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# INCREASED CLEARANCE OF DIGOXIN IN RABBITS DURING REPEATED ADMINISTRATION[1,2]

HERMANN R. OCHS, GUNTHER BODEM, GERD BALES,[3] DAVID J. GREENBLATT AND THOMAS W. SMITH

*Medizinische Universitätsklinik, Bonn, West Germany, Clinical Pharmacology Unit, Massachusetts General Hospital, Boston, and Department of Medicine, Harvard Medical School, Boston, Massachusetts*

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## ABSTRACT

OCHS, HERMANN R., GUNTHER BODEM, GERD BALES, DAVID J. GREENBLATT AND THOMAS W. SMITH: Increased clearance of digoxin in rabbits during repeated administration. J. Pharmacol. Exp. Ther. 204: 262-270, 1978.

The pharmacokinetics of digoxin were studied in three groups of rabbits. In the first two groups, radioimmunoassay was used as the analytic technique. After single i.v. doses of 0.8 mg (0.239 mg/kg) of digoxin, disappearance of digoxin from serum had three exponential phases. The mean (±S.E.) apparent elimination half-life for radioimmunoassayable digoxin was 14.8 ± 3.2 hours. With daily 0.8-mg injections of digoxin to eight female rabbits, mean (±S.E.) serum concentrations at 12 and 24 hours after the first dose were 0.62 ± 0.06 and 0.21 ± 0.03 ng/ml, respectively. After the seventh dose, these values fell to 0.23 ± 0.09 and 0.09 ± 0.04 ng/ml, respectively, significantly lower than the predicted concentrations ($P < .01$). The third group of animals received 0.2 mg/kg of digoxin daily for 13 days. The first and 13th doses included 216 $\mu$Ci of [$^3$H]digoxin, labeled in the $C_{12}$ position. Disappearance of total serum radioactivity had three exponential phases after both doses, and kinetic parameters as determined from $^3$H-counts were not significantly different. The apparent elimination half-life of serum radioactivity was 33.9 ± 1.8 hours. Recovery of radioactivity was 15% in urine and 75% in the feces over 96 hours after both doses. The amount of chloroform-extractable radioactivity in serum and urine tended to be higher after the first dose, but the differences generally did not reach significance. After incubation of urine with hydrochloric acid and with glucuronidase-sulfatase, chloroform-extractable radioactivity more than doubled after both doses. Thus, digoxin is extensively metabolized by the rabbit. Digoxin clearance measured by the more specific radioimmunoassay is greater than that determined from total serum radioactivity. During repeated administration, plateau levels of immunoassayable digoxin progressively fall, suggesting that digoxin stimulates its own clearance. This change in clearance is not reflected by the pharmacokinetics of total radioactivity.

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**Send reprint requests to:** Dr. David J. Greenblatt, Harvard Medical School, Massachusetts General Hospital, Boston, Mass. 02114.

[2] Presented in part at the 33rd Annual Meeting of the American Federation for Clinical Research, Atlantic City, N.J., May, 1976.

[3] Part of doctoral thesis of G.B., Universität Bonn, 1977.

Many aspects of the pharmacokinetics of digoxin in mammalian species remain poorly understood. We studied the disposition of intravenous digoxin in the rabbit after single and multiple doses, using both radioimmunoassay and tritiated [$^3$H]digoxin methods, with particular attention to the question of whether digoxin in submilligram doses is capable of inducing changes in its own metabolism.

## Methods

**Studies of nonradioactive digoxin.** *Single-dose study.* Five adult mixed-breed female rabbits (Fastnacht, Bonn, West Germany) (mean weight: 3.35 kg) received 0.8 mg of digoxin (Novodigal, Beiersdorf, Hamburg, West Germany) (0.239 mg/kg) i.v. as a single bolus dose. Venous blood samples (3–4 ml each) were drawn before drug administration and at the following times after the dose: 0.5, 1.0, 1.5, 2.0, 2.5, 4.0, 4.5, 5.0, 5.5, 6, 7, 8, 10, 12, 14, 21 and 24 hours. Serum was separated and frozen until the time of assay.

*Multiple-dose study.* Eight female rabbits (mean weight: 3.36 kg) and five male rabbits (mean weight: 3.52 kg) received single 0.8-mg i.v. doses every 24 hours for 7 consecutive days. Venous blood samples were drawn 12 and 24 hours after each dose. Serum was separated and frozen until the time of assay.

