeled β-methyldigoxin sampled from three volunteers shortly after intravenous administration when minimum metabolites were present. The plasma water concentrations were assayed after separation by centrifugation at 1500 rpm for 15 min. The concentrated red blood cells after two consecutive centrifugations at 2500 rpm for 20 min had hematocrits of 0.80–0.90 and were hemolyzed at −4°. The ghost cells were removed by centrifugation, and radioactivity was analyzed in the hemolysate.

RESULTS AND DISCUSSION[22]

Protein Binding of β-Methyldigoxin—The percent of drug bound to protein was calculated from:

$$f = \frac{100([A_p] - [A_p{}^u])}{[A_p]} = \frac{100[A_p{}^b]}{[A_p]} \qquad \text{(Eq. 1)}$$

where $[A_p]$, $[A_p{}^u]$, and $[A_p{}^b]$ are the concentrations of total, free, and bound drug in plasma, respectively. The value of $[A_p]$ was measured in plasma after the drug was added and before ultrafiltrations were performed.

The average f values for the parenthetical total plasma concentrations of drug, $[A_p]$, were 10.9% (76.0 ng/ml), 7.6% (30.4 ng/ml), 9.9% (6.00 ng/ml), 10.2% (2.40 ng/ml), and 11.4% (0.49 ng/ml), with an overall mean of 9.8 ± 1.1% for the concentration-independent protein binding of β-methyldigoxin. This amount is close to the 14% value of Dengler *et al.* (14) from equilibrium dialysis of radiolabeled drug in human plasma. Both do not agree with the reported 28.8% of Kramer *et al.* (15), obtained by dual closed-loop dialysis. By using their reported equation (15) to calculate protein binding, a value of 15% was obtained.

Red Cell Partitioning of β-Methyldigoxin—The red cell–plasma partition coefficient, D, of a drug has been defined as the ratio of the total drug concentration in the erythrocytes, $[A_{RBC}]$, to the free or unbound concentration of drug in plasma, $[A_p{}^u]$ (16):

$$D = [A_{RBC}]/[A_p{}^u] \qquad \text{(Eq. 2)}$$

The $[A_p{}^u]$ was calculated from $[A_p{}^u] = (1 - f)[A_p]$, with $f = 0.10$ for blood and $f = 0$ for red blood cell suspensions in plasma water.

The average D values obtained for the parenthetical concentrations in whole blood were: 0.86 ± 0.05 (45.0 ng/ml), 0.93 ± 0.04 (30.0 ng/ml), 0.91 ± 0.04 (20.0 ng/ml), and 0.87 ± 0.05 (10.0 ng/ml). Similar D values resulted with red blood cell–plasma water suspensions: 0.84 ± 0.04 (45.2 ng/ml) and 1.03 ± 0.06 (5.5 ng/ml). Therefore, D is invariant with changing concentrations of β-methyldigoxin. The drug equilibrated rapidly between red blood cells and plasma water, at least in the first sample that was taken 6 min after drug addition. The addition of digoxin to give molar ratios of 2.4 and 3.20 of β-methyldigoxin to digoxin had no effect on the rate or the extent of partitioning in red blood cells: 0.91 ± 0.02 (5.53 ng of β-methyldigoxin/ml–2.27 ng of digoxin/ml) and 1.11 ± 0.03 (5.53 ng of β-methyldigoxin/ml–1.73 ng of digoxin/ml).

Plasma Pharmacokinetics of ^{3}H-β-Methyldigoxin after Intravenous Administration—The concentrations of plasma β-methyldigoxin and digoxin were determined in three subjects at the two different dose levels for intravenous and oral administrations. They were calculated from the fraction of the total radioactivity of the TLC plate that could be assigned to the radioactivities of their respective spots. The fractions bound to plasma proteins were taken as 0.10 and 0.25 (17), respectively.

The concentrations of unbound β-methyldigoxin, $[A_p{}^u]$, in plasma were fitted against time by "feathering," the method of residuals (Figs. 1 and 2):

$$[A_p{}^u] = A_1'e^{-\alpha_1 t} + A_2'e^{-\alpha_2 t} + B'e^{-\beta t} + C'e^{-\gamma t} \qquad \text{(Eq. 3)}$$

The evaluated parameters for the various studies are given in Table I.

[19] Clay Adams Co., New York, N.Y.
[20] Amicon Corp., Lexington, Mass.

[21] Microhematocrit centrifuge model MB, International Equipment Co., Needham Heights, Mass.; tubes, Yankee, Clay Adams Division of Becton-Dickinson & Co., Parsippany, N.J.; microcapillary reader, International Equipment Co., Needham Heights, Mass.

[22] The plus and minus values for such mean values given in the text refer to the standard error, $\sigma/\sqrt{n}$, of such means, where σ is the standard deviation given in Table I and n is the number of values considered and was 6 unless otherwise specified.

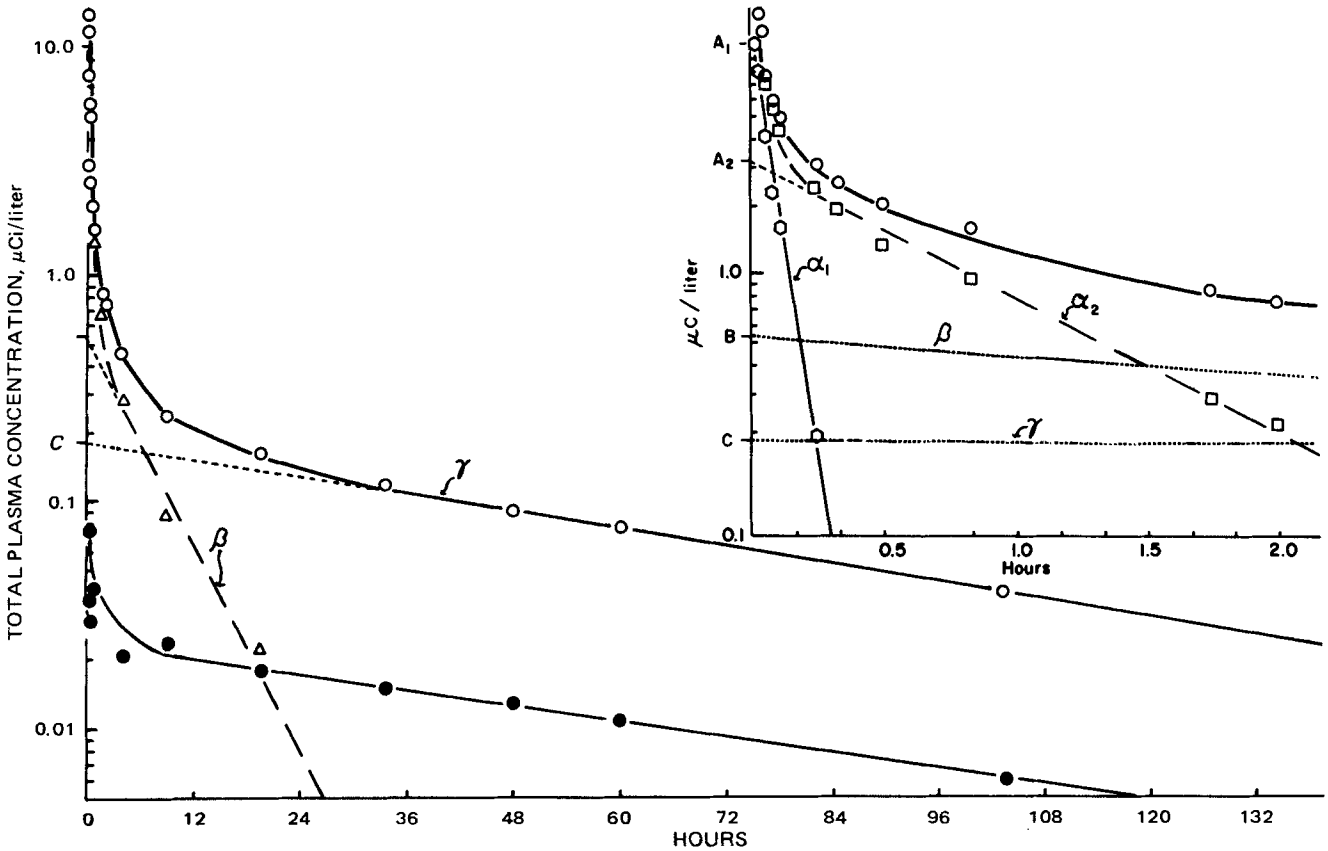


Figure 1—*Typical semilogarithmic plots of plasma β-methyldigoxin (○) and digoxin (●) concentrations, [A_p], against time for intravenous administration: 113.8 μCi = 264 μg of ^{3}H-β-methyldigoxin to Subject C. The inset is a plot of the initial β-methyldigoxin data on an expanded time axis. The data were treated by the method of residuals to obtain the parameters of* $[A_p] = A_1e^{-\alpha_1 t} + A_2e^{-\alpha_2 t} + Be^{-\beta t} + Ce^{-\gamma t}$, *where the data points* △ *represent* $[A_p] - Ce^{-\gamma t} = A_1e^{-\alpha_1 t} + A_2e^{-\alpha_2 t} + Be^{-\beta t}$, □ *represent* $[A_p] - Be^{-\beta t} - CE^{-\gamma t} = A_1e^{-\alpha_1 t} + A_2e^{-\alpha_2 t}$, *and* ○ *represent* $[A_p] - A_2e^{-\alpha_2 t} - Be^{-\beta t} - Ce^{-\gamma t} = A_1e^{-\alpha_1 t}$, *so that the straight lines that fit the terminal semilogarithmically plotted data have slopes* $\gamma/2.303$, $\beta/2.303$, $\alpha_2/2.303$, *and* $\alpha_1/2.303$ *and intercepts log C, log B, log* A_2, *and log* A_1, *respectively.*

Attempts to fit with a linear sum of only three exponentials consistently underestimated plasma data in the 3–30-hr time interval (Fig. 2).

