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Cardiac Glycosides

Part II: Pharmacokinetics and Clinical Pharmacology

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CHAPTER 11

The Positive Inotropic Action of Cardiac Glycosides on Cardiac Ventricular Muscle
M. REITER

The title of the above-mentioned chapter should read as follows:

The Positive Inotropic Action of Cardioactive Steroids on Cardiac Ventricular Muscle
M. REITER

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Part I: Experimental Pharmacology

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Pharmacokinetics – Distribution, Metabolism, and Elimination

CHAPTER 1

Pharmacokinetics of Digitoxin

LIV STORSTEIN

A. Introduction

Digitoxin is the main cardioactive glycoside in *Digitalis purpurea* and was purified by NATIVELLE (1864). Although it was the first cardiac glycoside introduced into clinical medicine, our knowledge of its pharmacokinetics and metabolism originates from research done during the last decades.

Experiments on isolated organs and intact animals have established important pharmacodynamic, pharmacokinetic, and metabolic principles. They have further demonstrated great species differences in the rate of elimination from the body, protein binding, metabolism, and drug tolerance. Basic experiments in animals have pointed to fields of research in humans. Information on the pharmacokinetics and metabolism of digitoxin in humans and in various animal species is far from complete and therefore does not allow a comparative survey of digitoxin pharmacokinetics. This chapter will therefore concentrate on the human pharmacology of digitoxin and include data from animal studies insofar as they have a direct bearing on the clinical pharmacology of digitoxin.

B. Drug Uptake and Tissue Distribution

I. Rate of Distribution and Distribution Half-Life

Digitoxin is distributed to tissues at about the same rate as digoxin but more slowly than ouabain. LÜLLMANN et al. (1969) determined the rate of distribution for the uptake process in isolated guinea-pig atria and found a half-life ($t_{1/2\alpha}$) of 21.5 min for digitoxin as compared with 16 min for digoxin and 6.5 min for ouabain. In adult humans (Table 1) we found a serum distribution half-life of 37.3 min, which is in good agreement with the findings of OKITA et al. (1955a). The onset of effect is related to serum distribution half-life and one can therefore expect a slowly increasing contractile response if the receptor sites are localized within the myocardial cells. A successive increase in contractility has been reported in dogs (STEINESS and VALENTIN, 1976) and was found by measuring left ventricular ejection parameters in humans (FORESTER et al., 1974).

II. Tissue Compartments and the Apparent Volume of Distribution

The uptake of digitoxin is high in isolated perfused hearts (DUTTA and MARKS, 1972). Free drug concentrations are most relevant for tissue uptake as shown by

LÜLLMANN et al. (1969) who found the same tissue: medium ratios whether atria were suspended in aqueous medium or in oxygenated whole blood when free drug concentrations were used for the calculations. A myocardial: serum ratio of 5.4 was found in humans (STORSTEIN, 1977 b) but the ratio was approximately 200 when free drug concentration was used for the calculation, pointing to the high. tissue affinity of digitoxin.

The apparent volume of distribution (V_D) calculated with total serum digitoxin concentrations was found to be 0.6 l/kg (VÖHRINGER and RIETBROCK, 1974; STORSTEIN, 1974 a), while a slightly lower value was reported by WIRTH et al. (1976). The apparent volume of distribution was 2.5 times higher in dogs than in humans (AMLIE et al., 1979) and species differences in digitoxin distribution are substantial.

III. Tissue Distribution

OKITA et al. (1955 a, b) measured the digitoxin content of human tissue after intravenous injections of radioactively labeled drug (Fig. 1). They found no preferential uptake of digitoxin in heart tissue. The colon and its contents had the highest digitoxin concentrations followed by gall bladder contents, kidney, and jejunum contents. Human fetuses had the highest concentrations in the heart and kidney followed by lung, liver, and intestine. LUKAS (1971) found tissue: serum ratios of 8.7, 6.7, 5.5, and 2.9 for kidney, ventricular myocardium, liver, and skeletal muscle respectively.

IV. Passage Across Biologic Membranes

Digitoxin is rapidly absorbed across the gastrointestinal tract with a peak value in serum after 1 h (STORSTEIN and JOHNSGARD, 1981; VÖHRINGER et al., 1977) and biologic availability is high. Drug uptake in some organs, like for instance the heart, is clearly dependent on free drug concentration while hepatic uptake was found to be independent of drug protein binding in conscious guinea-pigs by MARZO et al. (1977).

1. Blood–Brain Barrier

The blood–brain barrier acts as a regulatory interface between blood and the central nervous system and drug passage occurs across the brain capillaries. Permeability to drugs is regulated by transendothelial diffusion in proportion to their lipid solubility. The free unbound drug fraction is accessible for diffusion. After administration of identical doses of four glycosides to dogs (beagles) for 10 days, KUHLMANN et al. (1978) found higher concentrations of digoxin than of digitoxin in most organs while brain tissues had equivalent concentrations of the two drugs, indicating preferential uptake in the brain of digitoxin owing to its higher lipid solubility. Similar findings have been reported by BENTHE (1975) and FLASCH and HEINZ (1976) for various animal species. Cerebrospinal fluid (CSF) concentrations of digitoxin have recently been determined in humans (STORSTEIN et al., 1979). The mean CSF concentration was 0.84 ± 0.22 ng/ml giving a mean CSF: serum ratio of 0.07 ± 0.03. When free serum digitoxin levels were used for the calculations, how-

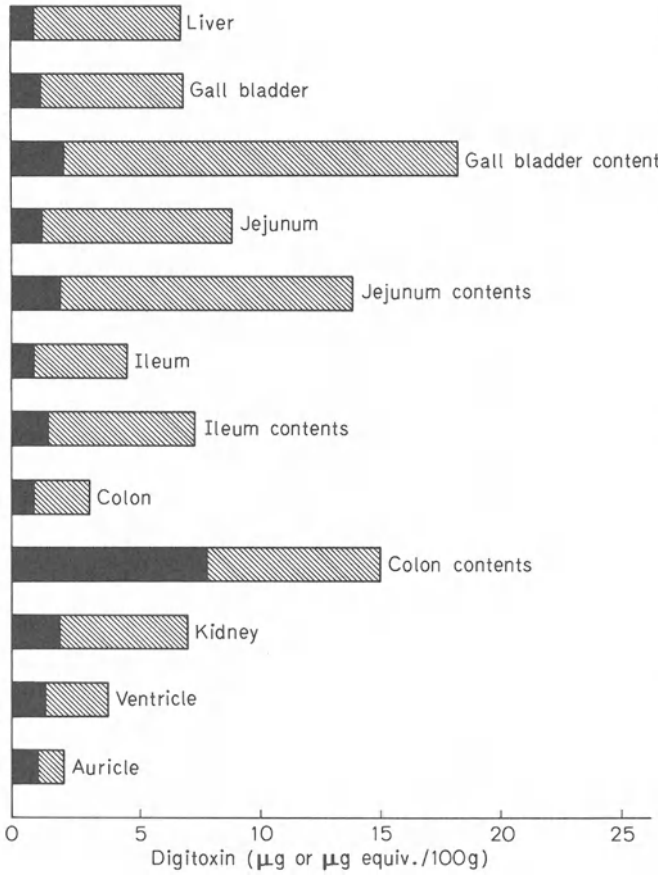


Fig. 1. Distribution of radioactive digitoxin *(solid bar)* and metabolites *(hatched bar)* in human tissue. (OKITA et al., 1955b)

ever, the mean ratio was 2.9. This ratio better reflects actual passage across the barrier and indicates that the penetration of digitoxin into the central nervous system is higher than for most other glycosides.

2. Placental Transfer

Drugs pass through the placenta from the maternal arteries by way of the intervillous space into the umbilical veins. Free, nonprotein-bound drug is available for passage and protein binding is therefore one important determinant for placental transfer. OKITA studied the placental transfer of digitoxin in guinea-pigs and rats and demonstrated placental passage of the drug. Guinea-pig fetal heart furthermore had six times the concentration of digitoxin in the maternal heart. These findings initiated a study in four pregnant women (OKITA et al., 1956) who were given radioactively labeled digitoxin before therapeutic abortion or delivery. Only a small percentage of the injected dose crossed the placental barrier but concen-

trations in fetal tissue were two to six times those in maternal tissue calculated on a weight basis. The fetal heart and kidneys had relatively higher concentrations of digitoxin and its metabolites than did other organs. Hepatic excretion of digitoxin and its metabolites could be demonstrated in the near-term fetus by their presence in the liver, gallbladder, and intestine. In two patients on maintenance treatment with digitoxin the drug concentration was higher in cord blood than in maternal blood at the time of delivery without the neonates exhibiting signs of digitoxin toxicity (STORSTEIN, unpublished). More information is obviously needed on the uptake, metabolism, and elimination of digitoxin in the human fetus.

C. Metabolism

The metabolism of digitoxin is complex, involving a number of enzymatic processes and has been the subject of numerous studies in the last decades. The following models have been used:

1) Studies with tissue preparations (HERRMANN and REPKE, 1963, 1964a, b, c, 1970; KOLENDA et al., 1971; LAUTERBACH and REPKE, 1969; REPKE and SAMUELS, 1964; WRIGHT, 1960; and others).
2) Animal studies (BROWN et al., 1955; CASTLE and LAGE, 1973, 1974; DOMSCHKE et al., 1969; FISCHER et al., 1952; FRIEDMANN et al., 1954; FÖRSTER and GADKE, 1957; GADKE and VAN ZWIETEN, 1969; GRIFFIN et al., 1971; INGWERSEN, 1974; KATZUNG and MEYERS, 1965, 1966; KUHLMANN et al., 1973; REPKE, 1958, 1959, and others).
3) Single dose studies after administration of radioactively labeled digitoxin to humans (OKITA, 1957; OKITA et al., 1953, 1955a, b, 1956; VÖHRINGER and RIETBROCK, 1974; WIRTH et al., 1976) and after administration of unlabeled drug (STORSTEIN and AMLIE, 1977a, b).
4) Studies in humans on maintenance treatment (STORSTEIN, 1977a, c; BODEM and UNRUH, 1978).

I. Basic Studies with Tissue Preparations and in Animals

The first two approaches led to the isolation of digitoxin metabolites by FISCHER et al. (1952), ASHLEY et al. (1958), and WRIGHT (1960). Hydroxylation transforms digitoxin to digoxin (REPKE, 1959). Metabolites with less sugar molecules are successively formed by hydrolysis. All these metabolites are lipid-soluble and cardioactive. Water-soluble metabolites are synthetized by conjugation to glucuronic and sulfuric acids. The genins of digitoxin and digoxin are epimerized via keto derivatives (REPKE and SAMUELS, 1964).

II. Single-Dose Studies in Humans

Marked species differences exist for digitoxin metabolism and elimination necessitating investigations in normal and diseased humans. OKITA et al. (1955a, b) studied digitoxin phamacokinetics, tissue distribution, and the relationship between unchanged digitoxin and metabolites in body fluids and tissues after a small single

dose of radioactively labeled drug. They found that the ratio of metabolite to unchanged drug was high in all tissue studies and furthermore the concentration in tissue was high when compared with blood. During a period of 3 weeks approximately 70% of the radioactivity was excreted through the kidneys, mostly as metabolized digitoxin and only 6%–10% as unchanged drug.

VÖHRINGER and RIETBROCK (1974) studied digitoxin metabolism in plasma, urine, and feces in six healthy volunteers after a single dose of radioactively labeled drug. In plasma the ratio between chloroform-soluble and water-soluble metabolites decreased with the time after injection but the amount of lipid-soluble substances was at all times at least three times higher than the amount of water-soluble substances. Unchanged digitoxin formed the major part of the lipid-soluble substances. Unchanged digitoxin was also the main lipid-soluble substance present in urine and feces although small amounts of other cardioactive metabolites could be spotted. The nature of the hydrophilic compounds in urine and feces was investigated. In urine 80% of the total hydrophilic radioactivity was nonconjugated polar metabolites of unknown identity while 16% was glucuronides and 4% sulfates. In feces, 79% of the chloroform-insoluble fraction appeared to be nonconjugated compounds while 13% were sulfates and 8% were glucuronides. After splitting of conjugation bonds, several metabolites were detected and digitoxigenin-mono-digitoxoside was identified as the main conjugation partner in urine. During the 8-day observation period 21.3% of the administered dose appeared in urine and 13% in feces.

WIRTH et al. (1976) studied urinary excretion and serum levels of digitoxin 20 days after a single dose of labeled drug. In contrast to OKITA et al. they found that unchanged digitoxin constituted 79% of total urinary radioactivity; 5% was unidentified lipid-soluble metabolites, small amounts were found of known cardioactive metabolites and 12% was water-soluble compounds. During 20 days 45% of the injected dose was excreted in the urine with 35% as unchanged digitoxin.

LUKAS and PETERSON (1966) using a double-isotope dilution derivative assay were unable to find any digitoxigenin after acid hydrolysis of urine from patients on maintenance treatment with digitoxin. STORSTEIN and AMLIE (1977a) studied the pattern of cardioactive and conjugated metabolites in urine of two subjects, 1, 2, 4, 6, and 8 days after a single dose utilizing thin layer chromatography, enzymatic splitting of conjugation bonds, and the ^{86}Rb method. Conjugation to glucuronic and sulfuric acids was found to be a rapid enzymatic process with maxima of 55% and 35% conjugated substances in blood within the first 2 h following injection. Unchanged digitoxin was the main substance present in serum (56.3% and 52.2%) and urine (48.3% and 45.6%) 24 h after a single dose in each of the two subjects. The relative amount of unchanged digitoxin decreased with time. Metabolites resulting from one enzymatic process like hydroxylation, hydrolysis, or conjugation had the following maxima: (1) hydroxylation on the sixth day in subject 1 and fourth day in subject 2; (2) hydrolysis on the second day in both subjects; (3) conjugation on the first day in subject 1 and the second day in subject 2. Conjugation was thus the most rapid process followed by hydrolysis and hydroxylation. Metabolites resulting from two enzymatic processes had maxima after 4–6 days while metabolites resulting from three enzymatic processes had maxima after 8 days in both subjects. Although only two subjects were studied, the metabolic

pattern showed marked similarities with positive t values for all days when the Kendall rank correlation coefficient was used to compute the number of agreements and disagreements between two rankings. It could thus be demonstrated that drug metabolism is progressive after a single dose, leading to less unchanged digitoxin and more metabolites resulting from several enzymatic processes with time. After 8 days only 28% and 29.1% was unchanged digitoxin, in agreement with the findings of OKITA et al. (1953, 1955 a, b) who found only 6%–10% unchanged digitoxin in urine over a period of 3 weeks. The results further emphasize that the time of sampling is of prime importance for the extent of drug metabolism.

In a similar study (STORSTEIN and AMLIE, 1977 b) the metabolic pattern in bile and urine was studied in two patients with T tube drainage after cholecystectomy. The parent compound was the main single excretory product in bile after digitoxin administration. All cardioactive metabolites were present and all were conjugated. Digitoxin metabolites predominated over digoxin metabolites. Hydrolyzed and conjugated metabolites were excreted in greater amounts than hydroxylated metabolites. The ratio between metabolite and digitoxin varied between 2 and 5 during the observation period while OKITA et al. (1955 a, b) found ratios of 5.8–8.9 in three patients studied on autopsy.

Animal experiments have shown that a number of metabolites are present in bile or feces after the administration of digitoxin (CASTLE and LAGE, 1974; RIETBROCK and VÖHRINGER, 1974), that the biliary excretion of digitoxin can be influenced by bile salts (GREENBERGER and THOMAS, 1973), and that drugs can alter both the biliary excretion and metabolic pattern of digitoxin (KLAASSEN, 1974; VÖHRINGER et al., 1975). After interruption of the enterohepatic circulation in the two biliary fistula patients all metabolites were still present in urine. The metabolic course, however, no longer led to less unchanged digitoxin and more metabolites with time as in the control patients. Water-solubility is supposed to facilitate biliary excretion of drugs. Contrary to what could be expected, bile contained mostly lipophilic metabolites.

III. Digitoxin Metabolism in Humans on Maintenance Treatment

Preliminary data on digitoxin metabolism in patients on maintenance treatment has been reported (STORSTEIN, 1973). A comparison between digitoxin metabolism on a maintenance regimen and after a single dose (STORSTEIN, 1977 a) showed that the mode of administration was of major importance for the pattern of digitoxin metabolites. Digitoxin is primarily used for maintenance therapy and the metabolic pattern on this regimen is therefore of prime clinical importance. Unchanged digitoxin is the predominant cardioactive substance in both serum and urine, and more important than any metabolic subgroups. Conjugated metabolites account for approximately 30% in serum and urine. Contrary to the previous concept of digitoxin metabolism, all cardioactive metabolites were found to be present in the conjugated forms. Animal experiments have demonstrated the presence of digitoxose-containing derivatives of digitoxigenin as the conjugates excreted in urine and bile (CASTLE and LAGE, 1974; KATZUNG and MAYERS, 1966; KUHLMANN et al., 1974). Recently BODEM and UNRUH (1978) have shown the presence of dihydrodigitoxin in patients on maintenance therapy. It is feasible that other digitoxin deriv-

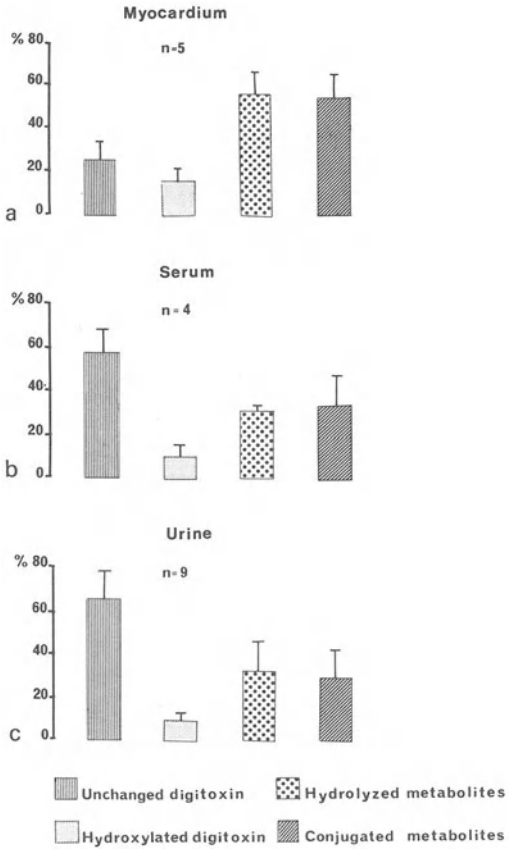


Fig. 2 a–c. Unchanged drug, all hydroxylated, all hydrolyzed, and all conjugated metabolites in **a** myocardium, **b** serum, and **c** urine from control patients. (STORSTEIN, 1977 b)

atives may also be present in the reduced form, as previously shown for digoxin by CLARK and KALMAN (1974). The concept of digitoxin metabolism has been changed to incorporate new knowledge. Information on epi and keto derivatives and metabolites with opened ring structures is still lacking in humans. Although unidentified metabolites were found in the studies by VÖHRINGER and RIETBROCK (1974) and WIRTH et al. (1976), the major pathways in human digitoxin metabolism are accounted for by hydrolyzed, conjugated, and hyrdroxylated metabolites both on maintenance treatment and after single dose.

Digitoxin metabolites in myocardial tissue from patients on maintenance treatment up to the time of death were determined by STORSTEIN (1977 b). The metabolic pattern in the myocardium (Fig. 2), differed significantly from that of serum and urine. Unchanged digitoxin was still the main cardioactive substance present but more hydrolyzed and conjugated metabolites were found in myocardial tissue compared with serum. Similar findings have been reported by CASTLE and LAGE (1973) who found that blood contained more unchanged digitoxin (51%) than heart tissue (9.6%) while the metabolic pattern was similar in heart, lung, liver, and kidney tis-

sue of rats. The liver is supposedly the main site of digitoxin metabolism (HERRMANN and REPKE, 1970). OKITA et al. (1955b) showed that the liver had a higher content of digitoxin metabolites than did other tissues but little is known of possible extrahepatic sites of digitoxin metabolism.

D. Enterohepatic Circulation

Enterohepatic circulation of digitoxin in humans was first proposed by OKITA et al. (1955b), and by OKITA (1967). On the basis of studies showing an increased ratio of water-soluble to lipid-soluble metabolites in the distant part of the gut compared with the duodenum, they concluded that lipid-soluble metabolites were partly reabsorbed. Enterohepatic circulation of digitoxin has been shown in several animals, the rat (LAUTERBACH, 1964), cat (IZUMI et al., 1968), and dog (KATZUNG and MEYERS, 1966). OLIVER et al. (1978) showed that the dog has the ability to absorb water-soluble digitoxin metabolites from the duodenum. CALDWELL et al. (1971) found a decrease in serum half-life of both chloroform-soluble and chloroform-insoluble metabolites after administration of the steroid-binding resin cholestyramine to humans. On the other hand VAN BEVER et al. (1976) failed to demonstrate any change in serum elimination half-life after administration of cholestipol to a group of patients with high initial digitoxin concentrations. Biliary excretion of digitoxin and metabolites in man was demonstrated by BEERMAN et al. (1971). The role of the enterohepatic circulation for the long serum half-life of digitoxin was investigated in a group of patients with T tube drainage after cholecystectomy (STORSTEIN, 1975). Interruption of biliary excretion into the gut by suction drainage led to marked reduction in serum elimination half-life when compared with a control group (Table 1). The renal clearance and excretion of digitoxin and cardioactive metabolites were the same in the biliary fistula and control groups. The marked increase in total digitoxin clearance was thus produced by an increased metabolic clearance of the drug, indicating enhanced loss through the feces when the enterohepatic circulation was interrupted. Peak bile concentrations were seen 15–60 min after injection and were higher than mean serum and urine concentrations through the greater part of the study.

E. Elimination and Excretion Pathways

I. Serum Elimination Half-Life

Serum elimination half-lives will partly depend on the method used for analysis of serum digitoxin concentrations. Four different methods have been used for the calculation of $t_{1/2\beta}$ (Table 2). After injection of a dose of radioactively labeled digitoxin, total radioactivity was counted (CALDWELL et al., 1971; VÖHRINGER and RIETBROCK, 1974; KRAMER et al., 1970; WIRTH et al., 1976). The average mean elimination half-life in these four studies was 9.5 days. CALDWELL et al. (1971) also calculated $t_{1/2\beta}$ with chloroform-extracted samples, containing digitoxin and its lipid-soluble and mostly cardioactive metabolites and found a mean $t_{1/2\beta}$ of 6.0 compared with 11.5 when total radioactivity was counted. These findings indicate that

Table 1. Influence of age and disease processes on pharmacokinetic parameters after a single intravenous dose of 0.6 mg digitoxin

Group (reference)	n	V_D (L/kg)	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (days)	$t_{1/2u}$ (days)	Cl_T (ml min^{-1} kg^{-1})	Cl_R (ml min^{-1} kg^{-1})	Cl_M (ml min^{-1} kg^{-1})	Protein binding (%)	Protein binding N
Control (STORSTEIN, 1974a, 1976a)	5	0.57 (0.15)	37.3 (33.3)	8.2 (2.6)	7.2 (2.7)	0.036 (0.014)	0.012 (0.004)	0.023 (0.016)	97.3 (0.5)	51
Children (LARSEN, and STORSTEIN, 1978)	5	0.59 (0.21)	69.5[a] (16.8)	5.9 (2.3)	—	0.065 (0.039)	0.045 (0.019)	0.020 (0.029)	97.0[a] (0.5)	32
Old age (STORSTEIN et al., 1980)	5	0.60 (0.14)	22.1 (10.5)	5.9 (2.2)	4.9 (2.2)	0.054 (0.021)	0.007[a] (0.005)	0.047[a] (0.021)	96.4[c] (1.0)	5
Biliary fistula STORSTEIN, 1975)	5	0.71 (0.47)	—	4.3[b] (1.2)	10.4 (4.0)	0.083[a] (0.034)	0.012 (0.009)	0.071[b] (0.029)	—	—
Thyrotoxicosis (STORSTEIN, 1979b)	6	0.58 (0.15)	27.4 (14.4)	4.6[b] (0.9)	4.5[a] (1.8)	0.064[a] (0.020)	0.014 (0.009)	0.050[a] (0.020)	97.3 (0.3)	6
Chronic active hepatitis (STORSTEIN, and AMLIE, 1979)	6	0.62 (0.14)	16.0 (9.3)	4.4[b] (1.6)	4.9[a] (0.6)	0.075[c] (0.007)	0.012 (0.007)	0.064[b] (0.014)	96.2[c] (1.0)	6
Uremia (STORSTEIN, 1974b, 1976a)	5	0.42[a] (0.05)	17.5 (9.1)	3.9[c] (0.9)	8.0 (1.7)	0.050 (0.012)	0.005[a] (0.004)	0.045[a] (0.012)	97.5 (0.4)	15
Nephrotic syndrome (STORSTEIN, 1976b)	5	1.00[a] (0.34)	54.5 (31.8)	4.8[a] (2.3)	5.0 (1.7)	0.112[a] (0.059)	0.031[b] (0.012)	0.080[a] (0.051)	96.7[a] (0.9)	7

V_D Apparent volume of distribution; $t_{1/2\alpha}$ serum distribution half-life; $t_{1/2\beta}$ serum elimination half-life; $t_{1/2u}$ urine concentration half-life; Cl_T total body clearance; Cl_R renal clearance; Cl_M metabolic clearance; [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.005$

Table 2. Serum elimination half-lives of digitoxin by various methods

Method	Serum elimination half-life (days)			
	n	Mean	SD	Range
Total ^3H radioactivity				
KRAMER et al. (1970)	6	8.2	1.7	6.0–10.2
CALDWELL et al. (1971)	8	11.5	2.3	8.4–16.4
VÖHRINGER and RIETBROCK (1974)	6	6.8	1.2	5.6– 8.6
WIRTH et al. (1976)	6	10.7		9.6–12.0
Total ^3H radioactivity with $CHCl_3$ extraction				
CALDWELL et al. (1971)	8	6.0	0.9	4.9– 7.8
^{86}Rb method				
RASMUSSEN et al. (1971)	13	6.2	2.2	3.7–11.3
GJERDRUM (1972)	20	6.9	2.7	2.4–11.5
STORSTEIN (1974a)	5	8.2	2.6	5.9–11.3
Radioimmunoassay				
STOLL et al. (1973)	8	8.0		6.5– 9.9
VAN BEVER et al. (1976)	11	6.8	1.0	5.4– 9.2
PETERS et al. (1977)	8	7.6	1.6	
Double-isotope dilution derivative assay				
LUKAS (1973)	10	5.0	0.7	4.3– 6.2

SD standard deviation

the relative amount of water-soluble metabolites increase with time after drug application and thus prolong $t_{\frac{1}{2}\beta}$ when included by the assay. The findings of STORSTEIN and AMLIE (1977a) that water-soluble metabolites increase with time are in good agreement with the results obtained by CALDWELL et al. (1971).

The ^{86}Rb method measures the biologic activity of lipid-soluble substances, i.e., digitoxin and its cardioactive metabolites. Serum elimination half-lives with this method were calculated by RASMUSSEN et al. (1971), GJERDRUM (1972), and STORSTEIN (1974a) and an average mean elimination half-life of 6.8 days was found in the three investigations.

Metabolites with an unchanged steroid nucleus show high cross reactivity with radioimmunoassays (FLASCH et al., 1977) among them glucuronides and sulfates. Hydroxylated metabolites on the other hand cross-react to a minor degree. The average half-life in three studies utilizing radioimmunoassay was 7.4 days.

The method which most specifically investigates unchanged digitoxin is the double-isotope dilution derivative assay by LUKAS and PETERSON (1966) which yields a mean serum elimination half-life of 5.0 days. These results confirm that metabolites contribute to the longer $t_{\frac{1}{2}\beta}$ obtained with less specific methods.

II. Serum Digitoxin Concentrations on Maintenance Treatment

Serum digitoxin levels on maintenance dosage with 0.1 mg digitoxin daily show remarkably similar mean values of 18.1–18.2 ng/ml (Table 3) with the double-iso-

Table 3. Relationship between dose and serum digitoxin concentration by various assays

Method	Serum digitoxin concentration (ng/ml)			
	0.05 mg/day Mean	0.07 mg/day Mean	0.10 mg/day Mean	0.15 mg/day Mean
Double-isotope dilution derivative				
LUKAS (1971)			18.2 (6.0) ($n=5$)	
^{86}Rb uptake				
RASMUSSEN et al. (1971)	12.0 (1.3[b]) ($n=17$)	16.7 (1.8[b]) ($n=17$)	18.2 (1.1[b]) ($n=32$)	19.2 (2.6[b]) ($n=5$)
GJERDRUM (1972)	11.2 (5.4)[a] ($n=33$)	16.1 (6.3)[a] ($n=24$)	18.1 (6.6)[a] ($n=81$)	
STORSTEIN (1979a)	12.8 (5.4)[a] ($n=102$)	16.4 (7.9)[a] ($n=193$)	18.1 (8.3)[a] ($n=282$)	
Radioimmunoassay				
MORRISON and KILLIP (1970)			25 (8)[a] ($n=74$)	
VÖHRINGER et al. (1976)			21.1 ($n=25$)	
PETERS et al. (1977)			26. 5 (7.3)[a] ($n=29$)	
KRAMER (1977)			23.6	
Na^+, K^+-ATPase				
BENTLEY et al. (1970)	13 ($n=10$)		22 ($n=115$)	30 ($n=17$)

[a] Standard deviation
[b] Standard error of mean

tope dilution derivative assay and the ^{86}Rb method in agreement with the fact that unchanged digitoxin constitute more than 90% of the cardioactive substances measured with the ^{86}Rb method. Close agreement between the various investigations was seen also for the lower maintenance dosage. On the other hand higher serum digitoxin concentrations were measured with radioimmunoassay or Na^+-K^+-ATPase assay in patients on 0.1 mg digitoxin a day (21.1–26.5 ng/ml). The individual variation in each dosage level is high for all methods, in agreement with marked individual variations in the rate of elimination.

III. Excretion Pathways

Total body clearance was calculated to be 0.036 $ml \cdot min^{-1} \cdot kg^{-1}$ (STORSTEIN, 1974a), 0.045 $ml \cdot min^{-1} \cdot kg^{-1}$ (VÖHRINGER and RIETBROCK, 1974), and 0.021 $ml \cdot min^{-1} \cdot kg^{-1}$ (WIRTH et al., 1976). Renal clearance was 0.012 $ml \cdot min^{-1} \cdot kg^{-1}$ (Table 1) after a single dose of digitoxin, a value similar to the renal clearance calculated in patients on maintenance treatment (STORSTEIN, 1974a). Whereas OKITA et al. (1955a) found that 70% of the dose was excreted in the urine over a period of 3 weeks, WIRTH et al. (1976) found that 45% was excreted in the urine

in the same period. Similar renal excretions of 13% (VÖHRINGER and RIETBROCK, 1974) and 16% (STORSTEIN, 1974a) were found during collection periods of 8 days which would probably have led to a 26% and 32% recovery respectively if urine collections had been complete. BEERMAN et al. (1971) recovered approximately 20% as unchanged digitoxin in 3 weeks while LUKAS (1971, 1973) recovered 28% in urine and 20% in feces after a single dose. GREEFF et al. (1979) found that the renal excretion was higher the first day after an oral than after an intravenous (i.v.) dose of digitoxin. The 8-day excretion was 12.4% after i.v. and 14.4% after oral medication. They calculated a 25% renal excretion after a single dose, the remaining 75% being fecal excretion.

On maintenance treatment LUKAS (1971, 1973) found that 18% was excreted in the urine and 14% in the feces as unchanged digitoxin. A higher renal excretion of unchanged digitoxin (30%) was found by STORSTEIN (1974a) with a further 3% as other cardioactive metabolites. Half the daily dose was excreted in urine as cardioactive and conjugated metabolites, indicating that the two main excretory pathways are of equal importance for the elimination of digitoxin in patients on maintenance therapy. Lymph drainage has only been studied in the rat (BEERMAN and HELLSTRÖM, 1971) and only 4% of the radioactivity was collected in the lymph in the 48 h following administration of the drug.

F. Modifications by Age

I. Neonates, Infants, and Children

Digitoxin has been used extensively in pediatric patients before the era of serum digitoxin concentration determinations. Recommended doses in neonates have been 0.002–0.003 $mg \cdot kg^{-1} \cdot day^{-1}$, in infants 0.003–0.006 $mg \cdot kg^{-1} \cdot day^{-1}$, and in children 0.002-0.004 $mg \cdot kg^{-1} \cdot day^{-1}$. GIARDINA et al. (1975) found a mean serum level of 30 ng/ml in infants on a mean maintenance dose of 0.0043 $mg \cdot kg^{-1} \cdot day^{-1}$ and similar mean digitoxin levels in children (34 ng/ml) on a lower maintenance dose of 0.0031 $mg \cdot kg^{-1} \cdot day^{-1}$. These findings support the concept of a higher maintenance dosage in infants than in children. The digitoxin concentrations measured by GIARDINA et al. (1975) in pediatric patients were significantly higher than in adults. Toxicity in a small number of infants and children was associated with serum digitoxin concentrations in the range 50–85 ng/ml, the values being higher than toxic concentrations observed in adults. These data indicate that children tolerate higher serum digitoxin levels before exhibiting symptoms or signs of digitoxin toxicity.