**Studies of [$^3$H]digoxin.** Six female and two male rabbits received 0.2 mg/kg of digoxin i.v. every 24 hours for 13 consecutive days. In the first and 13th doses, 0.1 mg of unlabeled drug was replaced with 0.1 mg of [$^3$H]digoxin (Sandoz, Basel, Switzerland) labeled in the $C_{12}$ position. The specific activity (before mixing with unlabeled digoxin) was 2160 $\mu$Ci/mg. After the first and 13th (radioactive) doses, venous blood samples were drawn at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4, 6, 8, 12, 14, 24, 30, 36, 48, 72 and 96 hours. Serum was separated and frozen until the time of assay. All urine and stool were collected during the 96 hours after each radioactive dose in samples fractionated as follows: 0 to 12, 12 to 24, 24 to 36, 36 to 48, 48 to 72, and 72 to 96 hours.

**Analysis of biological specimens.** Serum concentrations of nonradioactive digoxin were determined by radioimmunoassay as described previously (Smith *et al.*, 1969).

Total radioactivity in serum, urine and stool specimens was measured using a liquid scintillation counter (Isocap 300, Nuclear-Chicago, Des Plaines, Ill.); 0.1-ml aliquots of serum were added to 10 ml of Insta-Gel (Packard Instrument International, S.A., Zurich, Switzerland). Automatic external standardization was used to correct for quenching. One-half milliliter urine aliquots were added to 10 ml of Unisolve (Werner Zinsser, Frankfurt, West Germany) and counted as were the serum samples. Stool aliquots were combusted in a Packard sample oxidizer no. 306 (Packard Co., Frankfurt, West Germany) after which radioactivity was measured using Monophase (Packard) as a solvent.

*Fractionation of urine radioactivity.* After filtration (Selecta Faltenfilter no. 595½, Schleicher and Schüll, West Germany) a 10-ml aliquot of urine was adjusted to pH 7.4 with 1 N HCl. After addition of 20 ml of chloroform the mixture was stirred magnetically for 20 minutes. The extraction was repeated using another 20 ml of chloroform. After each extraction, radioactivity was measured in the aqueous phase after centrifugation at 4000 rpm for 5 minutes. Five thousand Fishman units (50 $\mu$l) of a commercial glucuronidase-sulfatase preparation (Glusulase, Endo Laboratories, Garden City, N.Y.) were then added to the aqueous phase and the urine was incubated for 24 hours at 37°C after adjustment to pH 4.2 with 1 N HCl. After incubation, double extraction with chloroform was repeated and radioactivity was determined as described above. The pH of the aqueous phase remaining was adjusted to 1.0 with hydrochloric acid and hydrolyzed at 100°C for 30 minutes. The hydrolysate was again extracted twice with chloroform, and radioactivity was determined.

*Fractionation of serum radioactivity.* Three grams of XAD-2 resin (particle size 50–100 $\mu$) were suspended in water to make a final volume of 5 ml, transferred to a column with a glass filter (size GII), and washed with 10 ml of distilled water. One milliliter of serum and 10 ml of 0.9% sodium chloride solution were added to the column. Five milliliters of 96% ethanol served as the eluate. Recovery of radioactivity was 95%.

The eluate was evaporated at room temperature and atmospheric pressure, and the residue was redissolved in 5 ml of 10% ethanol. Recovery was 100%. This solution was extracted twice with 10 ml of chloroform, and radioactivity was determined in the combined extracts.

**Analysis of data.** Data points for individual animals, as well as for "composite" points calculated as across-animal mean serum concentrations at corresponding times, were analyzed by computer using weighted iterative nonlinear least-squares regression analysis (Usanis, 1972; Marquardt, 1963). Data points were fitted to the following two functions:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

$$C = Ae^{-\pi t} + Pe^{-\alpha t} + Be^{-\beta t} \quad (2)$$

where $C$ is the serum concentration at time $t$ after the dose. The coefficients $A$, $P$, $B$, $\alpha$, $\pi$ and $\beta$ are "hybrid" quantities related to parameters of standard two- or three-compartment open pharmacokinetic models (Gibaldi and Perrier, 1975; Wagner, 1975; Greenblatt and Koch-Weser, 1975). The choice between equations 1 and 2 as functions of best fit was made by comparison of the sum of squares of weighted residual errors and by assessment of randomness of scatter of the actual data points about

the fitted functions. The following pharmacokinetic parameters were then calculated: distribution half-life ($t_{1/2\alpha}$), apparent elimination half-life ($t_{1/2\beta}$), volume of central compartment ($V_1$), total apparent volume of distribution using the "area" method ($V_d$) and total clearance. An intermediate "pi" half-life ($t_{1/2\pi}$) was calculated when triexponential solutions were used.

## Results

**Nonradioactive digoxin.** *Single doses.* Disappearance of digoxin after single i.v. doses was described by a triexponential function in four of five individual animals (table 1), and in the "composite" data (table 1; fig. 1). A biexponential solution was found more appropriate for animal 8 (table 1), possibly because several data points were not available. The across-animal mean parameter values were very close to those found from analysis of the composite data points.