The apparent volumes of distribution of the central compartment, $V_p{}^u$, referenced to the unbound drug in the plasma were estimated (16) from the individual $[A_p{}^u]_0$, 8.4 ± 1.9% of the dose per liter of plasma calculated at $t = 0$ from Eq. 3 and the intravenously administered dose, D = 100%:

$$V_p{}^u = D/[A_p{}^u]_0 \qquad \text{(Eq. 4)}$$

In 65% of the intravenous experiments, the plasma sample value taken at 90 sec after the start of the 1-min drug infusion was less than that taken at 120 sec. In some cases, the plasma maximum was not reached until 180 or 300 sec (Fig. 2). Thus, the injection and sampling sites could not be considered as being in the same kinetic compartment, and simultaneous penetration into and distribution from the central compartment must be postulated. The apparent volumes of distribution, $V_p{}^u$, as estimated by application of Eqs. 3 and 4, would be overestimated in these cases; large variability existed in the estimates of α_1 and A_1'.

The $V_p{}^u$ values were reasonably consistent within an individual for the two dose levels (Table I). If the α_1 phase, with its inherent variability in parameter estimations, were excluded, the apparent volume of distribution, $(V_p{}^u)'$, estimated on the premise that α_1 is infinite and A_1' is zero in Eq. 3 to produce an $[A_p{}^u]_0'$ value for Eq. 4, represented the volume of a central compartment larger than $V_p{}^u$. These volumes, $(V_p{}^u)'$ = 38.5 ± 3.7 liters, were remarkably consistent among individuals for all doses (Table I).

Plasma Pharmacokinetics of Digoxin after ^{3}H-β-Methyldigoxin Administration—Digoxin was the only metabolite of β-methyldigoxin found in significant concentrations in plasma, with a peak of 0.15 ± 0.03% of the dose per liter of plasma at 32.3 ± 7.0 min after intravenous administration of ^{3}H-β-methyldigoxin (Figs. 1–3). The plasma digoxin levels decreased subsequently by processes that could be described by the sum of two exponentials: a β-phase, $\beta = 0.68 \pm 0.21$ hr^{-1}, and a slower terminal γ-phase, $\gamma = 0.012 \pm 0.001$ hr^{-1}, that was reasonably linear in log plasma concentration–time and log digoxin to be excreted in urine–time plots (Figs. 1–3). Obviously, these values do not fully characterize the pure pharmacokinetic parameters since digoxin is being simultaneously formed from β-methyldigoxin.

When the concentration of total radioactivity in the plasma was plotted against time, a small anomalous increase in the plasma level with a subsequent decrease was consistently observed between 18 and 24 hr after both oral and intravenous administrations. This result could indicate enterohepatic circulation consistent with the observations of Doherty *et al.* (18) for digoxin. The time at which this increase occurred was consistent with such absorption being effected in the lower small intestine but was not of sufficient magnitude to perturb greatly the pharmacokinetic analyses.

Excretion Pharmacokinetics—The total radioactivity excreted in a time interval was the product of the concentration, microcuries per liter or gram, and the volume of urine or weight of feces collected. The amounts of ^{3}H-β-methyldigoxin and its individual metabolites excreted in the urine were calculated from the respective fractions of total radioactivity assignable to each on TLC analysis for the same three subjects for which β-methyldigoxin and digoxin had been analyzed in plasma. These amounts of materials in urine, U_t, were plotted cumulatively as the percent of dose against time (Fig. 4) to estimate the total recovery, U_∞, and were reasonably superimposable for all doses.

The semilogarithmic plots of the amounts of drug or metabolite not yet excreted in the urine, ln $(U_\infty - U_t)$, and the rate of excretion, ln $\Delta U/\Delta t$, against time (Figs. 3 and 5) exhibited the apparent terminal phases observed previously in the plasma (Figs. 1 and 2).

The percentages of the ^{3}H-β-methyldigoxin dose renally excreted as β-methyldigoxin, digoxin, and water-soluble metabolites are given in Table I. The percentages of the total administered radioactivity renally and totally (renal plus fecal) excreted also are given.

There were no apparent dose-dependent pharmacokinetics. When the plasma concentrations and cumulative urine content of β-methyldigoxin and digoxin were plotted against time in terms of the percent of the

Table I—Parameters for Intravenously Administered ^{3}H-β-Methyldigoxin Pharmacokinetics by Graphical Methods

Parameter	Subject A (66.3 kg, 169 cm, 1.76 m² Surface Area)		Subject B (71.3 kg, 179 cm, 1.89 m² Surface Area)		Subject C (67.4 kg, 166 cm, 1.75 m² Surface Area)		Average ± *SD*
D, dose, μg	294	589	273	633	264	631	
α_1, hr^{-1} [a]	13.9	9.2	16.7	8.3	15.1	33.3	16.1 ± 9.1
α_2, hr^{-1} [a]	2.8	1.7	2.4	1.7	1.5	2.7	2.1 ± 0.6
$10^2\beta$, hr^{-1} [a], intravenous, plasma (urine)	17 (23)	17 (28)	16 (23)	31 (28)	18 (28)	11 (35)	18 ± 7 (28 ± 4)
$10^3\gamma$, hr^{-1}, intravenous, plasma (urine)	16 (22)	16 (22)	18 (19)	20 (22)	15 (20)	19 (20)	17.3 ± 2.0 (20.8 ± 1.3)
A_1, % of dose/liter of plasma [a]	1.85	1.42	6.12	4.62	7.90	12.14	5.68 ± 4.02
A_2 [a]	1.78	2.02	1.76	1.35	2.53	3.44	2.15 ± 0.74
B [a]	0.57	0.50	0.33	0.53	0.43	0.45	0.47 ± 0.09
C [a]	0.12	0.12	0.09	0.12	0.15	0.14	0.12 ± 0.02
$V_p{}^u$, liters [b]	23.1	24.6	12.0	15.1	9.1	6.2	15.0 ± 7.5
$(V_p{}^u)'$, liters [c]	40.5	37.7	45.9	50.0	32.2	24.7	38.5 ± 9.2
10^2 area/*D*, hr/liter [d]	14.6	18.5	8.9	10.3	16.8	16.4	14.3 ± 3.8
Clearances, ml/min							
$(Cl_p{}^u)_{tot}$ [e]	114	90	187	162	99	102	126 ± 39
$(Cl_p{}^u)_r$ [f]	53	50	82	79	52	40	59 ± 17
$(Cl_p{}^u)_{mg}$ [g]	61	40	105	83	47	62	66 ± 24
$V^u_{pD_{ss}}$, liters [h]	427	338	621	486	396	321	432 ± 111
Percent of β-methyldigoxin dose, *D*, in urine from							
$10^2\ U^{BMD}_{\infty}/D$ [i]	43.8	44.4	55.1	46.4	51.0	40.4	46.9 ± 5.3
$10^2 U^{BMD}_{144\ hr}/D$ [j]	39.1	41.6	50.5	43.4	46.5	38.1	43.2 ± 4.7
$10^2\ U^{DIG}_{144\ hr}/D$ [k]	36.7	30.5	34.2	28.8	37.0	22.7	31.7 ± 5.5
$10^2\ U^{H_2O}_{144\cdot hr}/D$ [l]	5.9	5.9	3.2	5.0	3.4	8.3	5.3 ± 1.9
Recovery of administered radioactivity at 144 hr [m]							
Fecal, %	12.6	12.3	11.7	13.1	8.7	12.2	11.8 ± 1.6
Fecal + urinary, %	84.0	83.0	87.1	83.1	89.1	88.2	85.8 ± 2.7
144-hr recovery of β-methyldigoxin dose, %	94	90	100	90	96	81	91.8 ± 6.5

[a] Parameters of the sum of exponentials to express the unbound β-methyldigoxin concentration in percent of dose per liter of plasma: $10^2[A_p{}^u]/D = A_1e^{-\alpha_1 t} + A_2e^{-\alpha_2 t} + Be^{-\beta t} + Ce^{-\gamma t}$, where $[A_p{}^u]$ is the concentration in micrograms per liter at time *t*. Values were obtained by the method of residuals (35) from urine or plasma. [b] Estimated apparent volume of distribution of the central compartment referenced to unbound β-methyldigoxin in plasma from $D/[A_p{}^u]_0 = 10^2/(A_1 + A_2 + B + C)$ for $t = 0$ in equation of footnote *a*, where $[A_p{}^u]_0$ estimates the concentration of drug in plasma at zero time. [c] Estimated apparent volume of distribution of the equilibrated combined α-compartments from $D/[A_p{}^u]_0' = 10^2/(A_1 + B + C)$ from $t = 0$ in equation of footnote *a*, where $[A_p{}^u]_0'$ estimates the concentration of drug in plasma at zero time on assumption of instantaneous equilibration in the α_1- and α_2-compartments. [d] Determined by graphical integration of the area in micrograms-hour per liter under the unbound β-methyldigoxin plasma concentration–time curve; the area is divided by the administered dose in micrograms. [e] Total clearance from dose, *D*, divided by the area under the unbound plasma concentration–time plots. [f] Renal clearance from slope of plots of renal elimination rates of β-methyldigoxin, $\Delta U/\Delta t$, against plasma concentrations of unbound drug. [g] Metabolic clearance from the difference of total and renal clearances, $(Cl_p{}^u)_{tot} - (Cl_p{}^u)_r$. [h] Estimated equilibrated apparent volume of distribution during the psuedo-steady state or γ-phase, $(Cl_p{}^u)_{tot}/\gamma$. [i] Calculated from the micrograms of β-methyldigoxin renally excreted at infinite time, U^{BMD}_{∞}, which was estimated by adding to the amount excreted at 144 hr that amount that would have been excreted for five more half-lives if the slope of the natural logarithm of the excretory rate, ln $\Delta U/\Delta t$, against time was a constant, $-\gamma$. [j] Calculated from the micrograms of β-methyldigoxin renally excreted at 144 hr, $U^{BMD}_{144\ hr}$. [k] Calculated from the micrograms of β-methyldigoxin equivalent of the digoxin renally excreted at 144 hr, $U^{DIG}_{144\ hr}$. [l] Calculated from the micrograms of β-methyldigoxin equivalent of water-soluble metabolites renally excreted at 144 hr, $U^{H_2O}_{144\ hr}$. [m] Percent of total radioactivity administered excreted by 144 hr by specified route(s). [n] Percent of dose of β-methyldigoxin excreted by 144 hr renally as unchanged drug, digoxin, and water-soluble metabolites plus fecal excretion.