In a recent study (LARSEN and STORSTEIN, 1978) we have investigated digitoxin pharmacokinetics in five children, mean age 7.6 years. Digitoxin was given as a single dose of 0.02 mg/kg and serum samples and 24-h urine samples were collected for 7 days. As can be seen from Table 1, children had a longer serum distribution half-life than adults while no significant changes were observed for the other pharmacokinetic parameters. Children did, however, excrete digitoxin mostly through the kidneys whereas adults had higher metabolic than renal clearances (Fig. 3). Serum digitoxin protein binding was slightly reduced in children.

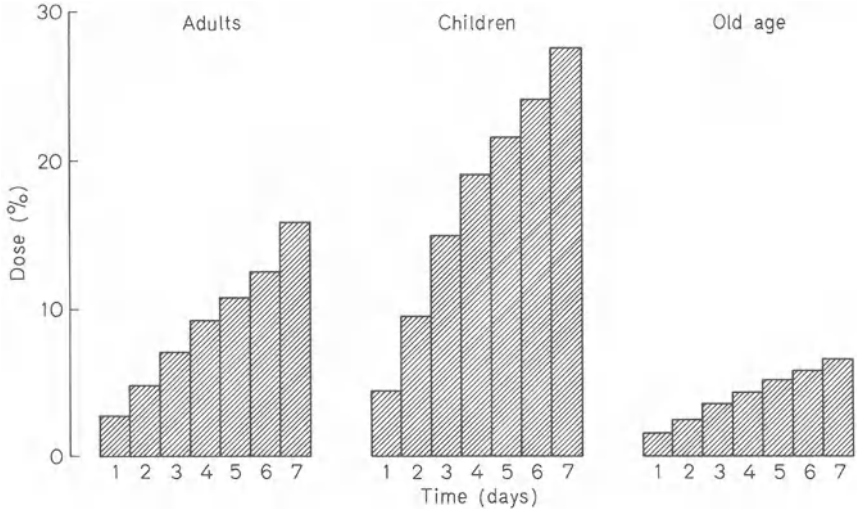


Fig. 3. Cumulative renal excretion of digitoxin and cardioactive metabolites in different age groups

Children tolerate high initial digitoxin concentrations without electrocardiographic disturbances. The slow distribution between serum and tissue in this phase may reflect a saturation of the uptake process and can explain the tolerance of high initial serum concentrations. The question why children tolerate higher serum concentrations on maintenance treatment than adults is not yet solved. A prospective study of digitoxin intoxication in children could clarify this problem. It has been shown for digoxin (KRASULA, 1974) that the ratio between myocardial and serum concentrations is the same in children as in adults. A reduced myocardial uptake of digitalis glycosides in pediatric patients is therefore probably not the explanation for the proposed tolerance of higher serum digitalis concentrations.

II. Old Age

Old age is an important risk factor for digoxin intoxication (BELLER et al., 1971) owing to the reduction in creatinine clearance with age. Digitoxin serum levels have been studied in 17 patients above 75 years of age and compared with serum levels in 14 control patients, all subjects receiving a digitoxin maintenance dose of 0.1 mg/day (KOKENGE et al., 1978). Mean serum digitoxin concentrations were the same in the two groups.

In a recent study on digitoxin pharmacokinetics in five patients above 75 years of age (STORSTEIN et al., 1980) we found no significant changes in the apparent volume of distribution (Table 1), $t_{1/2\alpha}$, $t_{1/2\beta}$, or urine concentration half-life. The geriatric group did, however, have a reduced renal clearance of digitoxin which was compensated for by a 100% increase in the metabolic clearance.

The diminished renal excretion in geriatric patients (Fig. 3) was adequately compensated for by an increased biliary excretion of the drug. On the evidence of

pharmacokinetic studies geriatric patients should thus tolerate the same dosage of digitoxin per unit body weight as younger adult patients. Whether digitoxin sensitivity is changed in old age, however, remains to be clarified.

G. Modifications by Disease States

I. Gastrointestinal Disease

TAKANASHI et al. (1978) studied the absorption of digitoxin in a group of patients with chronic diarrhea due to hyperthyroidism, cancer of the pancreas, liver cirrhosis, and various other disorders. They found that absorption was decreased, with significantly lower serum digitoxin concentrations during the 6 h observation period than in a control group. Patients with acute diarrhea could have normal or decreased absorption of digitoxin. Evaluation of their data is rendered difficult by the fact that some of the underlying disorders like hyperthyroidism and cirrhosis of the liver will lead to decreased serum digitoxin levels.

MYHRE et al. (unpublished) studied the absorption of digitoxin in one group of patients with malabsorption due to celiac disease and one group of patients with rapid intestinal passage due to dumping-syndrome after gastrectomy. The data were compared with data obtained from healthy control subjects. Digitoxin 0.6 mg was given as a single oral dose in the fasting state to five sprue and six gastrectomized patients while three patients with celiac disease were given the same dose intravenously. Absorption was rapid in both patient groups with maximum serum concentrations after 1 h, but serum digitoxin concentrations were lower than in the control group. The absolute biologic availability over a period of 24 h was 40.6% in celiac disease and 50.4% in gastrectomized patients. On the other hand intravenous administration of digitoxin to three patients with celiac disease also resulted in lower serum digitoxin levels than in healthy control subjects.

The protein binding was normal in both patient groups. These data point to malabsorption of digitoxin in patients with decreased absorptive function and rapid gastrointestinal passage. Malabsorption is not the only factor responsible for the reduced biologic availability in these patients as drug distribution and/or elimination may also be changed.

II. Thyroid Disease

Clinical observations point to the necessity of larger than usual doses of digitalis glycosides in hyperthyrodism and reduced doses in hypothyroidism. DOHERTY and PERKINS (1966) first showed that hyperthyroid patients had decreased and hypothyroid increased serum digoxin levels when compared with control subjects. EICKENBUSCH et al. (1970) injected radioactively labelled ouabain and digitoxin into patients with thyroid disease and also found lower glycoside levels in thyrotoxicosis and increased levels in myxedema. Samples were collected for 72 h and these data did not allow calculation of serum elimination half-lives for digitoxin.

In a recent study of six patients with thyrotoxicosis (STORSTEIN, 1979 b) a single dose of 0.6 mg digitoxin was injected intravenously (Table 1). Serum and urine

were collected for 8 days. Serum digitoxin protein binding was unchanged in the thyrotoxic patients as were parameters of drug distribution (V_D and $t_{1/2\alpha}$). Serum elimination was significantly enhanced and urine concentration half-life was shortened. Total body clearance was significantly increased owing to changes in metabolic clearance while renal clearance remained unchanged. The metabolic pattern of cardioactive and conjugated inactive metabolites was studied in serum and urine 24 h after the dose (Fig. 4). Thyrotoxic patients have significantly less unchanged digitoxin in serum than the control group and more digitoxigenin-bis-digitoxoside, digitoxigenin-mono-digitoxoside, and digitoxigenin. The hyperthyroid patients had less hydroxylated metabolites, more hydrolyzed, and more conjugated and hydrolyzed metabolites in serum than control subjects. This leads to an overall change in the enzymatic process towards less digitoxin, more hydrolyzed and conjugated metabolites in serum. These data on digitoxin pharmacokinetics confirm the experience that thyrotoxic patients need increased doses of digitoxin. The enhancement in digitoxin elimination is due both to an increased fecal excretion and enhanced metabolic degradation of digitoxin. Data on digoxin on the other hand (DOHERTY and PERKINS, 1966; CROXSON and IBBERTSON, 1975; GILFRICH, 1976; LAWRENCE et al., 1977; SHENFIELD et al., 1977) point to an increased renal elimination of digoxin. Although serum digitalis concentrations are lowered in thyrotoxicosis, different mechanisms seem to be responsible for ouabain and digoxin on the one hand and digitoxin on the other hand.

III. Hepatic Disease

Hepatic disease may interfere with drug pharmacokinetics by changing drug protein binding, drug metabolism, or biliary excretion. The development of edema and ascites may change drug distribution by the addition of new volumes into which drug can diffuse. Advanced hepatic dysfunction is also associated with changes in renal function. As digitoxin is extensively metabolized, caution has been advised when administering digitoxin in hepatic disease. LAHRTZ et al. (1969) followed serum digitoxin concentration and renal and fecal excretion of digitoxin 72 h after injection of radioactively labeled drug. They studied a group of patients with mixed hepatic disease and found that these patients had lower serum digitoxin levels than control subjects. In another study of ten patients with hepatic disease (ZILLY et al., 1976), four with cirrhosis, four with acute hepatitis, and two with extrahepatic cholestasis, a normal or increased clearance of digitoxin was found in most patients. One patient with concomitant renal disease had a marked prolongation of serum digitoxin half-life. Patients with hepatic disease tended to excrete more polar metabolites than control subjects. Digitoxin pharmacokinetics in patients with chronic active hepatitis was investigated by STORSTEIN and AMLIE (1979). These patients had a slightly lowered serum digitoxin protein binding and shortened serum distribution half-life compared with control subjects (Table 1). Serum elimination was enhanced and the urine concentration half-life was shortened. The increased rate of elimination was due to an increased total body clearance of digitoxin. As renal clearance was unchanged, the increase in total clearance was solely due to a marked increase in metabolic clearance of the drug. To clarify whether increased biliary secretion or increased metabolic conversion of digitoxin was re-

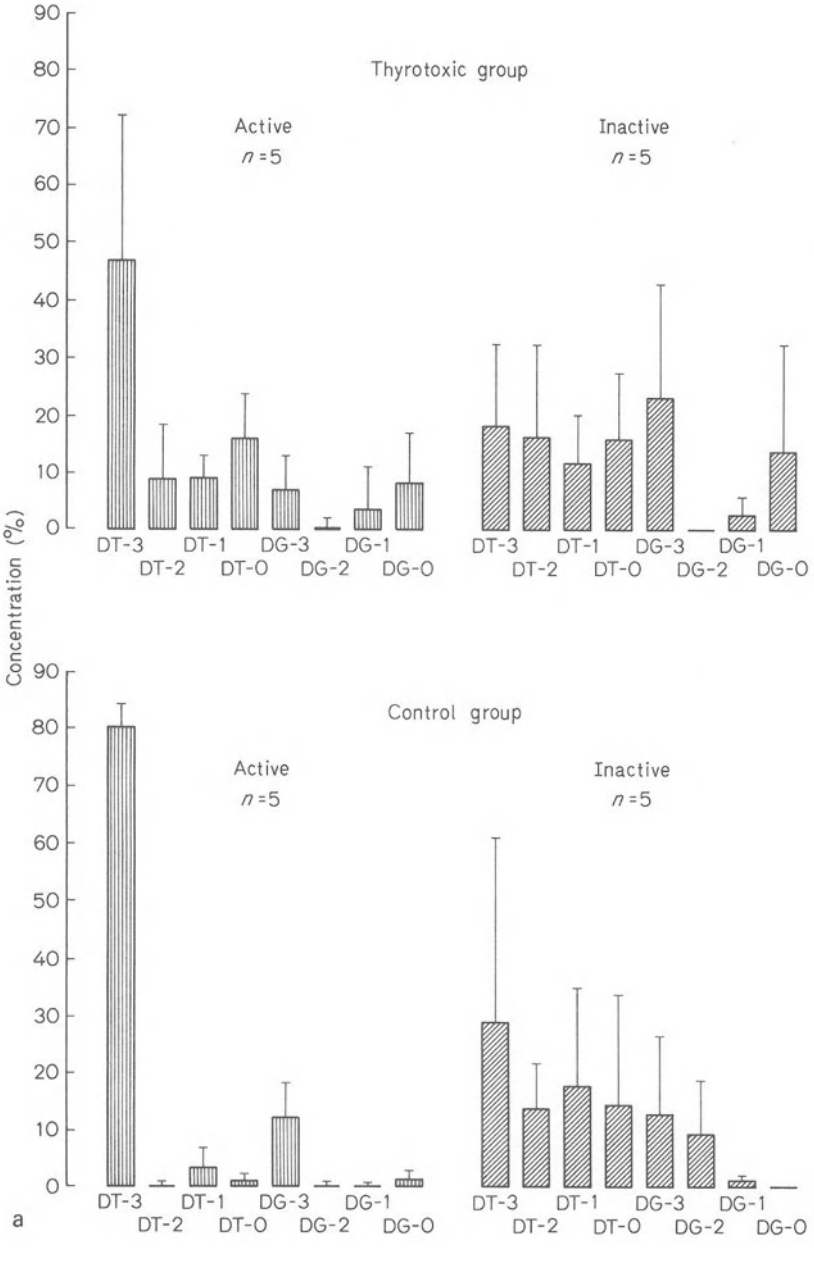
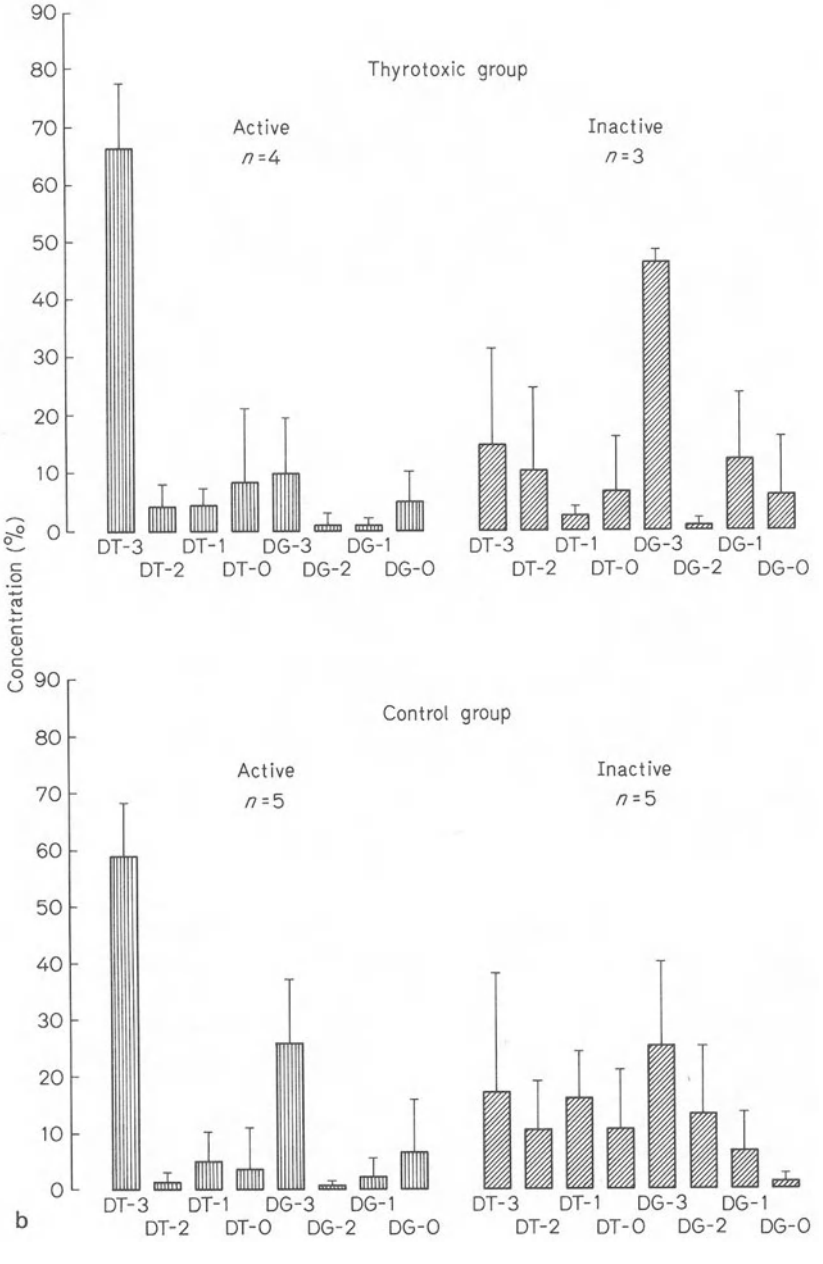


Fig. 4 a, b. Distribution of cardioactive metabolites (100%) and inactive conjugated metabolites (100%) in serum **a** and urine **b** after a single dose of digitoxin to patients with thyrotoxicosis and a control group. DT: Digitoxin metabolites. DG: Digoxin metabolites. Arabic members signify sugar molecules


Thyrotoxic group
Active
n=4
Inactive
n=3
Control group
Active
n=5
Inactive
n=5
Concentration (%)
0
10
20
30
40
50
60
70
80
90
DT-3
DT-2
DT-1
DT-0
DG-3
DG-2
DG-1
DG-0
b

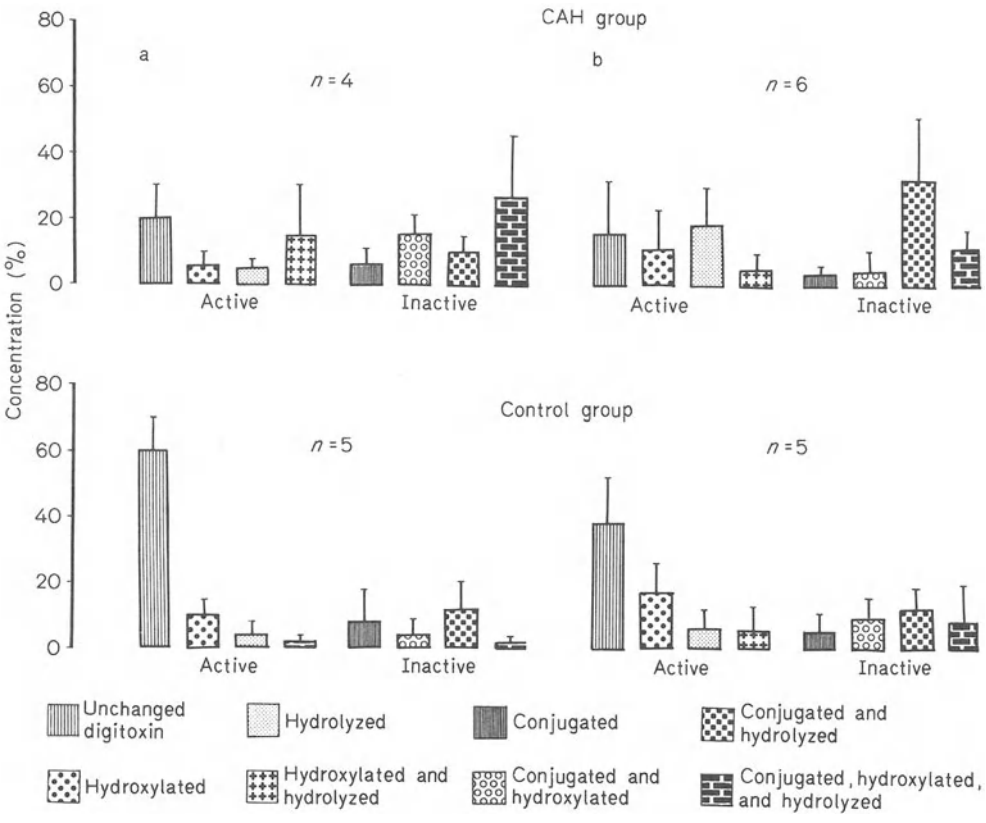


Fig. 5 a, b. Presentation of enzymatic subgroups resulting from one, two, and three enzymatic processes in **a** serum and **b** urine from patients with chronic active hepatitis (CAH group) and a control group

sponsible for the enhancement in elimination a study was made of the metabolic pattern in serum and urine 24 h after the dose. The chronic active hepatitis group had less unchanged digitoxin in serum, more digitoxigenin-bis-digitoxoside and digitoxigenin and more digoxigenin in serum than the control group (Fig. 5). These changes lead to an overall increase in hydroxylated, hydrolyzed, and conjugated metabolites.

It can thus be stated that digitoxin elimination is not impaired in various forms of hepatic disease and that it is even enhanced in patients with chronic active hepatitis.

IV. Renal Disease

1. Uremic Patients on Hemodialysis

FINKELSTEIN et al. (1975) found that absorption of digitoxin was not impaired in patients on hemodialysis. The serum concentration and excretion of radioactively labeled digitoxin was studied by LAHRTZ et al. (1969) in five patients treated with

Table 4. Serum elimination half-lives in patients with renal disease and patients on hemodialysis compared with control subject

Reference	Method	Uremic group Time (days)[a]	Control group Time (days)	p
RASMUSSEN et al. (1972)	^{86}Rb	5.2 (2.1) ($n=10$)	6.1 (1.7) ($n=10$)	NS[c]
STORSTEIN (1974b)	^{86}Rb	3.9 (0.9) ($n=5$)	8.2 (2.6) ($n=5$)	<0.01
PETERS et al. (1977)	RIa[b]	5.7 (0.9) ($n=4$)	7.6 (1.6) ($n=8$)	<0.05
VÖHRINGER et al. (1976)	^{3}H-Digitoxin	8.0 (2.6) ($n=6$)	6.8 (1.2) ($n=6$)	NS[c]
		Hemodialysis group	Control group	
KRAMER et al. (1970)	^{3}H-Digitoxin	9.3 (7.7) ($n=5$)	8.2 (1.7) ($n=6$)	NS[c]

[a] Mean values are given with standard deviations in parentheses
[b] RIA = radioimmunoassay
[c] NS = not significant

peritoneal dialysis during the investigation period. Their patients were followed for 72 h after the dose. Patients with renal disease had higher serum levels than control subjects, but the data did not allow calculation of serum half-life. No significant difference in serum elimination half-life was found by KRAMER et al. (1970) after administration of radiolabeled digitoxin intravenously to four patients on hemodialysis and one on peritoneal dialysis (Table 4). Serum concentrations on maintenance dosage are lower in patients on hemodialysis than in control patients as can be seen from Table 5. The daily renal excretion of digitoxin and cardioactive metabolites is only 2% of the daily dose in hemodialysis patients compared with 32.9% in the control group (STORSTEIN, 1977a; STORSTEIN and AMLIE, 1977b). The extent of conjugation was the same in hemodialysis and control patients and only minor changes were found in the pattern of cardioactive metabolites in urine in comparison with the control group. It can thus be concluded that although renal excretion of digitoxin is severely impaired in patients on treatment with hemodialysis, serum elimination is not decreased.

2. Uremia Per Se

Serum digitoxin levels were lower in the absorption phase after oral doses of digitoxin to patients with uremia (RASMUSSEN et al., 1972), but serum concentrations 24 h after the dose were not significantly changed (STORSTEIN, 1974b). PETERS et al. (1977) found no change in the biologic availability in the 24 h after an oral dose. The volume of distribution was significantly smaller in uremic patients than in control subjects (Table 1) when calculated per unit body weight. Serum elimination half-life is shortened (STORSTEIN, 1974b; PETERS et al., 1977) or unchanged (RASMUSSEN et al., 1972; VÖHRINGER et al., 1976). The decreased serum elimination half-

Table 5. Serum digitoxin concentrations in uremic patients (with and without treatment with hemodialysis) compared with control subjects

Reference	Method	Uremic group			Control group			
		Dose	*n*	[Digitoxin] (ng/ml)[a]	Dose	*n*	[Digitoxin] (ng/ml)	*p*
RASMUSSEN et al. (1972)	^{86}Rb	0.05	15	8.5 (0.9)	0.05	17	12.0 (1.3)	<0.05
		0.07	12	9.6 (1.5)	0.07	17	16.7 (1.8)	<0.01
		0.10	24	16.1 (1.1)	0.10	32	18.2 (1.1)	NS[c]
STORSTEIN (1974a, b)	^{86}Rb	0.07	5	17.9 (12.6)	0.07	11	21.1 (10.5)	NS[c]
VÖHRINGER et al. (1976)	RIA[b]	0.10	30	19.4	0.10	25	21.1	NS[c]
PETERS et al. (1977)	RIA[b]	0.10	21	23.0 (6.4)	0.10	29	26.5 (7.3)	<0.05
STORSTEIN et al. (1977)	^{86}Rb	0.067	26	12.1 (5.5)	0.083	544	17.0 (8.1)	<0.003
		Hemodialysis group						
PETERS et al. (1977)	RIA[b]	0.10	27	24.6 (7.9)	0.10	29	26.5 (7.3)	NS[c]
KRAMER (1977)	RIA[b]	0.10		15.2	0.10		23.6	

[a] Mean values are given with standard deviations or standard errors in parentheses
[b] RIA = radioimmunoassay
[c] NS = not significant

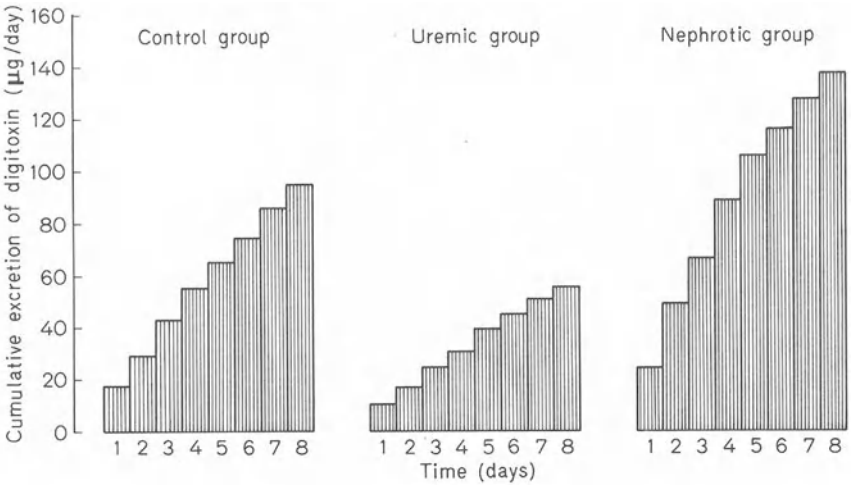


Fig. 6. Cumulative renal excretion of digitoxin and cardioactive metabolites after a single dose administered to patients with uremia and nephrotic syndrome in comparison with a control group

life was due to a marked increase in metabolic clearance of digitoxin and cardioactive metabolites and with a concomitant decrease in the renal clearance of the drug. This decrease in renal clearance is reflected in a lower 8-day cumulative renal excretion of digitoxin (56.7 µg) compared with 94.9 µg in the control subjects (Fig. 6). VÖHRINGER et al. (1976) found a reduced renal excretion of digitoxin over a period of 192 h following an oral dose of radioactively labeled digitoxin in uremic patients compared with five control subjects. The mean fecal excretion was higher in the uremic patients. PETERS et al. (1977) also found a lower excretion of digitoxin in three patients with uremia compared with three patients with heart insufficiency ($p < 0.005$). We suggested (STORSTEIN, 1973, 1974b) that compensatory mechanisms were operative in uremic patients, accounting for the reduction in serum elimination half-life. Increased fecal excretion was demonstrated by VÖHRINGER et al. (1976). We have studied the metabolic pattern of cardioactive and inactive conjugated metabolites of digitoxin in serum and urine in uremic patients on maintenance treatment with the drug and compared these results with data from control subjects (Fig. 7). Uremic patients had less unchanged digitoxin and more hydroxylated and hydrolyzed metabolites than the control group whereas the extent of conjugation was the same in the two groups (STORSTEIN, 1977c). BODEM and UNRUH (1978) found that uremic patients have markedly increased levels of dihydrodigitoxin when compared with patients without renal failure. VÖHRINGER et al. (1976) on the other hand were not able to demonstrate significant changes in digitoxin metabolism.

We showed that uremic patients had lower serum concentrations on maintenance therapy than control patients (Table 5). Similar findings were reported by PETERS et al. (1977) and VÖHRINGER et al. (1976). Urine concentrations are markedly reduced in uremic patients on maintenance treatment (STORSTEIN, 1974b)

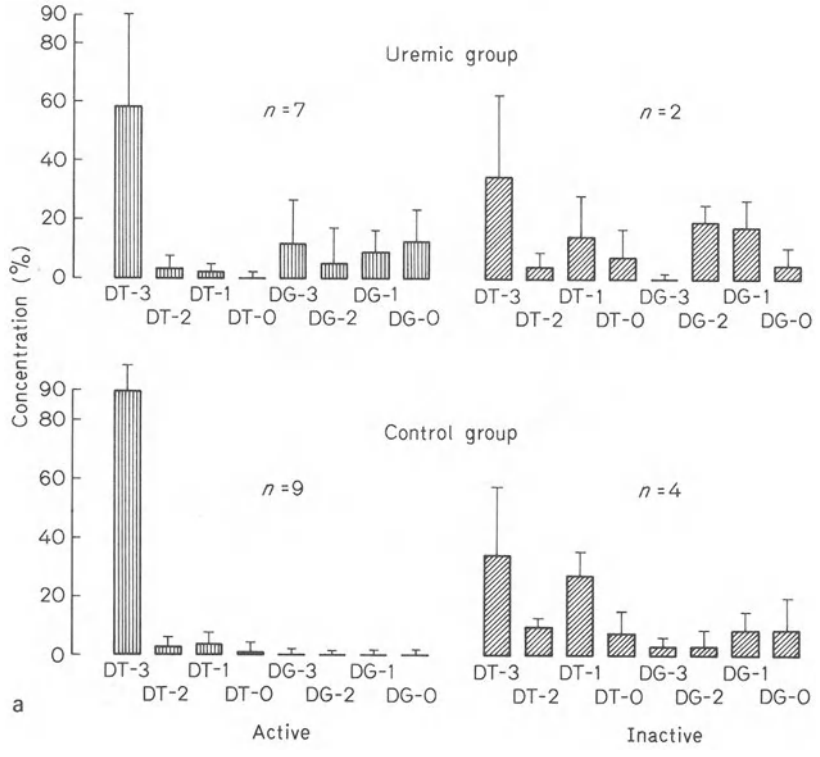
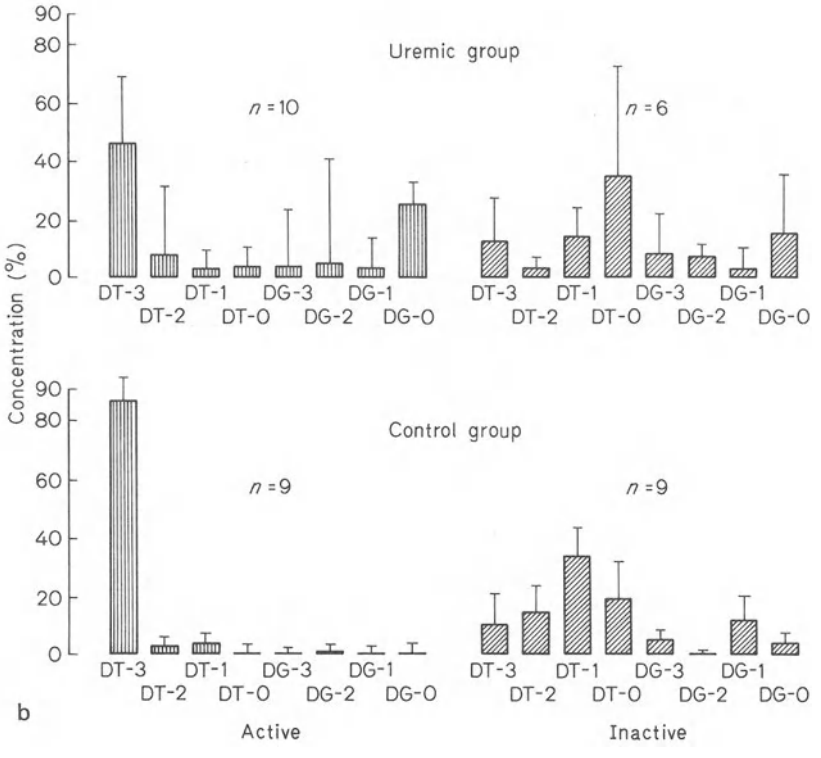


Fig. 7 a, b. Distribution of cardioactive metabolites (100%) and inactive conjugated metabolites (100%) in **a** serum and **b** urine from uremic and control patients. (STORSTEIN, 1977c). DT: Digitoxin metabolites. DG: Digoxin metabolites. Arabic members signify sugar molecules

leading to a concomitant decrease in the renal excretion of the drug. Renal clearance of digitoxin and cardioactive metabolites is 0.58 ml/min in uremic compared with 0.98 ml/min in control patients on maintenance treatment.

3. Nephrotic Syndrome

Single-dose kinetics was studied in five patients with nephrotic syndrome (STORSTEIN, 1976b). As can be seen from Table 1 these patients had an increased volume of distribution and lowered serum protein binding compared with control subjects. Serum elimination half-life was enhanced owing to a marked increase in total body clearance. This increase in total body clearance was due to enhancement of both renal and metabolic clearance of the drug. Nephrotic syndrome is unique in allowing passage of substances with high molecular weight through the glomeruli. Digitoxin excretion in urine was composed of free and protein-bound drug (urine digitoxin protein binding 60.1%). In accordance with these findings PETERS et al. (1974) found lowered serum digitoxin values in nephrotic patients on maintenance treatment.