Distribution of digoxin was extensive. The apparent volume of the central compartment and of the total distribution space both exceeded body weight. The mean (± standard error) apparent elimination half-life was 14.8 ± 3.1 hours and the mean total clearance was 142 ± 14 ml/min. As discussed below, renal clearance of the unchanged drug contributed very little to the total clearance.

*Multiple doses.* Table 2 shows the mean serum digoxin levels measured 12 and 24 hours after each dose in the eight female and five male rabbits. Predicted values for each group at each time point were calculated by application of multiple-dose kinetics to the mean concentrations observed after the first dose, assuming that terminal elimination or "beta" phase of the curve had been reached. Observed concentrations progressively fell during chronic doses, eventually reaching levels significantly lower than predicted (table 2). The serum level measured 24 hours after the second dose in male rabbits was significantly lower than predicted ($P < .05$). The difference was even greater after the seventh dose, when the observed serum concentration was less than 10% of the predicted ($P < .005$). The serum level 12 hours after the seventh dose reached only 28%

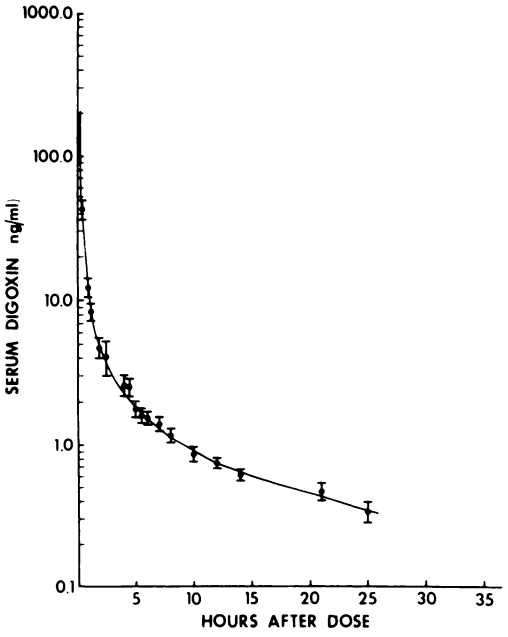


FIG. 1. Serum concentrations of immunoassayable digoxin after single i.v. injections of 0.8 mg to five female rabbits. Each point is the mean (± S.E.) serum concentration of all five animals at corresponding points in time. Solid line is the computer-generated function of best fit.

TABLE 1

*Pharmacokinetics of intravenous digoxin in the rabbit as measured by radioimmunoassay*

| Parameter | Animal No. | | | | | Mean ± S.E. | Composite[a] |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 5 | 8 | | |
| Body weight (kg) | 3.33 | 3.64 | 3.09 | 3.61 | 3.07 | 3.35 ± 0.12 | 3.35 |
| $t_{1/2\alpha}$ (hr) | 0.181 | 0.193 | 0.263 | 0.162 | 0.454 | 0.251 ± 0.05 | 0.248 |
| $t_{1/2\pi}$ (hr) | 2.24 | 1.55 | 1.51 | 1.72 | | 1.76 ± 0.17 | 1.69 |
| $t_{1/2\beta}$ (hr) | 10.33 | 18.49 | 14.30 | 24.41 | 6.29 | 14.76 ± 3.16 | 13.05 |
| $V_1$ (liters/kg) | 0.88 | 1.65 | 2.47 | 0.59 | 2.97 | 1.71 ± 0.45 | 1.68 |
| $V_d$ (area) (liters/kg) | 37.26 | 73.50 | 62.29 | 52.32 | 28.70 | 50.81 ± 8.15 | 50.34 |
| Total clearance (ml/min) | 138.5 | 166.9 | 155.3 | 89.3 | 161.8 | 142.4 ± 14.12 | 149.1 |

[a] Composite data points were calculated as across-animal mean values at corresponding times. Composite kinetic parameters were determined from analysis of the composite data points (fig. 1).

of the predicted value for the male rabbits and 33% of predicted for the females.

**[$^3$H]Digoxin.** Disappearance of serum radioactivity after both the first and 13th doses of digoxin was best described by a triexponential function (tables 3 and 4). The only exception was animal 6, after the first dose. Mean and composite pharmacokinetic parameters again were in close agreement. None of the kinetic parameters for the first and 13th doses were significantly different ($P > .05$) (tables 3 and 4). Figures 2 and 3 show actual composite data points and computer-generated functions of best fit.