^{3}H-β-methyldigoxin dose per liter of plasma and the percent of dose, respectively, for the two dose levels of ^{3}H-β-methyldigoxin, the curves were superimposable (Fig. 4).

It can be estimated (Table I) that 92 ± 2.7% of the intravenous ^{3}H-β-methyldigoxin dose was eliminated by all processes at 144 hr. Of this percent, renal excretion accounted for fractions that were 0.470 unchanged, 0.345 digoxin, and 0.058 water-soluble metabolites. The fraction in the feces was 0.127. As will be justified later, the biliary excretion of unchanged β-methyldigoxin was negligible. The biliary excretion of digoxin has been estimated as 0.16 of its renal excretion (18). Thus, the fraction of digoxin formed from the dose of ^{3}H-β-methyldigoxin was estimated on the premise of negligible enterohepatic reabsorption as 0.16 (0.345) + 0.345 = 0.40. The fraction of the other metabolites of β-methyldigoxin would then be 0.127 [fecal] − 0.16 (0.345) [biliary digoxin] + 0.058 [urinary water solubles] = 0.130, of which 0.67 (0.058) = 0.039 would be renally excreted sulfates and glucuronide conjugates of β-methyldigoxin.

Clearances of β-Methyldigoxin—Total clearances of plasma-unbound β-methyldigoxin $(Cl_p{}^u)_{tot}$ of 126 ± 4 ml/min were estimated from the areas (Table I) under the plasma unbound concentration–time curves extrapolated to infinite time on the presumption of terminal first-order decay of plasma levels. Total clearance does not necessarily imply pharmacodynamic inactivation, since it includes the metabolic clearance of β-methyldigoxin where pharmacodynamically active digoxin is the major metabolite. There was no dose dependency. Thus:

$$(Cl_p{}^u)_{tot} = fD/\text{area} \qquad \text{(Eq. 5)}$$

where *D* = administered dose, and *f* = 1 for intravenously administered β-methyldigoxin.

The renal clearances of β-methyldigoxin, $(Cl_p{}^u)_r$, were determined from the slopes of plots (Fig. 6) of the rates of urinary excretion, $\Delta U/\Delta t$, against the plasma concentrations of unbound drug, $[A_p{}^u]$, taken at the midtime of the urine collection period. There was no apparent dose dependency (Table I). The average renal clearance was 59 ± 7 ml/min. The renal clearances appeared to be independent of urine flow between 0.9 and 5.5 ml/min and of urinary pH between 5.5 and 7.4. Literature values of renal clearances corrected for protein binding were 73 ml/min based

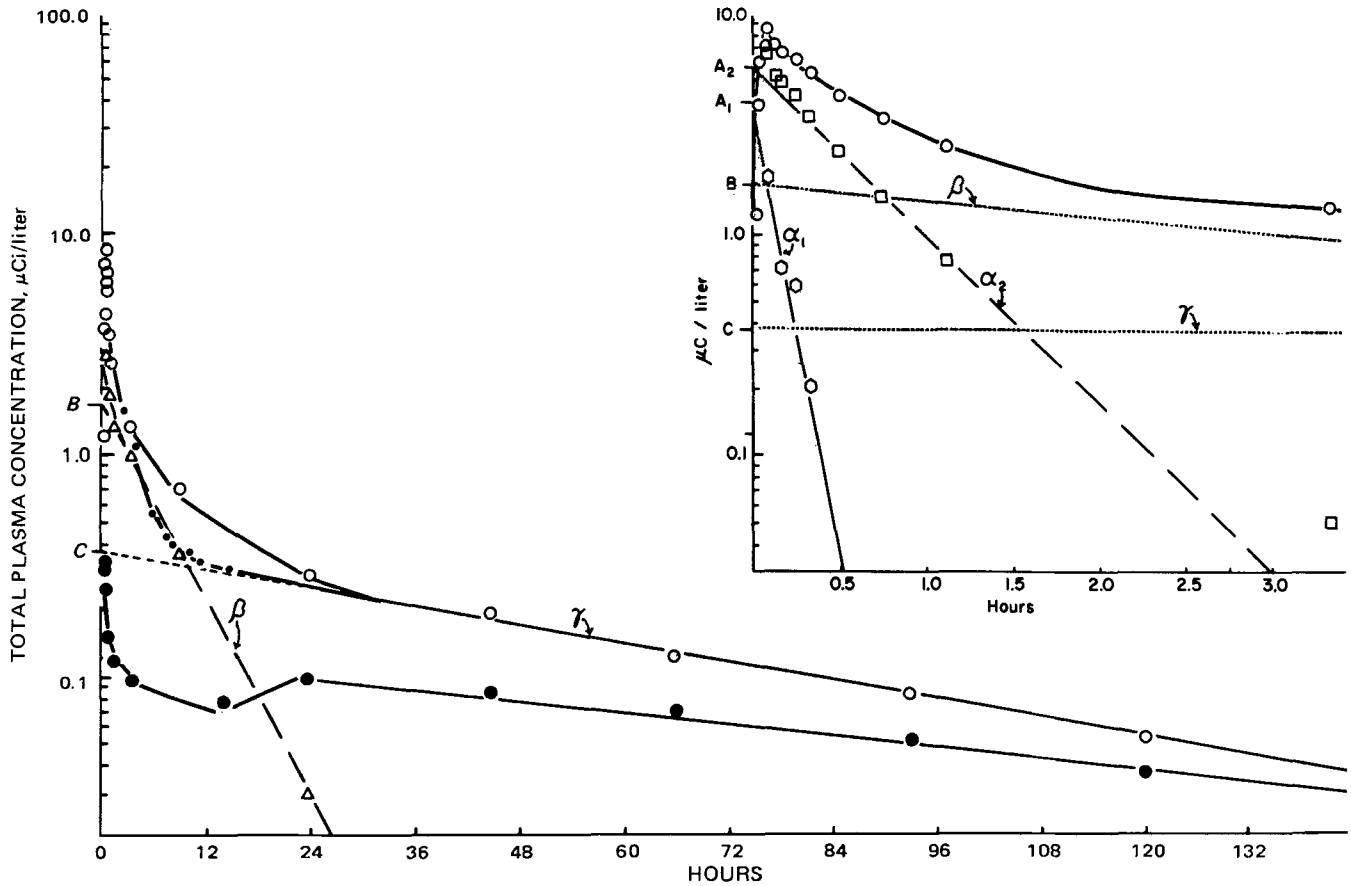


Figure 2—*Typical semilogarithmic plots of plasma β-methyldigoxin (○) and digoxin (●) concentrations, [A_p], against time for intravenous administration: 254.1 μCi = 589 μg of ^{3}H-β-methyldigoxin to Subject A. The various lines and symbols obtained by the method of residuals are as specified in Fig. 1. The dotted segment (·····) between 3 and 24 hr represents the best fit if [A_p] was the sum of only three exponentials. In this subject, there was a time delay in obtaining the maximum plasma concentration of β-methyldigoxin after intravenous administration.*

on total radioactivity (19) and 84 ml/min based on radioimmunoassay (20). These latter values overestimate the true renal clearance of β-methyldigoxin because they are modified by the simultaneous clearance of the major metabolite, digoxin, which is significantly higher than that of the parent drug.

The fraction of the total radioactivity administered intravenously that was fecally excreted in the first 48 hr was 0.083 ± 0.012 (n = 3) and agreed with the data of Abshagen *et al.* (21) for the fraction of total radioactivity excreted biliary. These investigators obtained the value of 0.105 ± 0.027 (n = 3) for the fraction of total radioactivity administered intravenously as tritiated β-methyldigoxin that appeared in the bile within 48 hr from patients with biliary tract disease in whom a biliary cannula was inserted. The average biliary clearance, $(Cl_p{}^u)_G$, of unbound β-methyldigoxin was estimated from the quotient of the average rates, dG/dt, of biliary excretion of β-methyldigoxin for the three patients given in the literature (21), dG/dt = 0.32, 0.032, and 0.0092% of the total dose of radioactivity excreted per hour during 0–4-, 4–24-, and 24–48-hr intervals, respectively, and the average plasma concentrations of unbound drug, $[A_p{}^u]$ = 2.21, 0.187, and 0.066% of the dose of unbound β-methyldigoxin per liter of plasma at the 2-, 14-, and 36-hr midpoints of these intervals in the three subjects of this present study who were given the drug intravenously. The respective individual quotients, $(Cl_p{}^u)_G = dG/dt/[A_p{}^u]$, were 0.145, 0.171, and 0.135 liter/hr and averaged 2.5 ± 0.2 ml/min (n = 3).

Since this biliary clearance would make a negligible contribution to the total clearance, the average metabolic clearance of unbound β-methyldigoxin, $(Cl_p{}^u)_m$ = 66 ± 10 (intravenous), was determined only from the differences between the total and renal clearances (Table I). If β-methyldigoxin metabolism occurs only in the liver and the average hepatic plasma flow is 800 ml/min (22), the metabolic efficiency is 66/800 = 0.083.

The endogenous creatinine clearances were normal (23) and were 129 ± 6 ml/min (n = 7), corrected for a body surface of 1.73 m^2 (n = 7) at urine flows of 1.2 ± 0.2 ml/min. The ratio of the renal clearance of β-methyldigoxin to creatinine was 0.42 ± 0.02 and implies an excess of tubular reabsorption for β-methyldigoxin.