H. Concluding Remarks

The highly protein-bound drug digitoxin is rapidly and well absorbed. Unbound drug has a high affinity to tissue and crosses both the blood–brain and placental barrier. Digitoxin is metabolized to a great number of active and inactive metabolites, but unchanged drug is the main cardioactive substance present. Renal and fecal pathways are responsible for drug excretion and the enterohepatic circulation plays an important role in the long elimination half-life of digitoxin. Digitoxin pharmacokinetics is influenced by age as the renal excretion of the drug is increased in children and decreased in old age although serum elimination is unchanged. Gastrointestinal, thyroid, hepatic, and renal disease influence digitoxin pharmacokinetics and metabolism, but the investigated disease states do not lead to any accumulation of the drug.

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CHAPTER 2

Pharmacokinetics of Digoxin and Derivatives

N. RIETBROCK and B. G. WOODCOCK

A. Tissue Distribution

In the absence of sensitive analytical methods, estimation of the quantity of glycoside in the myocardium was at one time possible only by observing the pharmacologic effect in the intact animal, or in a heart–lung preparation, or in the embryonic chick heart (WEESE, 1928, 1929; LENDLE, 1935; ROTHLIN, 1944; FRIEDMAN and BINE, 1947; BINE et al., 1951; ROTHLIN and BIRCHER, 1954). Later, the direct detection of digitalis glycosides in various organs and tissues of the rat was achieved using the xanthydrol or m-dinitrobenzol reaction (REPKE, 1958). Fluorescence techniques enabled additional inferences on the structure of the steroid nucleus to be obtained (JENSEN, 1953; WELLS et al., 1961; JELLIFFE, 1967). The main disadvantage of the foregoing methods is the relatively low sensitivity.

More precise investigations on the tissue distribution and binding of cardiac glycosides to cell components became possible with the introduction of radiolabeled glycosides (see this Handbook, Vol. 56/I, Chap. 4). Subsequently, the introduction of radioimmunoassay methods enabled glycoside distribution studies in humans to be achieved without having to administer radiolabeled drug (KARJALAINEN et al., 1974; ANDERSSON et al., 1975; KIM et al., 1975).

Naturally, and apart from a few exceptions, investigations on glycoside concentrations in tissue have confined themselves primarily to the estimation of glycoside concentration in the myocardium. The glycoside distributes itself differently in the different regions of the heart. In the muscle of the left ventricles glycoside concentrations are definitely higher than in the musculature of the right ventricles and auricles. KUSCHINSKY et al. (1968) have reported that no more than 10% of digoxin in the myocardium is bound to specific receptors. PFLEGER et al. (1975) have suggested that histologically detectable differences in structure between auricles and ventricular musculature are associated with the different distribution pattern. In the animal the T tubules are fully developed and the lower content of strophanthin G in the guinea-pig heart can be explained by a reduction in the specific binding sites due to the T tubules which occupy the surface of the cell membrane.

The estimation of the glycoside concentration in the myocardium is the first and most obvious means of providing an answer to the question – does a constant relationship exist between the concentration of cardiac glycoside in blood and that in the myocardium? Almost all investigators in this field have measured the digoxin concentration in the whole muscle. A summary of the findings from such studies on biopsy material taken during heart surgery or specimens of heart removed postmortem etc. is given in Tables 1 and 2. A striking observation is the extreme vari-

Table 1. Relationship between plasma glycoside concentration and the concentration in different regions of the heart obtained in patients during surgery

Reference	Region of heart	Distribution ratio
COLTART et al. (1972; 1974; 1975)	Papillary muscle	39:1–155:1
HAASIS et al. (1977)	Papillary muscle	46.6 ± 8.96:1 ($r=0.844$)[a]
HÄRTEL et al. (1976)	Papillary muscle	46.9:1–90:1 ($r=0.967$)
GÜLLNER et al. (1974)	Right atrium	23.9 ± 3.2
CARRUTHERS et al. (1975)	Papillary muscle, right atrium	39.3:1–114:1 3.8:1–124:1
REDFORS et al. (1973)	Right and left ventricle	19:1–177:1 ($r=0.83$)
CHAMBERLAIN (1973)	Left ventricle	39:1–155:1
CARROLL et al. (1973)	Right atrium	25:1–128:1
BINNION et al. (1969)	Left atrium	350:1

[a] r = regression coefficient for the relationship between plasma glycoside concentration and the glycoside concentration in the tissue

Table 2. Relationship between plasma glycoside concentration in different regions of the heart obtained post-mortem

Reference	Region of heart	Distribution ratio
HAASIS et al. (1977)	Left ventricle	46.2 ± 9.5:1
	Right ventricle	33.0 ± 7.4:1
JUSKO and WEINTRAUB (1974)	Left ventricle	20.1–60:1 mean 35.6:1
GORODISCHER et al. (1976)	Left ventricle	84:1–325:1 mean 146.1:1
WEINMANN et al. (1979)	Left ventricle	51.9 ± 59.1 ($r=0.379$)[a]
	Right ventricle	26.5 ± 21.9 ($r=0.490$)
	Left atrium	25.0 ± 15.2 ($r=0.861$)
	Right atrium	16.4 ± 10.5 ($r=0.681$)
ANDERSSON et al. (1975)	Ventricle, atrium (children)	47:1–174:1 mean 104:1
KIM et al. (1975)	Ventricle, left atrium, right atrium (children)	Mean 99:1 (newborn) mean 114:1

[a] r = regression coefficient for the relationship between plasma glycoside concentration and the glycoside concentration in the tissue

ation in glycoside concentration in different areas of heart. Only a small percentage of the glycoside present can be involved specifically with the effects on the heart arising from binding to glycoside receptors of Na^+, K^+-activated ATPase of the cell membrane, the greater percentage of the digoxin content being bound nonspecifically. Also the digoxin concentration in tissue homogenates is highly dependent

on the ratio of muscle tissue to fat and connective tissue etc. in the specimen taken. Possible reasons for the differences in the estimates of the digoxin content of cardiac tissue reported by the various laboratories are: size of the patient groups; type of glycoside used, dosage as well as compliance factors; time of blood and tissue sampling (steady state); different parts of the same organ sampled (e.g., ventricle); histologic variations between tissue samples; different methods of tissue handling and preparation; differences in extraction procedures; differences in the assay methods used; variations in electrolyte concentrations in serum; and finally alterations in acid-base status and oxygen tension in the tissue. Undoubtedly one of the most important causes of variations in the glycoside concentration in the heart must be general or local changes in the myocardium resulting from the various pathologic states. To what extent dilatation or hypertrophy, fibrosis, lipomatosis, or interindividual differences in the ratio of connective tissue to muscle fiber tissue can influence the distribution of digoxin in the heart is difficult to assess because such changes seldom occur in isolation from other events. With regard to the dependence of glycoside distribution on morphological structure under different metabolic conditions of the heart, animal studies involving acute ischemia and necrotic changes in the heart tissue indicate that the digoxin content of ischemic tissue in comparison with adjacent normal myocardium is definitely lower (BELLER et al., 1972; THOMPSON et al., 1972; HOPKINS and TAYLOR, 1973; KUHLMANN et al., 1975). In view of these possible factors the extreme variability in the glycoside concentration of myocardium is more understandable. However, there does exist a complex relationship between the glycoside concentration in the myocardium and the concentration in serum such that estimations of serum glycoside concentration in regard to clinical observations are sensible and useful guides in the control of digitalis therapy.

Considerable differences in the distribution of digoxin and β-methyldigoxin have been found in the central nervous system. As early as 1952, FRIEDMAN et al. established that digitoxin was taken up by the brain very much more slowly than by other tissues but remained there longer. The main reason for the slower diffusion of digoxin, β-methyldigoxin, and also other glycosides into the central nervous system (CNS) is the blood–brain barrier which retards the free diffusion of glycoside from plasma water into the extracellular fluid of brain. BENTHE (1975) found a strong correlation between the distribution coefficient for brain–plasma water and the lipid solubility of different glycosides. Similar concentration differences between polar and lipophilic glycosides were found after repeated dosage in cats (FLASCH and HEINZ, 1976), in dogs (RIETBROCK and KUHLMANN, 1977; KUHLMANN et al., 1979), and in humans (HAASIS and LARBIG, 1976). ANDERSSON et al. (1975) found a mean digoxin concentration of 32 ng/g wet weight in the telencephalon region of the brain in humans during steady-state treatment. This concentration was comparable to that in skeletal muscle but three times higher than in fatty tissue. A seven times higher concentration than in the telencephalon was recorded for the choroid plexus where the concentration exceeded even that in the myocardium by a factor of 1.7 (Fig. 1; KUHLMANN et al., 1979).

Digoxin distribution studies on various brain tumors by WILLIAMS et al. (1976) indicate the importance of the blood–brain barrier and Na^+, K^+-ATPase in glycoside uptake. Corresponding to the significantly higher ATPase activity in menin-

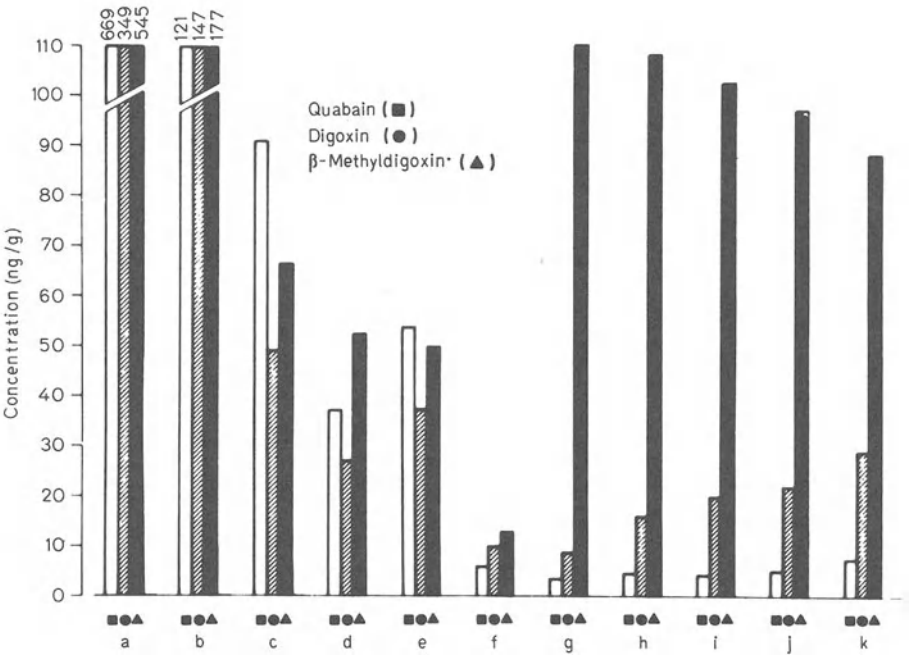

Fig. 1a–l. Comparison of digoxin (●), β-methyldigoxin (▲), and ouabain (■) (ng/g wet weight) in various regions of the brain and in other tissues of dogs 24 h after daily intravenous administration of 0.0125 mg/kg ^{3}H-digoxin, β-methyldigoxin, and ouabain over 10 days. **a** renal cortex; **b** renal medulla; **c** myocardium; **d** liver; **e** adrenal; **f** skeletal muscle; **g** telencephalon; **h** thalamus/hypothalamus; **i** mesencephalon; **j** cerebellum; **k** medulla

giomas in comparison with malignant blastomas (LAWS and O'CONNOR, 1970; ÅGREN et al., 1971) the concentrations of digoxin were 21.8 ± 7.3 ng/g and 5.7 ± 5.2 ng/g wet weight respectively. In addition both types of tumor were observed to alter the nature of the blood–brain barrier and the blood flow in the adjacent non-tumor tissue of the brain cortex was raised.

For digoxin in dogs there is a distinct concentration difference between the cerebrum and the cerebellum and between different sections of the brain stem, whereas the more lipophilic β-methyldigoxin is more evenly distributed over the whole brain (Fig. 1; KUHLMANN et al., 1979). Investigations on the time course of digoxin and β-methyldigoxin distribution have shown that in contrast to most tissue compartments where there is a rapid exchange with blood, the equilibrium with the brain compartment is slow (RIETBROCK and KUHLMANN, 1977; KUHLMANN et al., 1979). On a daily maintenance dose, the glycoside accumulation in dog brain is higher than in other tissues. Corresponding to the longer accumulation half-life in the brain (Fig. 2), the steady-state concentration for digoxin would be expected after 12–15 days and for β-methyldigoxin after 24–30 days (Fig. 3). Also it appears that the larger part of the glycoside content in brain is bound nonspecifically, and although the concentration of β-methyldigoxin is higher than that for digoxin, the frequency of central toxicity effects using data on four β-methyldigoxin patients was not significantly different from that in digoxin patients (RIETBROCK and ALKEN, 1980).

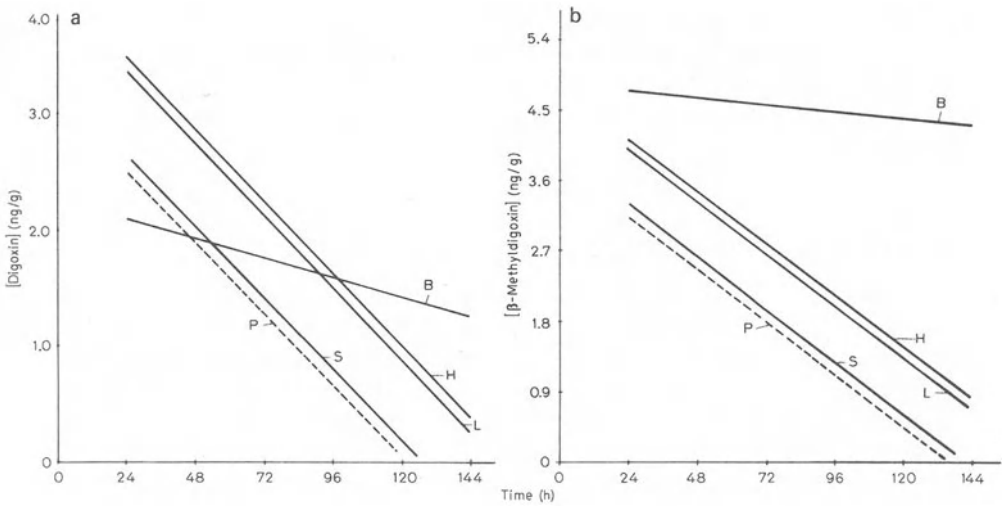

Fig. 2 a, b. Elimination of digoxin **a** and β-methyldigoxin **b** in plasma *(P)*, skeletal muscle *(S)*, heart *(H)*, liver *(L)*, and brain *(B)* in dogs in the post steady state. Concentrations are expressed as logarithms. The *broken lines* indicate that plasma concentrations are measured in 10 × ng/ml (RIETBROCK et al., 1977a)

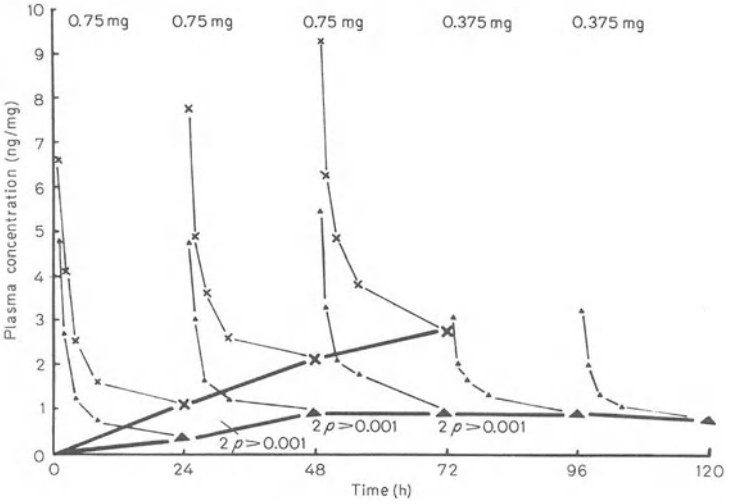

Fig. 3. Glycoside concentration in plasma for each 24 h period following the intravenous administration of 0.75 mg ^3H-digoxin △ or ^3H-β-methyldigoxin x daily for 3 days and 0.375 mg ^3H-digoxin for a further 2 days in patients with acute hepatitis (ZILLY et al., 1975)

The reported values for cerebrospinal fluid : serum ratios in patients on maintenance doses with digoxin vary between 0.03 and 0.33 (ALLONEN et al., 1977; BODEM et al., 1977; GAYES et al., 1978; SCHOTT et al., 1976; SOMOGYI et al., 1972). Children have the same ratios as adults (ALLONEN et al., 1977). β-Methyldigoxin has a six times higher penetration into the cerebrospinal fluid, where the ratio is

0.6 compared with 0.1 for digoxin (BODEM et al., 1977). These human data are in accordance with observations in animals which show a higher penetration into the brain for β-methyldigoxin (KUHLMANN et al., 1979). Drug concentrations in the cerebrospinal fluid do not necessarily reflect concentrations in various parts of the cerebrum.

B. Apparent Distribution Volume

The digoxin concentration–time curve after intravenous injection or infusion indicates that the pharmacokinetics of digoxin should be described by a model containing at least two kinetically distinct compartments (DOHERTY et al., 1968; GREENBLATT et al., 1974; NYBERG et al., 1974; REUNING et al., 1973). A three-compartment model has been proposed by KRAMER et al. (1974) and by SUMNER and RUSSELL (1976).

The first very rapid decrease of concentration is mainly the result of dilution in blood and is complete within a few minutes. The next step is the distribution phase of digoxin during which the half-life varies between 20 and 60 min. The rapid disappearance of digoxin from the central compartment is mainly due to a rise of concentration in the peripheral compartment rather than to the elimination of the glycoside. Extensive distribution of digoxin to the peripheral compartment is indicated by an average volume of distribution at steady state which greatly exceeds the average volume of the central compartment (REUNING et al., 1973; NYBERG et al., 1974; KOUP et al., 1975), and by the fact that the peripheral compartment is much larger than the volume of body water. The central compartment for digoxin includes principally the plasma. The peripheral compartment is mainly the skeletal muscle. The deep compartments include erythrocytes and brain where the equilibrium concentration is reached later than in most other tissue (GORODISHER et al., 1976; KUHLMANN et al., 1979). The extensive distribution of digoxin in tissue is reflected by the large volume of distribution. The total volume of distribution, calculated at steady state (V_{Dss}), varies in healthy subjects between 5.1 and 8.1 l/kg; in patients with cardiac failure is approximately 5.0 l/kg; and in renal failure 3·3 and 4·4 l/kg (REUNING et al., 1973; NYBERG et al., 1974; KOUP et al., 1975, 1976). The volume of distribution in neonates and infants is higher than that found in adults. The mean V_{DSS} in infants, 2–81 days old, is 9.9 l/kg (WETTRELL et al., 1974). The basis for this difference seems to be due, at least partially, to an increased tissue binding of digoxin in the young age group. Among other possible factors leading to the quantitative differences in volume of distribution is a change with age in body composition.

C. Elimination

I. Metabolism

Extensive investigations on the metabolism of digoxin show that structural modifications, including conjugation reactions with glucuronate and sulfate take place, which lower the lipid solubility and raise the water solubility. Metabolic steps in-

clude: (a) cleavage of the digitoxose residues; (b) conjugation reactions; (c) reduction of unsaturated lactone rings.

1. Cleavage of Digitoxose Residues

The cleavage of cardiac glycosides into the genin and sugar moiety by enzymatic hydrolysis has already been reported in the very early literature (FISCHER, 1928; KELSEY, 1951). The first conclusive analytical investigations, however, came from the research activities of OKITA et al. (1955) and REPKE (1959, 1966, 1970). Further confirmation of the catabolism pathways and identification of bis- and mono-digitoxosides of digoxin and digitoxin was provided by HAACK et al. (1957) and KAISER et al. (1957) with successful isolation studies on extracts obtained from the fermented leaves of *Digitalis purpurea* and *Digitalis lanata.*

The liver is the principal site for the cleavage of the β-glycoside–glycoside bond. The precise nature of the enzyme however is not yet clearly explained. A cleavage by β-glucosidase is unlikely since this enzyme has an absolute specificity for carbon atom 3 which in digoxin and digitoxin has the OH group linking the sugars in the epimer form (REPKE, 1970). The cleavage of a sugar chain is therefore probably not a simple hydrolysis step. A preliminary oxidation through NADPH-dependent enzyme systems of the liver must also be considered. Investigations on rat liver microsomal preparations have shown that the digitoxose groups of digitoxin are only released in the presence of NADPH and that the reaction can be inhibited by the microsomal drug metabolism inhibitor SKF 525A (Prodiafen; β-diethylaminoethyl diphenylpropyl-acetate) and can be inhibited by carbon monoxide (SCHMOLDT et al., 1975). Neonate liver is unable to carry out the cleavage reaction, thus supporting the participation of these postnatally acquired enzymes (HERRMANN and REPKE, 1963 a).

What conclusions regarding the pharmacokinetics and pharmacodynamics can be deduced? The first factor of importance is that the 50% inhibitory concentrations for inhibition of Na^+, K^+-ATPase by tris-, bis-, and mono-digitoxosides of digoxigenin and digitoxigenin lie between 0.1 and 1 nmol/l (REPKE, 1966, 1971). As to the quantitative differences in clinical efficacy, for example, for mono-digitoxosides and the genins in comparison with the tridigitoxosides, these are not solely attributable to differences in the activity spectrum. More important deciding factors are alterations in the pharmacokinetics of the sugar-deficient metabolites. There are three aspects to note.

The first aspect refers to the quantity of the various metabolites derived from digoxin or methyldigoxin. Moving from plasma to urine to feces the major fraction, unchanged glycoside, progressively falls. Furthermore the smaller quantities of sugar-deficient metabolites consist almost entirely of conjugates of mono- and bis-digitoxosides (Table 3).

The second aspect is the lipophilicity. The different absorption properties of digoxin and digitoxin are due to differences in their solubilities. The monoglycosides are strongly nonpolar as shown by their considerably higher chloroform solubilities and *n*-octanol/water distribution coefficients (Table 4). Thus it would be expected that the penetration of the mono-digitoxosides through lipid membranes would be at least equal and possible higher.

Table 3. Percentage of unchanged drug and metabolites following administration of digoxin and β-methyldigoxin

Drug		Plasma	Reference	Urine	Reference	Feces	Reference
Digoxin	Digoxin	80–90	[2]	68	[4]	44	[4]
				40–93	[1]		
				90	[8]		
	Digoxin-mono-digitoxoside			3.5	[4]	19	[4]
				0– 6	[1]		
	Digoxin-bis-digitoxoside			11	[4]	20	[4]
				0– 6	[1]		
	Dihydrodigoxin	0–40	[3]	7–47	[1]		
		0–30	[7]				
	Polar	0–17	[2]	1– 5	[4]	9–20	[4]
				2–10	[1]		
				5–10	[8]		
β-Methyl digoxin	β-Methyldigoxin	90	[5]	54	[5]	7–13	[6]
				31[a]	[6]		
				51[b]	[6]		
				59–69	[8]		
	Digoxin	10	[5]	40	[5]	26–34	[6]
				31[b]	[6]		
				50[a]	[6]		
				20–35	[8]		
	Digoxin-mono-digitoxoside			1	[6]	13–18	[6]
				1.5– 2	[8]		
	Digoxin-bis-digotoxoside			3	[6]	15–18	[6]
				2– 4	[8]		
	Polar	0	[5]	5–10	[8]	26[b]	[6]
				6	[5]	18[a]	[6]
				10	[6]		

[a] After oral dose; [b] after intravenous dose

[1] Clark and Kalman (1974)
[2] Rietbrock and Abshagen (1973)
[3] Peters et al. (1978)
[4] Marcus et al. (1966a)
[5] Hinderling et al. (1977)
[6] Rietbrock et al. (1975)
[7] Watson et al. (1973)
[8] Zilly et al. (1975)

Table 4. Physicochemical properties of digoxin, β-methyldigoxin, digitoxin, and digitoxigenin-mono-digitoxoside. (PETERSEN et al., 1977)

Glycoside	H_2O solubility	$CHCl_3$ solubility	*n*-Octanol/H_2O distribution coefficient
Digoxin	0.04	0.25	18
Methyldigoxin	0.13	45	54
Digitoxin	0.008	14	70
Digitoxigenin-mono-digitoxoside	0.092	107	390

Table 5. Half-life in plasma, total excretion, and fraction as polar metabolites of various cardiac glycosides. (RIETBROCK et al., 1977a)

Glycoside	Urine		Feces		$t_{1/2}$ (days)	Period (days)
	Daily dose (%)	Polar fraction (%)	Daily dose (%)	Polar fraction (%)		
Digoxin	70.3±2.4	5	14.1±2.5	18	1.5±0.3	0–7
Digoxigenin-mono-digitoxoside	19.6±5.0	57	59.2±7.0	21	0.7±0.2	0–6
Digitoxin	22.5±1.6	23	16.1±1.7	21	6.8±0.5	0–8
Digitoxigenin-mono-digitoxoside	44.2±7.6	87	47.5±6.4	12	2.3±0.2	0–8

The third aspect refers to the elimination rate. While the bis-digitoxosides have a similar pharmacokinetic profile to the original compounds (REPKE, 1972), this is changed considerably on formation of the mono-digitoxosides (Table 5). The half-life becomes significantly shortened. Simultaneously, the metabolism rate is elevated because of the stronger intervention of the liver in the elimination process. The elimination of glycoside thus becomes more independent of the renal function (RIETBROCK et al., 1977a).

2. Conjugation Reactions

Conjugation reactions with sulfate and glucuronide must be considered as factors in the biotransformation and rapid elimination of the mono-glycosides and aglycones (HERRMANN and REPKE, 1963a, b; KUHLMANN et al., 1974).

For a long time the view was held that these conjugation reactions could only be effected after completely splitting off the digitoxosides and subsequent epimerization of the hydroxyl group on carbon 3 of the aglycone. Measurements at the epimerization position by HERRMANN and REPKE (1963a) established however that this is already restrictively fixed, and that the ability of the human liver to carry out elimination of digitoxigenin through initial epimerization is unlikely. How do these observations fit in with the rapid biotransformation of mono-digitoxosides?

For a number of years the possibility of direct conjugations with tri-digitoxosides and their sugar-deficient metabolites has been considered. Almost all the relevant studies left room for contradictory interpretations. Characterization of the metabolites on the basis of solubility and estimations following conjugate hydrolysis with β-glucuronidase and sulfatase were the methods utilized in nearly all the investigations reported. Up to the present time, direct structural analysis of the chloroform-soluble metabolites of plasma, urine, and feces has been frustrated owing to difficulties in isolation and the small quantities recovered. Despite the reservations already mentioned and a deficiency in definitive methodological alternatives, chromatographic analysis reveals two distinct pathways: the direct conjugation of the mono-digitoxoside and the conjugation, (possibly confined to the rat only) of the 3 epimer of the aglycone (HERRMANN and REPKE, 1963 a, b; RIETBROCK and VÖHRINGER, 1974). Both reaction steps lead to a considerable increase in elimination rate (REPKE, 1970).

It is to be hoped that further progress in the clarification of biologic efficacy and pharmacokinetics will result from the recent synthesis of some specific cardenolide glucuronides and cardenolide suphates by PETERSEN et al. (1977). In the case of digoxin-16′-glucuronide for example it was observed that the water solubility is increased by a factor greater than 10^3 and the chloroform solubility decreased by a factor greater than 10^4 (PETERSEN et al., 1977).

The cardioactivity of conjugates retaining at least one digitoxose group is comparable to digoxin. In contrast, the cardioactivity of the cardenolide–genin conjugate is at least 10% lower. The higher polarity of these compounds allows further conjecture that their diffusion properties are similar to those of ouabain, their elimination rates high, and their accumulation therefore comparatively low (PETERSEN et al., 1977).

3. Hydrogenation

In the last few years several reports have been published on the occurrance of dihydro compounds in plasma and urine of humans (LUCHI and GRUBER, 1968; WATSON et al., 1973; CLARK and KALMAN, 1974; PETERS et al., 1978). The saturation of the double bound between C20 and C22 is effected by intestinal microorganisms (HERRMANN and REPKE, 1968). Application of appropriate experimental procedures excluded the involvement of a hepatic process or a transformation through enzymes of the intestinal wall. Whether the resulting cardanolide has the 20α or the 20β configuration is unknown at the present time.

The microorganisms responsible are probably anaerobic since saturation of the lactone ring becomes completely suppressed, when for example, lanatoside C is incubated with cecum contents in the presence of oxygen (HERRMANN and REPKE, 1968). Since the hydrogenation occurs in the lower intestine the appearance of the cardanolide in urine is retarded with a clearly defined lag phase. Formation and absorption of hydrogenated compounds is subject to considerable individual variation depending on the nature of the bacterial flora of the gut and the bioavailability of the parent glycoside. The cardioactivity of dihydrodigoxin is only 1/20 of that for digoxin (HERRMANN and REPKE, 1968).

At about the same time LUCHI and GRUBER (1968) published a case study on a 57-year-old patient who required a daily dose of 2–3 mg digoxin for the maintenance of compensation. Whilst the half-life of total radioactivity following a dose of radiolabeled digoxin was 35 h and the fraction excreted in the urine in the first 24 h was 26%, these values being within the limits usually encountered, the degree of metabolism was extremely high with a value of 57% of which 15% was dihydrodigoxigenin.

WATSON et al. (1973) succeeded in the determination of dihydrodigoxin in plasma and urine using gas chromatography–mass spectroscopy (GC–MS). Dihydrodigoxin is reduced digoxin and its detection showed that glycolysis is not a necessary prerequisite for reduction. A high dihydrodigoxin concentration in plasma was detected in 3 of 150 patients. In one case the concentration amounted to 30% of the total glycoside and it was surprising, in view of the earlier report from LUCHI and GRUBER (1968), that none of the patients required an unusually high daily dose of digoxin. The highest daily dose administered was 1 mg and the lowest 0.25 mg. CLARK and KALMAN (1974) examined the distribution and excretion of metabolites in 50 patients on maintenance therapy with 0.125–0.75 mg digoxin daily. From 2% to 10% of the glycosides excreted in the urine were polar, water-soluble metabolites. 50% of the patients excreted dihydrodigoxin, mean value 13%, with a range of 1%–47%, of the total glycoside output.

The clinical importance of such dihydro compounds must be evaluated from two aspects. The first is with regard to the radioimmunoassay of glycoside concentration in plasma and urine. It is known that digoxin and digitoxin antibodies are "far-sighted" in their discriminating capacity so long as no structural alterations have taken place in the basic steroid nature of the molecule (LARBIG and KOCHSIEK, 1972). After hydrogenation of the lactone ring however, the affinity almost completely disappears (RIETBROCK et al., 1977 b). Out of nine antibodies, six of which were obtained from commercial sources, one commercial preparation with a cross reactivity against dihydrodigoxin of more than 10% should not be used for the estimation of cardiac glycoside fractions in plasma and urine (RIETBROCK et al., 1977 b). The second aspect is the large variation in glycoside plasma concentration for a given dose, where the coefficient of variation normally ranges from 0.2 to 0.6. After an intravenous dose of digoxin and after an oral dose of β-acetyldigoxin only 61% and 39% of the total variance (ng^2/ml^2) respectively can be explained in terms of differences in body weight, age and serum creatinine (RIETBROCK et al., 1978).

It remains to be seen in further investigations whether individual differences in transformation of digitalis glycosides into their dihydro compounds has an effect on the variation of glycoside plasma concentrations. Animal experiments have shown that dihydrodigoxin persists in the tissue of the dog for an extremely short period (KUHLMANN, 1978). Furthermore, the presence of a plasma–tissue concentration gradient of approximately 1.0 indicates the absence of an organ-specific distribution.