Pharmacokinetic parameters based on total serum radioactivity differed considerably from

TABLE 2

*Serum digoxin concentrations measured at 12 and 24 hours after daily intravenous doses of digoxin to male and female rabbits*

| Dose No. | Male Rabbits ($n$ = 5) | | | | Female Rabbits ($n$ = 8) | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 hr after dose | | 24 hr after dose | | 12 hr after dose | | 24 hr after dose | |
| | Observed (mean ± S.E.) | Predicted | Observed (mean ± S.E.) | Predicted | Observed (mean ± S.E.) | Predicted | Observed (mean ± S.E.) | Predicted |
| 1 | 0.53 ± 0.08 | 0.53 | 0.22 ± 0.09 | 0.22 | 0.62 ± 0.06 | 0.62 | 0.21 ± 0.03 | 0.21 |
| 2 | 0.60 ± 0.08 | 0.62 | 0.11 ± 0.05[a] | 0.26 | 0.82 ± 0.17 | 0.69 | 0.26 ± 0.06 | 0.23 |
| 3 | 0.45 ± 0.09 | 0.64 | 0.05 ± 0.03[b] | 0.27 | 0.64 ± 0.05 | 0.70 | 0.16 ± 0.07 | 0.24 |
| 4 | 0.41 ± 0.09 | 0.64 | 0.11 ± 0.03[c] | 0.27 | 0.69 ± 0.12 | 0.70 | 0.19 ± 0.06 | 0.24 |
| 5 | 0.38 ± 0.11 | 0.64 | 0.09 ± 0.04[b] | 0.27 | 0.57 ± 0.16 | 0.70 | 0.17 ± 0.06 | 0.24 |
| 6 | 0.25 ± 0.06[b] | 0.64 | 0.11 ± 0.04[d] | 0.27 | 0.34 ± 0.07[b] | 0.70 | 0.16 ± 0.08 | 0.24 |
| 7 | 0.19 ± 0.07[b] | 0.64 | 0.02 ± 0.02[b] | 0.27 | 0.23 ± 0.09[b] | 0.70 | 0.09 ± 0.04[c] | 0.24 |

Result of one-tailed t-test of difference between observed and predicted values:
[a] $P < .05$.
[b] $P < .005$.
[c] $P < .01$.
[d] $P < .025$.

TABLE 3

*Pharmacokinetics of [$^3$H]digoxin in rabbits after single intravenous injection*

| Parameter | Animal No. | | | | | | | | Mean ± S.E. | Composite[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | |
| $t_{1/2\alpha}$ (hr) | 0.16 | 0.15 | 0.16 | 0.11 | 0.17 | | 0.18 | 0.28 | 0.18 ± 0.02 | 0.30 |
| $t_{1/2\pi}$ (hr) | 4.17 | 5.07 | 2.08 | 3.54 | 5.92 | 1.51 | 8.21 | 2.48 | 4.12 ± 0.79 | 4.01 |
| $t_{1/2\beta}$ (hr) | 29.05 | 28.88 | 41.04 | 39.03 | 31.08 | 34.94 | 41.91 | 30.54 | 33.92 ± 1.81 | 30.14 |
| $V_1$ (liters/kg) | 5.85 | 9.53 | 8.80 | 5.80 | 6.97 | 18.34 | 5.85 | 6.50 | 8.46 ± 1.50 | 10.29 |
| $V_d$ (liters/kg) | 54.79 | 65.37 | 107.62 | 74.92 | 65.34 | 59.25 | 59.62 | 68.22 | 69.39 ± 5.88 | 62.47 |
| $k_e$ ($hr^{-1}$) | 0.22 | 0.16 | 0.21 | 0.26 | 0.20 | 0.06 | 0.17 | 0.27 | 0.196 ± 0.023 | 0.140 |
| Clearance (ml/min) | 84.97 | 86.28 | 98.52 | 94.12 | 82.56 | 78.37 | 61.6 | 96.52 | 85.36 ± 4.22 | 85.50 |

[a] Composite data points were calculated as across-animal mean values at corresponding times. Composite kinetic parameters were determined from analysis of the composite data points (fig. 2).