Clearances of Digoxin after Intravenous ^{3}H-β-Methyldigoxin Administration—The average renal clearance, $(Cl_p{}^u)_r$ = 222 ± 32 ml/min (n = 5), of plasma-unbound digoxin was determined by the method described previously for β-methyldigoxin (Fig. 6) and can be compared with the values calculated for unbound digoxin of 163 (24), 133 (25), 120 (26), and 205 (27) in normal subjects. However, these estimates were based on total radioactivity or immunoassay rather than pure di-

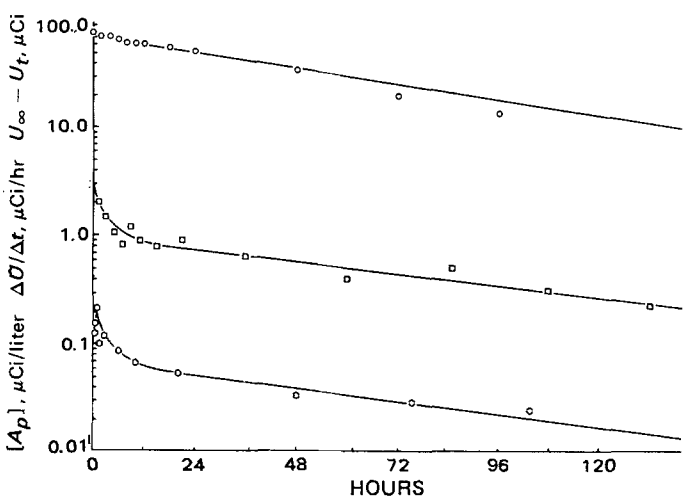


Figure 3—*Typical semilogarithmic plots of plasma concentrations of digoxin, [A_p] (○); amounts of digoxin yet to be excreted, $U_\infty - U_t$ (○); and rates of urinary excretion of digoxin, $\Delta U/\Delta t$ (□), against time for intravenous administration: 272.8 μCi = 633 μg of ^{3}H-β-methyldigoxin to Subject A.*

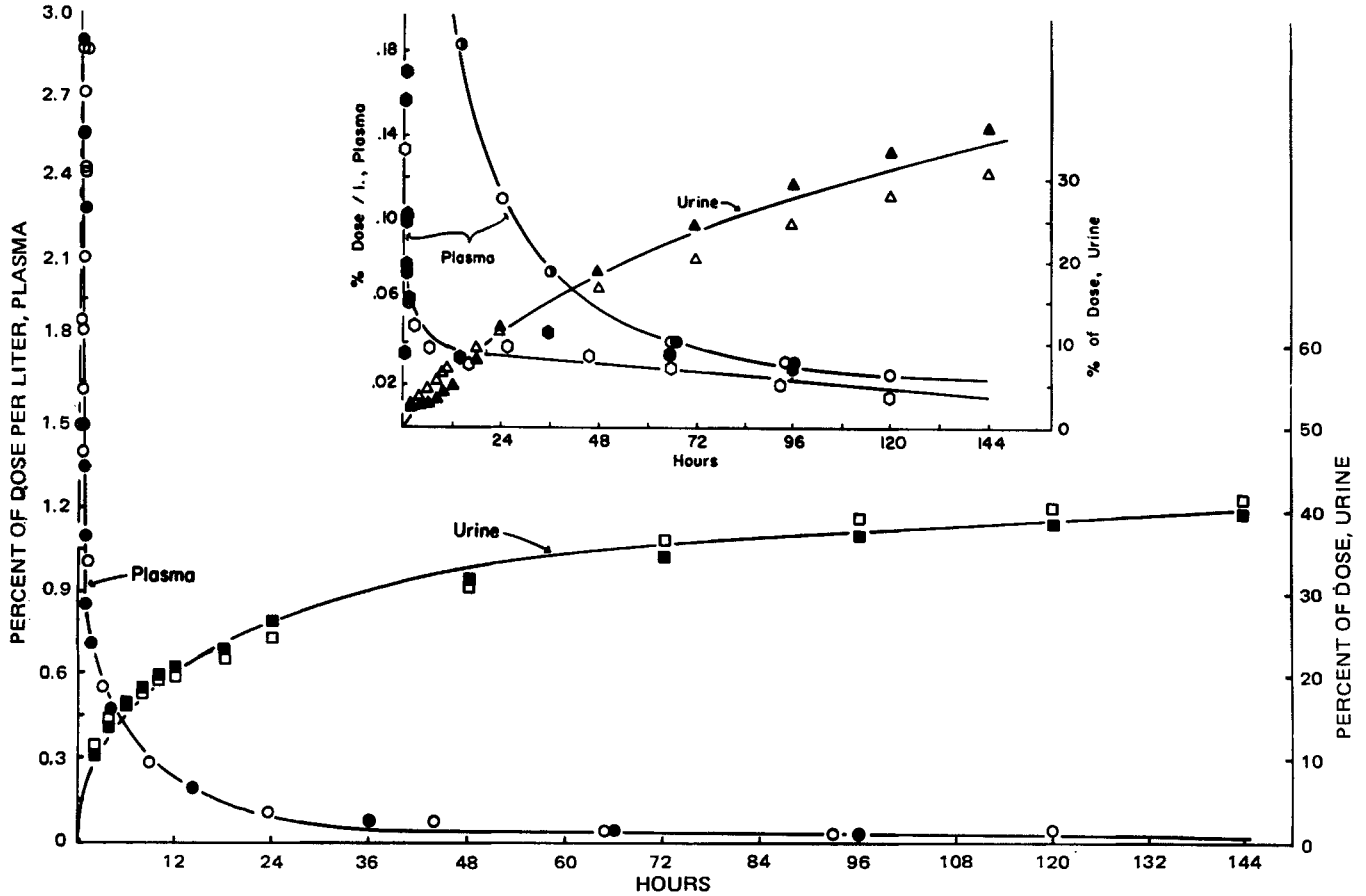


Figure 4—*Typical plasma concentrations and cumulative urine excretions in percent of β-methyldigoxin dose per liter of plasma and percent of dose, respectively, against time for intravenous administration: 126.7 μCi = 294 μg (open symbols) and 254.1 μCi = 590 μg (solid symbols) of ^{3}H-β-methyldigoxin to Subject A. Key: ○, ●, plasma levels of β-methyldigoxin; □, ■, urinary amounts of β-methyldigoxin; ○, ● (inset), plasma levels of digoxin; and △, ▲ (inset), urinary amounts of digoxin.*

goxin in plasma and underestimated the renal clearance when the plasma values were not corrected for metabolites, particularly if the metabolites had a smaller volume of distribution than digoxin. The urine flows in these studies were not specified. Evidence for urine flow dependency of the renal clearance of digoxin was reported (28). In these studies, the renal clearance of the metabolite appeared to be independent of urine flow between 0.9 and 5.5 ml/min and of urinary pH between 5.5 and 7.4.

An alternative rationale for the apparent discrepancy between the renal clearance values is a pharmacokinetic interaction between β-methyldigoxin and digoxin. β-Methyldigoxin could alter the renal clearance of digoxin by increasing its volume of distribution and/or its rate constant

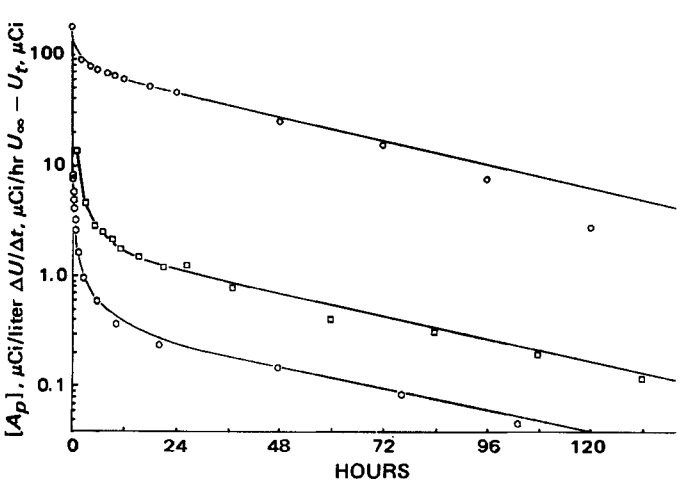


Figure 5—*Typical semilogarithmic plots of plasma concentrations of β-methyldigoxin, [A_p] (○); amounts of β-methyldigoxin yet to be excreted, $U_\infty - U_t$ (○); and rates of urinary excretion of unchanged drug, $\Delta U/\Delta t$ (□), against time after intravenous administration: 272.8 μCi = 633 μg of ^{3}H-β-methyldigoxin to Subject B.*

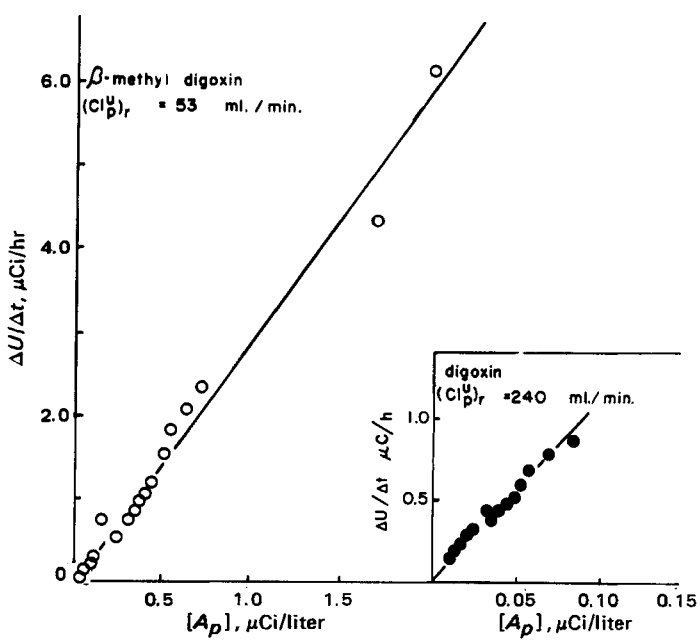


Figure 6—*Typical renal clearance plots for β-methyldigoxin and digoxin (inset) after ^{3}H-β-methyldigoxin intravenous administration of 126.7 μCi = 294 μg and 117.5 μCi = 272.6 μg to Subjects A and B, respectively. The renal clearances, (Cl$_p^u$)$_r$, of unbound drug were obtained by multiplying the slopes by the respective fractions of drug unbound to plasma protein.*