II. Excretion

The principle route of digoxin and β-methyldigoxin elimination is via renal excretion. After a single intravenous or oral dose the glycosides can be detected in sig-

Table 6. Renal excretion of digoxin, β-methyldigoxin, α-acetyldigoxin, and β-acetyldigoxin

Reference	Preparation (mg)	Dose	Renal excretion (days/%)	Comments
HUFFMANN and AZARNOFF (1972)	Digoxin	0.5 i.v.	10/57	
	Digoxin	0.5 p.o.	10/40	Fasting
GREENBLATT et al. (1973)	Digoxin	0.75 i.v.	6/76	1 h Infusion
	Digoxin	0.25 p.o.	6/41	
DENGLER et al. (1973)	^3H-Digoxin	0.25 i.v.	6/70	
	^3H-Digoxin	0.25 p.o.	6/50	Fasting
SANCHEZ et al. (1973)	Digoxin	0.25 p.o.	5/39	Fasting
	Digoxin	0.25 p.o.	5/38	
GREENBLATT et al. (1974)	Digoxin	0.75 p.o.	6/41	Fasting
	Digoxin	0.75 p.o.	6/36	
GREENBLATT et al. (1973)	Digoxin	0.75 i.v.	6/69	
	Digoxin	0.75 i.v.	6/76	Infusion
MARCUS et al. (1976)	Digoxin	0.4 i.v.	6/60	1 h Infusion
	Digoxin	0.4 i.v.	6/70	3 h Infusion
	Digoxin	0.4 p.o.	6/52	Fasting
LINDENBAUM (1973)	Digoxin	0.5 p.o.	1/25	Fasting
FLASCH (1975)	Digoxin	0.6 i.v.	6/50	
	Digoxin	0.75 p.o.	6/32	Fasting
	β-Acetyldigoxin	0.6 p.o.	6/41	Fasting
KLOTZ et al. (1976)	Digoxin	0.6 i.v.	8/39	1 h Infusion
	β-Acetyldigoxin	0.6 p.o.	8/32	Fasting
BOCHNER et al. (1977)	Digoxin	0.5 i.v.	2/35	
	Digoxin	0.5 p.o.	2/23	Fasting
BONELLI et al. (1977)	β-Acetyldigoxin	1.0 p.o.	4/41	Fasting
DANON et al. (1977)	Digoxin	0.5 p.o.	5/44	Fasting
GREEFF et al. (1977)	Digoxin	0.5 i.v.	7/67	
	Digoxin	0.5 p.o.	7/28	
	Digoxin	0.5 p.o.	7/37	Fasting
	β-Acetyldigoxin	0.6 p.o.	7/42	
	β-Acetyldigoxin	0.6 p.o.	7/45	Fasting
	β-Acetyldigoxin	0.5 p.o.	7/45	
	β-Methyldigoxin	0.5 i.v.	7/64	
	β-Methyldigoxin	0.5 p.o.	7/41	
	β-Methyldigoxin	0.5 p.o.	7/40	Fasting
RIETBROCK et al. (1975)	^3H-β-Methyldigoxin	0.2 i.v.	7/62	
	^3H-β-Methyldigoxin	0.2 p.o.	7/55	
ECKSTEIN and KÜHNE (1976)	^3H-β-Acetyldigoxin	0.29 p.o.	7/58	
	^3H-α-Acetyldigoxin	0.27 p.o.	7/57	

i.v. = intravenous; p.o. = oral

nificant quantities in urine over a period of about 6 days (DOHERTY and PERKINS, 1962; RIETBROCK et al., 1975). On maintenance doses the deep compartments of the body accumulate digoxin with the result that the elimination of digoxin on discontinuation of treatment is slower than after a single oral dose (RIETBROCK et al., 1976).

Biliary secretion of digoxin, methyldigoxin, and acetyldigoxin makes a smaller but significant contribution to total elimination (MARCUS et al., 1966a; DOHERTY

Table 7. Cumulative urinary excretion of cardiac glycoside (% dose): comparison of intravenous and oral doses

Urine collection period (days)	Digoxin		β-Methyldigoxin	
	i.v. sol.	Oral sol.	i.v. sol.	Oral sol.
2	45 [1]		33 [1]	
7	74 [3]	34 [3]	62 [2]	55 [2]
	64 [1]		64 [1]	
14		50 [5]	82 [4]	75 [4]
Extrapolated to infinite time	67 [1]		78 [1]	

1) GREEFF et al. (1977)
2) RIETBROCK et al. (1975)
3) DOHERTY et al. (1970)
4) BEERMANN (1972)
5) BEERMAN et al. (1972)

et al., 1969; ECKSTEIN and KÜHNE, 1976; RIETBROCK et al., 1977a). It may be the dominant elimination pathway in patients with severe renal insufficiency and partly compensates for the reduction in renal elimination (BLOOM and NELP, 1966; MARCUS et al., 1966b; DOHERTY et al., 1967).

Prediction of the digoxin elimination rate has received much attention in recent years for the development of prescribing aids such as nomograms (ARONSON, 1978). It is becoming increasingly apparent that the elimination of digoxin is a complex process, readily modified by physiologic, pharmacologic, and pathologic factors as explained in the following discussion.

1. Renal Excretion

After a single intravenous dose of digoxin or methyldigoxin given in a radiolabeled form as a trace dose, or as a therapeutic dose of unlabeled drug, the proportion of the dose recovered in the urine in patients with normal renal function, is in the region of 60%–70% over a collection period of 5–7 days, in most studies reported (Table 6). The urinary excretion of cardiac glycoside varies considerably between the different studies and also between individual subjects. Differences between studies reflect mainly bioavailability differences between the pharmaceutical preparations. In a group of six healthy subjects, digoxin renal elimination varied from 50% to 80% of the dose during maintenance dosage. This variation represented differences in bioavailability for the same preparation in different patients (KONGOLA et al., 1976; MAWER, 1980). After oral doses the quantity of glycoside recovered in the urine is less than after intravenous administration, even when the oral dose is in solution (Table 7).

2. Renal Excretion of Metabolites

Most of the dose of digoxin or methyldigoxin appears in urine as unchanged drug (Table 3). DOHERTY and KANE (1975) reported that 95%–98% of digoxin recovered in a 7-day urine collection was unchanged drug but other studies (WATSON et al., 1973; CLARK and KALMAN, 1974) utilizing GC–MS, indicate that the degree

of metabolism is more variable. After oral doses of digoxin and methyldigoxin, metabolites in the urine include small amounts of digoxigenin, mono-digitoxosides, and bis-digitoxosides (Table 3) and occasionally appreciable quantities of reduced digoxin and corresponding metabolites. Reduced digoxin and the dihydrometabolites may, in some patients, be the most abundant group of metabolites. Of these dihydrodigoxin is the principal metabolite. These derivatives appear to be formed in the gastrointestinal tract by microorganisms and absorbed as such (see Sect. C.I).[1] They can account for as much as 50% of the total extractable digoxin and metabolites in urine and a mean value of about 15% of total glycoside can be expected (CLARK and KALMAN, 1974; PETERS et al., 1978; PETERS and KALMAN, 1978). Occurrence of significant quantities of dihydrometabolites in urine is not influenced by age, sex, dose, or blood level of digoxin and could be dependent on diet or gut flora (PETERS et al., 1978). Half-lives for disappearance of dihydrodigoxin were very short, 1.2 h in patients with normal renal function. This can be compared with 11.5 h, 8.5 h, and 2 h for digoxin-bis-digitoxoside, digoxin-mono-digitoxoside, and digoxigen respectively, obtained after a single dose of radiolabeled digoxin (GAULT et al., 1979) and with 16.8 h for the half-life of digoxin-mono-digitoxoside following maintenance digoxin treatment (Table 5).

Urinary excretion of ^3H-α-acetyldigoxin and ^3H-β-methyldigoxin by healthy volunteers was, in one study, 60% and 50% respectively of which 85%–90% was digoxin (ECKSTEIN and KÜHNE, 1976). HINDERLING et al. (1977) also studied ^3H-methyldigoxin elimination in volunteers after administration of an intravenous bolus. Six day urinary excretion was 80% of the dose of which 32% was digoxin and 43% unchanged β-methyldigoxin. Almost identical data were reported in an earlier study by RIETBROCK et al. (1975) and Table 3.

3. Factors Influencing Renal Elimination

BLOOM and NELP (1966) were the first to publish data showing a relationship between the renal clearance of digoxin and creatinine. Since the major part of digoxin is excreted by the kidney and a good correlation between creatinine and digoxin clearance is apparent, it has been generally accepted that digoxin is excreted by glomerular filtration, where 60%–70% of the total plasma digoxin concentration passes into the ultrafiltrate, and creatinine clearance is used as a measure of digoxin renal clearance (IISALO, 1977; see Sect. C.II.6). However, STEINESS (1974) has reported that half of the urinary digoxin in humans is excreted by active tubular secretion and half by glomerular filtration, and a similar finding was later reported for the rat (ROMAN and KAUKER, 1976). These observations do not in themselves reduce the usefulness of creatinine clearance as an index of digoxin clearance. Indeed, creatinine is an nonpolar molecule like digoxin and presumeably equally susceptible, as judged from clearance studies on nonpolar compounds, to active transport by anion and cation transport systems or both (WEINER, 1973). DOHERTY et al., (1969) reported evidence for the tubular reabsorption of digoxin at both the proximal and distal sites in the nephron of the dog. Evidence based on micropunc-

1 Dihydrodigoxin can be detected in urine of patients after intravenous digoxin administration (PETERS, personal communication)

ture studies has shown that ^{3}H-labeled digoxin in rats is absorbed from the proximal convoluted tubule but not from the Loop of Henle (ROMAN and KAUKER, 1976).

STEINESS (1974) showed that spironolactone (Aldactone), an aldosterone antagonist, inhibits the tubular secretion and thereby the renal clearance of digoxin. In a later study from the same group of investigators (WALDORFF et al., 1978) the renal clearance of digoxin was measured after a single 0.75 mg dose of digoxin given intravenously. It was alleged that in the period 0–24 h spironolactone is without significant effect on digoxin renal clearance, because the fraction eliminated by tubular secretion is small in comparison with the fraction excreted by glomerular filtration and because the secretion process is saturated at the high serum digoxin concentrations. In contrast, renal digoxin clearance in the period 24–48 h and 48–144 h is lowered by spironolactone because the active renal secretion process at the much lower digoxin concentrations prevailing carries a relatively larger proportion of the total digoxin eliminated by the kidney. Corresponding to these changes, the plasma digoxin clearance was decreased by a mean value of 26% but the range was large (+2% to −74%). An average increase of about 30% in the steady-state plasma concentration was to be anticipated (WALDORFF et al., 1978). These studies refer to single doses of digoxin and their significance with regard to patients on maintenance therapy is uncertain. Moreover, the *absence* of an effect of spironolactone on digoxin elimination has also been reported (OHNHAUS and MASSON, 1977). This study brings into question the former interpretation since OHNHAUS and MASSON used small intravenous doses (0.1 mg) of labeled digoxin thus avoiding the problem, if it exists, of saturation of the secretion process.

That hypokalemia may lead to the development of digitalis toxicity in digitalis patients receiving a normal digoxin dose and with plasma concentrations in the therapeutic range is widely appreciated. STEINESS (1978) however has reported that digoxin toxicity in hypokalemic patients is compounded by a reduced active renal tubular secretion of digoxin in humans, an effect already observed earlier in dogs (MARCUS et al., 1971). All patients were receiving both digoxin and furosemide maintenance treatment and inulin clearance was used as the baseline for changes in clearance. When patients received potassium supplements, the tubular secretion was restored to normal. These changes are regarded by the authors as significantly increasing the risk of digoxin-induced arrhythmias by lengthening the plasma half-life of digoxin and raising the plasma digoxin concentration. MALCOLM et al. (1977) measured the urinary excretion of a single 0.75 mg oral dose of digoxin in eight healthy subjects before and during the administration of oral furosemide 40 mg daily, and observed no significant effect for paired comparisons on the digoxin renal excretion. The authors compared digoxin excretion with the urine flow and put forward their observations as confirmation of the results of BISSETT et al. (1973) that there exists no dependence of digoxin excretion on the urine flow rate. Although the validity of their data is supported by the studies of BROWN et al. (1976), TILSTONE et al. (1977), and TSUTSUMI et al. (1979) where no significant effect of furosemide on digoxin renal elimination was found and by the report from BACZ'YNSKI and KOKOT (1978) with a similar finding in the case of β-methyldigoxin, their conclusions can be criticised. The possibility that augmented urine flow could abbreviate the time for tubular reabsorption and result in the elimination of more

digoxin in the urine was originally put forward by BISSET et al. who subsequently, and possibly prematurely, refuted their own hypothesis. BISSET et al. obtained their data on two patients who had nephrogenic diabetes insipidus and MALCOLM et al., TILSTONE et al., and TSUTSUMI et al. studied healthy subjects. All these studies involved an increase in urine flow, over and above the normal flow rate which cannot be equated with changes in urine flow that occur in patients with congestive cardiac failure. Congestive cardiac failure patients usually have a low urine flow rate and a digoxin tubular reabsorption higher than normal might thus be expected. Little weight can be given to the report of BROWN et al. who studied a small mixed group of subjects, four cases with heart disease and two volunteers without heart disease. In the case of the β-methyldigoxin study, the characteristics of the urinary elimination of β-methyldigoxin are known to differ from those for digoxin (RIETBROCK et al., 1975; ZILLY et al., 1975; HINDERLING et al., 1977). Moreover TSUTSUMI et al. produced evidence that furosemide *inhibits* the tubular secretion of digoxin [see STEINESS, (1978) above], thus casting doubt on the value of furosemide studies as a basis for conclusions on the relationship between urine flow rate and digoxin renal elimination. Thus, HALKIN et al., (1975) in studies on 35 patients under treatment in hospital for congestive cardiac failure or for atrial fibrillation found that variation in digoxin clearance in these patients was more related to urine flow and urea clearance than to creatinine clearance. Because of the effect of urine flow on digoxin clearance, it was suggested by HALKIN et al. that urea clearance may therefore be a better way of inferring what the situation is at any time in the patient with cardiac conditions requiring digoxin (see Sect. C.II.6).

Other drugs having actions on digoxin renal elimination are quinidine, which produces a reduction in renal clearance and an increase in serum digoxin concentrations of 2.5 times by an action which may involve inhibition of digoxin secretion (DOERING, 1979; HAGER et al., 1979), and alcohol, which may act indirectly through a reduction in digoxin biovailability when consumed simultaneously with digoxin tablets (SCHWABE et al., 1979).

4. Extrarenal Excretion

KOUP et al. (1975) found a mean value for total body clearance of digoxin of 188 ± 44 ml/min/1.73 m^2 which was significantly higher than that for digoxin renal clearance 144 ± 41 ml/min/1.73 m^2, despite the difference being relatively small in comparison with the standard deviation of the mean. These data together with similar data from SUMNER and RUSSELL (1976), RIETBROCK et al., (1977a) give fractions of 18%–28% for digoxin and 27% for methyldigoxin eliminated by non-renal routes in patients with normal renal function.

According to CALDWELL and CLINE (1976), about 30% of an intravenous dose of ^3H-digoxin reaches the digestive tract in 24 h. About 45%–50% of the radioactivity recovered in the stools after a dose of labeled digoxin consists of unchanged drug, from 10%–20% digoxigenin-bis-digitoxoside and 25% digoxigenin-mono-digitoxoside (DOHERTY and KANE, 1975).

In patients with renal failure the reduced renal elimination rate (Table 6) is partly compensated by extrarenal elimination. BLOOM and NELP (1966) found a

much larger quantity of digoxin in the stools of patients with renal failure than in normal subjects, without any indication of impaired gastrointestinal absorption. In anephric subjects, fecal elimination of ^3H-digoxin increased several fold (DOHERTY et al., 1968) indicating that this pathway is usable when renal excretion is unusable or limited by disease.

5. Effect of Extrarenal Excretion on Bioavailability

A low biovailability of digoxin and methyldigoxin was reported some years ago (LINDENBAUM, 1973; for a review see KELLER and RIETBROCK, 1977). Although numerous studies on new tablet formulations have subsequently been made and a variety of methods for estimating bioavailability applied, bioavailability values much greater than 60% for digoxin and 70% for methyldigoxin have not been achieved. Furthermore, even in the case of digoxin and methyldigoxin solutions the bioavailability is below 100% (Table 6; GREENBLATT et al., 1976). RIETBROCK et al., (1975) determined the absorption of ^3H-β-methyldigoxin in 12 healthy subjects after single oral and intravenous doses given as a solution. After the oral dose the urine and fecal excretion of total radioactivity was $62.2\% \pm 2.1\%$ and $29.0\% \pm 5.2\%$ of the dose respectively. After the intravenous dose the urine and fecal excretion was $55.2\% \pm 2.8\%$ and $28.6\% \pm 5.7\%$ respectively thus indicating almost complete absorption of the glycoside when given orally as a solution although considerable quantities of the drug or its metabolites were found in the stools. Methyldigoxin and digoxin thus appear to be completely absorbed and to have some enterohepatic recycling similar to that reported for mono-digitoxosides (KUHLMANN et al., 1974; VÖHRINGER, 1978).

6. Prediction of Digoxin Elimination

Like many other drugs used in clinical practice digoxin elimination can be described by a first-order kinetic model. In a single-compartment model the rate of drug elimination, dC/dt, after a single dose is given by $dC/dt = -KC$ where K is the elimination constant and C is the total drug in the body at any given time. From integration we obtain the equation for the elimination half-life $t_{1/2}$ which expresses the rapidity with which the drug is eliminated from the body. $t_{1/2} = \ln 2/K$.

As descriptions of the rate at which a drug is eliminated from the body, both K and $t_{1/2}$ are dependent on the apparent volume of distribution V_D. Thus the primary pharmacokinetic parameter that rigorously characterises the kinetics of drug elimination is the elimination clearance *(Cl)*. Since $K = Cl/V_D$ we obtain the equation $t_{1/2} = 0.693\ V_D/Cl$ showing the relationship between the apparent volume of distribution, clearance and the half-life. For digoxin the global clearance, Cl, is the sum of the renal clearance and the extrarenal or fecal clearance.

Since digoxin has a narrow therapeutic index, is eliminated predominantly in the urine, and is used frequently in patients with renal insufficiency the assessment of digoxin clearance is of paramount importance in maintenance dosage estimation. Several workers have published equations and nomograms intended to aid the physician in deciding on the most suitable dosage regimen for the individual

Table 8. Failure of serum creatinine measurements to predict creatinine clearance in patients with severe cardiac failure (DOBBS et al., 1976)

Degree of cardiac failure	Number of patients	Creatinine clearance (ml/min)[a]		Paired *t* test	
		Measured	Predicted	*t*	*p*
Severe	20	34.5±8.5	46.6±10.5	3.1	<0.01
Less than severe	31	55.2±6.5	55.4± 6.8	0.1	>0.9

[a] Standard errors of mean are given

patient (DETTLI et al., 1972; SHEINER et al., 1972; BÄTTIG et al., 1974; DOBBS et al., 1976; SUMNER and RUSSELL, 1976; GAULT et al., 1976). All the nomograms or equations require knowledge of the creatinine clearance from which the clearance of digoxin is estimated and utilized to compute the appropriate maintenance dose to arrive at the predicted concentration. A comparison of the different equations and nomograms has been made by ARONSON (1978) who observed that the equations of TOZER (1974) and DOBBS et al. (1976) satisfactorily predicted the correct maintenance dose in a group of 24 patients with atrial fibrillation or cardiac failure and predicted creatinine clearances in the range 10–138 ml/min.

DOBBS et al. (1976) examined the relationship between measured creatinine clearance and the corresponding predicted creatinine clearance based on the data of SIERSBAEK-NIELSEN et al. (1971) utilizing serum creatinine measurements. The predicted creatinine clearance was not valid in 20 patients with more severe symptoms out of the total of 51 cardiac failure patients examined (Table 8). The 20 patients with more severe cardiac symptoms had a 25% lower mean measured clearance in comparison with the remaining 31 patients with less severe cardiac failure and the predicted clearance was approximately 35% higher than the measured values. In patients with severe cardiac failure it is therefore necessary to measure, rather than predict the creatinine clearance before computing the appropriate dose. These results however indicate a qualitative and quantitative change in handling of creatinine by the body and therefore a possible source of nonparallelism between creatinine and digoxin handling by the kidney.

Whilst recognizing that attention to the most appropriate method for the determination of creatinine in various disease states will improve digoxin prescribing, there is a significant body of evidence to indicate that renal clearance of creatinine may not always be the best index of digoxin renal clearance. In Sect. C.II.3 reference was made to the studies of HALKIN et al. (1975) who reported a better correlation in congestive cardiac failure patients between digoxin renal clearance and urine flow rate and between digoxin renal clearance and urea clearance. Some years earlier BAYLISS et al. (1972) in observations on elderly ambulent patients found no significant correlation between digoxin clearance and creatinine clearance and suggested that the lack of correlation probably reflected tubular secretion of creatinine and reabsorption of digoxin. In 1972 KRAMER and SCHELER observed that digoxin clearance is mainly a function of the glomerular filtration rate (expressed as creatinine clearance) and serum protein binding but LUCCHINI et al. (1979) using

the more accurate ^{131}I-clearance technique for measurement of glomerular filtration rate found that in 14 hospitalized adult patients with compensated chronic heart failure the correlation between digoxin renal clearance and glomerular filtration was better than for creatinine clearance. LUCCHINI et al. however could only account for about 60% of the variation in digoxin clearance leaving 40% unexplained. This shortfall in the predictive capacity of glomerular filtration is however no better than that for most other previously reported models based on creatinine clearance (ARONSON, 1978) and therefore the possible introduction of glomerular filtration as an index of digoxin renal elimination must await further investigations.

The previous discussion on the elimination of digoxin by renal and extrarenal routes has indicated that changes in renal tubular secretion, tubular absorption, and biliary secretion of digoxin are possible factors in patients with cardiac failure that lead to difficulties in predicting digoxin clearance.

7. Acceleration of Digoxin Elimination

The therapeutic attempt to shorten the duration of toxicity of cardiac glycosides in the body has resulted in a variety of possible procedures but all have their limitations, in most instances because of the high tissue binding of cardiac glycosides. The prompt treatment by intravenous furosemide may be beneficial in the management of massive digitalis overdose (ROTMENSCH et al., 1978), but hemodialysis and similar physical techniques are probably more reliable. Mean dialyzance for digoxin of up to 28 ml/min (IISALO and FORSSTROM, 1974; VAN DER VIJGH, 1977; GILFRICH et al., 1978) and for methyldigoxin 25 ml/min (KRAMER et al., 1972) have been obtained. The efficacy of hemodialysis is low (3%–5%) if estimated in relation to a single dose injected before dialysis and high (30%–50%) if estimated in relation to the excretory capacity of normal kidneys during a period corresponding to the duration of dialysis (KRAMER, 1977). In practical terms this relatively low efficacy means however that hemodialysis is only of limited value in life-threatening digoxin intoxication (GAULT et al., 1976; RISLER et al., 1979).

Hemoperfusion over charcoal was applied on an experimental basis in the clinical field for barbiturate intoxication as early as 1965 (YATZIDIS et al., 1965). Recently its application in the treatment of digitalis toxicity has received attention (GILFRICH et al., 1978; KRAMER et al., 1977; BISCHOFF et al., 1977; RISLER et al., 1979). In dogs with chronic digitalis intoxication the clearances of digoxin, β-methyldigoxin, and β-acetyldigoxin using hemoperfusion over charcoal ranged from 36–43 ml/min and marked improvement of cardiac arrythmias were observed. These data are comparable to the mean clearance values obtained from in vitro studies where at a blood flow rate of 100 ml/min the digoxin clearance over charcoal was 51 ml/min (GILFRICH et al., 1978). RISLER et al. (1979) reported that the non-ionic polymeric absorbent Amberlite XAD 4 can remove as much digoxin as normal human kidneys, but that the amount of digoxin removed was only a small percentage of the total body pool. After use of the technique in a patient, these authors concluded that compared with the risks of hemoperfusion as an invasive treatment, its effect was small and accordingly could not be recommended as a standard therapeutic procedure for severe digoxin intoxications.

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CHAPTER 3

Pharmacokinetics of Strophanthus Glycosides

K. GREEFF and K. E. WIRTH

A. Introduction

Of the therapeutically useful strophanthus glycosides, only those of strophanthidin K or ouabagenin (strophanthidin G) are now used in a pure form. They are present in a variety of plants but are mainly extracted from the seeds of the African *Strophanthus kombé* or *S. hispidus* (strophanthoside K), the seeds of *S. gratus*, or from the wood of the ouabaio tree *Acocanthera schimperi* (ouabain). Chemically they differ from the digitalis glycosides in a different substitution of the aglycone and sugar residues (Fig. 1).

Ouabain, in contrast to digitoxin, possesses a CH_2OH group at C10, a hydroxyl group at both C1 and C5, and rhamnose instead of 3-digitoxose as sugar residue, thereby making ouabain highly polar or water soluble. Dihydroouabain (hydroxylated at the lactone ring) has been used for certain experimental purposes; despite being 1,000 times less potent than ouabain, its onset of action and subsequent washout in isolated heart preparations are, however, more rapid.

Strophanthoside K (γ-strophanthin-K), in contrast to ouabain, possesses a CHO group at C10, no hydroxyl group at C1 and C11 and a cymarose and two glucose residues (Fig. 1). β-strophantin-K amd α-strophantin-K (cymarin) are formed by splitting off the glucose residues. Cymarol, a metabolite of cymarin, contains a CH_2OH group instead of the CHO group. Dihydrocymarin has a saturated lactone ring.

Convallatoxin (from *Convallaria majalis*) and Helveticosol (from *Erysimum canescens*) are composed of the aglycone strophanthidin K and the sugar residues rhamnose and digitoxose, respectively. The short-acting acetylstrophanthidin, which is of only experimental value, is strophanthidin K with the acetyl group at C3.

B. Enteral Absorption

I. Human Investigations

1. Ouabain

Since 1885 when FRASER recommended the use of a tincture made of strophanthus seeds for the treatment of cardiac insufficiency the discussion on the oral efficacy of strophanthus glycosides has continued. FRAENKEL (1906) introduced intravenous (i.v.) injection of ouabain, because he found this glycoside to be relatively ineffective on oral administration. LINSENMEIER (1909) observed that high oral doses of 10–30 mg ouabain were necessary to compensate for cardiac insufficiency,

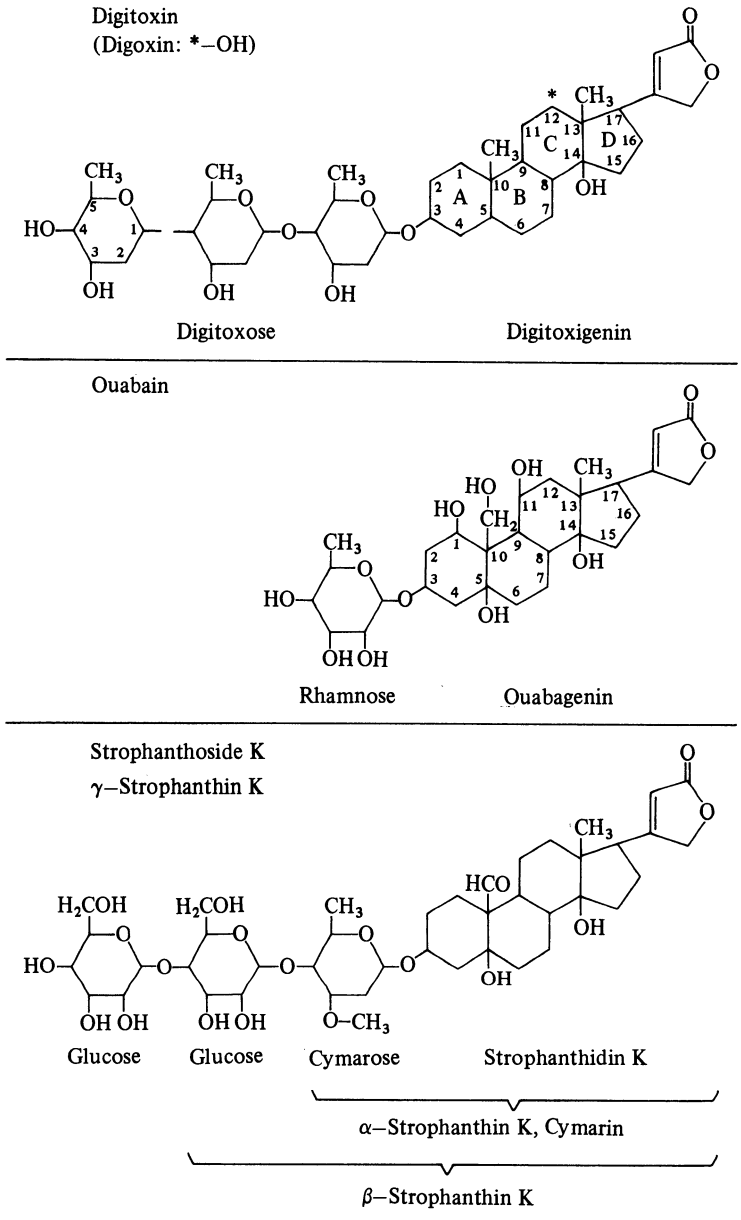


Fig. 1. Chemical constitution of ouabain and the glycosides of strophanthidin K; for derivatives see also Fig. 6

FRAENKEL, however, in 1933 maintained that this therapy was unreliable and sometimes ineffective. HOCHHEIM (1906) reported therapeutic success with high oral doses of strophanthin G up to 30 mg/day. He further noted no accumulation of strophanthin G. EGGLESTON and WHITE (1927) found that ouabain had no effect in doses of up to 5.2 mg perlingual. SARRE (1952) found that an oral dose of 3 or 1.5 mg perlingual resulted in the same therapeutic effect as 0.25 mg i.v. REINDELL

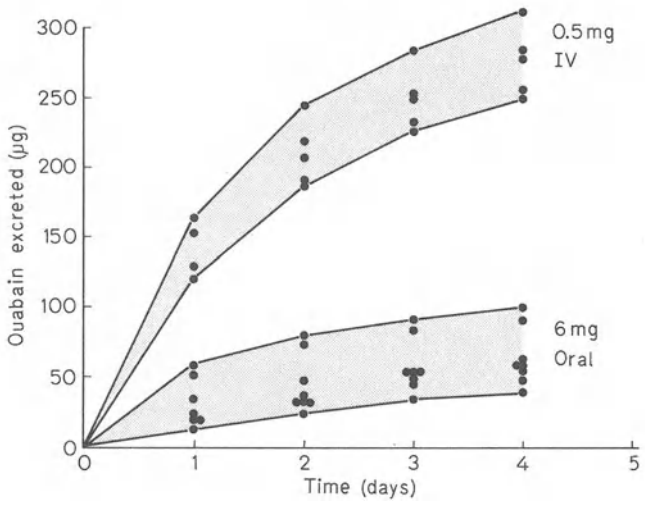


Fig. 2. Renal excretion of ouabain after intravenous injection of 0.5 mg or oral administration of 6 mg in healthy volunteers. Renal ouabain excretion was calculated by extrapolation to infinity after i.v. injection with 328 µg (66%) and after oral application with 79 µg (1.3%). (GREEFF et al., 1974)

et al. (1952) confirmed on the one hand the oral efficacy of ouabain, but its unreliable absorption on the other. PISCITELLO and MAGGI (1973) investigated the efficacy of an alcoholic ouabain solution and found with 75–170 µg/kg given orally, a dose-dependent shortening of the left ventricular ejection time and ejection time index measured according to WEISSLER and SCHOENFELD (1970). A case of lethal ouabain poisoning was reported in 1952 in a patient, who was receiving a daily oral dose of 6–12 mg ouabain. By mistaking ouabain tablets for methadone, an extra single dose of 7.5–10 mg led to stenocardia, atrioventricular block, and cardiac arrest (NEUGEBAUER, 1960).

LAHRTZ et al. (1968) were unable to measure radioactivity in the serum of patients, who had received ^{3}H-ouabain orally and intraduodenally, whereas MARCHETTI et al. (1971) found an absorption rate of 7% after 5 h. The enteral absorption of ouabain was measured, using radioimmunoassay (RIA), comparing renal excretion after i.v. and oral administration (VERSPOHL, 1973; GREEFF et al., 1974; GREEFF, 1977). Smaller amounts of the glycoside were found in urine after a single oral dose of 6 mg ouabain, than after an i.v. injection of 0.5 mg (Fig. 2). Absorption quotients of 0.7%–3.0% (mean 2.2%) were calculated. Using ^{3}H-ouabain ERDLE et al. (1979) confirmed the low and irregular absorption of the glycoside when administered sublingually or orally.

2. Strophanthoside K

GHIRARDI et al. (1973) measured a mean absorption of 31% within 24 h after the rectal application of 250, 500, and 1,000 µg ^{3}H-strophanthoside K to humans. There seem to be no further investigations on the absorption of strophanthoside K in humans.

3. Cymarin

Recent clinical experience has shown that cymarin is absorbed more efficiently than ouabain. This glycoside was brought on to the market in tablet and ampule form as early as 1913 (SCHUBERT, 1913) and clinically investigated (ALLARD, 1913). GEISSBERGER (1961) and SCHWARZBACH and HERMSTEIN (1967) calculated an absorption quotient for cymarin of 15%–25% with a dose of 1.5–3.0 mg. KRUEGER (1969) found a maintenance dose of 1 mg cymarin to be sufficient for therapy. GROBEL and MOTTAHEDIN (1966) determined an absorption quotient of 20% for cymarin, HANDRICK (1966) and STORZ (1969) calculated a mean absorption quotient of 33% and 36%, respectively, and a disappearance rate of 39%. Values of 29% absorption and 35% disappearance rate were determined by KRÄMER et al. (1972); these were calculated from clinical parameters and ECG analysis.