TABLE 4

*Pharmacokinetics of [$^3$H]digoxin in rabbits after intravenous injection given after 13 days of chronic therapy*

| Parameter | Animal No. | | | | | | | | Mean ± S.E. | Composite[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | |
| $t_{1/2\alpha}$ (hr) | 0.16 | 0.06 | 0.16 | 0.18 | 0.21 | 1.22 | 0.11 | 0.26 | 0.29 ± 0.13 | 0.19 |
| $t_{1/2\pi}$ (hr) | 3.01 | 5.97 | 2.08 | 2.98 | 3.63 | 9.74 | 4.32 | 1.24 | 2.21 ± 0.67 | 3.69 |
| $t_{1/2\beta}$ (hr) | 33.58 | 42.87 | 41.01 | 36.10 | 32.70 | 78.77 | 216.61 | 36.87 | 43.12 ± 6.1[b] | 35.4 |
| $V_1$ (liters/kg) | 5.53 | 3.27 | 8.88 | 7.27 | 8.28 | 18.5 | 4.04 | 7.11 | 7.35 ± 1.78 | 8.01 |
| $V_d$ (liters/kg) | 69.10 | 78.73 | 108.56 | 87.82 | 80.18 | 101.25 | 42.37 | 80.13 | 86.54 ± 5.23[b] | 71.87 |
| $k_e$ ($hr^{-1}$) | 0.258 | 0.385 | 0.206 | 0.232 | 0.205 | 0.048 | 0.034 | 0.197 | 0.196 ± 0.040 | 0.176 |
| Clearance (ml/min) | 92.7 | 69.38 | 99.37 | 103.97 | 96.32 | 59.38 | 8.48 | 77.21 | 85.47 ± 6.39[b] | 83.80 |

[a] Composite data points were calculated as across-animal mean values at corresponding times. Composite kinetic parameters were determined from analysis of the composite data points (fig. 3).
[b] Animal 7 not used in calculation of means.

those based upon digoxin radioimmunoassay. This is not surprising, since blood was sampled for much longer after the dose in the [$^3$H]digoxin studies than in the radioimmunoassay study due to the sensitivity of the latter technique. The duration of sampling can greatly influence pharmacokinetic results (Gibaldi and Weintraub, 1971). Furthermore, the radioimmunoassay is a relatively specific analytic technique, whereas quantitation of total radioactivity is less specific.

After the first dose of [$^3$H]digoxin, mean 96-hour recovery of radioactivity was 15.3 ± 1.0% of the dose in urine and 75.5 ± 1.9% in stool (fig. 4). After the 13th digoxin dose (second radioactive dose), recovery was nearly identical, being 15.2 ± 0.9% of the dose in urine and 76.9 ± 2.4% in the stool (fig. 4).

**Fractionation of radioactivity.** *Urine.* After the first [$^3$H]digoxin dose, 96-hour cumulative chloroform-extractable urinary radioactivity averaged 3.3 ± 0.2% of the dose, or about 22% of total urinary radioactivity. After enzymatic cleavage with glucuronidase-sulfatase and strong acid hydrolysis, another 5.6 ± 0.8% of the dose (36% of urinary radioactivity) was extracted into chloroform (fig. 5). After the 13th digoxin dose, extraction of radioactivity into chloroform before enzymatic cleavage and hydrolysis (3.0 ± 0.2% of the dose) was slightly lower than after the first dose, but the difference was not significant. Extraction of radioactivity after these procedures was higher (5.9 ± 0.5% of the dose) after the 13th dose than after the first dose (5.6 ± 0.8%) (fig. 5). Again, the difference was not significant. However, the findings suggest a trend toward an increase in urinary excretion of conjugated digoxin metabolites, and a decreased excretion of unconjugated drug, associated with chronic treatment. It should be emphasized that only a small fraction of the total dose was excreted in the urine, and that less than 60% of urinary radioactivity was extractable into chloroform regardless of degradation procedures.

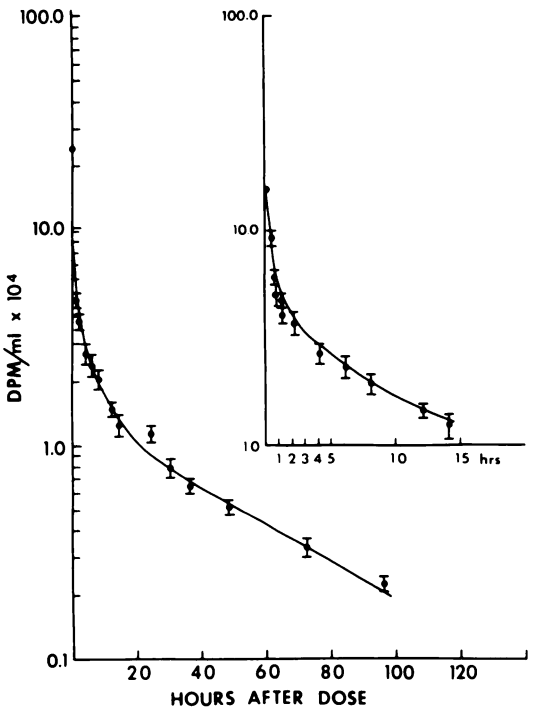


FIG. 2. Serum concentrations of total radioactivity after a single i.v. injection of [$^3$H]digoxin to eight rabbits. Each point represents the mean (± S.E.) concentration for all eight animals at corresponding points in time. Solid line is the computer-generated function of best fit.