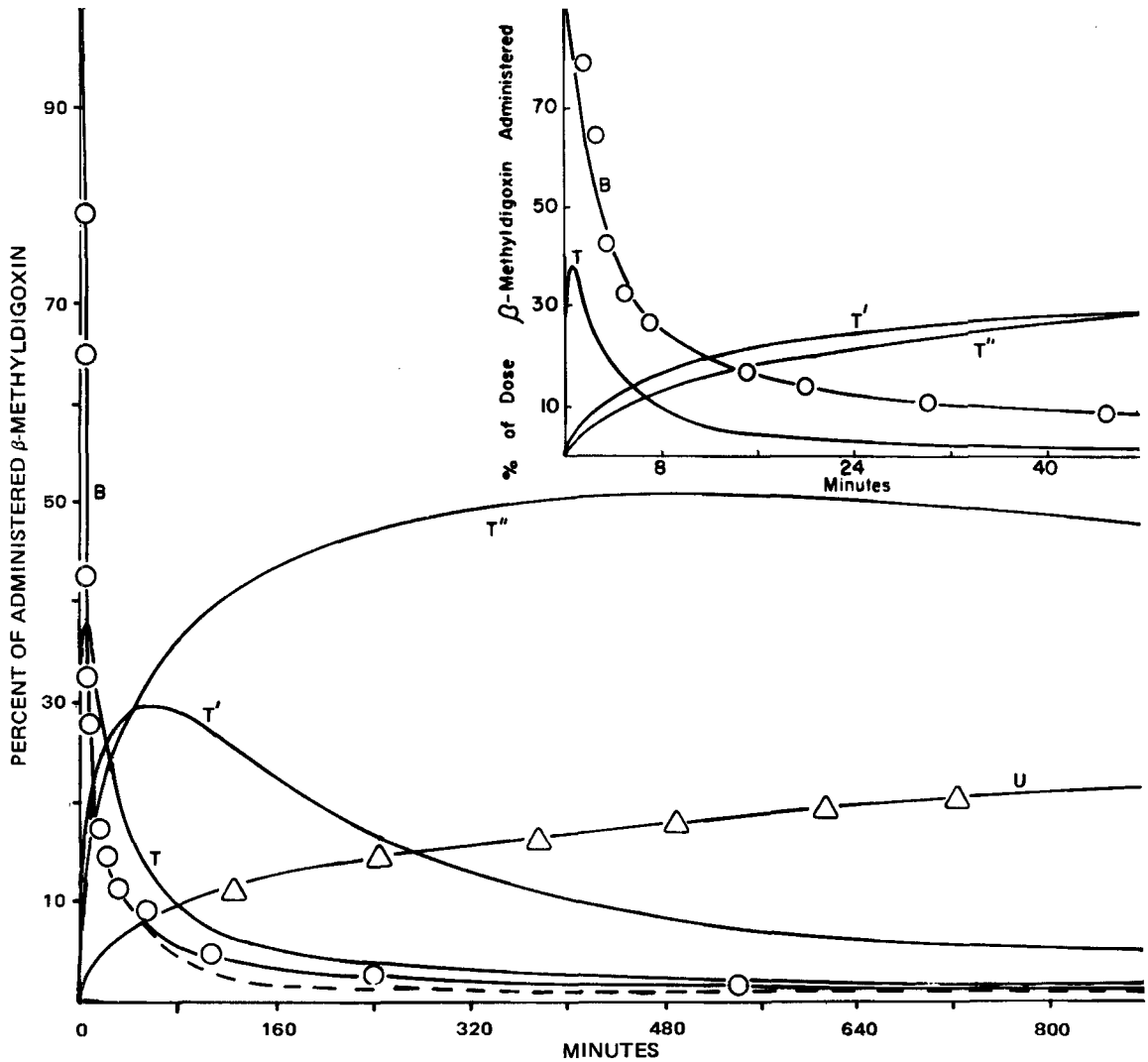


Figure 7—*Typical analog computer fitting of plasma water (unbound drug),* B (○), *and urine,* U (△), *data of β-methyldigoxin to the four-compartment body model for the 0.3-mg iv administration to Subject C over 13 hr with the parameters of Table II. The amounts of drug in various tissues* T, T′, *and* T″ *are generated in terms of percent of administered dose where the concentration of drug at zero time is taken as 100%. The inset is for an expanded time scale and shows the fit for the early plasma data. The dashed line represents the best attempt to fit the plasma data to a three-compartment body model.*

of elimination. The latter would imply saturable renal elimination of digoxin, but the renal clearances for digoxin were constant over wide ranges of plasma concentration ratios of β-methyldigoxin to digoxin (Fig. 6).

A more plausible rationale is to postulate renal metabolism of β-methyldigoxin to digoxin, which could account for the apparent discrepancy between the apparent renal clearances of digoxin observed when the drug *per se* or β-methyldigoxin was administered.

The biliary clearances of plasma-unbound digoxin were determined by the method described previously for β-methyldigoxin. The average rates, dG/dt, of biliary excretion of digoxin for the three patients given in the literature (21) were dG/dt = 0.188, 0.052, and 0.029% of the total dose of radioactivity excreted per hour during 0–4-, 4–24-, and 24–48-hr intervals, respectively. The average plasma concentrations of unbound digoxin were $[A_p{}^u]$ = 0.070, 0.026, and 0.016% of the dose of ^{3}H-β-methyldigoxin per liter of plasma at the 2-, 14-, and 36-hr midpoints of these intervals in the three subjects of this present study who were given ^{3}H-β-methyldigoxin intravenously. The respective individual quotients for the biliary clearance of digoxin, $(Cl_p{}^u)_G = dG/dt/[A_p{}^u]$, were 2.69, 2.00, and 1.83 liters/hr and averaged 36 ± 4 ml/min (n = 3). The ratio of these estimates of biliary and renal clearances of digoxin is 0.16, which agrees well with the ratio of drug biliary excreted to drug renally excreted of 0.16 obtained by Doherty *et al.* (18).

The total clearances of digoxin were also calculated from Eq. 5 from the areas up to the 144 hr studied, where the average area divided by the dose was 0.025 ± 0.005 hr/liter (n = 5) for the area under the plasma unbound concentration–time curves of digoxin for intravenously administered ^{3}H-β-methyldigoxin. However, the f in Eq. 5 is that fraction of the dose, D, of intravenously administered ^{3}H-β-methyldigoxin that is transformed to digoxin in 144 hr. It can be estimated from the ratio of the metabolic clearance, $(Cl_p{}^u)_m$, of β-methyldigoxin to its renal clearance, $(Cl_p{}^u)_r$, which is equal to the ratio of metabolites formed to renally excreted unchanged β-methyldigoxin. Thus, the average fraction transformed to metabolites is:

$$f = (U_{144}^{BMD}/D)(Cl_p{}^u)_m/(Cl_p{}^u)_r = 0.48 \pm 0.05 \qquad \text{(Eq. 6)}$$

where U_{144}^{BMD}/D is the fraction of ^{3}H-β-methyldigoxin dose renally excreted unchanged at 144 hr. To be completely rigorous, the fraction of β-methyldigoxin that is biliary excreted or changed to other metabolites than digoxin (such as conjugates of β-methyldigoxin) should be subtracted from f. It can be estimated from the experimentally determined composition of the water-soluble metabolite fraction of urine and the biliary excretion (29) that this amount comprises only 0.09 of the total ^{3}H-β-methyldigoxin dose; a better estimate of f is 0.39 ± 0.05, which agrees well with the previous estimates of 0.40 from the excretion pharmacokinetics.

The total clearance of digoxin was estimated as 290 ± 73 ml/min (n = 5). Subtraction of an estimated renal clearance of 222 ± 32 ml/min (n = 5) and a biliary clearance of 36 ml/min implies that the metabolic clearance of digoxin is 34 ± 16 ml/min. If digoxin metabolism occurs only in the liver, the metabolic efficiency is 34/800 = 0.043. Total and extrarenal unbound digoxin clearances of 277 and 72 ml/min, respectively,