Using RIA, WIRTH et al. (1979) compared the renal excretion of cymarin after administration of 0.6 mg i.v. and 6 mg orally. Total excretion, extrapolated to infinity, was calculated as 210 µg (35% of the given dose) following i.v. administration and 394 µg (13% of the given dose) on oral administration. An absorption quotient of 37% was calculated from these values (Fig. 3). An interesting conclusion from these observations was that the kinetics of cymarin varied according to its route of administration. Most of an i.v. injection of cymarin was excreted within 12 h, and 77% of the total amount excreted was found in the urine within 24 h (Fig. 3). Upon oral administration, however, 42% of the total amount excreted was found within 1 day and 81% after 2 days in the urine. This demonstrates that cymarin is effective for longer following oral administration than after i.v. injection. These observations raise the question as to whether other cardiac glycosides exhibit different kinetics upon oral or i.v. administration. Digitoxin for instance seems to be eliminated to a higher degree after oral than after intravenous administration (GREEFF et al., 1979).

4. Cymarol

This glycoside is a metabolite of cymarin. FIEHRING et al. (1970) calculated from clinical parameters, after i.v. and oral doses of cymarol, an absorption quotient of 40% and a daily disappearance rate of about 50%. GUNDERT-REMY et al. (1978) found 30% of the radioactivity of an i.v. dose of ^{3}H-cymarol in the urine within 6 days and 68% in the feces, upon oral application however, only about 18% of radioactivity was found in the urine. An absorption quotient of 87% was calculated from blood levels and a value of 57% was determined from urinary excretion data.

5. Helveticoside Derivatives

A new strophanthin derivative, cyclopentanone helveticoside, has recently been investigated in humans. This glycoside is composed of strophanthidin K coupled with D-digitoxose. STORZ (1974), comparing clinical parameters upon i.v. and oral administration, determined an absorption quotient of 35% and a disappearance rate of 48%.

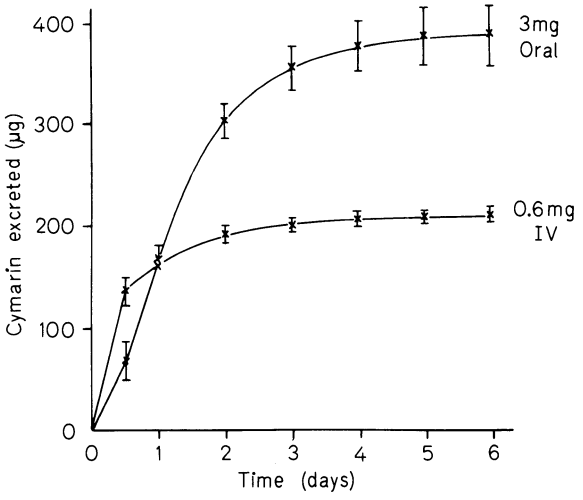


Fig. 3. Renal excretion of cymarin after oral or intravenous application in healthy volunteers. (WIRTH et al., 1979)

II. Animal Experiments

1. Ouabain

HATCHER and EGGLESTON (1919) found on oral administration of 80–984 mg/kg ouabain to rats, only slight traces in the urine but up to 75% of the given dose in the feces. Using the increased potassium excretion as an extracardiac parameter for effects of cardiac glycosides, GREEFF (1958a) was unable to detect any enteral absorption in the rat upon oral administration of up to 10 mg/kg. BUCHTELA et al. (1970) determined an absorption rate of 5%–10% in rats upon oral administration of 1 mg/kg ^3H-ouabain.

LAUTERBACH (1967a) observed that the absorption quotient in isolated rat intestine decreased with increasing ouabain concentrations; the absorption was described as a transport process with saturation kinetics, whereas diffusion did not take place under physiologic conditions (see Chap. 6). FORTH et al. (1969a), using blood-perfused duodenum in situ of rat and guinea pig, also found that the absorption or transport rate was dependent on the glycoside concentration. A dose-dependent ouabain absorption was determined in the perfused small intestine of the rat (OHLMEIER and RUIZ-TORRES, 1972).

In guinea pigs LÜLLMANN et al. (1971) found on oral administration of ^3H-ouabain only a very slight absorption, whereas MARCHETTI et al. (1971) determined an absorption quotient of 10% after 15 h, HAASS et al. (1972) of 2% after 1 h and MARZO et al. (1974) about 36% 5 h after intraduodenal administration.

In cats VON NYÁRY (1932) determined an absorption quotient for ouabain of 45%, using a method still of value today; an i.v. lethal dose of ouabain was injected into the duodenum, followed by an i.v. infusion 3–6 h later to bring about death by cardiac arrest. REINERT (1952), using the same method, measured an absorption quotient of about 60% 5 h after an intraduodenal injection of the i.v. lethal dose; this value, however, varied greatly from animal to animal. It should be noted that,

when administering digitoxin, maximal, i.e., 100% absorption takes place within 2 h, whereas the time needed for the maximal absorption of ouabain is up to 5 h. LAHRTZ et al. (1968), using ^3H-ouabain in cats found, after oral and intraduodenal administration such a variation in absorption rates that no statistical analysis was possible. FORTH et al. (1969 b) injected ^3H-ouabain into a sutured duodenal loop of cat in situ and calculated an absorption of 10% within 1 h. LAUTERBACH and VOGEL (1968) also carried out experiments on cat intestine, the results are described in Chap. 6.

2. Strophanthoside K

According to the investigations of GREEFF (1958 b) and ENGLER et al. (1958), strophanthoside K is absorbed in rats only after bacterial transformation into cymarin. LINGNER et al. (1963 b) reported an absorption quotient of 10% in cats after intraduodenal administration of strophanthoside K. MARZO et al. (1973) administered ^3H-strophanthoside K rectally to guinea pigs and found an absorption quotient of about 55%, while measuring excretion in the urine over a period of 15 h. These same authors (MARZO et al., 1974) reported an absorption of about 38% upon intraduodenal administration of ^3H-strophanthin K in the same species.

3. Cymarin

In unanesthetized rats 33% of the orally given dose of cymarin is absorbed (GREEFF, 1958 a). GEISSBERGER (1961) and LINGNER et al. (1963 b) calculated an absorption of 27% upon intraduodenal administration to cats and subsequent titration. Upon measuring chemically the amount of cymarin remaining after an intraduodenal administration, LINGNER et al. (1963 a) calculated an absorption quotient of 94%; these authors have suggested that a large amount of the absorbed cymarin was rendered inactive by its passage through the liver. Analysis of the intestinal contents of the test animals showed the presence of desmethylcymarin (helveticoside); it was further shown that the cat, like the rat, was able to demethylate cymarin. LINGNER et al. (1963 a) investigated in cats the enteral efficacy of some semisynthetic cymarin derivatives. Compared with cymarin, acetylcymarin for example, was equally effective while diacetalcymarol was 18 times less so. According to BOUTAGY and THOMAS (1977), diacetalcymarol is absorbed in the rat more efficiently than its parent glycoside cymarol, but is metabolized more quickly in the liver; in spite of improved absorption, an improved therapeutic effect cannot be expected.

4. Convallatoxin

LAUTERBACH (1964) noted that in rats the absorption of convallatoxin is dependent upon the dose administered; thus, absorption rate was about 15% after a dose of 15 μmol/kg, the absorption quotient decreasing with increasing dose. This phenomenon was also observed by LAUTERBACH and VOGEL (1968) and by LAUTERBACH (1967 b) in isolated rat intestine as well as in perfused rat small intes-

tine in vivo (LAUTERBACH, 1968). A transport mechanism with saturation kinetics has been described (LAUTERBACH, 1967a) (for details see Chap. 6). LINGNER et al. (1963a) found an absorption rate of about 36% in the anesthetized cat.

5. Other Derivatives of Strophanthidin K

SCHAUMANN and WEGERLE (1969) assayed the efficacy and calculated the relative toxicity and enteral activity of several esters and ethers of helveticoside and helveticosol by intravenous and intraduodenal administration to cats and guinea pigs. They did not report exact calculations of the absorption; the ratio of the toxic doses upon intravenous or intraduodenal infusion varied between 20% and 50%.

ZIELSKE et al. (1969) calculated the absorption rates 2 h after i.v. and intestinal administration of ^3H-helveticosol (strophanthidol K-mono-digitoxoside) and some of its esters and ethers in guinea pigs. They obtained a value of 7%, as measured from the contents of the intestine and about 33%, as measured from blood levels. The different esters and ethers demonstrate in most cases much higher absorption rates, i.e., up to 45% and 65%, respectively.

C. Blood Level and Tissue Distribution

I. Human Investigations

1. Ouabain

Concentrations of this glycoside in human investigations have almost always been measured in blood plasma or urine. The plasma concentration curves obtained may in most cases be fitted to a two-compartment model (Fig. 4). After an i.v. injection of ouabain its plasma level falls rapidly, with a half-life of distribution (α

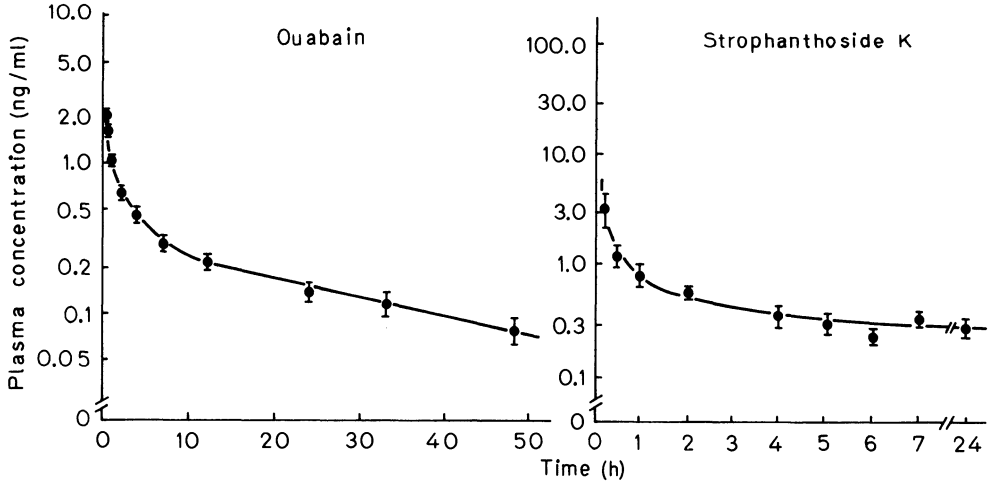


Fig. 4. Plasma level (mean values ± standard errors) following single intravenous injection of ouabain or strophanthoside K (0.5 mg) in healthy male subjects according to SELDEN and SMITH (1972) (ouabain) and GREEFF, STROBACH, WIRTH (unpublished) (strophanthoside K)

Table 1. Half-life of elimination $t_{1/2}$ of ouabain in healthy volunteers and patients with normal renal function. Calculation from blood levels after i.v. injection

Reference	Method	$t_{1/2}$(h)
MARKS et al. (1964)	^{3}H	5
VERSPOHL (1973)	RIA	$\leqq$ 8.5
ERDLE et al. (1979)	^{3}H	11
KRÄMER et al. (1972)	^{3}H	12–17
KRAMER and SCHELER (1972)	^{3}H	13.9
SELDEN and SMITH (1972)	RIA	21.8
SELDEN et al. (1974)	^{3}H	22
LAHRTZ et al. (1969)	^{3}H	$\simeq$ 50
SELDEN and HAYNIE (1975)	^{3}H	50

phase) of 2.3 min (MARKS et al., 1964), 4 min (ERDLE et al., 1979), and approximately 20 min (LAHRTZ et al., 1969). The rapid decrease in plasma concentration during the α phase to values that lie near the limit of detection for RIA complicates the estimation of the half-life of the slow β phase, which represents the excretion. Even when using ^3H-ouabain, which can be detected over a longer period, the half-life for the β phase varies, according to various authors between 11 and 50 h (Table 1). MARKS et al. (1964) measured a half-life of 5 h, though this value was calculated from measurements made during 2 h after an i.v. injection of ^3H-ouabain. SELDEN and SMITH (1972) measured half-lives of 18–25 h (mean 22 h) in seven healthy volunteers, 7–48 h after administration of 0.5 mg ouabain; the plasma ouabain concentration after 48 h was lower than 0.2 ng/ml. The distribution value was calculated by VERSPOHL (1973) as 16 l/kg after i.v. injection of 0.5 mg ouabain.

MARKS et al. (1964) measured the difference in ^3H-ouabain concentration between coronary artery and venous blood in five patients undergoing open heart surgery. ^3H-ouabain (25 µCi) was administered i.v. after coronary bypass. Samples of arterial and coronary sinus blood were taken at frequent intervals. A difference in the ouabain concentration between coronary arterial and venous blood existed only during the first 4 min; after this period of time the heart tissue and blood perfusing it seemed to be in equilibrium. This is consistent with observations on the rapid onset of positive inotropic effects after ouabain administration. In three of the patients, right auricle tissue samples were obtained 30–90 min after ouabain administration: these samples contained approximately 5–10 times the plasma concentration. The authors assumed that a period of extracorporeal circulation does not remove ouabain from its binding sites in heart muscle. SELDEN and NEILL (1975) injected ouabain into patients undergoing coronary sinus catheterization and measured a coronary arteriovenous difference for the first 9–12 min. From these results the authors calculated a left ventricular ouabain concentration of 30 ng/g of myocardium.

2. Strophanthoside K

The pharmacokinetics of strophanthoside K, like those of ouabain, have been measured almost without exception in the central compartment (BRASS and PHIL-

IPPS, 1970; GHIRARDI et al., 1973; MARZO et al., 1976; WIRTH et al., 1979). No obvious differences were found between the two glycosides (Fig. 4). GREEFF and STROBACH (1979) found in three patients who had died from an overdose of strophanthoside K, a cardiac muscle concentration of between 133 and 290 ng/g wet weight, with a plasma concentration of between 21 and 55 ng/ml; the concentration quotients for cardiac muscle/plasma, were calculated as 5, 6, and 8.

3. Acetylstrophanthidin

According to SELDEN et al. (1973) this aglycone has a plasma half-life of 2.3 h. LOWN and LEWIN (1954) and LUCCHESI and SHIVAK (1964) used acetylstrophanthidin (because of its rapid elimination) for a tolerance test in digitalized patients in order to determine the optimal individual digitalis levels (see also Chap. 8). Further information on the uptake of the strophanthus glycosides in cardiac muscle can be found in Chap. 7.

II. Animal Experiments

1. Ouabain

In dogs VERSPOHL (1973) determined the distribution of ouabain after an injection of 60 μg/kg; the value, 18 l/kg, was of the same order of magnitude as that determined by the same author in humans. The radioimmunologically determined blood levels over a period of 24 h gave a half-life of 8.5 h or less. SELDEN and SMITH (1972) also determined plasma oubain concentrations by RIA over a longer period of 48 h and calculated, in seven dogs, half-lives of between 17 and 24 h (mean 18 h). Using the tolerance test of repeated intravenous infusion, GREEFF et al. (1969) calculated a half-life for ouabain of 26 h.

In rats the highest concentrations of radioactivity 16 min after the administration of ^3H-ouabain was found in the hypophysis, followed by liver, ventricular muscle, kidney, and skeletal muscle. After 6 h, the sequence of radioactive concentration was as follows: hypophysis, adipose tissue, adrenal glands, and skeletal muscle, followed by kidney and hypothalamus (DUTTA and MARKS, 1966). These observations indicate a fast hepatic clearance for ouabain in the rat. The excretory capacity of the rat liver is dependent on age. The investigations of KLAASSEN (1972) showed a liver concentration of 4 μg/g, 15 min after administration of 4 mg/kg i.v. ^3H-ouabain in 7-day-old animals, compared with a liver concentration of 20 μg/g in 39-day-old animals. The plasma half-life for the younger animals was calculated as 30 min and that of 39-day-old animals as 5 min. KUPFERBERG and SCHANKER (1968), in agreement with the above results, showed that in rats, with ligatured kidneys, 85% of a 1 mg/kg i.v. ^3H-ouabain dose was excreted by the bile within 90 min. The plasma : liver concentration ratio was calculated as 33 : 117, indicating an active transport of the glycoside from the blood into the liver. They further confirmed this by showing an active uptake of ^3H-ouabain into rat liver slices against a four-fold concentration gradient.

RUSSELL and KLAASSEN (1972) found that ouabain disappeared from the plasma much more rapidly in the rat than in rabbit and dog and that there are differ-

Table 2. Quabain concentration in plasma, liver, and bile in rats, rabbits, and dogs 20 min after intravenous injection of 80 μg/kg ^3H-ouabain. In rats plasma concentration is lower than in rabbits and dogs but bile concentration is higher. Thus, the concentration gradient of ouabain from bile to plasma is more than 100 times higher than in rabbits or dogs. (RUSSELL and KLAASSEN, 1972)

[Ouabain]	Rat	Rabbit	Dog
Plasma (ng/ml)	22.1 ± 8.2[a]	65.8 ± 12.6	65.1 ± 31.7
Liver (ng/g)	368 ± 51	144 ± 20	230 ± 28
Bile (ng/ml)	24,300 ± 1,730	173 ± 29	630 ± 35
Bile/plasma	1,510 ± 328	2.89 ± 0.65	9.27 ± 1.72
Liver/plasma	19.9 ± 2.8	2.49 ± 4.51	3.30 ± 0.75
Bile/liver	70.8 ± 9.7	1.31 ± 0.29	2.69 ± 0.11

[a] Mean ± standard error of two to four animals

Table 3. Concentration of ^{3}H in heart and heart/plasma ^{3}H concentration gradients after administration[a] of ^{3}H-glycosides to rats, rabbits, and dogs (RUSSELL and KLAASSEN, 1973)

	Concentration in heart[b]		
	Rat	Rabbit	Dog
Ouabain	37.0 ± 1.7[c]	350 ± 51.0	561 ± 29.0[d, e]
Digoxin	94.6 ± 6.4	74.6 ± 13.5	402 ± 57.0[d, e]
Digitoxin	106 ± 24.5	118 ± 1.4	251 ± 24.8[d, e]
	Concentration in heart[b]/ Concentration in Plasma[f]		
	Rat	Rabbit	Dog
Ouabain	2.21 ± 0.52	5.54 ± 0.78[d]	13.7 ± 5.31[d]
Digoxin	4.21 ± 0.64	1.26 ± 0.33[d]	6.31 ± 1.68[e]
Digitoxin	2.01 ± 0.98	0.47 ± 0.01	1.36 ± 0.01[e]

[a] Tissue samples were taken 20 min after an intravenous dose of 0.08 mg/kg of each ^3H-glycoside
[b] Glycoside ng equiv./g heart
[c] Each value represents the mean ± standard error of two to four animals
[d] Significantly different from rats, $P < 0.05$
[e] Significantly different from rabbits, $P < 0.05$
[f] Glycoside ng equiv./ml plasma

ences in the concentration gradient of ^3H-ouabain from bile to plasma in the three species (Table 2). They found that 20 min after ^3H-ouabain was administered the rat exhibited an overall bile : plasma concentration gradient of 1,500 whereas the same gradient was much less for the rabbit (2.9) and dog (9.3). The authors suppose that the relative inability of the rabbit and dog to excrete ouabain into the bile is due to a low capacity for transferring ouabain from the plasma to liver and from the liver to bile (Table 2). RUSSELL and KLAASSEN (1973) further observed differences in the ouabain concentration in the heart of rat, rabbit, and dog (Table 3).

They also found differences in the concentration of digoxin and digitoxin (Table 3).

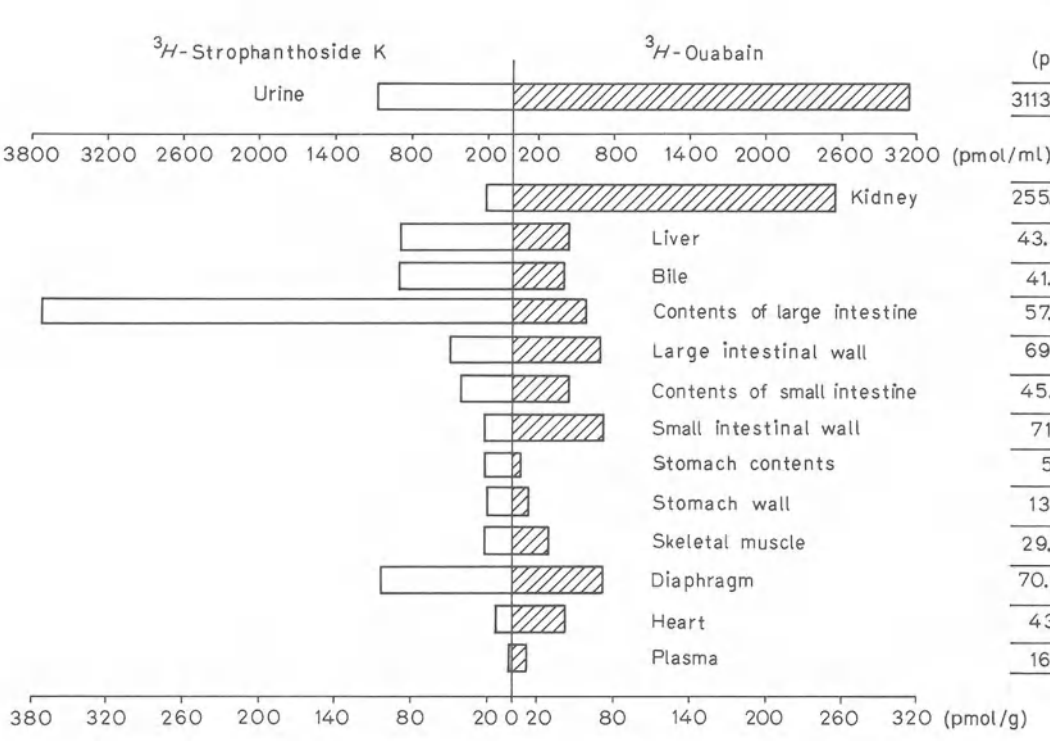
The uptake of ^3H-ouabain into the myometrium also seems to differ between the rat and the rabbit (MURTHY et al., 1972): the uptake in the rat, in contrast to the rabbit, is independent of the concentration of Na^+ and ATP and is not antagonized by K^+ or by a lowering of temperature.

In guinea pigs DUTTA and MARKS (1966), using ^3H-ouabain, found after 16 min the highest concentration of radioactivity in the kidneys, followed by ventricular muscle, hypophysis, atria, and liver. MARCHETTI et al. (1971), GROPE (1978), and MERK (1980) found the highest concentration of radioactivity in the kidneys and urine, demonstrating that the main pathway of excretion for ouabain in guinea pigs is via the kidneys. MERK (1980) found, 6 h after administering ^3H-ouabain, 82% in various organs and a different distribution compared with strophanthoside K (Fig. 5). GROPE (1978) also compared the ouabain concentration of various organs after 8 days of treatment with 5 µg/kg i.p. daily (Table 4). About the same concentration of ouabain was found in heart and skeletal muscle, but the total amount found in the heart was 100 times higher than in the skeletal muscle. Traces of ouabain were detectable for longer (72 h) in heart and liver than skeletal muscle and kidney.

FRICKE et al. (1969) working with the Langendorff preparation of the isolated guinea pig heart, found only a slight accumulation of ^3H-ouabain bound to nuclear, membrane, and mitochondrial fractions. The highest radioactivity was found in the microsomal fractions. LÖHR et al. (1971) using autoradiography, found that in contrast to digoxin, ouabain was bound extracellularly to the cell membrane. PFLEGER et al. (1975) found, however, an intracellular uptake of this glycoside in guinea pig heart preparations, that was seven times lower than that for digitoxin. They suggested, according to the analysis of the efflux kinetics, two compartments for ouabain, which they were, however, unable to localize. LÜLLMANN et al. (1975) proposed the existence of a small, saturable compartment for ouabain in the guinea pig papillary muscle, at the cell membrane.

Ouabain uptake in guinea pig liver was investigated by KOLENDA et al. (1971). They detected an uptake, which resulted in a liver : medium concentration gradient of about 3, no excretion being detected via the bile, as in the case of digoxin and digitoxin. It was supposed, that the hydrophilic ouabain molecule was not able to penetrate into the active liver cell compartment and could not therefore be metabolised.

In the cat BENTHE (1975) investigated the tissue distribution of ^3H-ouabain: 5 h after administration of a single dose (100 µCi, i.e., 0.1 mg/2 kg), 1.2% of the given dose was found in both the myocardium and the kidney, 0.45% in the liver, 0.003% in brain, and 0.01% in skeletal muscle. The biliary excretion during the experiment was measured as 0.6%. The high myocardial binding of ouabain, 2–3 times higher than for other glycosides investigated by the above authors (digitoxin, digoxin, β-acetyldigoxin, and β-methyldigoxin), could be explained by a high affinity of the myocardium for this glycoside. FLASCH and HEINZ (1976) administered daily 21.6 µg/kg (32.5 µCi/kg) of ^3H-ouabain over 5 days to the cat and measured the radioactivity of certain organs 5 h after the last administration: 1.8% of the daily dose per g wet weight was found in the myocardium and 0.02% in the cerebrum



	[^3H-Ouabain]		[^3H-Strophanthoside K]	
	(pmol/g)	(ng)	(pmol/g)	(ng)
Urine	3113.9 ± 271.9	9080.0	1228.5 ± 93.4	6756.8
Kidney	255.9 ± 15.8	596.8	19.9 ± 3.1	60.9
Liver	43.8 ± 5.9	618.9	88.2 ± 13.9	1447.6
Bile	41.3 ± 9.3	18.1	89.7 ± 9.7	54.8
Contents of large intestine	57.5 ± 11.7	1462.3	371.1 ± 7.0	10659.6
Large intestinal wall	69.8 ± 19.2	514.1	50.4 ± 7.3	448.8
Contents of small intestine	45.7 ± 12.5	359.6	41.0 ± 6.5	257.0
Small intestinal wall	71.5 ± 5.5	380.3	22.3 ± 2.9	176.5
Stomach contents	5.8 ± 1.3	10.1	19.6 ± 7.6	88.9
Stomach wall	13.3 ± 2.7	49.0	17.5 ± 1.8	52.0
Skeletal muscle	29.8 ± 4.5	3423.4	20.9 ± 1.6	2892.7
Diaphragm	70.8 ± 3.4	77.4	104.0 ± 10.9	99.0
Heart	43.1 ± 0.5	47.1	11.5 ± 1.5	13.0
Plasma	16.0 ± 1.7	193.6	1.0 ± 0.1	15.0

Fig. 5. Distribution of ^3H-strophanthoside K and ^3H-ouabain in guinea pigs 6 h after i.p. injection of 69 μmol/kg. ng refers to the total organ. (MERK, 1980)

and cerebellum; in plasma 0.02%/ml of the daily administered dose could be found.

SELDEN and NEILL (1975) investigated the myocardial uptake of ^3H-ouabain after a single i.v. dose in humans (0.5 mg) and dogs 8–36 μg/kg). As in humans, the ouabain concentration difference between coronary artery and coronary sinus narrowed rapidly during the first 1–3 min and thereafter at a slower rate in an exponential fashion for the next 4–12 min, with a mean half-life of 1.4 min. The exponential phase of ouabain removal from the coronary circulation accounted for 49% of that directly measured in left ventricular plasma samples, obtained shortly after ouabain uptake was complete. Left ventricular plasma ouabain concentration in these samples was about 12-fold higher than in simultaneously obtained plasma. Thus, the initial rapid efflux of ouabain from the coronary circulation would appear to reflect movement into interstitial fluid, the subsequent exponential removal reflecting binding to myocardial cells.

The following quotients were determined by ABSHAGEN et al. (1971) for the distribution of ^3H-ouabain between erythrocytes and plasma: cow, cat, and guinea pig 0.09, rat 0.04, human 0.03, and dog 0.01. This uptake seems, in the case of the cat to be independent of the presence of Na^+, K^+-ATPase, as no evidence of this enzyme has been found in cat erythrocytes (GREEFF et al., 1964).

2. Strophanthoside K

In guinea pigs GIERTZ et al. (1954) measured, upon administration of unlabeled glycosides, in contrast to digitoxin, a higher activity in the kidneys and somewhat less in the liver. MARZO et al. (1974) found, 24 h after an i.v. or intramuscular administration in guinea pigs, the highest tritium concentration in the intestinal contents, bile, liver, and skeletal muscle (Table 5). At this point 31% of the administered tritium radioactivity was still to be found in the animals. Of particular interest is the high concentration gradient from kidney to liver for ouabain compared with the low gradient for strophanthoside K (MARZO and GHIRARDI, 1977), which is confirmed by MERK (1980) and GROPE (1978) (Table 6). In the heart a higher concentration of tritium was found in the ventricular compared with the atrial muscle. Upon investigating the subcellular distribution of the glycoside in the heart of guinea pigs, the highest specific concentration was found in the microsomal fraction, lower concentrations being evident in the nuclear and mitochondrial fractions (see also MARZO and GHIRARDI, 1974). Following rectal administration of tritium-labeled glycoside, the highest activity was found in the intestinal contents and less in the walls of the rectum, the bile, and the urine (MARZO et al., 1973). MERK (1980) also found in guinea pigs after intraperitoneal (i.p.) administration of labeled and unlabeled strophanthoside K, the largest amount in the intestinal contents, less in the urine and in skeletal muscle (Fig. 5). It was found that 6 h after administration, 92% of the labeled and 80% of the unlabeled glycoside was still to be found in the body of the animals. These results demonstrate that strophanthoside K has a distribution different from that of ouabain. BRASS (1971) administered tritiated glycoside to mice, and found, 30 min later, the highest activity in the liver, approximately half of that in the kidney, and less activity in heart and skeletal muscle.

Table 4. Distribution of ouabain in guinea pigs after treatment for 8 days by i.p. injection. The values were determined by RIA 6, 24, 48, or 72 h after the last injection. Mean values ± standard errors of eight animals (mean body weight 300 g). Calculations for total organs were made on the basis of 4% for plasma, 40% for skeletal muscle, or the measured wet weight. GROPE (1978)

	Ouabain							
	(ng/g tissue)				(ng/total organ)			
Time (h)	6	24	48	72	6	24	48	72
Blood	0.58 ± 0.05	< 0.1	< 0.1	< 0.1	7.0 ± 0.6	< 0.1	< 0.1	< 0.1
Heart	3.98 ± 0.2	0.94 ± 0.05	0.79 ± 0.2	0.82 ± 0.2	4.5 ± 0.28	0.97 ± 0.09	0.78 ± 0.14	1.05 ± 0.24
Kidney	12.56 ± 1.65	1.0 ± 0.16	0.94 ± 0.17	< 0.1	37.94 ± 5.27	2.98 ± 0.51	2.9 ± 0.5	< 0.1
Liver	3.0 ± 0.49	5.4 ± 0.6	4.3 ± 0.4	4.3 ± 0.17	37.97 ± 5.20	69.49 ± 8.91	55.65 ± 5.42	51.59 ± 2.49
Skeletal muscle	3.9 ± 0.3	1.3 ± 0.1	< 0.1	< 0.1	470.0 ± 41.7	154.0 ± 16.2	< 0.1	< 0.1
Adrenal gland	7.93 ± 0.75	3.38 ± 0.34	< 0.1	< 0.1	0.85 ± 0.08	0.37 ± 0.03	< 0.1	< 0.1

Table 5. Concentration of ^3H-strophanthoside *K* in body organs, tissues, and fluids of guinea pigs after i.v. administration of 250 μg/kg of the drug, at different times. (MARZO et al., 1974)

	15 min	30 min	1 h	2 h	5 h	10 h	24 h
Heart	586 ± 65	404 ± 37	297 ± 12	133 ± 7	95 ± 2	81 ± 3	43 ± 3
Liver	1,092 ± 92	1,392 ± 79	1,611 ± 75	851 ± 51	334 ± 31	192 ± 24	105 ± 8
Kidneys	1,748 ± 191	1,136 ± 180	587 ± 42	301 ± 24	194 ± 7	132 ± 7	64 ± 5
Skeletal muscle	224 ± 19	224 ± 11	217 ± 5	206 ± 14	189 ± 3	159 ± 11	148 ± 4
Skin	174 ± 10	144 ± 7	122 ± 11	69 ± 10	40 ± 10	22 ± 4	15 ± 2
Intestine wall	158 ± 26	120 ± 12	130 ± 5	80 ± 8	115 ± 10	106 ± 7	85 ± 10
Intestine contents	103 ± 16	119 ± 10	189 ± 34	279 ± 12	538 ± 47	834 ± 82	1,076 ± 81
Blood	307 ± 20	212 ± 17	149 ± 13	45 ± 4	16 ± 1	8 ± 0.4	7 ± 0.3
Urine (cumulative values)	5,124 ± 2,341	9,898 ± 1,813	12,923 ± 3,232	10,466 ± 922	9,076 ± 1,052	4,140 ± 753	2,110 ± 169
Bile	547 ± 104	810 ± 113	1,252 ± 174	1,218 ± 195	1,012 ± 245	707 ± 102	979 ± 429

Mean values ± standard errors in ng/g or ng/ml. Each group consists of seven findings

Table 6. Different distribution of ouabain and strophanthoside K regarding their concentrations in kidney and liver in guinea pigs. Strophanthoside K is concentrated in liver tissue to a higher degree than in kidney tissue whereas ouabain is bound to a higher degree in kidney tissue

Reference	Method	Dose (μg/kg)	Time after application (h)	Kidney concentration (ng/g)	Liver concentration (ng/g)	Q
		Strophanthoside K				
MARZO et al. (1974)	^{3}H	250 i.v.	5	±194 ± 7	334 ± 31	0.58
MARZO et al. (1974)	^{3}H	250 i.m.	5	213 ± 31	506 ± 63	0.42
MERK (1980)	^{3}H	60 i.p.	6	17.4 ± 2.7	77.0 ± 12.1	0.23
MERK (1980)	RIA	60 i.p.	6	16.3 ± 4.5	28.7 ± 4.5	0.57
		Cymarin				
MERK (1980)	RIA	38 i.p.	6	0.8 ± 0.1	3.0 ± 0.3	0.27
		Ouabain				
MARZO and GHIRARDI (1977)	^{3}H	100 i.v.	5	317 ± 12	54 ± 2	5.87
GROPE (1978)	RIA	50 i.p.	6	31.5	8.0	3.94
MERK (1980)	^{3}H	50 i.p.	6	186.5 ± 11.5	31.9 ± 4.3	5.85

i.v. = intravenous, i.m. = intramuscular, i.p. = intraperitoneal

3. Cymarin

MOERMAN (1965) investigated the distribution of cymarin in the rat 5 min after iv administration of 5 mg/kg and found 47% in skeletal muscle, 14% in the small intestine, and 11% in the liver. The concentrations in blood were 7 μg, in heart muscle 10 μg, and in skeletal muscle 5.8 μg/g. The total recovery at that time was 87%. MERK (1980) found in guinea pigs, 6 h after i.p. administration of 69 nmol/kg, 6% of a total recovery of 21% in the large intestine. The concentrations in the plasma were less than 1 ng, in heart muscle 2.1 ng, in skeletal muscle 2.8 ng, in the kidneys 0.8 ng, and in the liver 3.0 ng/g. The concentration gradient from kidney to liver is similar to that of strophanthoside K being lower than 1, indicating a high intestinal excretion.