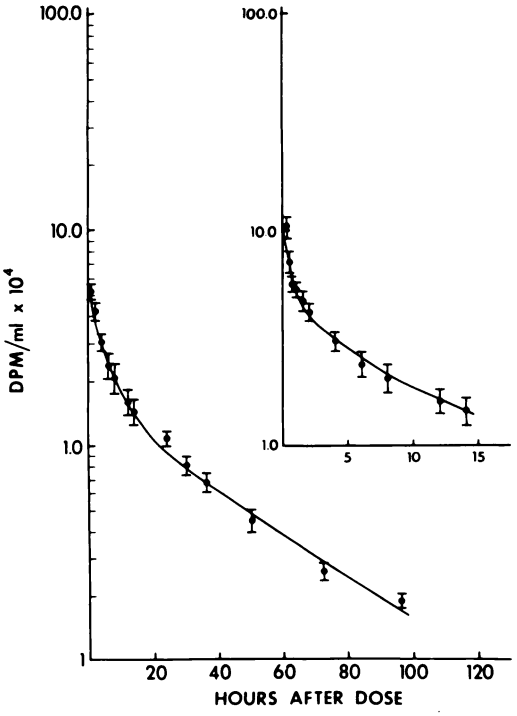


FIG. 3. Serum concentrations of total radioactivity after an i.v. dose of [$^3$H]digoxin to eight rabbits. [$^3$H]Digoxin was included in the last of 13 daily i.v. doses. Each point represents the mean (± S.E.) concentration for all eight animals at corresponding points in time. Solid line is the computer-generated function of best fit.

*Serum.* Concentrations of chloroform-extractable radioactivity in serum were lower after the 13th than after the first digoxin dose (fig. 6), particularly in the range of 1 to 8 hours after the injection. The differences reached significance at 4 and 8 hours.

## Discussion

Use of a pharmacokinetic model to describe pharmacological data necessarily oversimplifies the complex physiological processes involved in drug distribution and elimination. The present study suggests that single bolus doses of intravenous digoxin to rabbits, regardless of whether or not the drug is tagged with a radioactive label, confers upon the organism the characteristic of a three-compartment open pharmacokinetic model. This model is neither mathematically unique nor physiologically accurate, but is more consistent with the data than is the customary two-compartment model. Triexponential kinetics of intravenous digoxin have also been reported in human studies (Doherty and Perkins, 1962; Kramer *et al.*, 1974; Sumner *et al.*, 1976; Dengler *et al.*, 1973a,b).

Pharmacokinetic parameters of digoxin in the rabbit indicate that distribution of the drug to body tissues is very extensive. Apparent volumes of distribution are many times greater than body weight. Thus, tissue uptake of digoxin in this species appears to be more extensive than in humans, for which total apparent volumes of digoxin distribution are in the range of 5 to 8 liters/kg (Koup *et al.*, 1975; Kramer *et al.*, 1974; Nyberg *et al.*, 1974). The elimination half-life of immunoassayable digoxin in this study was approximately 14 hours, and that of total radioactivity approximately 35 hours. The difference is probably explained by the greater specificity of the radioimmunoassay, and by the fact that blood sampling continued for a longer period of time after the [$^3$H]digoxin doses than after administration of the unlabeled drug. In another study of [$^3$H]digoxin in rabbits, Schmidt *et al.*, (1974) observed an apparent elimination half-life of total radioactivity in the range of 3.4 days. However, their