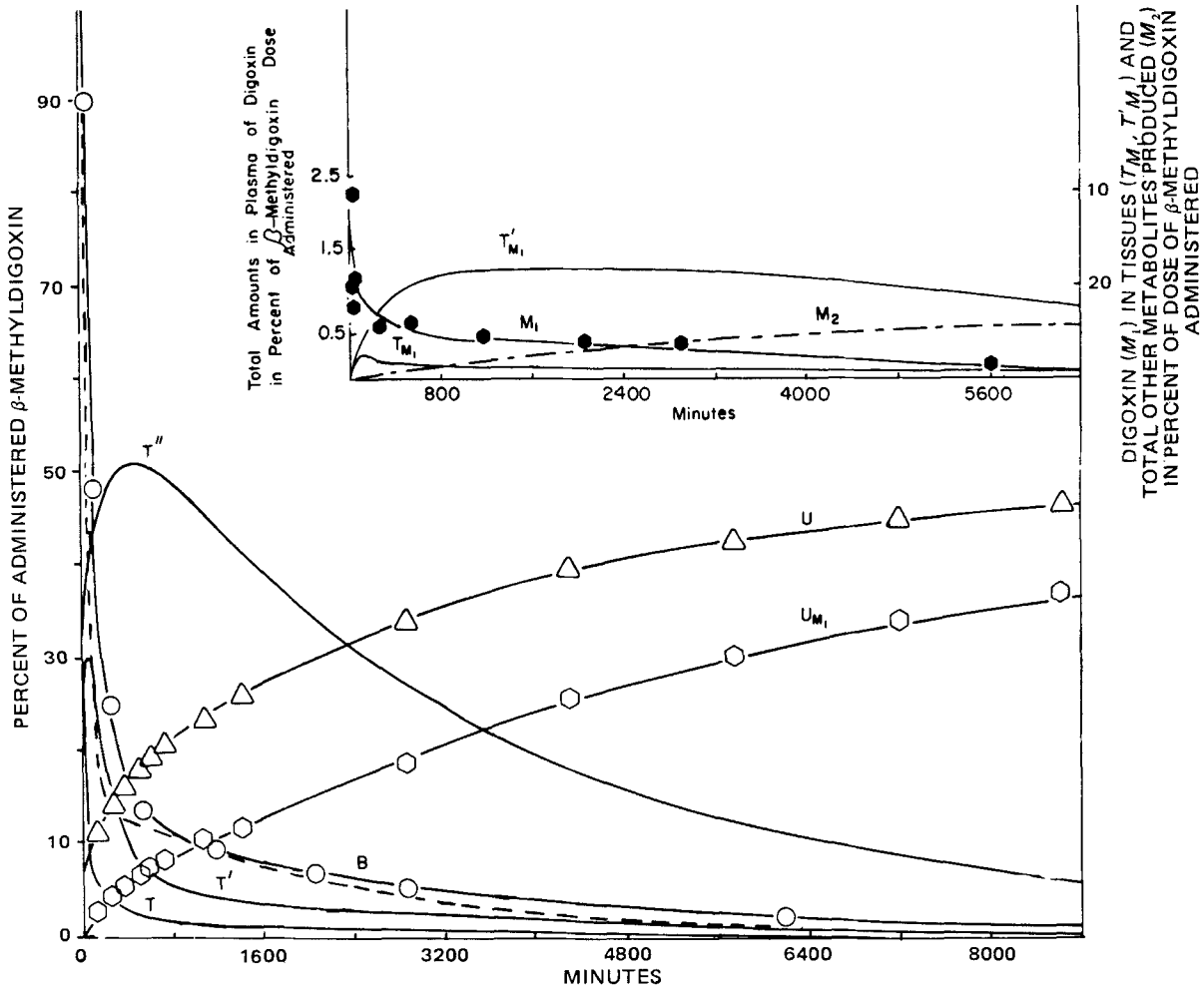


Figure 8—*Typical analog computer fitting of plasma water (unbound drug), B (○), and urine, U (△), data of β-methyldigoxin for the four-compartment body model for the 0.3-mg iv administration to Subject C over the complete time of the study. The digoxin (○) urine values, U_{M_1}, are fitted, and the amounts of drug in various tissues T, T′, and T″ are generated in terms of percent of administered dose where the concentration of drug at zero time is taken as 100%. The plasma β-methyldigoxin values (○) are shown multiplied by a factor of 10. The inset demonstrates the fitting to the total plasma data of formed digoxin, M_1 (●), in accordance with the necessary three-compartment body model (left scale); the amounts of digoxin in the various tissues are generated (T_{M_1} and T'_{M_1}) (right scale). The amounts of other metabolites, M_2, produced are also shown in the inset (right scale). The dashed line represents the best attempt to fit the plasma β-methyldigoxin data to a three-compartment body model.*

were reported for digoxin administration (27), in agreement with the values of this study.

The ratio of the renal clearance of digoxin to creatine was 1.52 ± 0.10 (n = 5) and implies an excess of tubular secretion in addition to glomerular filtration. Evidence for the tubular secretion of digoxin was reported previously (27, 30).

Pharmacokinetic Models for Intravenously Administered ^{3}H-β-Methyldigoxin and Digoxin and Their Fitting by Analog Computer—The model with the minimum number of compartments consistent with the experimental data was sought. The four-compartment body model postulated for plasma β-methyldigoxin levels with time by graphical analysis was challenged by both digital[23] and analog computation. A three-compartment body model (Scheme II) failed consistently to fit the terminal portion of the data (Figs. 1, 2, 4, 7, and 8) by both computer methods. Residual plots (31) showed definite trends about the mean with the three-compartment body model but random distribution with the four-compartment body model (Scheme III) to confirm the latter; typical best fits on this premise are given in Figs. 7 and 8.

Similar procedures were used in analog computer fitting of the digoxin metabolite, M_1, generated from β-methyldigoxin. A two-compartment body model (Scheme IV) failed consistently to fit the initial plasma data of the metabolite, M_1, and a three-compartment body model (Scheme V) was preferred (Fig. 7). This result agrees with the findings of Kramer *et al.* (32), who obtained better overall fits with a three- than with a two-compartment body model for digoxin on digoxin administration.

Typical computer programs and details were given previously (16). The initial conditions were placed on the central compartment, B, to obtain the initial drug concentration, $[A_p{}^u]_0$, from the best analog computer fit to Scheme III of the unbound plasma concentrations of β-methyldigoxin against time (Figs. 7 and 8). This voltage was considered as 100% of the drug in the central compartment that contained the plasma and associated fluids on the assumption of instantaneous mixing.

The cumulative urinary excreted amounts of the parent drug and metabolite, M_1, in microcuric-equivalent metabolite were plotted in terms of percent of dose administered. The simultaneous analog computer fitting of the plasma and urine data generated drug and metabolite amounts in the peripheral tissues and the total body, respectively, with time. Good and consistent fits were obtained with the four-compartment body model for the parent drug in plasma and urine and with the three-compartment body model for M_1 in urine. A typical fitting is shown in Figs. 7 and 8. The experimental plasma data of digoxin metabolite M_1 showed greater variability. Sets of microscopic rate constants that gave the best analog computer fittings to a four-compartment body model and the derived apparent volumes of distribution are given in Table II. The route of M_1 to bile M_1 in Scheme V was omitted in the analog computer fitting; therefore, $k_{M_1M_2}$ in the program and Table II was actually $k_{M_1M_2} + k_{M_1\,\text{bile}}$, which overestimates the former by 12% since the biliary clearance of digoxin is this percent of its total clearance.

Significance of Apparent Volumes of Distribution—The apparent

[23] Nonlinear regression program, Wang calculator 720, typewriter 702, Wang Laboratories, Tewksbury, MA 01876.

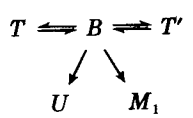
Table II—Parameters[a] for ^{3}H-β-Methyldigoxin and Digoxin Pharmacokinetics on Intravenous Administration Obtained by Analog Computer Fitting

Parameter	Subject A (66.3 kg, 169 cm, 1.79 m² Surface Area)		Subject B (71.3 kg, 179 cm, 1.89 m² Surface Area)		Subject C (67.4 kg, 166 cm, 1.75 m² Surface Area)		Averages ± *SD*
D, dose, μg	294	589	273	633	264	631	—
$10^4\ k_{BT}$, sec^{-1}	23.9	22.6	21.8	24.4	25.2	21.7	23.3 ± 1.4
$10^4\ k_{TB}$	15.4	26.1	12.2	11.9	16.0	11.3	15.5 ± 5.6
$10^4\ k_{BT'}$	7.85	6.05	5.27	5.91	7.17	6.91	6.53 ± 0.95
$10^4\ k_{T'B}$	1.78	3.15	0.766	1.18	1.64	1.67	1.70 ± 0.81
$10^4\ k_{BT''}$	1.49	2.10	3.14	3.43	5.45	3.84	3.24 ± 1.39
$10^4\ k_{T''B}$	0.109	0.146	0.185	0.125	0.156	0.115	0.139 ± 0.029
$10^4\ k_{BU}$	0.771	0.635	1.12	1.16	1.37	1.63	1.11 ± 0.37
$10^4\ k_{BM}$	1.08	0.776	0.906	1.30	1.38	2.38	1.30 ± 0.57
$10^4\ k_{MT}$	13.0	14.1	7.75	7.95	7.37	12.9	10.5 ± 3.1
$10^4\ k_{TM}$	13.0	7.81	3.40	4.23	2.62	2.41	5.58 ± 4.13
$10^4\ k_{MT'}$	11.4	11.0	5.58	6.12	5.15	11.10	8.38 ± 3.04
$10^4\ k_{T'M}$	0.130	0.109	0.198	0.135	0.151	0.0833	0.134 ± 0.0390
$10^4\ k_{M_1U}$	3.01	3.83	2.50	1.82	2.59	1.86	2.60 ± 0.76
$10^4\ (k_{M_1M_2} + k_{M_1\text{bile}})$[b]	0.615	1.54	0.260	0.703	0.474	1.15	0.79 ± 0.47
$(V_p{}^u)_{\text{BMD}}$, liters[c]	13.6	15.2	15.1	11.8	7.0	7.0	11.6 ± 3.8
$(V_{pT}{}^u)_{\text{BMD}}$[d]	21.0	13.2	27.0	24.1	12.0	13.5	16.9 ± 6.2
$(V^u_{pT'})_{\text{BMD}}$[d]	59.9	29.3	103.8	58.9	30.7	29.1	56.8 ± 26.2
$(V^u_{pT''})_{\text{BMD}}$[d]	186	219	256	322	245	234	244 ± 46
$(V_{pD}{}^u)_{\text{BMD}}$[e]	280	277	402	417	295	284	326 ± 65
$(V^u_{pD_{ps}})_{\text{BMD}}$[f]	514	458	757	676	566	573	570 ± 128
$(V_p)_{\text{DIG}}$[g]	9.38	10.3	22.1	74.6	32.8	14.9	27.3 ± 24.7
$(V_D)_{\text{DIG}}$[h]	838	1058	695	3596	1241	2079	1585 ± 1099