4. Convallatoxin

MERK (1980) investigated the distribution of convallatoxin 6 h after an i.p. dose of 69 nmol/kg in guinea pigs and found 13% in the urine and 10% in the large intestine. In skeletal muscle the concentration was 6.6 ng, in heart muscle 2.3 ng, in liver 4.4 ng, and in the kidney 7.4 ng/g tissue.

D. Metabolism

I. Human Investigations and Animal Experiments

1. Ouabain

Owing to the short-lived action of ouabain in humans and animals, HATCHER and EGGLESTON (1919) supposed that transformation in the liver played an important role in the elimination of this glycoside. FARAH (1946b) found 80%–85% of a 4–6 mg dose of ouabain, 2–4 h after infusion, in the bile of rats; as a biologic assay method was used to determine the ouabain concentration, no information could be obtained on its biologic transformation. COX et al. (1959) re-examined the biliary excretion of ouabain in rats by means of paper chromatography and made comparative investigations with regard to biliary and renal excretion. Thus 88% of an i.v. injection of 1 mg/kg ouabain was found in the bile within 5 h and 5% in the urine within 24 h after the injection. Extracts of bile or urine, examined chromatographically, did not show the presence of metabolites even at these high doses, no compound with an saturated lactone ring being detectable. Only upon administration of an even higher dose of 10 mg/kg i.p. did these authors find, apart from ouabain, traces of two compounds of a less polar nature, which appeared to be metabolites. According to these authors, the high biliary excretion is characteristic of ouabain because of its polarity, as in previous investigations. COX and WRIGHT (1959) found, using the same experimental procedure, after a dose of the relatively nonpolar digitoxin, only 10% within 5 h in the bile. Of this 10%, a large portion had been metabolized (e.g., to digoxin); this is in contrast to the behavior of ouabain.

RUSSELL and KLAASSEN (1972) confirmed the high biliary excretion of ouabain in rats, even at low doses; 54.6% of an i.v. injection of 0.08 mg/kg ^3H-ouabain was found within 12 h, whereas the biliary excretion was much lower in rabbits (4.4%) and dogs (1.3%). These observations confirm the assumptions of HATCHER and EGGLESTON (1919), that the high resistance of the rat to ouabain is due to a preferential elimination via the liver. This is not as believed, due to metabolism, but to an excretion of the unchanged molecules. Up to now, there is no information on any possible metabolic transformation of ouabain in the rat. Indeed, the fast biliary excretion is only partly responsible for the high resistance of this species, as it is well known that the heart and also the cardiac Na^+, K^+-ATPase of the rat are particularly insensitive to ouabain (REPKE et al., 1965; GREEFF and SCHLIEPER, 1967; DRANSFELD et al., 1966, 1967).

Neither were traces of ouabain metabolites found in other species, e.g., in a heart–lung preparation of the dog (FARAH, 1946a), in the ^3H-ouabain perfused, isolated guinea pig liver, by investigating the bile (LÜLLMANN et al., 1971; KOLENDA et al., 1971), or in experiments with cats (LAHRTZ et al., 1968). In humans, the only investigations have been on the renal excretion of ouabain; no metabolites were found in these experiments (MARKS et al., 1964; LAHRTZ et al., 1968; LÜLLMANN et al., 1971).

2. Strophanthidin K Derivatives

The first indication of a metabolic transformation of strophanthoside K (γ-strophanthin-K) to cymarin (α-strophantin-K) was found in experiments on rats (for

chemistry see Fig. 1). It was observed that strophanthoside K becomes pharmacologically active upon oral application after the splitting off of the terminal glucose residue and transformation into cymarin (GREEFF, 1958 a, b; ENGLER et al., 1958). This transformation is apparently brought about by the intestinal flora, as the effectiveness of orally administered strophanthoside K could be completely prevented by oral pretreatment with antibiotics or sulfonamides (GREEFF, 1958 b). This interpretation was confirmed by the chromatographic identification of cymarin in the urine of rats and also by the observation, that the in vitro incubation of γ-strophanthin-K with feces of rat, dog, cow, guinea pig, or human produced cymarin (ENGLER et al., 1958; ENGLER, 1958). It may hence be concluded that, not only with the rat, but also with humans and other species, cymarin is produced from strophanthoside K in the intestine and is absorbed as such. Cymarin could also be found in urine and feces after a subcutaneous injection of strophanthoside K; therefore, the investigators have assumed that cymarin may also be produced within the enterohepatic circulation or after elimination via the intestinal wall.

STOLL and RENZ (1951) investigated the metabolism of β-strophanthin-K using enzyme preparations from the tissues of various species, e.g., cardiac muscle of pig, calf, cow, and horse. This glycoside was found to be unmetabolized or metabolized only to a slight extent. STOLL et al. (1951) showed on the other hand, that enzyme preparations from the lower fungi (*Aspergillus oryzae* and *Claviceps purpurea*) split β-strophanthin K into glucose and cymarin.

MARZO et al. (1973) applied ^{3}H-strophanthoside K rectally to guinea pigs, and found 45% unchanged glycoside and 55% cymarin in the rectal contents, whereas in urine, the ratio was 90 : 10 and in the bile 78 : 22. They concluded that only the rectal contents are able to transform strophanthoside K into cymarin to a significant extent.

GILLISSEN et al. (1964) investigated the metabolism of cymarin in isolated frog and cat liver. In the frog liver a metabolite was detected, besides cymarin, which was more polar but unidentifiable, whereas in the cat liver cymarin was largely demethylated to helveticoside; small amounts of strophanthidin K as well as other metabolites with high polarity were also found.

WEISS-BERG and TAMM (1963) described a microbial reduction of strophanthidin K to produce strophanthidol. Strophanthidin-19-carbonic acid or 10β-hydroxy-19-norperiplogenin derivatives are produced by a process of autoxidation (BINKERT et al., 1962; VON WARTBURG et al., 1962; LAUTERBACH, 1964). MOERMAN (1965) applied cymarin i.v. to the rat in vivo and upon thin layer chromatographic analysis, cymarin itself as well as cymarol and strophanthidin and several other unidentifiable metabolites were found.

LAUTERBACH and REPKE (1960) investigated the metabolism of cymarin in rat liver slices: small amounts of strophanthidin were detected as well as four further metabolites, one of them probably helveticoside. Allocymarin was apparently not metabolized. In further experiments with rat liver slices and liver homogenate, LAUTERBACH (1964) investigated the metabolism of cymarin, helveticoside, convallatoxin, and strophanthidin. In all cases a reduction of the 19-oxocardenolide took place, producing the 19-hydroxy derivatives, cymarol, helveticosol, convallatoxol, and strophanthidol. This reduction does not result in an inactivation, as 19-hydroxy derivatives may be more potent than their mother substances, (for review see LAUTERBACH, 1964) it could actually mean an increase in polarity and therefore an

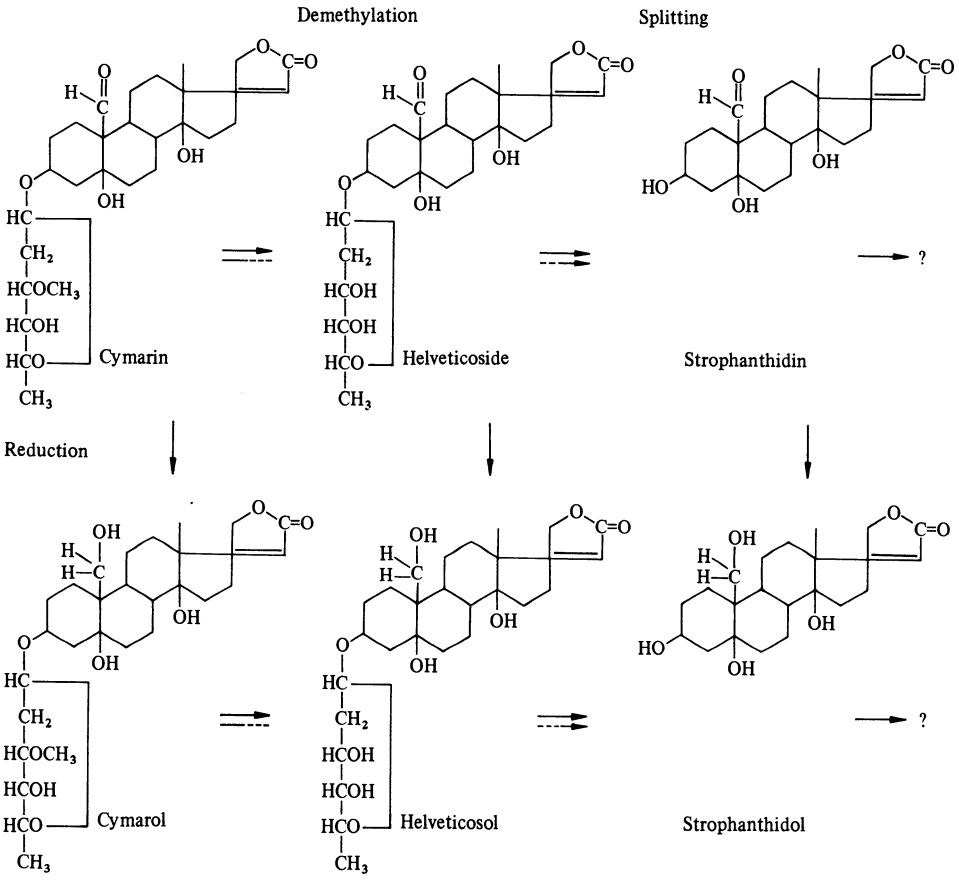


Fig. 6. Metabolic pathways of cymarin. (LAUTERBACH, 1964)

acceleration in renal excretion or fecal elimination. The primary alcohol groups formed do not take part in further reactions. It may hence be concluded that strophanthidin derivatives are not inactivated as previously supposed, via a fermentative oxidation of their aldehyde groups. The various metabolic pathways, according to LAUTERBACH (1964), can be seen in Fig. 6. Alternatively, a demethylation of cymarose may occur with additional splitting of the sugar residues. A subsequent inactivation may take place by the epimerization or conjugation of the C3 hydroxyl group (LAUTERBACH and REPKE, 1960; THOMAS and WRIGHT, 1965; LAUTERBACH, 1969).

E. Excretion

Ouabain and strophanthin G when given orally are absorbed slowly but incompletely, so that their excretion can be quantitatively estimated only after parenteral administration. Following i.v. injection the blood level of strophanthus glycosides falls quickly owing to the rapid distribution (Fig. 4). As such, the half-life for ex-

cretion is difficult or impossible to measure by RIA using plasma levels. Excretion of strophanthus glycosides takes place via the kidneys, the bile, or the intestine and exhibits species variation.

I. Human Investigations

1. Ouabain

In investigating the excretion of ouabain in humans, the tritium-labeled glycoside has been most useful. Renal ^{3}H excretion after i.v. injection has been measured over periods varying from 1 to 4 days. In spite of this variation, most authors have calculated similar excretion rates, as the major portion of the total amount excreted leaves the body within the first 24 h (Table 7).

MARKS et al. (1964) found for ouabain a renal excretion rate on the first day of 45%–63%. In two patients, 2%–8% of the administered radioactivity was excreted in the bile within the first 24 h. LAHRTZ et al. (1969) calculated a renal tritium excretion in patients with healthy kidneys of 61% within 48 h; in patients with renal insufficiency the excretion rate was only about 28% in the same period. A further value for renal tritium excretion upon i.v. administration of ^3H-ouabain of 52% within 48 h was reported by EICKENBUSCH et al. (1970). KRAMER and SCHELER (1972) found an excretion of 34% within 24 h and 44% within 72 h. SELDEN et al. (1974) determined excretions of 46% or 48% in 6 days using ^3H-ouabain or RIA, respectively; 5.4% was found by SELDEN et al. (1974) in the bile of four cholecystectomized patients and 34% in the feces in a total excretion of 77% (three patients). ERDLE et al. (1979) found a value of 50% within 3 days. Using RIA, VERSPOHL (1973) calculated a renal excretion rate of 29% in 24 h after i.v. injection of 0.5 mg, 56% after 4 days and 66% upon extrapolating to infinity (VERSPOHL, 1973; GREEFF, 1977; GREEFF et al., 1975).

Much smaller amounts of ^3H-ouabain have been found in the urine following oral or intraduodenal administration, due to slight absorption (see Sect. B.II); 0.2%–2% of the given dose was found by LAHRTZ et al. (1968) and 1.0%–1.5% by MARCHETTI et al. (1971) within 24 h, 0.6%–2.5% by ERDLE et al. (1979) within 72 h. After oral administration of 6 or 8 mg of ouabain, VERSPOHL (1973) and GREEFF (1977) calculated 0.5%–4.5% of the given dose in the urine using RIA.

2. Strophanthoside K

BRASS and PHILIPPS (1970) found in volunteers 68% of the i.v. dose of 0.25 mg ^3H-strophanthoside K in the urine within 7 days, most of the excretion, i.e., 90%, taking place within the first 24 h (Table 7). In contrast, patients with terminal renal insufficiency excreted only 12%–40% of the given dose in 7 days. MARZO et al. (1976) extrapolating to infinity measured 53% of a single i.v. injection of 0.5 mg ^3H-strophanthoside K in the urine and 51% after intramuscular administration. The major part was also excreted within the first 24 h. These authors measured a biliary excretion of 3.8%–7.6% in six cholecystectomized patients. WIRTH et al. (1979), using RIA determined a renal excretion of an i.v. injection of 0.5 mg strophanthoside K of 70%. A study of excretion after rectal administration was

Table 7. Renal excretion of strophanthus glycosides in healthy volunteers and patients without renal failure as a percentage of administered dose in different collection periods. ∞ Means of cumulative excretion calculated by extrapolation to infinity

Reference	Method	Dose		Renal excretion (% dose)	(h)
		Ouabain			
MARKS et al. (1964)	^{3}H	25 μCi and unlabeled	i.v.	45–63[a]	24
LAHRTZ et al. (1968)	^{3}H	1 μCi/kg (0.6 μg/kg)	p.o.	0.5–2	24
LAHRTZ et al. (1969)	^{3}H	0.66 μCi/kg (≅0.04 mg/ 70 kg)	i.v.	61	48
EICKENBUSCH et al. (1970)	^{3}H	1 μCi/kg	i.v.	52	48
MARCHETTI et al. (1971)	^{3}H	50–150 μg/kg	p.o.	1.0–1.5	24
KRAMER and SCHELER (1972)	^{3}H	0.1 μCi/0.09 mg	i.v.	34 44	24 72
VERSPOHL (1973)	RIA	0.5 mg	i.v.	29 56	24 96
SELDEN et al. (1974)	^{3}H RIA	≅73 μCi and unlabeled	i.v. i.v.	46[b] 48	144 144
GREEFF et al. (1974)	RIA	8 mg 6 mg	p.o.	0.5–4.4 0.7–3.0	∞ ∞
ERDLE et al. (1979)	^{3}H	27 μCi (0.25 mg) and unlabeled	i.v.	≅ 50	72
		Strophanthoside K			
BRASS and PHILIPPS (1970)	^{3}H	48 μCi (0.25 mg)	i.v.	68	168
GHIRARDI et al. (1973)	^{3}H	250–1,000 μg	p.r.	10–12	24
MARZO et al. (1976)	^{3}H	131 μCi (0.25 mg)	i.v.	37–42 53	24 ∞
			i.m.	32–33 51	24 ∞
			i.v.	62[c]	∞
WIRTH et al. (1979)	RIA	0.5 mg	i.v.	70	∞
		Cymarin			
WIRTH et al. (1979)	RIA	0.6 mg 3.0 mg	i.v. p.o.	35 12	∞ ∞
		Acetylstrophanthidin			
SELDEN et al. (1973)	RIA	1.0 mg	i.v.	22	24

[a] In 2 of 15 persons 2%–8% radioactivity were excreted in the bile in 24 h
[b] Excretion of 5.4% radioactivity in the bile in 120–164 h, of 21%–40% in the feces in 144 h
[c] Excretion of 3%–5% radioactivity in the bile in 24 h, 4%–8% extrapolated to infinity in six cholecystectomized patients
i.v. = intravenous, p.o. = oral, p.r. = rectal, i.m. = intramuscular

carried out by GHIRARDI et al. (1973). Upon application of 250, 500, or 1,000 μg ^3H-strophanthoside K, 10.4%–11.9% of the tritium radioactivity could be measured in the urine within the first 24 h.

3. Cymarin

The renal excretion of cymarin is lower than that of strophanthoside K or ouabain. After i.v. injection of 0.5 mg to healthy volunteers WIRTH et al. (1979) found 35% of the given dose in the urine (Table 7, Fig. 3).

4. Acetylstrophanthidin

A total renal excretion of 22% was measured after i.v. injection of 1.0–1.5 mg of this short-acting cardenolide in two healthy subjects, by means of RIA (SELDEN et al., 1973).

II. Animal Experiments

1. Ouabain

The first animal experiments for measurements of the excretion of ouabain were carried out in rats, dogs, and cats by HATCHER and EGGLESTON (1919). A greater amount of the i.v. dose was found in the bile of rats and cats than in the dogs. FARAH (1946 b) also found that the greater part of a given dose of ouabain was excreted in the rat via the liver and bile. These observations, that the lethal dose of ouabain in the rat after partial and total hepatectomy decreased in proportion to the amount of liver removed, confirmed the importance of the liver in the excretion of ouabain. As described before, COX et al. (1959) found a predominantly biliary excretion of ouabain in rats upon i.v. injection of high doses (1 mg/kg). DUTTA et al. (1964) used ^3H-ouabain and therefore smaller doses. They found about 12% of a single i.p. injection of 0.2 mg/kg (50 μg/animal) in the urine after 72 h and about 77% in the feces. In contrast, i.v. injection of 250 or 400 μg/animal (25–30 kg body weight) in sheep resulted within the first 64 min in a predominantly renal excretion; the ratio of excretion of ouabain in urine and bile was 30:1 (Table 8).

Detailed investigations of species variations in the biliary excretion of ouabain were carried out by RUSSELL and KLAASSEN (1972, 1973). It was found that, 20 min after an i.v. injection of 80 μg/kg ^3H-ouabain the biliary concentration was much higher in rats than in the rabbit or dog (Table 2). Especially noticeable was the high bile : plasma concentration gradient of 1,500 for rats compared with 2.9 for rabbits and 9.3 for dogs, suggesting that ouabain does not pass so easily from the plasma into the liver or bile in the rabbit and dog as it does in the rat. The species variation in biliary excretion of ouabain is said by the authors to be an important factor in the differing toxicity. These differences in biliary excretion are peculiar to ouabain, as digoxin and digitoxin may be excreted in the bile in large amounts in rabbits and dogs, as well as in rats. IGA and KLAASSEN (1979) found in rats a hepatic extraction of ouabain of about 50% comparing the area under the plasma concentration–time curve and cumulative biliary excretion, respectively, after intraportal and intravenous administration.

SELDEN et al. (1974) investigated the excretion of ^3H-ouabain in dogs and found, 4–6 days after an i.v. injection, 54% in the urine and 5% of the given dose in the bile. Renal excretion also apparently plays an important role in the guinea pig; GARBE and NOWAK (1968) found a total excretion of 91% 26 h after i.v. administration of ^3H-ouabain with only traces found in the feces. MERK (1980) found 43% of an i.p. injection of 69 nmol/kg ^3H-ouabain in the urine, 0.1% in the bile and 9% of the given dose in the intestinal contents. LÜLLMANN et al. (1971) also reported that ouabain is excreted predominantly by the kidneys in guinea pigs. Investigations which fail to show any excretion of ^3H-ouabain in isolated perfused guinea pig liver (KOLENDA et al., 1971) are in agreement with the above findings. MARZO et al. (1974) determined a renal excretion of 24% 5 h after an i.v. injection of 90 μg/kg ^3H-ouabain and an excretion of 4% via the bile (Table 8).

Very few experiments along these lines have been performed with cats. HATCHER and EGGLESTON (1919) failed to demonstrate the presence of any glycoside in the urine of cats. LAHRTZ et al. (1968) in experiments with eight cats, found 3 h after oral or intraduodenal administration of ^3H-ouabain a predominantly renal rather than a biliary excretion.

2. Strophanthoside K

MARZO et al. (1973, 1974) carried out investigations on the pharmacokinetics of this substance in guinea pig. Upon rectal administration of 250, 500, or 1,000 μg/kg ^3H-strophanthoside K, 9% of the radioactivity was found in the urine within 5 h and 18% within 15 h after application (1973). Of the excreted glycoside, 90% remained unmetabolized, the rest being cymarin. The concentration ratio for the parent compound: cymarin in the bile was found to be 78 : 22. Intravenous administration of ^3H-strophanthoside K to guinea pigs with bile fistulae resulted in 19% of the dose being excreted via the kidneys and 23% in the bile. MARZO et al. (1974) found that six times more strophanthoside K was excreted in the bile in guinea pigs than ouabain. Qualitatively similar results were reported by MERK (1980): 27% of the dose were found in the urine 6 h after the administration of 69 nmol/kg strophanthoside K intraperitoneally but 43% in the intestinal content (Table 8). The differences in distribution and excretion of ouabain and strophanthoside K are seen in Fig. 5.

3. Cymarin

The elimination of this glycoside was investigated by MOERMAN (1965) in rats: 10% of an i.v. dose of 5 μg/g was excreted via the kidneys within 2 h. In guinea pigs MERK (1980) found by RIA 2.2% in the urine, and 16% in feces 6 h after i.p. injection of 69 nmol.

4. Acetylstrophanthidin

SELDEN et al. (1973) measured a renal excretion of 13% and an excretion in the bile of 1.5%–2.1% 24 h after the i.v. administration of 1 mg to dogs (17–25 kg).

Table 8. Animal experiments on the excretion of strophanthus glycosides by urine, bile, and feces given in the percentage of administered dose in different collection periods

Reference	Species	Dose		Excretion					
				Urine		Bile		Feces	
				(% dose)	(h)	(% dose)	(h)	(% dose)	(h)
		Ouabain							
Cox et al. (1959)	Rat	1 μg/g	i.v.			88	5		
	Rat	1–2 μg/g	i.p.	≅ 5	24				
Dutta et al. (1964)	Rat	29.5 μCi (0.05 mg)	i.p.	≅12	42			≅77	72
	Sheep	88 and 147 μCi (250 and 400 μg)	i.v.	≅ 3.2	≅ 1	≅ 0.1			
Russell and Klaassen (1972, 1973)	Rat	10 μCi/kg (80 μg/kg)	i.v.			≅55	12		
	Rabbit	5 μCi/kg (80 μg/kg)	i.v.			≅ 4	12		
	Dog	2 μCi/kg (80 μg/kg)	i.v.			≅ 1	12		
			i.v.						
Selden et al. (1974)	Dog	≅73 μCi and unlabeled	i.v.	54	≅100	≅ 5	≅85	≅14	144
Garbe and Nowak (1968)	Guinea pig		i.v.	91	24			10	3
Marzo et al. (1974)	Guinea pig	15 μCi/kg (90 μg/kg)	i.v.	24	5	4	5		
Merk (1980)	Guinea pig	69 n mol/kg	i.p.	43	6	0.09	6	9	6
		Strophanthoside K							
Marzo et al. (1974)	Guinea pig	40 μCi/kg	i.v.	34	24	23	5	32	24
		(250 μg/kg)	i.m.	39	24			29	24
Merk (1980)	Guinea pig	69 n mol/kg	i.p.	27	6	0.2	6	34	6
		Dihydroouabain							
Dutta et al. (1964)	Rat	77.5 μCi (50 μg)	i.p.	5	36			23	36
	Sheep	78–155 μCi (2–50 μg)	i.v.	2.45	≅ 1	0.05	≅ 1		

The experiments were carried out with ^{3}H-labeled glycosides with exception of those of Cox et al. (1959) using thin layer chromatography and bio assay.
i.v. = intravenous, i.p. = intraperitoneal, i.m. = intramuscular

5. Dihydroouabain

DUTTA et al. (1964) measured in rats a 5% renal excretion and an excretion of 23% via the intestine after an i.p. dose of 50 µg/animal ^3H-dihydroouabain. An anesthetized sheep with canulated ureters and biliary tract, excreted 2.5% of a given dose within 64 min; excretion in the bile was 50 times less than in the urine.

6. Convallatoxin

LAUTERBACH (1964) measured in the rat a mean biliary excretion rate of 14% within the first 3 h after intraduodenal doses of 0.5–32 µmol/kg. Of the amount excreted, 70% was the parent compound and the rest convallatoxol. MERK (1980) found by RIA 13% in the urine and 10% in the feces after i.p. injection of 69 nmol.

F. Conclusions

Strophanthus glycosides, in contrast to most glycosides from digitalis plants are distributed faster in the peripheral tissues and their blood levels fall more rapidly after i.v. injection of a single dose.

Ouabain (strophanthin G) is not metabolized and is hence, rapidly excreted via the kidneys, whereas strophantoside K is metabolized, the main metabolite being cymarin. Strophantoside K and its metabolites are predominantly eliminated via liver and/or bile, this elimination occuring more slowly than that of ouabain. Metabolism and excretion patterns of strophanthus glycosides are very different in the various species. Thus, for instance in dogs ouabain is eliminated more slowly than digitoxin while in rats it is eliminated rapidly via liver and bile.

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CHAPTER 4

Pharmacokinetics of Squill Glycosides

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A. Introduction

Among the native squill glycosides, only proscillaridin A has so far found considerable clinical application. In addition, the semisynthetic meproscillarin (4'-methylproscillaridin A) was recently introduced. Pharmacokinetic knowledge of these glycosides has been obtained by indirect techniques based on clinical parameters and by measurements in plasma and other body fluids by use of ^{86}Rb uptake inhibition assay or by ^{3}H-labeled glycosides. Generally, there is agreement between the results from the two approaches.

Separation of intact glycoside from polar metabolites, mainly conjugates, has usually been made by extraction with dichloromethane or chloroform. This raises some doubt concerning the validity of the results found because the extraction step of the ^{86}Rb procedure is not specific for the intact glycosides. Metabolites, such as the aglycone scillarenin, are also extracted and might contribute to the inhibition of ^{86}Rb transport, although it is known that hydrolysis and subsequent epimerization decrease the inhibitory effect of squill glycosides to a large extent (BELZ et al., 1973; BERGDAHL and ANDERSSON, 1977). It has been found that some of the glucuronides of digoxin and digitoxin exhibit considerable inotropic activity in isolated guinea pig papillary muscle (BELZ and HEINZ, 1977). It is possible that also conjugated squill glycosides are cardioactive; this remains to be studied.

B. Distribution After Intravenous and Oral Administration

I. Proscillaridin A

BELZ et al. (1974b) administered proscillaridin A intravenously during 2 h to five male volunteers. During the 1st h after the administration, plasma concentrations measured by ^{86}Rb technique were found to decrease rapidly. This suggested a rapid initial distribution of the glycoside to the tissues. Plasma concentration data after oral intake further supported this view and also revealed two maxima in the plasma concentration time curve. Thus, after giving 2.5 mg as tablets, BELZ et al. (1974c) measured a peak concentration in the first plasma sample taken (after 0.5 h), with a median value of 0.41 ng/ml (range 0–1.5 ng/ml). A second maximum of about the same magnitude was found after 10 h, and there was a distinct minimum at 3 h (median value $\sim$ 0.10 ng/ml). The existence of two peaks in the plasma concentration time curve after oral administration to healthy subjects was confirmed by ANDERSSON et al. (1977a) also using the ^{86}Rb technique for estimation of gly-

coside plasma concentration. In that study, the first maximum was found after 20–45 min, and higher values were observed (range 1.64–3.20 ng/ml) than in the investigation by BELZ et al. (1974c) despite similar dosage. Some difference might be attributed to a more frequent plasma sampling during the first 2 h after drug intake, thereby better defining the true maxima, but the magnitude of the differences suggests other influences, e.g., dosage form factors and subject variability.

The second peak in the concentration time curve could be caused either by redistribution of the glycoside or by hepatoenteric recycling. An analysis of the proscillaridin concentrations in portal and peripheral blood after oral intake of 1.5 mg of the drug (tablets) was performed in four patients undergoing diagnostic portal vein catheterization (ANDERSSON et al., 1977b). The individual portoperipheral differences were small and reached a maximum within 10–25 min; at 4 h no differences were found. Reoccurring portoperipheral differences were registered after 6–10 h in three patients, suggesting new absorption of glycoside. An extensive biliary excretion of proscillaridin A, mainly in conjugated form, was demonstrated by ANDERSSON et al. (1977d) in patients with biliary drainage. In vitro experiments demonstrated splitting of proscillaridin conjugates by enteric contents (ANDERSSON et al., 1977d), opening up a possibility for reabsorption of the drug. These findings suggest that hepatoenteric recycling, and not redistribution of the glycoside from initial binding sites, is the most probable cause of the second peak in the plasma concentration time curve.

Measurement of proscillaridin concentrations in the thoracic duct lymph in two patients after oral intake of the glycoside showed that the concentration in the lymph closely followed that in the plasma (ANDERSSON et al., 1977c), which should exclude proscillaridin transport in the lymph as an important alternative pathway to the systemic blood after absorption.

II. Meproscillarin

The plasma concentration of meproscillarin, after oral and intravenous administration of the glycoside, exhibited a pattern qualitatively similar to that found with proscillaridin A (BELZ et al., 1976). Thus, after intravenous administration (Fig. 1), there was an initial steep decrease in the plasma concentrations occurring for about 3 h. From 4 to 10 h, no further decrease, but rather a small increase in plasma concentration was observed in most of the 16 subjects investigated. After oral administration, the plasma concentrations generally decreased sharply after the first sample taken at 1 h, and a second peak was more distinct (Fig. 1). RIETBROCK and STAUD (1975) administered 0.5 mg ^{3}H-methylproscillaridin orally to healthy volunteers and found maximum plasma concentrations of total radioactivity (2.5% of the dose/liter) within 1–2 h after oral intake. They also observed a second peak of activity (1.1% of the dose/liter) in two of five subjects investigated between 6 and 12 h. In four patients with biliary drainage given the same dose, STAUD et al. (1975) found maximum radioactivity (2.8%–6% of the dose/liter plasma) within 1–3 h after intake. A second maximum was seen in one patient, which might indicate incomplete drainage.