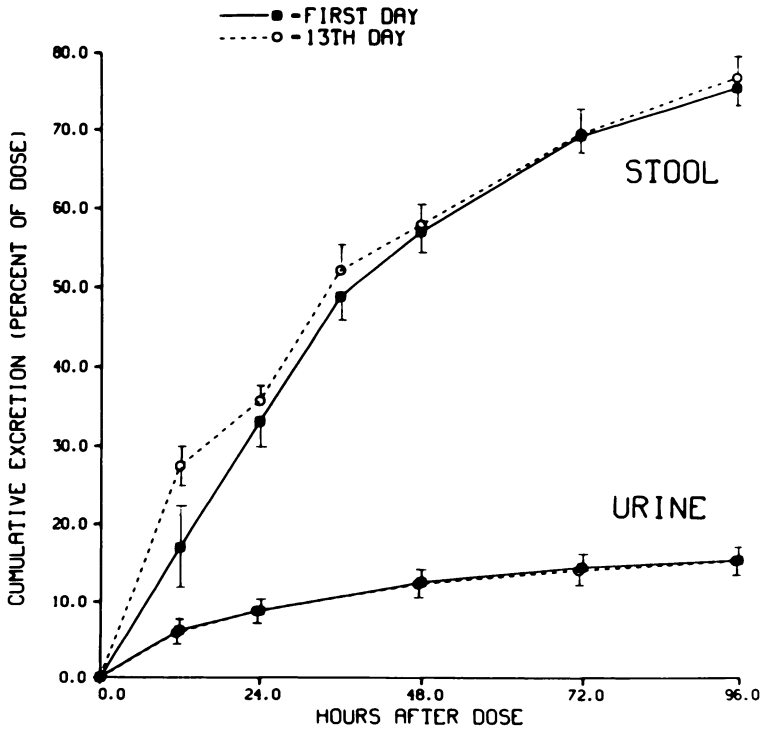


FIG. 4. Cumulative excretion of total radioactivity in stool and urine during 96 hours after an i.v. injection of [$^3$H]digoxin on the first and 13th days of daily i.v. injections. Each point is the mean (± S.E.) for all eight animals.

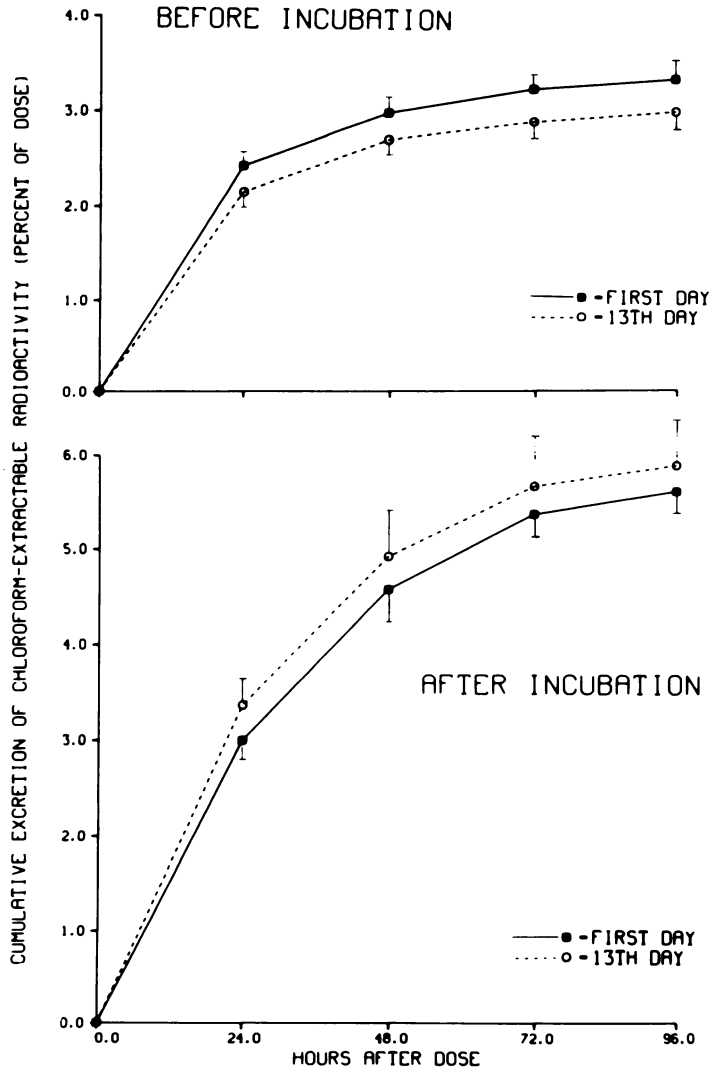


FIG. 5. Chloroform-extractable radioactivity in urine during 96 hours after i.v. injection of [$^3$H]digoxin on the first and 13th days of daily intravenous injections. Top: before incubation with glucuronidase-sulfatase and hydrochloric acid. Bottom: after incubation. Each point is the mean (± S.E.) for five animals.

radioactive compound was randomly rather than specifically labeled, and they sampled blood for 20 days after the dose.

Only 15% of administered radioactivity was recovered in the urine over 96 hours after [$^3$H]digoxin. Furthermore, only a relatively small proportion of this radioactivity was extractable into chloroform, the rest being conjugated and/or polar metabolites. These findings indicate that unlike the rat (Okita, 1967), guinea pig (Okita, 1967), dog (Harrison *et al.*, 1966) and human (Greenblatt *et al.*, 1973), in rabbits renal elimination of unchanged digoxin contributes relatively little to the total clearance of the drug. About 75% of radioactivity was recovered in the stool. Attempts to extract radioactivity in stool samples from three animals into chloroform, both before and after enzymatic cleavage and/or hydrolysis, yielded essentially no recovery of radioactivity. The findings strongly suggest extensive biotransformation and subsequent fecal excretion of metabolic products by the rabbit.