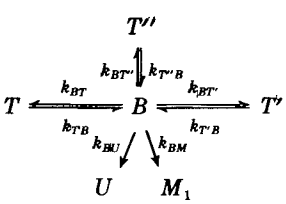
[a] Values for the microscopic rate constants in second^{-1} of distribution and elimination were obtained from the best simultaneous analog computer fits of the plasma and urine data in accordance with Schemes III and V. [b] The value is 112% of $k_{M_1M_2}$, since the biliary clearance of digoxin is 12% of its total clearances. [c] Values for the apparent volumes of distribution of the central compartment referenced to unbound β-methyldigoxin in plasma were calculated from the estimate of $[A_p{}^u]_0$ obtained from the initial conditions placed on B to give the best analog computer fit of the plasma data according to Scheme III, where $V_p{}^u = [A_p{}^u]_0/\text{dose}$. [d] The volumes of distribution for β-methyldigoxin at steady state for the different tissue compartments were calculated from $V_{pT}{}^u = V_p{}^u(k_{BT}/k_{TB})$, $V^u_{pT'} = V_p{}^u(k_{BT'}/k_{TB})$, and $V^u_{pT''} = V_p{}^u(k_{BT''}/k_{T''B})$. [e] The values of the overall steady-state volume of distribution for β-methyldigoxin were calculated (35) from $V_p{}^u[1 + (k_{BT}/k_{TB}) + (k_{BT'}/k_{T'B}) + (k_{BT''}/k_{T''B})]$. [f] The values of the overall pseudo-steady-state volume of distribution (36) for β-methyldigoxin were calculated from $(\text{Cl}_p{}^u)_{\text{tot}}/\gamma$, where $(\text{Cl}_p{}^u)_{\text{tot}} = (k_{BM} + k_{BU})V_p{}^u$ on the presumption that this value is $\gamma V^u_{pD_{ps}}$ in the terminal pseudo-steady state. The value of γ was estimated from the terminal slope of the semilog plot of the terminal analog computer fit of the plasma data. [g] Values for the apparent volume of distribution of the central compartment referenced to total (bound and unbound) digoxin in plasma were obtained from the best analog computer fit of the data for a three-compartment body model. [h] The values of the overall steady-state volumes of distribution of digoxin were calculated (35) from $(V_p)_{\text{DIG}}[1 + (k_{MT}/k_{TM}) + (k_{MT'}/k_{T'M})]$. The mean value is 1182 ± 542 if the anomalous 3596 of Subject B is excluded.

volume of distribution, $V_p{}^u$, of the central compartment referenced to unbound β-methyldigoxin in the plasma is 15 ± 3 liters by graphical methods (Table I) and 12 ± 2 liters by analog computer fitting (Table II). This amount is approximately the 15-liter extracellular body water of a young man (22).

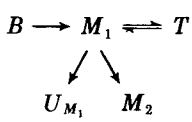
The overall volumes of distribution at steady state (Table II) of β-methyldigoxin, $(V_{pD}{}^u)_{\text{BMD}} = 326 \pm 27$ liters, and digoxin, $(V_D)_{\text{DIG}} = 1182 \pm 245$ liters ($n = 5$) if one anomalous value of 3596 liters is excluded, are larger than the total body water volume of 40 liters (22) and imply significant binding to and/or partitioning into tissues. A similar mean $(V_D)_{\text{DIG}}$ for digoxin of 800 liters can be calculated from the data obtained in healthy volunteers after digoxin administration by Kramer *et al.* (32). The overall volume of distribution at pseudo-steady state of β-methyldigoxin is significantly larger than the overall volume of distribution at steady state for β-methyldigoxin ($V^u_{D_{ps}} = 1.75 \times V^u_{D_{ss}}$). This result is a normal consequence of the kinetic properties of a slowly equilibrating deep compartment, T'', of large capacity (33) and implies that, after administration of a single dose of β-methyldigoxin, a relatively larger fraction of the dose in the body is in the tissue compartment than after chronic dosing with steady-state conditions.



Scheme II

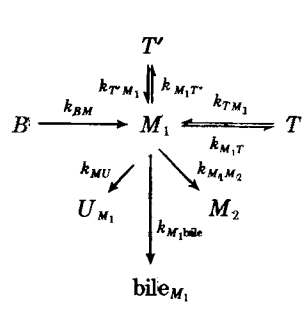


Scheme III



Scheme IV

Criteria for Determination of Terminal Half-Lives of β-Methyldigoxin Disposition—The magnitudes of the microscopic rate constants of equilibration for the shallow compartments T and T' (Scheme III), imply that these are relatively rapidly equilibrating with the central compartment and that the model ultimately degenerates to a two-compartment body model consisting of a new "central compartment," B +



Scheme V

Table III—Data for Estimates of "True" Renal Clearances of 0.6-mg Doses, D, of β-Methyldigoxin on Premise of Renal Metabolism

Subject	$Cl_{app\ BMD}$[a]	$(U_{BMD})144\ hr/D$[b]	$(U_{DIG})144\ hr/D$[b]	AUC_{DIG}/D[c]
A	50	0.416	0.305	1.808×10^{-3}
B	79	0.434	0.288	0.923×10^{-3}
C	40	0.381	0.227	1.178×10^{-3}
Mean ± *SD*	56 ± 20	0.410 ± 0.027	0.273 ± 0.041	$1.30 \pm 0.46 \times 10^{-3}$

[a] Obtained from slopes of plots of the rates of urinary excretion of β-methyldigoxin against unbound plasma concentrations at the midtime of the urine collection interval. [b] Fractions of total dose excreted into urine at 144 hr. The individual doses are given in Table I. [c] Areas under the plasma concentrations of plasma unbound digoxin concentrations with time are expressed as micrograms per milliliter per minute.

$T + T'$, and the peripheral compartment, T''. It is practical and useful to ascertain the time of pseudo-steady-state equilibration between this new central compartment and the tissue, T'', so that the terminal slopes, $-\gamma$, of the natural logarithm of the plasma level against time can be considered as constant and truly representative of the overall half-life of drug elimination from the body, a constant of importance in the prediction of blood level on chronic administration.

Representative analog computation (Subject B, 0.6 mg iv of ^{3}H-β-methyldigoxin) using the appropriate pharmacokinetic parameters (Table II) showed that changes in the ratios, r, of the amounts in the peripheral compartment to the amounts in this new central compartment:

$$r = T''/(B + T + T') = T''/C \qquad \text{(Eq. 7)}$$

were relatively small, <7.5%, 1600 min after drug administration and that the semilogarithmic plot of plasma level, $[A_p{}^u]$, against time of slope $-\gamma$ was reasonably linear after that time.

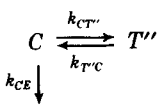
Thus, Eq. 7 for the expression of plasma level of β-methyldigoxin as a sum of exponentials can be reduced to two exponentials:

$$[A_p{}^u] = A'e^{-\alpha' t} + C'e^{-\gamma t} \qquad \text{(Eq. 8)}$$

where after 1600 min the term $A'e^{-\alpha' t}$ should become negligible. It was stated previously (34) that the final pseudo-steady state would be achieved for all practical purposes when, as applied to this case of β-methyldigoxin, the fractional contribution, $f_{\alpha'}$, of the α' term to the total plasma concentration of Eq. 8 is:

$$f_{\alpha'} = A'e^{-\alpha' t}/(A'e^{-\alpha' t} + C'e^{-\gamma t}) < 0.01 \qquad \text{(Eq. 9)}$$

The amounts of drug in the $C = B + T + T'$, T'', and $E = U + M$ compartment were generated on the analog computer for the four-compartment body model of Scheme III using the parameters listed in Table II for Subject B at the 633-μg dose (0.6 mg). The generated data were then fit by analog computation to the degenerated two-compartment body model (Scheme VI).



Scheme VI

The values of $k_{CT''} = 3.72 \times 10^{-5}\ sec^{-1}$, $k_{T''C} = 1.18 \times 10^{-5}\ sec^{-1}$, and $k_{C,E} = 2.85 \times 10^{-5}\ sec^{-1}$ fit the C, T'', and E compartments generated from the four-compartment body model of Scheme III excellently after 1600 min with the same terminal half-life of 42 hr. The ratios, r (Eq. 7), generated by the parameters of the two-compartment body model were definitely coincidental with those generated by the parameters of the four-compartment body model after 4000 min at 5.18; r was 5.17 compared to 5.10 at 2200 min and 5.10 compared to 4.90 at 1600 min for the two- and four-compartment body models, respectively. The $f_{\alpha'}$ was less than 0.01 (Eq. 9) subsequent to this 1600 min.

Valid estimates of the overall half-life of β-methyldigoxin must be based on satisfactory numbers of points adequately placed in time at least 1600 min or 27 hr after drug administration. This fact casts doubts on the validity of estimates of half-lives of all digoxin-type drugs based on plasma levels obtained within the 1st day after acute drug administration.

Effect of Renal Metabolism on Apparent Renal Clearances of a Drug and Its Metabolite—If a drug is metabolized by the liver *and* the kidney, the apparent renal clearances of both the drug and the metabolite are erroneous and the renal clearances of the metabolite derived from the administered drug and the metabolite administered *per se* differ significantly.