Available data thus suggest that the distribution patterns of proscillaridin A and meproscillarin are similar. Both show second maxima in the plasma concen-

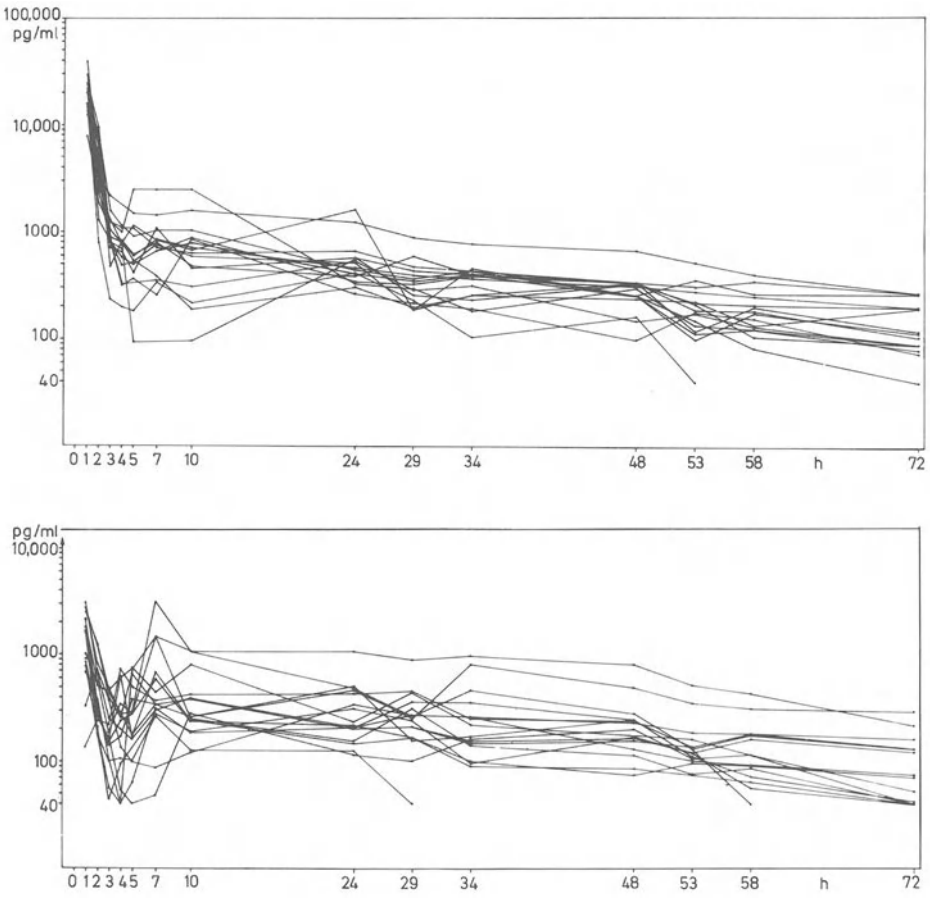

Fig. 1. Plasma meproscillarin concentrations at different times after intravenous *(upper panel)* and oral *(lower panel)* administration of 1.2 mg to 16 healthy volunteers. BELZ et al., 1976

tration profile, probably because of hepatoenteric recycling. As judged from the area under the plasma concentration time curve after intravenous administration and the slope of the terminal part of the curve, squill glycosides, like most other cardiac glycosides, have a large volume of distribution in the body. Except for in vitro results showing a high binding of squill glycosides to plasma proteins (BELZ and SCHREITER, 1974), there are no data that allow conclusions to be drawn about the affinity of the squill glycosides to different tissues in the body.

C. Metabolism and Excretion Pathways

I. Proscillaridin A

Evidence for an extensive metabolic inactivation of proscillaridin A was found by ANDERSSON et al. (1975). They gave repetitive oral doses of the drug to healthy vol-

unteers and found less than 1% of the daily administered dose in feces and urine during 24-h sampling periods, measured as proscillaridin extractable with dichloromethane. The concentration of extractable proscillaridin found in bile after single oral doses to patients with biliary drainage was 10–100 times higher than that in plasma (ANDERSSON et al., 1977 d). The amount of intact drug found in bile during 24 h was estimated to be less than 1% of the dose, but after incubation of the bile samples with β-glucuronidase and sulfatase, this amount increased 100- to 200-fold. Thus, conjugation of the glycoside to glucuronic and/or sulfuric acid seems to be a major metabolic pathway.

After single oral doses of the glycoside to patients undergoing diagnostic portal vein catheterization, low peak concentrations of proscillaridin were found in the portal vein as well as in peripheral veins (ANDERSSON et al., 1977 b). However, after enzymatic hydrolysis of the plasma samples of one patient, the extractable proscillaridin as well as the portoperipheral concentration differences increased markedly. The peaks in the portal blood at 30 min after administration were 2.4 before and 47 ng/ml after the deconjugation. The corresponding peaks in the peripheral plasma were 0.8 and 18 ng/ml.

After single oral doses of 2.5 mg of the glycoside to male volunteers, pooled plasma samples had a proscillaridin concentration of 0.56 ng/ml. Enzymatic treatment increased the concentration to about 50 ng/ml (BERGDAHL, 1977). When treating venous samples taken 30 min after the dose had been administered, glycoside concentrations as high as 150 ng/ml were found after deconjugation (BERGDAHL, 1977). Deconjugation of plasma samples from patients on maintenance dose treatment, taken just before the dose of proscillaridin, increased the concentration from 0.66 ± 0.43 ng/ml (mean $\pm$ SD) to 9.2 ± 6.6 ng/ml (BERGDAHL, 1977). A large proportion of conjugated proscillaridin is obviously present in both portal and peripheral blood. Its existence in portal blood suggests that conjugation already takes place in the gut wall.

II. Meproscillarin

Using tritiated meproscillarin, WEYMANN et al. (1978) performed a study of the disposition of the glycoside in rats and dogs. After intravenous administration of the drug to rats, an average of 55% of the dose was recovered in feces during the first 48 h; about 30% of this fraction was soluble in chloroform. During the same time, only 6% of the dose was recovered in urine, and of this more than 90% was chloroform-soluble. The large amount of drug in feces seems to be explained by biliary excretion: within 5 h an average of 25% of an intraduodenal dose was recovered in bile. Half of this amount was extractable by chloroform (bile/chloroform ratio 1 : 2, three extractions).

The same authors also found a rapid metabolism of meproscillarin in dogs. Thus, 30 min after an oral dose almost half of the radioactivity in plasma was bound to polar metabolites of the glycoside. At this time, the chloroform-extractable activity (plasma/chloroform ratio 1 : 1, three extractions) consisted of approximately 65% meproscillarin, the rest was scillarenin, the corresponding aglucone. During the following hours, the amount of meproscillarin gradually decreased and was replaced by metabolites. Unchanged glycoside was not detectable

after 7 h when the chloroform fraction consisted of 70% proscillaridin and 30% 3α, 12β-hydroxyscillarenin.

As in rats, biliary excretion of meproscillarin was also predominant in dogs. After an oral dose of 20 μg/kg, 25% appeared in bile within 6 h; 4% of the dose was found in the chloroform phase. The metabolites in the water-soluble fraction consisted mainly of glucuronides (70%–80%) and sulfates (10%). During 5 days after an oral dose of meproscillarin, an average of 70% was recovered in feces. Only about half of this amount was said to be extractable with chloroform. During the first 48 h of sampling, mainly unchanged meproscillarin was found in the feces of the dogs. As the glycoside and its metabolites were excreted via the bile predominantly as conjugates, the authors concluded that a splitting of the conjugates occurred in the gut. However, this conclusion is valid only if the degree of absorption of the glycoside was high.

Detailed studies of the fate of tritiated meproscillarin after single oral doses to five healthy men were performed by RIETBROCK and STAUD (1975). Solubility in chloroform was determined by three extractions with a 2:1 ratio between organic and aqueous phase. About 45% of the radioactivity in plasma was chloroform-soluble, and this percentage remained constant for a period of 6 days. However, the low amount of radioactivity in plasma made quantitation of various metabolites possible only during the first 4 h. Initially, mainly meproscillarin and also proscillaridin, scillarenin, and two unidentified metabolites were detected. The kinetics of these two unknown metabolites suggested that one of them might be transformed into the other. At 4 h, no meproscillarin was found, and 70% of the radioactivity in plasma consisted of the more stable metabolite. When the water-soluble fraction of radioactivity in plasma was treated with β-glucuronidase, there was a 62% cleavage of the fraction; meproscillarin was the major cleavage product.

The investigators also found that during a sampling period of 7 days, an average of 56% of the administered meproscillarin dose was recovered in feces; 80% of this fraction was chloroform-soluble and consisted mainly of meproscillarin (90%) and small amounts of scillarenin (4%). The chloroform-insoluble fractions (about 12% of the given dose and probably conjugates) could not be hydrolyzed by treatment with β-glucuronidase.

In urine, a mean of 6% of the dose was recovered in the chloroform and 14% in the aqueous phase during 7 days. The former fraction consisted mainly of the two unknown metabolites, but small amounts of meproscillarin, proscillaridin, scillarenin, and another unidentified metabolite were also found. After treatment of the chloroform-insoluble fractions with β-glucuronidase, half of it was hydrolyzed and, as in plasma, mainly meproscillarin was recovered. The amount of meproscillarin excreted in urine in patients with biliary drainage (STAUD et al., 1975) was similar to that found in healthy subjects.

STAUD et al. (1975) found that on average 55% (range 29%–89%) of an oral dose of tritiated meproscillarin could be recovered in the bile of patients with biliary drainage; the sampling time varied from 72 to 120 h; 43% of the dose was excreted during the first 24 h. The chloroform-soluble fraction (extraction conditions as above) was about 12% of the dose. Within this fraction, quantitation of various metabolites was not possible, but scillarenin, meproscillarin, proscillaridin, and the above-mentioned three unidentified metabolites were found. Of the polar fraction,

80% could be split by enzyme treatment and found to be meproscillarin conjugated to glucuronic acid. Only 3% of the radioactivity was recovered in feces during 96–120 h. This finding strengthens the impression that biliary excretion is responsible for the large amount eliminated in feces in healthy volunteers (RIETBROCK and STAUD, 1975). As mainly unchanged meproscillarin was found in feces and polar conjugates predominated in the bile, deconjugation appears to have occurred during the intestinal passage. A marked increase in the glycoside concentration was found when bile samples from patients given proscillaridin were treated in vitro with enteric contents (ANDERSSON et al., 1977d). A similar deconjugation of meproscillarin conjugates by enteric contents therefore seems probable.

The extent of hepatoenteric recycling of meproscillarin was first shown by BELZ and BADER (1974) giving oral doses of activated charcoal together with an intravenous dose of the glycoside. They found that plasma concentrations of the drug 10 h after administration were about 60% of that of controls not given charcoal, suggesting adsorption to charcoal in the intestine after biliary excretion of drug. Thus, reabsorption of squill glycosides after biliary excretion reduces the elimination by this pathway. Nevertheless, more than half of the dose appears to leave the body in the feces. This fraction should be further increased when renal function is compromised (see below).

D. Elimination Rate

I. Proscillaridin A

Studies of the effects of proscillaridin A in patients with cardiac disease, mostly atrial fibrillation, have revealed that the glycoside has a moderate duration of action. The daily loss of glycoside during maintenance therapy was estimated at 20%–50% of the loading dose (MEIER and WAGNER, 1965; HÄNEL and MEIFFERT, 1966; BELZ, 1968; LÖSCHHORN, 1969; WÜLFING VON DER HEYDEN, 1969; BULITTA, 1974).

After intravenous administration of proscillaridin to male volunteers, BELZ et al. (1974b), using the ^{86}Rb uptake inhibition assay, found a mean terminal elimination half-life ($t_{\frac{1}{2}}$) of 46 h. This was evaluated during a period of 9–96 h after the dose. Using the same assay, BELZ and BRECH (1974) determined the plasma $t_{\frac{1}{2}}$ of proscillaridin after oral maintenance doses to male volunteers and patients with serious renal insufficiency. The elimination of proscillaridin was found to be independent of renal function; the median $t_{\frac{1}{2}}$ in the volunteers was 47 h (range 28–68 h) and in the patients 41 h (range 34–88 h). BERGDAHL (1979) also determined the plasma $t_{\frac{1}{2}}$ of proscillaridin by the ^{86}Rb assay after stopping maintenance dose treatment. In four young male volunteers the mean elimination constant corresponded to a $t_{\frac{1}{2}}$ of 23 h (range 21–29 h), which is shorter than that found by BELZ and BRECH (1974). The elimination of proscillaridin from plasma in 24 elderly patients with slight to moderate cardiac insufficiency (BERGDAHL, 1979) was in agreement with previous studies including those using indirect methods; a median $t_{\frac{1}{2}}$ of 49 h was found. A striking finding was the tenfold variation of the elimination rate; $t_{\frac{1}{2}}$ ranged 19–209 h, and seven patients had a $t_{\frac{1}{2}}$ longer than 69 h. A statistical evaluation showed that methodological factors were of minor importance for the variation observed. The true variation may be even greater, as some patients with

an evidently rapid elimination had to be excluded because the proscillaridin concentration was below the detection limit of the method in samples crucial for the evaluation. It should also be pointed out that the hepatoenteric recycling of the glycoside (cf. above) makes calculation of the elimination rate difficult as a true exponential decline of the plasma concentration cannot be taken for granted.

II. Meproscillarin

WEYMANN et al. (1978) investigated the elimination of tritiated meproscillarin in dogs. Plasma $t_{\frac{1}{2}}$ of total label averaged 18 h; the same value was found for the label that could be extracted by chloroform. The information on meproscillarin elimination obtained indirectly from studies of drug effects in patients with atrial fibrillation has shown a daily loss of glycoside of about 40% of the loading dose (HERKEN and BRANDES, 1978). Using tritiated meproscillarin, RIETBROCK and STAUD (1975) found that the plasma $t_{\frac{1}{2}}$ of label averaged 51 h in five healthy volunteers. STAUD et al. (1975), studying the drug in patients with biliary drainage, found a $t_{\frac{1}{2}}$ of label in plasma ranging 18–30 h. As discussed above, interruption of hepatoenteric recycling is the most probable explanation to the difference between the reports.

Data on the elimination of meproscillarin in volunteers and patients have also been obtained by measuring the glycoside concentration in plasma by means of the ^{86}Rb assay. After single oral doses of meproscillarin, an average $t_{\frac{1}{2}}$ of 33 h was found; the corresponding result after intravenous administration was 23 h (BELZ et al., 1976). After single intravenous doses of meproscillarin, the elimination of the glycoside from plasma was shown to be independent of renal function. In patients with uremia, the plasma $t_{\frac{1}{2}}$ averaged 29 h and that of controls was 27 h (BELZ et al., 1974a). When comparing the elimination of meproscillarin in male volunteers and patients with renal insufficiency, BECKMANN et al. (1978) observed average elimination half-lives of 46 and 49 h, respectively. As expected, no correlation was found between the elimination constant of the glycoside and creatinine clearance. During continuous treatment with meproscillarin in patients with impaired renal function and healthy subjects, no statistically significant differences were found between the plasma concentrations of the two groups (TWITTENHOFF et al., 1978). This further supports the view that meproscillarin is eliminated independent of renal function.

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Pharmacokinetics – Additional Pharmacokinetic Parameters of Cardiac Glycosides

CHAPTER 5

Plasma Protein Binding of Cardiac Glycosides

J. KRIEGLSTEIN

A. Introduction

As early as 1913 OPPENHEIMER reported that the toxic effects of digitoxin on the isolated frog heart were considerably attenuated when the glycoside was dissolved in serum rather than in Ringer's solution. He suggested that digitoxin is partially bound to a constituent of serum. About 20 years later HOEKSTRA (1931) and BRÜCKE (1934) considered this problem again and demonstrated a binding of digitoxin when various proteins were added to the perfusion medium of isolated frog heart preparations. Further studies carried out by LENDLE and PUSCH (1935) and HAARMANN et al. (1940 a, b) showed the first evidence of albumin being the binding protein in serum. The albumin binding of digitoxin was confirmed by the work of FAWAZ and FARAH (1944), FARAH (1945), and ROTHLIN and KALLENBERGER (1950). The first extensive quantitative study on the binding of cardiac glycosides to human plasma proteins was presented by SCHOLTAN et al. (1966) and by LUKAS and DEMARTINO (1969).

B. Characterization of Plasma Protein Binding

As shown in Table 1, there is a relatively large range in the binding data reported. Some of the differences may be partially explained by differences in protein concentration (albumin content), the temperature at which the study was performed, and the experimental procedure used. Nevertheless, it becomes evident that digitoxin is highly bound to plasma protein (i.e., 90%), digoxin is bound only to an intermediate extent (20%–30%), and ouabain reveals only very small if any binding.

BAGGOT and DAVIS (1973) compared the extent of plasma protein binding of digitoxin and digoxin in several species (Table 2). They used plasma of cat, dog, goat, horse, man, monkey, opossum, ox, pony, rabbit, rat, sheep, and swine. In every plasma studied, digitoxin is more avidly bound than digoxin although the extent of binding varies significantly among species. In addition, human plasma revealed the highest percentage of bound digitoxin as was also found in earlier studies (FARAH, 1945; PFORDTE and FÖRSTER, 1970; ABSHAGEN et al., 1971).

It is now generally accepted that cardiac glycosides in human plasma are mainly bound to albumin. LUKAS and DEMARTINO (1969) found 91% of digitoxin in human plasma to be bound to albumin, 6% to γ-globulin, and 3% to other globulins (α_2, β_1, β_2). EVERED (1972) found digoxin to be entirely bound to albumin in human serum. Using pure γ-globulin solutions, a considerable binding affinity of the glycosides to this protein was also demonstrated (SCHOLTAN et al., 1966; KUSCHIN-

Table 1. Binding of cardiac glycosides and some of their metabolites to human plasma protein

Drug	Protein		Binding		Total drug concentration (μg/ml)	Reference
	Type[a]	Concentration (g/100 ml)	%	Association constant (liter/mol)		
Digitoxin	HSA	0.8	30–42		10–20	HAARMANN et al. (1940a)
	HSA	0.5	48		3.3	FARAH (1945)
	HSA	4	90		100	ROTHLIN and KALLENBERGER (1950)
	HSA	0.5	44.5		33	SPRATT and OKITA (1958)
	HSA	0.5	43.0		0.133	
	HSA	1	57.5	1×10^4	30	SCHOLTAN et al. (1966)
	HSA	4	91.5		30	
	HS		93.6		0.25	KUSCHINSKY (1969)
	HSA	4	93.8	2.78×10^4	0.25	
	HSA	4	97	9.62×10^4	0.01–12	LUKAS and DEMARTINO (1969)
	HP	7.1–7.3	97		0.01–12	
	HSA	0.1	50		0.01–12	
	HP		88		0.106	KOBINGER and WENZEL (1970)
	HSA	3	92.6		0.025	SOLOMON et al. (1971)
	HP		90.1		0.0125	SHOEMAN and AZARNOFF (1972)
	HS		97.3		0.010–0.025	STORSTEIN (1976a)
	HP	6.8	92.3		0.050	BAGGOT and DAVIS (1973)
	HP	7.2	93.7		0.0007–0.001	KRAMER et al. (1974)
	HSA	0.05–0.1		5×10^4	0.01–0.1	BROCK (1975)
	HS	4–6		3.6×10^4		
	HS	7.2	93.3			WIRTH et al. (1976)
	HSA	4	93.2–94.2		0.02–0.26	RICHTER and HAUSTEIN (1977)
Digitoxigenin	HSA	1	81	3.37×10^4	30	SCHOLTAN et al. (1966)
	HSA	4	84.2	1.058×10^4	0.25	KUSCHINSKY (1969)
	HP	7.1–7.3	94		5	LUKAS and DEMARTINO (1969)
	HSA	4	94	4.06×10^4	5	
	HSA	0.69	78		5	
	HP		71		0.0919	KOBINGER and WENZEL (1970)
	HS		92.7		0.2	STORSTEIN (1976a)
Digitoxin bisdigitoxoside	HSA	4		9.7×10^4	20.3	LUKAS and DEMARTINO (1969)
	HS		96.5		0.2	STORSTEIN (1976a)

Table 1 (continued)

Drug	Protein		Binding		Total drug concentration (μg/ml)	Reference
	Type[a]	Concentration (g/100 ml)	%	Association constant (liter/mol)		
Digitoxin mono-digitoxoside	HSA	4		9.7×10^4	16.2	LUKAS and DEMARTINO (1969)
	HS		98.7		0.2	STORSTEIN (1976a)
Digoxin	HSA	1	46.2	7.03×10^3	30	SCHOLTAN et al. (1966)
	HS		18.6		0.25	KUSCHINSKY (1969)
	HSA	4	21.3	4.61×10^2	0.25	
	HP	7.1–7.3	23		0.005	LUKAS and DEMARTINO (1969)
	HP	7.1–7.3	10		30	
	HSA	4	23	9×10^2	2	
	HSA	1	13		2	
	HP		0		0.0372	KOBINGER and WENZEL (1970)
	HS		< 1			DOHERTY et al. (1971)
	HS	8	25	1.85×10^5	0.25–25	EVERED (1972)
	HS	6.3–7.05	30	6.8×10^4	0.000032–0.02129	OHNHAUS et al. (1972)
	HP	6.8	24.9		0.01	BAGGOT and DAVIS (1973)
	HSA	4	23.8		0.002–0.006	DENGLER et al. (1973)
	HSA	4.34		4.9×10^2	0.002	BROCK (1974)
	HP	7.2	29.4		0.004–0.006	KRAMER et al. (1974)
	HS		21.2		0.2	STORSTEIN (1976a)
	HP		22.1–24.1		0.003–0.06	HINDERLING (1977)
Digoxigenin	HSA	4	14		1	LUKAS and DEMARTINO (1969)
	HSA	2	10		1	
	HSA	1	7		1	
	HSA	0.1	1.1		1	
	HP		26		0.0546	KOBINGER and WENZEL (1970)
	HS		13.3		0.2	STORSTEIN (1976a)
Digoxin bis-digitoxoside	HS		16.3		0.2	STORSTEIN (1976a)
	HP		19.9–23.5		0.003–0.06	HINDERLING (1977)
Digoxin mono-digitoxoside	HS		18.5		0.2	STORSTEIN (1976a)
	HP		26.1–28.2		0.003–0.06	HINDERLING (1977)

Table 1 (continued)

Drug	Protein		Binding		Total drug concentration (μg/ml)	Reference
	Type[a]	Concentration (g/100 ml)	%	Association constant (liter/mol)		
Dihydrodigoxin	HP		22.8–24.4		0.003–0.06	Hinderling (1977)
Ouabain	HSA	0.78	13.4		20	Haarmann et al. (1940b)
	HSA	1	11.5	1.09×10^3	30	Scholtan et al. (1966)
	HS		2.4		0.25	Kuschinsky (1969)
	HSA	4	4.6	$< 10^2$	0.25	
	HP		0		0.0066	Kobinger and Wenzel (1970)
	HP	7.2	0.5		0.0003–0.0005	Kramer et al. (1974)
	HSA	4	4.8		0.02–0.25	Richter and Haustein (1977)
Ouabagenin	HSA	1	16.5	1.39×10^3	30	Scholtan et al. (1966)
	HP	1	10		0.1104	Kobinger and Wenzel (1970)
Acetyldigitoxin	HP		83		0.1217	Kobinger and Wenzel (1970)
α-Acetyldigitoxin	HSA	4	81–83		0.02–0.04	Bodem et al. (1975)
β-Acetyldigoxin	HP		24		0.0477	Kobinger and Wenzel (1970)
16-Acetyl-16α-gitoxin	HSA	4	93.1–95.8		0.08–0.49	Richter and Haustein (1977)
Convallatoxin	HSA	1	23	2.5×10^3	30	Scholtan et al. (1966)
	HP		16		0.0041	Kobinger and Wenzel (1970)
Fluor-α-acetyl-digoxin	HSA	4	26.2		0.002–0.004	Bodem et al. (1978)
16α-Gitoxin	HSA	4	72.5–75		0.012–4.9	Richter and Haustein (1977)
Hellebrin	HP		43		0.00538	Kobinger and Wenzel (1970)
Hellebrigenin	HP		34		0.00375	Kobinger and Wenzel (1970)
Lanatoside C	HP		46		0.0712	Kobinger and Wenzel (1970)
	HSA	4	25.1		0.002–0.006	Dengler et al. (1973)
β-Methyldigoxin	HP	7.2	29.8		0.003–0.005	Kramer et al. (1974)
	HP		9.8		0.00049–0.076	Hinderling et al. (1977)
Pengitoxin	HSA	7.7	96		30	Emmrich et al. (1969)
Proscillaridin A	HP		85		0.00899	Kobinger and Wenzel (1970)
Scillarenin A	HP		75		0.0136	Kobinger and Wenzel (1970)

[a] HP, human plasma; HS, human serum; HSA, human serum albumin

Table 2. Plasma protein binding of digitoxin and digoxin in several species of animals according to BAGGOT and DAVIS (1973)

Species	Percent bound		Plasma protein concentration (g/100 ml)
	Digitoxin 0.05 µg/ml	Digoxin 0.01 µg/ml	
Goat	85.2	22.5	6.7
Sheep	86.1	25.3	6.4
Ox	86.6	19.2	6.8
Horse	83.3	36.4	6.1
Pony	81.0	29.7	8.1
Swine	88.7	31.2	7.2
Dog	88.8	27.0	6.9
Cat	86.6	18.1	7.3
Man	92.3	24.9	6.8
Monkey	90.1	30.7	8.4
Rat	86.1	17.3	7.0
Rabbit	90.3	40.1	6.5
Opossum	72.3	21.9	6.3

SKY, 1969). However, in plasma or serum the binding of cardiac glycosides to globulins may be neglected. Plasma and serum bind the glycosides to the same extent as a 4% albumin solution does (SCHOLTAN et al., 1966; KUSCHINSKY, 1969).

The albumin binding reaction is spontaneous and reversible and follows the law of mass action (SCHOLTAN et al., 1966; LUKAS and DEMARTINO, 1969). When the albumin concentration is decreased, the binding of digitoxin and digoxin also diminishes (Table 1; SCHOLTAN et al., 1966; KUSCHINSKY, 1969; LUKAS and DEMARTINO, 1969). On the other hand, the albumin binding of the glycosides does not clearly depend on the total glycoside concentration. A constant protein-bound fraction of about 30% was found in human serum over a wide range of total digoxin concentration (0.032–21.29 ng/ml; OHNHAUS et al., 1972). Only when the total concentration of digoxin in human plasma was increased to 30 µg/ml did the extent of binding seem to be considerably reduced (LUKAS and DEMARTINO, 1969). In a similar dosage range (10–40 µg/ml) far above therapeutic plasma levels, the albumin binding of digitoxin depended on the total glycoside concentration (SCHOLTAN et al., 1966).

The binding affinity of the cardiac glycosides increases with their hydrophobic character (SCHOLTAN, 1968) and diminishes considerably with decreases in temperature (LUKAS and DEMARTINO, 1969; SCHOLTAN et al., 1966). Therefore, it has been concluded that these compounds are bound to albumin mainly by hydrophobic forces. Consequently, the binding affinity of digitoxin, digoxin, and ouabain decreases in the aforementioned order. Digoxin differs chemically from digitoxin only in an additional hydroxyl group at position 12-β of its steroid nucleus. Because of this hydroxyl group, digoxin is more polar and binds less avidly than digitoxin to albumin. Ouabain is even more polar than digoxin, and therefore, the binding affinity of this glycoside is even more reduced.

In contrast to this distinct relationship between polar groups on the steroid nucleus and binding affinity to albumin, the participation of the sugar residues in the

binding of the glycosides is unclear. Most authors demonstrate that digitoxin, digoxin, and even some of their digitoxosides bind more avidly to albumin than the corresponding genins (KUSCHINSKY, 1969; LUKAS and DEMARTINO, 1969; HINDERLING, 1977). Digitoxose seems to enhance binding of the genins to albumin. The increase of binding conferred on the genin does not appear to vary with the number of digitoxose residues; the binding constants of digitoxin, of monodigitoxoside, and of didigitoxoside to albumin are of a similar magnitude and more than twice as large as that of the genin (Table 1; LUKAS and DEMARTINO, 1969). However, SCHOLTAN et al. (1966) who carried out these experiments in a very high range of concentration reported a reduction of binding affinity by the digitoxose residues (Table 1).

There seems to be one major binding site for digitoxin on the albumin molecule with a relatively high affinity (LUKAS and DEMARTINO, 1969; KUSCHINSKY, 1970). However, the determination of the number of binding sites for the cardiac glycosides on the albumin molecule is surrounded by some uncertainty. The existence of additional binding sites with lower binding affinity cannot be excluded. Because of the low affinity of digoxin to albumin and its limited water-solubility, it is difficult to obtain reliable data for a Scatchard plot particularly for this glycoside. Thus, the values reported in the literature differ remarkably. Whereas LUKAS and DEMARTINO (1969) asssume one binding site on the albumin molecule also for digoxin, OHNHAUS et al. (1972) calculated an infinite number of digoxin binding sites. Digitoxin, digoxin, and their genins compete for their binding to the albumin molecule indicating that they are attached to the same binding sites (LUKAS and DEMARTINO, 1969).

Most cardiac glycosides, except digitoxin, have an intermediate or a low affinity to albumin (association constants $K < 10^3$ liter/mol; Table 1) and in addition, they have a relatively large apparent volume of distribution (VÖHRINGER and RIETBROCK, 1974; STORSTEIN, 1976b). Thus, the plasma protein binding of these glycosides is hardly relevant for pharmacokinetics in therapeutic circumstances. On the contrary, digitoxin reaches with an association constant $K = 9.62 \times 10^4$ liter/mol the category of strongly bound drugs (MARTIN, 1965; JUSKO and GRETCH, 1976). For instance, association constants in a similar order of magnitude are reported for phenylbutazone $K = 1 \times 10^5$ liter/mol (CHIGNELL, 1969), for warfarin $K = 8.8 \times 10^4$ liter/mol (SOLOMON et al., 1968), for tolbutamide $K = 9.04 \times 10^4$ liter/mol (HSU et al., 1974), and for chlorpromazine $K = 2.1 \times 10^4$ liter/mol (KRIEGLSTEIN et al., 1972). Therefore, the plasma protein binding of digitoxin may be of some pharmacokinetic significance.

C. Role of Albumin Binding in Pharmacokinetics

From the earliest findings, namely, those of OPPENHEIMER (1913), it may be supposed that the therapeutic effects of cardiac glycosides are influenced by their binding to plasma protein. Many experimental approaches were undertaken to describe quantitatively the role of plasma protein binding in the pharmacokinetics of cardiac glycoside. To achieve clear-cut experimental conditions, isolated heart preparations were frequently used (LÜLLMANN et al., 1969; KOBINGER et al., 1970; RIEGER and KUSCHINSKY, 1972). It is demonstrated in these in vitro studies that the uptake of the cardiac glycosides into heart tissue and their pharmacologic effects depend

on the extent of their binding to constituents of the perfusion or incubation media. However, the results of these experiments are scarcely transferable to in vivo conditions. Under conditions of therapeutic equilibrium, human plasma contains only 1% of all of the digoxin in the body and approximately 6% of the body pool of digitoxin (LUKAS, 1976). On the contrary, when an isolated guinea pig heart or isolated atrial tissue is treated with a protein solution containing the glycoside, the larger amount of the drug is present in the medium. This might be the reason why experimental studies on isolated organs reveal a marked influence of plasma protein binding on the pharmacodynamic effects of cardiac glycosides whereas in vivo this influence is not clearly demonstrable.

In vivo plasma *and* tissue binding regulate the concentration of free glycoside in the extracellular and plasma water. The final concentration of free glycoside at equilibrium is widely determined by the tissues because the binding capacity of the tissues far exceeds that of the plasma (DOHERTY et al., 1967; LUKAS and DEMARTINO, 1969; EVERED, 1972; LUKAS, 1976; BINNION, 1978). Nevertheless, there remains some evidence that the effects at least of digitoxin may be influenced by its strong binding to albumin. Because of its greater affinity to albumin, the plasma concentration of digitoxin in patients is higher than that of digoxin and exhibits less fluctuation during the course of the day. The total concentration of digitoxin is 14 times greater than that of digoxin; however, the concentration of free digitoxin is only half that of digoxin (LUKAS and DEMARTINO, 1969). Furthermore, plasma protein binding of digitoxin has been suggested as contributing significantly to its long duration of action (BIGGER and STRAUSS, 1972).

Disease states can affect the plasma protein binding of cardiac glycosides by altering the amount of protein available for drug binding and by altering the binding capacity of the proteins. Hypoalbuminemia and uremia were especially investigated in this regard.

It is easily demonstrable in vitro that lowering the protein concentration resulted in decreased binding of cardiac glycosides. Therefore, it was expected that hypoalbuminemia would affect the concentration of free glycosides in the plasma to a significant extent. Thus, plasma protein binding of digoxin was found to be diminished in kwashiorkor serum (BUCHANAN et al., 1976), and the binding of digitoxin was reduced in hypoalbuminemic sera of patients with active hepatitis (STORSTEIN, 1977) and with nephrotic syndrome (STORSTEIN, 1976b).

The findings on protein binding of cardiac glycosides in uremia are conflicting. Plasma protein binding of digitoxin, digoxin, and methyldigoxin was significantly reduced in uremic patients (KRAMER et al., 1974). Decreased binding of digotoxin to plasma proteins in uremia was also reported by SHOEMAN and AZARNOFF (1972) and by PETERS et al. (1977). However, addition of urea to plasma in vitro does not influence digitoxin binding (SHOEMAN and AZARNOFF, 1972). The altered binding observed in uremia was interpreted, therefore, as an alteration in plasma proteins or could also be caused by an inhibitor strongly bound to albumin (HAWLINA and RAHN, 1974). CRAIG et al. (1976) treated the sera from uremic patients with charcoal and afterward found a significant increase of drug protein binding. Digitoxin and several other drugs were investigated. They explained this enhancement of drug binding by the removal of an inhibitor that may accumulate in uremia.