Chronic administration of drugs leads to ac-

cumulation in the body. Drug accumulation should be essentially complete, and a steady-state achieved, during chronic therapy for a period exceeding four to five times the apparent elimination half-life of the drug, assuming that the clearance of the drug is not influenced by chronic dosage. In the present study, accumulation of digoxin during 7 consecutive days of administration was not consistent with this prediction. Instead of reaching a steady-state plateau value, concentrations of immunoassayable digoxin progressively fell, and by the 7th day were substantially lower than the predicted levels. The findings suggest that chronic digoxin administration stimulated its own clearance. Whereas digoxin antibodies tend to be specific for digoxigenin and its mono- and bis-digitoxosides as well as digoxin (Smith *et al.*, 1970), but not for other metabolites, total radioactivity tends to reflect the sum of digoxin and all of its metabolites. It is therefore not surprising that the kinetics of total radioactivity in serum, urine and stool did not appear to be influenced by repeated dosing of digoxin.

Fractionation of serum and urinary radioactivity showed a trend suggestive of a shift from chloroform-extractable substances to water-soluble conjugated metabolites associated with chronic digoxin therapy in the rabbit. However, the differences were small and generally did not reach significance. Some digoxin metabolites, such as the reduction product dihydrodigoxin, are also extracted into chloroform but tend not to be detected by radioimmunoassay. In any case, it appears that the fractionation techniques used in the present study are not sufficiently sensitive to elucidate completely changes in digoxin disposition due to chronic digoxin administration in the rabbit.

Thus, our data indicate that repeated administration of digoxin to rabbits appears to stimulate digoxin biotransformation. Many experimental examples are available which demonstrate that coadministration of other drugs (such as barbiturates, spironolactone or steroids) can increase the metabolic clearance of digitalis glycosides (Abshagen, 1973; Castle and Lage, 1973; Nevasaari *et al.*, 1976; Soly-

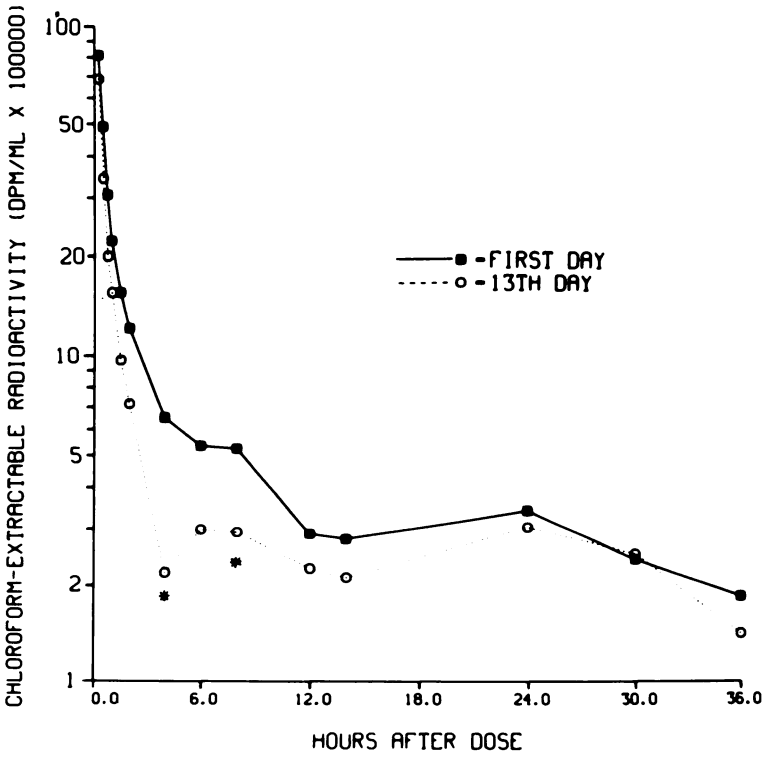


FIG. 6. Chloroform-extractable radioactivity in serum after a single i.v. dose of [$^3$H]digoxin on the first and 13th days of daily i.v. injections. Each point is the mean for five animals.* Statistically significant ($P < .025$) difference between mean values at that point in time.

moss *et al.*, 1972). Furthermore, it has been shown that chronic administration of any of a number of drugs can lead to increased clearance of that particular drug (Conney, 1967, 1969; Conney and Burns, 1972; Gelehrter, 1976; Remmer, 1964). The present study is the first demonstrating that digoxin, even in submilligram doses, can stimulate its own metabolism.

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