A model for both liver and kidney metabolism of β-methyldigoxin to digoxin from β-methyldigoxin content (B) in the central compartment is given in Scheme VII, where M_B is the amount of digoxin metabolite from hepatic metabolism, M_K is the amount of digoxin metabolite in the kidney that results from renal metabolism, and U_{DIG} and U'_{DIG} are the amounts of digoxin in the urine that result from the hepatic and renal processes, respectively. The area under a plasma level–time curve, AUC, can be related to the amount of drug provided to the body, D, and the total clearance, Cl_{tot}, of that drug provided that all elimination processes are first order (33). Thus, the AUC for digoxin on digoxin administration is:

$$AUC_{DIG} = D_{DIG}/(Cl_{tot})_{DIG} \qquad \text{(Eq. 10)}$$

where:

$$(Cl_{tot})_{DIG} = (Cl_{met})_{DIG} + (Cl_G)_{DIG} + (Cl_{ren})_{DIG} \qquad \text{(Eq. 11)}$$

and the amount of digoxin ultimately renally excreted, $(U_{DIG})_\infty$, from the amount in the body can be estimated from the fraction of the total clearance that is due to renal clearance:

$$(U_{DIG})_\infty = \frac{(Cl_{ren})_{DIG}}{(Cl_{tot})_{DIG}} D_{DIG} \qquad \text{(Eq. 12)}$$

Combination of Eqs. 10 and 12 gives:

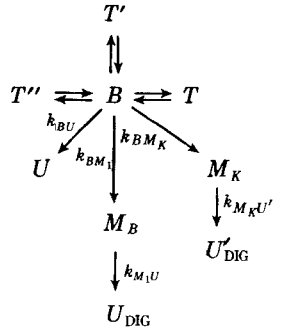
$$(U_{DIG})_\infty = (Cl_{ren})_{DIG}\, AUC_{DIG} \qquad \text{(Eq. 13)}$$

This equation also represents the amount of digoxin at infinite time appearing in the urine as a consequence of the renal clearance of digoxin produced by the hepatic metabolism of β-methyldigoxin.

Thus, the difference between the total digoxin excreted into the urine, $(U_{DIG})_{tot}$, and the value from Eq. 13 represents that amount of digoxin, $(U'_{DIG})_\infty$, that could be a product of renally metabolized β-methyldigoxin:

$$(U'_{DIG})_\infty = (U_{DIG})_{tot} - (U_{DIG})_\infty = (U_{DIG})_{tot} - (Cl_{ren})_{DIG} AUC_{DIG} \qquad \text{(Eq. 14)}$$

The ratio of the true renal clearance of β-methyldigoxin, $Cl_{true\ BMD}$, to its apparent renal clearance, $Cl_{app\ BMD}$, is equal to the ratio of the sum of the ultimate amount of β-methyldigoxin excreted in the urine, $(U_{BMD})_\infty$, and the ultimate amount of digoxin excreted in the urine that was formed from renal metabolism of the β-methyldigoxin filtered and secreted into the kidney, $(U'_{DIG})_\infty$, to the ultimate amount of β-methyl-



Scheme VII

digoxin excreted:

$$\frac{Cl_{true\ BMD}}{Cl_{app\ BMD}} = \frac{(U_{BMD})_\infty + (U_{DIG})_\infty}{(U_{BMD})_\infty} \quad \text{(Eq. 15)}$$

Thus, on consideration of Eqs. 14 and 15 with appropriate rearrangement:

$$Cl_{true\ BMD} = Cl_{app\ BMD}\left[\frac{(U_{BMD})_\infty + (U_{DIG})_{tot} - (Cl_{ren})_{DIG}AUC_{DIG}}{(U_{BMD})_\infty}\right] \quad \text{(Eq. 16)}$$

and if renal metabolism of β-methyldigoxin does exist, its true renal clearance can be calculated from the experimental values (Table III) of its apparent renal clearance, $Cl_{app\ BMD}$, appropriate values of the renal clearance of digoxin obtained on digoxin administration *per se*, $(Cl_{ren})_{DIG}$, and the area under the plasma digoxin level–time curve on intravenous β-methyldigoxin administration, AUC_{DIG}. The amounts of β-methyldigoxin and digoxin excreted into the urine at 144 hr on intravenous β-methyldigoxin administration were considered to be estimates of $(U_{BMD})_\infty$ and $(U_{DIG})_\infty$, respectively (Table III).

When the renal clearance of unbound digoxin, $(Cl_{ren})_{DIG}$, on digoxin administration was taken as 205 ml/min, close to the 222 value obtained in these studies and the average for all values on bolus and infusion digoxin administration given in the literature (27) after correction for protein binding, the calculated "true" renal clearance of β-methyldigoxin (Eq. 16) was equal to its apparent renal clearance, 56 ml/min. When $(Cl_{ren})_{DIG}$ was taken as 120 (26), 133 (25), and 163 (24) ml/min, the true renal clearances of β-methyldigoxin were 72, 70, and 64 ml/min, respectively, to suggest respective renal metabolisms of 16, 14, and 8 ml/min. Only if a true renal clearance for digoxin of 78 ml/min could be postulated would the true renal clearance of β-methyldigoxin be the same as this value.

REFERENCES

(1) R. G. Stoll, M. S. Christensen, E. Sakmar, and J. G. Wagner, *Res. Commun. Chem. Pathol. Pharmacol.*, **4**, 503 (1972).

(2) J. E. Doherty, W. M. Perkins, and G. K. Mitchell, *Arch. Intern. Med.*, **108**, 87 (1961).

(3) F. I. Marcus, G. Kapadia, and G. G. Kapadia, *J. Pharmacol. Exp. Ther.*, **145**, 203 (1964).

(4) H. J. Dengler, G. Bodem, and K. Wirth, *Arzneim.-Forsch.*, **23**, 64 (1973).

(5) A. von Wartburg, F. Kalberer, and J. Rutschmann, *Biochem. Pharmacol.*, **14**, 1883 (1965).

(6) F. Snyder, *Anal. Biochem.*, **9**, 183 (1964).

(7) L. A. Baillie, *Int. J. Appl. Radiat. Isotopes*, **8**, 1 (1960).

(8) L. R. Axelrod, C. Matthijssen, J. W. Goldzieher, and J. E. Pulliam, *Acta Endocrinol., Suppl.*, **49**, 99 (1969).

(9) H. Werbin, I. L. Chaikoff, and M. R. Imada, *Proc. Soc. Exp. Biol. Med.*, **102**, 8 (1959).

(10) A. Grollman, *J. Gen. Physiol.*, **9**, 813 (1926).

(11) A. Goldstein, *Pharmacol. Rev.*, **1**, 102 (1949).

(12) M. C. Meyer and D. E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).

(13) J. Savory, M. G. Heintges, and R. G. Sobel, *Clin. Chem.*, **17**, 301 (1971).

(14) H. J. Dengler, G. Bodem, and K. Wirth, *Cardiovasc. Res., VI, World Congr. Cardiol.*, **1970**, 119.

(15) P. Kramer, E. Köthe, J. Saul, and F. Scheler, *Eur. J. Clin. Invest.*, **4**, 53 (1974).

(16) E. R. Garrett and H. J. Lambert, *J. Pharm. Sci.*, **62**, 550 (1973).

(17) E. E. Ohnhaus, P. Spring, and L. Dettli, *Eur. J. Clin. Pharmacol.*, **5**, 34 (1972).

(18) J. E. Doherty, W. J. Flanigan, M. L. Murphy, R. T. Bulloch, G. L. Dalrymple, O. W. Beard, and W. M. Perkins, *Circulation*, **42**, 867 (1970).

(19) P. Kramer and F. Scheller, *Dtsch. Med. Wochenschr.*, **97**, 1485 (1972).

(20) R. Haasis, D. Larbig, and K. O. Kleck, *Klin. Wochenschr.*, **53**, 529 (1975).

(21) H. Abshagen, M. Rennekamp, R. Küchler, and N. Rietbrock, *Eur. J. Clin. Pharmacol.*, **7**, 177 (1974).

(22) A. C. Guyton, "Textbook of Medical Physiology," 5th ed., Saunders, Philadelphia, Pa., 1968, pp. 325, 422.

(23) G. J. Tobias, R. F. McLaughlin, Jr., and J. Hopper, Jr., *N. Engl. Med. J.*, **266**, 317 (1962).

(24) G. A. Ewy, G. G. Kapadia, L. Yao, M. Lullin, and F. I. Marcus, *Circulation*, **39**, 449 (1969).

(25) P. M. Bloom and W. B. Nelp, *Am. J. Med. Sci.*, **44**, 133 (1966).

(26) J. E. Doherty and W. M. Perkins, *Am. Heart J.*, **63**, 528 (1962).

(27) J. R. Koup, D. J. Greenblatt, W. J. Jusko, T. W. Smith, and J. Koch-Weser, *J. Pharmacokinet. Biopharm.*, **3**, 181 (1975).

(28) H. Halkin, L. B. Sheiner, C. C. Peck, and K. L. Melmon, *Clin. Pharmacol. Ther.*, **17**, 385 (1975).

(29) N. Rietbrock and U. Abshagen, *Dtsch. Med. Wochschr.*, **98**, 117 (1973).

(30) E. Steiness, *Circulation*, **50**, 103 (1974).

(31) C. Daniel and F. S. Wood, "Fitting Equations to Data," Wiley-Interscience, New York, N.Y., 1971, p. 27.

(32) W. G. Kramer, R. P. Lewis, T. C. Cobb, W. F. Forester, Jr., J. A. Visconti, L. A. Wanke, H. G. Boxenbaum, and R. M. Reuning, *J. Pharmacokinet. Biopharm.*, **2**, 299 (1974).

(33) E. R. Garrett, in "Klinische Pharmakologie und Pharmakotherapie," Kuemmerle H. P., E. R. Garrett and K. M. Spitzy, Eds., Urban & Schwarzenberg, München, West Germany, 1971, p. 38.

(34) P. H. Hinderling and E. R. Garrett, *J. Pharmacokinet. Biopharm.*, **4**, 199 (1976).

(35) D. S. Riggs, in "The Mathematical Approach to Physiological Problems," Williams & Wilkins, Baltimore, Md., 1963, pp. 146, 193.

(36) R. Nagashima, G. Levy, and R. A. O'Reilly, *J. Pharm. Sci.*, **57**, 1888 (1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 19, 1975, from *The Beehive, College of Pharmacy, University of Florida, Gainesville, FL 32610.*

Accepted for publication May 6, 1976.

Supported in part by Grant NIH-RR-82 from the National Institutes of Health, Bethesda, Md., and by an unrestricted grant from Searle Laboratories, Skokie, Ill.

The authors thank the nursing staff of the Clinical Research Center, Shands Hospital, University of Florida, for their assistance in performing the clinical studies.

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