From the relatively strong binding of digitoxin to one binding site on the albumin molecule, the question arises whether there are other avidly bound drugs that

may compete with digitoxin. Many experiments on the interaction of digitoxin with other drugs due to plasma protein binding were performed in vitro. For example, it was demonstrated that phenylbutazone, warfarin, tolbutamide, sulfadimethoxine, and clofibrate interfere with the albumin binding of digitoxin in vitro (SOLOMON et al., 1971). BROCK (1976) also found a displacement of digitoxin from human serum albumin in vitro by bile acids, cholic acid, desoxycholic acid, surface active organic anions, and free fatty acids. However, the clinical significance of all these in vitro effects is very doubtful because the concentrations of the displacing substances used are never found in clinical practice.

During the therapeutic maneuver of switching glycosides, significant displacement from albumin of one glycoside by the other could occur. Again from in vitro studies it is known that digitoxin, digoxin, and their genins can compete for their albumin binding site (LUKAS and DEMARTINO, 1969). However, even these competition phenomena were only demonstrable at concentrations of the displacing agents that are several thousand times higher than those attained in plasma under therapeutic circumstances.

The interaction of heparin with cardiac glycosides may be of some clinical interest (STORSTEIN and JANSSEN, 1976). Changes in glycoside binding were first observed in uremic patients during hemodialysis within 5 min after heparin injection. The reduction in plasma protein binding of digoxin and digitoxin was considerable. Similar changes were produced by heparin administered to patients with normal renal and hepatic function. Displacement of the cardiac glycosides from their albumin binding site directly by heparin may be excluded as no influence of heparin on digitoxin binding was measurable in vitro. STORSTEIN and JANSSEN (1976), therefore, suggested that the heparin-induced release in free fatty acids was responsible for the changes in digitoxin and digoxin binding during hemodialysis.

D. Conclusion

Digitoxin is highly bound to human serum albumin. This binding may be of some pharmacokinetic significance. However, clinical importance of albumin binding of digitoxin as well as of the other cardiac glycosides remains to be shown.

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CHAPTER 6

Intestinal Absorption and Secretion of Cardiac Glycosides

F. LAUTERBACH

A. Introduction

Absorption of cardiac glycosides has attracted considerable scientific interest and effort since their widespread introduction into therapy. Among others, two reasons for this phenomenon are obvious:

1) The small therapeutic index of these drugs made precise knowledge of their absorption behavior indispensable.
2) The great differences in the ratio of cardioactive doses after oral and intravenous (i.v.) glycoside administration – suggesting absorption of the strophanthins to be minimal and that of digitoxin to be almost complete – have been recognized for a long time (LENDLE, 1925; for a review of the older literature see LENDLE, 1935; WEESE, 1936).

Whereas LENDLE (1935) was surprised that the substance with the greatest solubility in water revealed the least effect after intestinal administration, just this relation was later regarded as evidence that the absorption of cardiac glycosides fitted into the theoretical framework which had been developed for the absorption of drugs in the meantime.

This framework dates back to the early studies by OVERTON (1899, 1902) and COLLANDER and BÄRLUND (1933) demonstrating the positive correlation between permeability and lipid solubility of solutes and hence suggesting that solubility in the membrane lipids was the rate-limiting step in membrane permeation. In 1937, HÖBER and HÖBER demonstrated this principle to hold true for intestinal absorption as well. Further observations by OVERTON (1896) and TRAVELL (1940) led to the conclusion that in the case of electrolytes – which most drugs are – only uncharged molecular species are able to cross the cell membrane. Therefore, this principle was later named "nonionic diffusion" (MILNE et al., 1958).

The compatibility of the intestinal absorption of numerous drugs with the theory of nonionic diffusion has been demonstrated especially by the work of BRODIE, HOGBEN, SCHANKER and co-workers (for reviews see BRODIE, 1964; SCHANKER, 1962, 1964, 1971; KURZ, 1975). The number of noncontradictory results as well as simplicity and intelligibility of this theory favored the widespread opinion that nonionic diffusion is the sole absorption mechanism of drugs.

Inclusion of cardiac glycosides in this concept was facilitated by the fact that they behave as nonelectrolytes under normal physiologic conditions and, therefore, direct influences of pH and of pK_a values are not to be expected. Furthermore, the inverse relation between polarity and absorption rate was highly suggestive. It has to be kept in mind, however, that correlation with polarity is only one

requirement to be met if absorption by diffusion is postulated. This is underlined by a few theoretical considerations.

Starting from Fick's first law of diffusion, permeation across a homogeneous membrane in the steady state can be described as

$$\frac{dn}{dt} = -\frac{D \times A}{\delta} \times \varkappa \times (c' - c'') \tag{1}$$

dn being the number of moles of solute passing through a membrane of area A during the time intervall dt, δ the thickness of the membrane, $\varkappa$ the partition coefficient of the solute between the membrane matrix, assumed to behave like a liquid phase, and the adjacent solutions, and c' and c'' the concentrations of the solute in the solutions facing the *cis* and *trans* sides of the membrane, respectively.

Since for biologic membranes normally neither δ nor $\varkappa$ are exactly known, the empirical "permeability coefficient" P has been introduced instead of D. Equation (1) is thus transformed to

$$\frac{dn}{dt} = -P \times A \times \Delta c \tag{2}$$

P depends on the properties of the membrane as well as on those of the solutes. In case of absorption studies, Eq. (2) would be further simplified by regarding the compartment on the *trans* side of the absorbing membrane as very large and, hence, neglecting c'', to

$$\frac{dn}{dt} = -P \times A \times c \tag{3}$$

c being the concentration in the intestinal lumen.

For practical purposes, calculation with the administered dose instead of the intestinal concentration is often preferable, hence

$$\frac{dn}{dt} = -\frac{P \times A}{V} n \tag{4}$$

n being the number of moles contained in the volume V, or, further

$$\frac{dn}{dt} = -k_{abs}\, n\,, \tag{5}$$

where k_{abs} is the absorption coefficient. As is realized by comparing Eqs. (4) and (5), it depends not only on the permeability P, but also on the experimental conditions determining the absorbing area A and the solvent volume V.

Absorption half-life is defined as

$$t_{1/2} = \frac{\ln 2}{k_{abs}}. \tag{6}$$

As long as the experimental conditions are kept constant, the amount absorbed is proportional to the intestinal pool of solute, i.e., absorption follows a first-order reaction. Equation (5) can be integrated to

$$n = n_0 \exp(-k_{abs}t) \tag{7}$$

n_0 being the dose administered at zero time. It follows from Eq. (7), that the intestinal pool n declines according to a falling exponential function.

The absorbed amount at time t is

$$(n_0 - n) = n_0 (1 - \exp(-k_{abs}t)) \tag{8}$$

and the absorption rate

$$\frac{n_0 - n}{n_0} \times 100 = (1 - \exp(-k_{abs}t)) \times 100 . \tag{9}$$

In summary, at least three necessary, but not sufficient conditions, have to be fulfilled to justify the conclusion on absorption by diffusion. (These conditions are also met by a transport system working within its proportionality range. For the importance of determining the flux ratio see Sect. D).

1) Absorption rate is positively correlated to lipid solubility, i.e., it decreases with increasing polarity. Since $\varkappa$ as a distribution coefficient between the membrane phase and aqueous solutions is difficult to determine, polarity of cardiac glycosides as well as that of other drugs may be inferred from partition coefficients between organic solvents and water (COHNEN et al., 1978) or the mobility in chromatographic systems (Fig. 1).
2) Absorption rate is independent of the intestinal concentration or, under constant experimental conditions, the intestinal dose.
3) The time course of absorption follows a first-order reaction, i.e., k_{abs} remains constant over the entire absorption period. Absorption is virtually complete after a sufficiently long period of time.

The main aim of this chapter is to reveal the complexity of the intestinal permeation of cardiac glycosides and to review our present state of knowledge of its mechanisms. Therefore, mere physical and galenic factors, summarized as "bioavailability," are beyond the scope of this review and have been treated elsewhere in this volume (see Chap. 8). Likewise, the numerous clinical investigations are included only insofar as they contribute to this intention. According to its purpose, the review deals with three topics.

First, it will give a critical review of the extent to which published results are compatible, or incompatible, with absorption of cardiac glycosides by diffusion. In this it faces several difficulties. Comparability of various author's results is severely hampered by the varying experimental conditions. Moreover, many of the older investigations drew conclusions about the absorption by a comparison of intestinal and i.v. equieffective doses. In fact, an intestinal efficacy was determined – and will be cited as such in this case – which is not necessarily congruent to an absorption rate. Though the situation has greatly improved during the last few years owing to the availability of radioactively labeled glycosides and sensitive radioimmunoassays, the difficulties in obtaining exact quantitative data on the

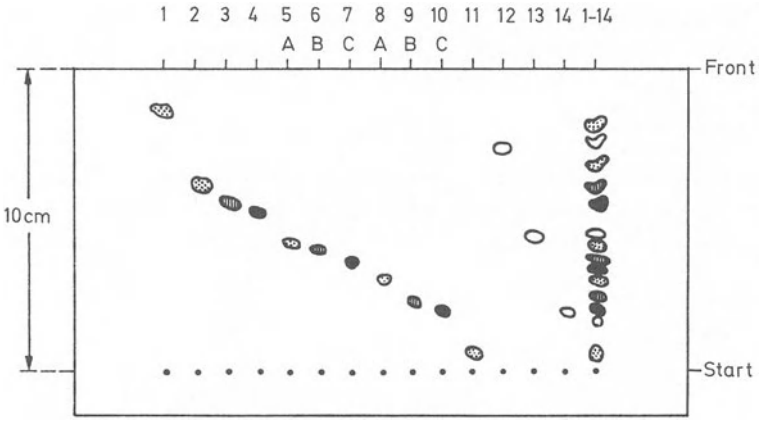

Fig. 1. Separation of cardiac glycosides by thin layer chromatography. *1* Acetyldigitoxin; *2* digitoxin; *3* gitoxin; *4* digoxin; *5* lanatoside A; *6* lanatoside B; *7* lanatoside C; *8* desacetyllanatoside A; *9* desacetyllanatoside B; *10* desacetyllanatoside C; *11* strophanthoside K; *12* cymarin; *13* proscillaridin; *14* scillaren A. R_F value of convallatoxin is between that of glycosides 8 and 9. – Methylene chloride/methanol/formamide 80:19:1; Silicagel G. (NEHER, 1967)

permeation process for a great variety of glycosides still exist. Finally, in humans absorption rates are calculated almost exclusively either by the pharmacokinetic method of comparing areas under plasma level curves (AUC) or determining the cumulative urinary excretion (CUE), neither method allowing direct conclusions on the permeation process itself.

Second, it will report on results demonstrating the existence of a transport mechanism in the intestine, capable of uphill secretion of cardiac glycosides into the gut lumen.

Third, it will try to develop a unifying concept for the diverging results, based on the intestinal permeation of cardiac glycosides by diffusion as well as *by* and *against* a secretory transport mechanism.

B. Intestinal Absorption of Cardiac Glycosides

I. Dependence on Polarity

1. Results Compatible with Diffusion

a) Natural Glycosides

Naturally occuring glycosides in therapeutic use can be arranged in order of increasing polarity: digitoxin < acetyldigoxin < digoxin < lanatoside C < convallatoxin < strophanthin. The importance of polarity for intestinal efficacy has been stressed already by WHITE and GISVOLD (1952) as well as by ROTHLIN and BIRCHER (1954). In further experiments the inverse correlation between absorption rate and polarity has been substantiated by several authors. HERRMANN et al. (1962) compared equieffective emetic doses in the dog and found intestinal efficacies to de-

crease in the order digitoxin ≈ acetylstrophanthidin > lanatoside E≫ouabain. By determination of the ratio of intraduodenal to i.v. toxicity intestinal efficacies could be arranged in the order digitoxin > digoxin > proscillaridin > ouabain in the cat (LENKE and SCHNEIDER, 1969; SCHAUMANN and WEGERLE, 1971) or digitoxin > digoxin > convallatoxin in the cat, pig, and guinea pig (VOGEL et al., 1970).

According to chemical determination of the unabsorbed residue the sequence of absorption rates was digitoxin ≈ cymarin > digoxin > lanatoside C ≈ convallatoxin ≈ strophantoside K in the cat (LINGNER et al., 1963b). Absorption of tritium-labeled glycosides from tied loops in situ decreased in the order digitoxin > digoxin > ouabain in the rat and in the cat (FORTH et al., 1969a, b). HEMPELMANN et al. (1978) determined the absorption rate of 15 glycosides in the cat and found a rough correlation with the octanol/water partition coefficient, though with some noteworthy exceptions (see Sect. B.I.2).

In humans, practically complete absorption of digitoxin has been inferred from a comparison of i.v. and oral equieffective doses by the classic studies of GOLD et al. (1940, 1944). This result has been confirmed by numerous investigators either by indirect methods or by direct estimations of the residual amount in the intestine by the intestinal tube technique (BEERMANN et al., 1971). In the guinea pig, the intestinal efficacy of digitoxin is unusually low (HOTOVY, 1951; ACHELIS and KRONEBERG, 1956; LORENZ and STOECKERT, 1958; VOGEL et al., 1970) or even negligible (HABERLAND, 1965). Possibly a pronounced first-pass effect is responsible for this phenomenon. The amount absorbed, as determined by chemical methods, exceeded the i.v. lethal dose 2- to 4-fold (LAUTERBACH, 1963) and with ^{3}H-digitoxin a 30-min absorption rate of 53% was observed (GREENBERGER et al., 1969). Digitoxigenin-mono-digitoxoside, possessing solubility characteristics very similar to digitoxin, had an intestinal efficacy of 95% in the cat (KRONEBERG et al., 1962).

Digoxin is probably the most intensively investigated cardiac glycoside in humans. Absorption rates of 40%–60% were found by gastric tube technique in the stomach, duodenum, or upper jejunum; 14-day CUE after intrajejunal instillation amounted to 45% (BEERMANN et al., 1972a). Comparable values were obtained by OCHS et al. (1975a) and GREEFF et al. (1977), whereas DOHERTY et al. (1961) and FLASCH et al. (1978) reported absorption rates up to 80%. (For further reports see the literature surveys in OCHS et al., 1975a; GREEFF et al., 1977; HINDERLING et al., 1977; FLASCH et al., 1978.)

Lanatoside C revealed absorption rates similar to or even identical with those of digoxin. Depending on the experimental set-up values between 40% and 80% have been reported (BLANKART and PREISIG, 1970; ALDOUS et al., 1972; BEERMANN, 1972a; DENGLER et al., 1973a). Owing to the appearance of a late second peak of plasma radioactivity as well as renal excretion of digoxin and acetyldigoxin after oral administration of ^{3}H-lanatoside C (BEERMANN, 1972a; DENGLER et al., 1973b; ALDOUS and THOMAS, 1977) the high absorption rate of the relatively polar lanatoside C has been ascribed to the formation of less polar glycosides in distal parts of the gastrointestinal tract by deacetylation and removal of the terminal glucose by intestinal bacteria, as demonstrated previously for other glucose-containing glycosides (ENGLER et al., 1958; LAUTERBACH and REPKE, 1960) and substantiated for lanatoside C (DENGLER et al., 1973b).

Estimations of the absorption of gitoxin are severely impeded by its outstandingly low solubility in water as well as in organic solvents (REPKE and MEGGES, 1963; BAUMGARTEN, 1966). Obviously depending on the solvents and solubilizers used, intestinal efficacies and absorption rates between 0% and 94% have been observed (HOTOVY, 1951; HACKENBERG, 1953; LINGNER et al., 1963 b; MEGGES, 1966). In humans, a 95% absorption from a solution of gitoxin in ethanol/glycerol/water has been claimed (LESNE, 1978). For the 16α isomer of gitoxin, 16-*epi*-gitoxin, HAUSTEIN (1977) determined a 3-day CUE of 70% in humans.

Proscillaridin is considerably more polar than digoxin as judged by its chromatographic behavior (Fig. 1) and its chloroform/water partition coefficient (GREENBERGER et al., 1969). Accordingly, intestinal efficacies of only 20%–35% have been estimated (KURBJUWEIT, 1964; BELZ, 1968; BELZ et al., 1974). Its low intestinal efficacy might be influenced, however, by extensive first-pass conjugation with glucuronic and sulfuric acid in the intestinal wall (ANDERSSON et al., 1977 a, b).

Absorption of the most polar glycoside to be dealt with, ouabain (strophanthin G), in humans is small and erratic. On the basis of radioimmunologic determination of 4-day CUE GREEFF et al. (1974) calculated an absorption rate of approximately 2% (range 0.5%–4.4%) for a swallowed dose and approximately 1.5% (range 0.7%–2.4%) after sublingual administration.

b) Semisynthetic Glycosides

The evidence accumulated at the beginning of the 1960s that the intestinal absorption of drugs is governed by polarity, stimulated various attempts to increase the absorption of cardiac glycosides by improving their lipid solubility. The main procedures employed, all involving free hydroxyl groups, mostly of the sugar moieties, were esterification with acyl residues of varying chain length, etherification with methyl groups, and formation of ketals by reaction with acetone. In general, augmented intestinal efficacies or absorption rates resulted from these measures (MEGGES and REPKE, 1961; LINGNER et al., 1963 a; HABERLAND, 1965; SCHAUMANN and WEGERLE, 1969; LENKE and BROCK, 1970; KAISER, 1971; ZIELSKE et al., 1971). Only a few of the numerous compounds synthesized have been introduced into therapy for various reasons. One reason might have been the decrease in cardiotonic activity accompanying the reduced polarity in practically all of the derivatives tested.

Pentaacetylgitoxin was intensively studied by Repke and co-workers. In the rat, the acetyl groups are split off during passage through the mucosal barrier or shortly thereafter and hence mainly gitoxin and gitoxin metabolites are excreted (REPKE and MEGGES, 1963; MEGGES, 1966; MEGGES et al., 1977). In contrast, in humans 16-acetylgitoxin was detected as the main metabolite in serum, bile, and urine. On the basis of the 4-day CUE, the absorption rate was estimated as 50% (HAUSTEIN et al., 1978).

Two different monoacetyl gitoxins have been tested in the rat jejunum in vitro (PFORDTE and FÖRSTER, 1970). 16-acetylgitoxin revealed a higher absorption rate than α-acetylgitoxin probably owing to its lower deacetylation rate. In humans, the intestinal efficacy of 16-acetylgitoxin has been given as 90% (HEUCHEL and COCH, 1967). Absorption of gitoxin was likewise improved by formylation. For pentaformyl gitoxin intestinal efficacies of 57% (tablets) and 90% (solution) have been reported (LESNE et al., 1978).

Acetylation of one of the two free hydroxyl groups of the terminal digitoxose of digoxin leads to α-acetyldigoxin (3‴-acetyldigoxin), occuring also as a genuine glycoside, or β-acetyldigoxin (4‴-acetyldigoxin). The intestinal efficacy of β-acetyldigoxin was superior to digoxin, in the cat and the rat (BENTHE and CHENPANICH, 1965; GREEFF et al., 1965). From the radioimmunologically determined 7-day CUE GREEFF et al. (1977) calculated an absorption rate for β-acetyldigoxin between 64% and 74% under various conditions as compared with 56% for digoxin. Values for β-acetyldigoxin absorption between 68% and 81% have also been reported by FLASCH (1975) and KLOTZ et al. (1976). The absorption rate of α-acetyldigoxin was determined as 78% from CUE by BODEM et al. (1974), almost identical as the value of 80% found for digoxin by the same team (DENGLER et al., 1973a). No difference between the intestinal efficacies of α- and β-acetyldigoxin was inferred from the observation of electrocardiographic changes (WATZKE and KLEPZIG, 1972). Deacetylation already occurs during the process of absorption. After oral administration of tritium-labeled β-acetyldigoxin in humans, 93% of the radioactivity in the portal blood was represented by digoxin (FLASCH et al., 1977). Human duodenal and jejunal mucosa formed digoxin from α-acetyldigoxin in vitro; hydrolysis was inhibited by esterase blockers like paraoxon (BODEM et al., 1974).

The use of ether instead of ester linkages for the attachment of nonpolar groups to the sugar residues of cardiac glycosides has the advantage that these auxiliary groups are less readily split off (KAISER and SCHAUMANN, 1969). In fact, β-methyldigoxin is absorbed unchanged in the guinea pig (VOIGTLÄNDER et al., 1972). In the rat, β-methyldigoxin was demethylated only slightly or not at all in the intestinal mucosa (BERGMANN et al., 1972). Intestinal absorption of digoxin is significantly improved by 4‴-methylation. Intestinal efficacy rose to 54% (1 h; digoxin 21%) in the guinea pig and to 73% (2.5–3 h; digoxin 50%) in the cat (SCHAUMANN and WEGERLE, 1971). In the rat, the intestinal absorption velocity of tritium-labeled β-methyldigoxin was about three times that of digoxin (RIETBROCK et al., 1972). The high, often almost complete, absorption of β-methyldigoxin has been repeatedly confirmed in humans (LARBIG et al., 1971; BEERMANN, 1972b; HÄRTEL et al., 1973; RIETBROCK et al., 1975; BOERNER et al., 1976; HAYWARD et al., 1978).

A significant rise in absorption was also achieved by 4′-methylation of proscillaridin, yielding methylproscillaridin (proscillaridin-4′-methylether). Its intestinal efficacy in the cat was determined as 85% as compared with 25% proscillaridin absorption in the same series (RASCHACK et al., 1978). In humans, determination of plasma levels by ^{86}Rb erythrocyte assay and comparison of AUC gave an absorption rate of 60% (BELZ et al., 1976); the same value was deduced from electrocardiographic changes (KRÄMER and HOCHREIN, 1976).

2. Results Incompatible with Diffusion

The strict relation between polarity and absorption rate is by no means generally observed. In three different species the tested glycosides ranked in three different orders (FORTH et al., 1969a, b). In the cat, in vivo absorption rates decreased in the order digitoxin > digoxin > peruvoside > ouabain; in the rat, in vitro, in the or-

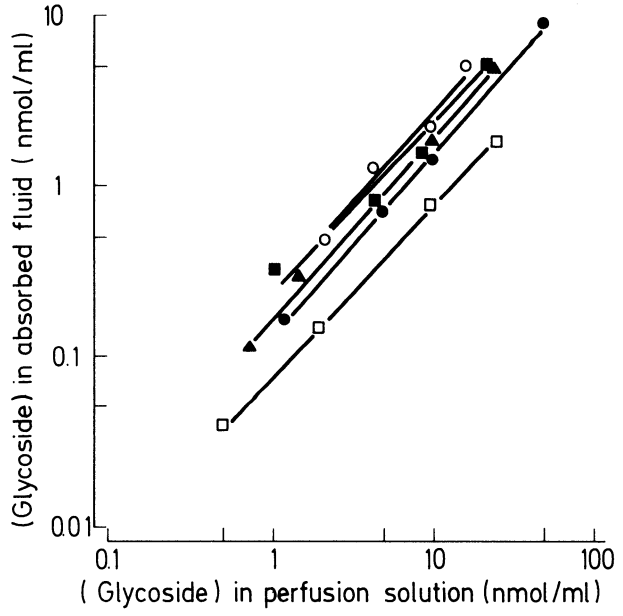

Fig. 2. Absorption of cardiac glycosides in perfused guinea pig jejunal loop in vitro. Absorption period 2 h. *Triangles* digitoxin; *solid squares* peruvoside; *open squares* digoxin; *open circles* proscillaridin; *solid circles* ouabain. (FORTH et al., 1969a)

der digitoxin > peruvoside > digoxin ≈ proscillaridin > ouabain; and in the guinea pig, in vitro, proscillaridin > peruvoside ≈ digitoxin > ouabain > digoxin (Fig. 2). It is particularly noteworthy that in the guinea pig the more polar proscillaridin was absorbed better than digitoxin and the highly polar ouabain proved to be superior to digoxin.

Yet another sequence was found by GREENBERGER et al. (1969) for the absorption of six glycosides from isolated intestinal loops in vivo. Neither in the rat nor in the guinea pig was the absorption rate correlated with the chloroform/water partition coefficients of the respective glycosides (Fig. 3). Comparable to the results of FORTH et al., in the guinea pig digoxin absorption was smaller than in the rat, and not better than that of the completely chloroform-insoluble ouabain. Convallatoxin and convallatoxol were absorbed to the same extent as digoxigenin-monodigitoxoside in the cat despite a 40- to 50-fold smaller octanol/water partition coefficient (HEMPELMANN et al., 1978).

II. Dependence on Dose

1. Results Compatible with Diffusion

There are only a few investigations under strictly controlled conditions which demonstrate proportionality between the intestinal concentration and the amount absorbed. In most cases, a rather narrow concentration range was investigated. In vitro perfusion of the jejunum for 2 h by the method of FISHER and PARSONS (1949)

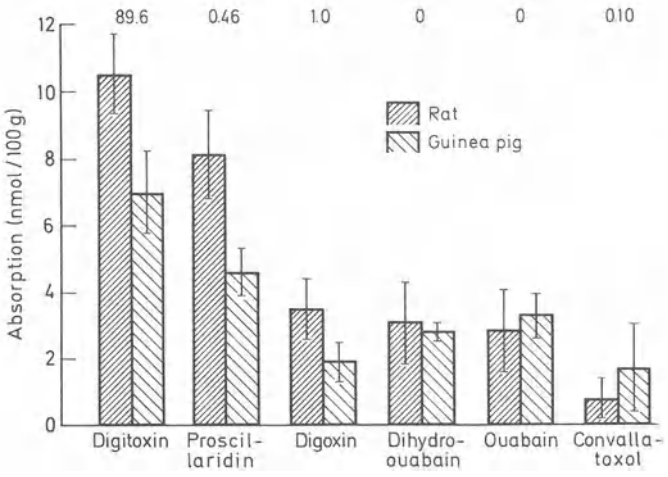

Fig. 3. Absorption of cardiac glycosides from tied-off intestinal loops in rats and guinea pigs in vivo. Absorption period 30 min. The glycosides were administered in a dose of 10 µg/100 g body weight. Note that absorption is expressed as nmoles/100 g. Means ± one standard deviation. The chloroform/water partition coefficient of the respective glycosides is shown above the columns. (GREENBERGER et al., 1969)

was used by FORTH et al. (1969a). The entire concentration range was $5 \times 10^{-7} - 2.5 \times 10^{-4}$ *M* within which the concentration of five glycosides tested varied between 1:7.5 and 1:166. No significant changes in the relative glycoside concentrations of the absorbed fluid were observed in the rat nor in the guinea pig (Fig. 2). Likewise, the same authors (FORTH et al., 1969a) observed no changes in the 20-min absorption rate from tied loops of the rat jejunum in vivo when the concentration of the same glycosides varied within a concentration range of $10^{-6} - 6 \times 10^{-5}$ *M* between 1:4–1:50.

CALDWELL et al. (1969) studied digoxin absorption in the rat in vitro by means of the everted sac technique (WILSON and WISEMAN, 1954) and in vivo. Linearity between intestinal concentration and amount absorbed was observed within 60 min in vitro, between 8.6×10^{-6} and 4.6×10^{-3} *M* (1:534), and in vivo within 30 min, between 9×10^{-6} and 1.3×10^{-4} *M* (1:14). By the same methods CALDWELL et al. (1970) observed constancy of the absorption rate of ouabain in vitro, between 10^{-8} and 10^{-3} *M* , and in vivo, between 8.5×10^{-6} and 1.7×10^{-4} *M* (1:20).

GREENBERGER et al. (1969) reported linearity of digitoxin absorption in the rat in vivo, between 4×10^{-6} and 1.3×10^{-4} *M* (1:33) and in everted sacs, between 2.5×10^{-8} and 10^{-6} *M* (1:40). The authors concluded that their results indicated digitoxin, digoxin, and ouabain to be absorbed by a passive, nonsaturable transport process in the rat. The validity of this conclusion will be discussed in Sect. D.

2. Results Incompatible with Diffusion

In contrast to the results just cited several authors detected a change in the intestinal efficacy or absorption rate when the intestinal glycoside dose or concentration

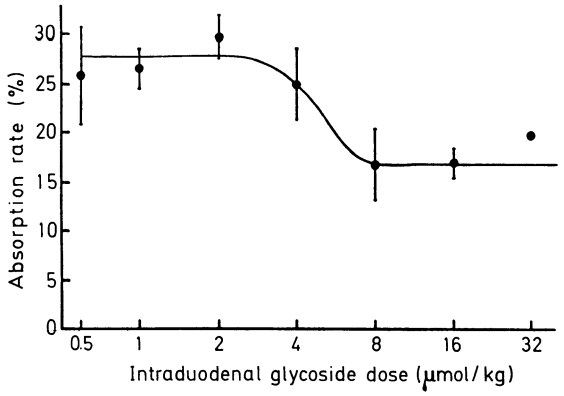

Fig. 4. Dose dependence of the absorption rate of convallatoxin in the rat. Absorption period 3 h. Means ± standard errors. (LAUTERBACH, 1964)

was changed under otherwise constant conditions. Unusually high efficacies of polar glycosides were observed in the cat when low doses were administered intestinally and the effective amount absorbed determined by subsequent estimation of the remaining i.v. lethal dose. The 3-h and 6-h intestinal efficacy of ouabain were determined as 30% and 45%, respectively, after administration of 0.15 mg/kg in an approximately 10^{-4} *M* solution (NYÁRY, 1932). REINERT (1952) reported a 5-h intestinal efficacy as high as 60% under comparable conditions. KOHLI and VOHRA (1960) observed a stepwise decrease in the intestinal efficacy of peruvoside from 60% to 35% to 20% after administration of 1, 2, and 4 Hatcherdoses, respectively. LAUTERBACH and VOGEL (1968) and LAUTERBACH (1969) infused between 2.5% and 80% of the previously determined intestinal lethal dose (LD_{100}). The intestinal efficacies of convallatoxin, convallatoxol, desacetyllanatoside C, and strophanthoside K decreased steeply from 40% to 50% after administration of 2.5%–5% of the LD_{100} to 8%–13% after administration of a full lethal dose. A less steep, though still pronounced fall of the intestinal efficacy was observed with ouabain. Only digoxin revealed an intestinal efficacy roughly independent of dose.

Direct evidence, derived from chemical glycoside determination, of the nonlinearity between intestinal glycoside dose and amount absorbed was first obtained by LAUTERBACH (1964). In the rat, the absorption rate of convallatoxin decreased significantly from a more or less constant plateau of approximately 26% to another, lower plateau of approximately 17% within a strikingly narrow dose range (Fig. 4). From these results the author concluded that absorption of convallatoxin proceeds not only by diffusion but also by a transport mechanism of limited capacity. A decrease in absorption rate with increasing intestinal concentrations was likewise demonstrated with a tritium-labeled congener of convallatoxin, convallatoxol (differing merely by the reduction of the C_{19} aldehyde to a primary alcohol group). Increasing the intestinal concentration from 10^{-6} to 10^{-4} *M* caused the 3-h absorption rate to fall from 12% to 4% (SEIDENSTÜCKER and LAUTERBACH, 1976; SEIDENSTÜCKER, 1978).

The participation of a saturable process in glycoside absorption was substantiated by in vitro perfusion of isolated rat small intestine with $5 \times 10^{-6} - 8 \times 10^{-4}$ *M*

cardenolide solutions. The absorption rates of the polar glycosides convallatoxin, convallatoxol, desacetyllanatoside C, and ouabain fell significantly with increasing concentrations, the magnitude of the effect being dependent on the concentration range studied and the respective experimental conditions. In extreme cases, absorption rate was diminished to 1/68 of its original value (LAUTERBACH, 1967).

An especially peculiar phenomenon was observed with three glycosides of low polarity, namely an increase in the absorption rate with rising doses. This holds true for digitoxin in the rat intestine in vivo as well as in vitro (LAUTERBACH, 1964, 1967) and for the mouse intestine in vitro (DAMM et al., 1975). A dose of β-methyldigoxin causing cardiac arrythmias in guinea pigs (1.18 mg/kg) had an intestinal efficacy of 38% whereas by comparison with the lethal dose (1.55 mg/kg) 55% was effective (SCHAUMANN and WEGERLE, 1971). Doses of 3 and 7 μmol/kg isopropylidene helveticosol (helveticosol acetonide) were absorbed to the extent of 44% and 59%, respectively (SCHAUMANN et al., 1970). An explanation for these peculiarities will be attempted in Sect. D where also further examples of concentration-dependent absorption rates will be dealt with.

III. Dependence on Inhibitors

1. Results Compatible with Diffusion

If a carrier-mediated transport process is involved in glycoside absorption, inhibition of absorption by structurally related compounds should be demonstrable. Attempts by CALDWELL et al. (1969, 1970) to inhibit the absorption of digoxin by an equimolar concentration of ouabain and vice versa in the everted sac of rat small intestine proved unsuccessful. The authors, therefore, concluded that both glycosides are absorbed by passive diffusion.

Moreover, if the transport mechanism in question is an active one, interference of metabolic inhibition would be expected. However, in the everted sac of the rat there appears to be no significant influence of anaerobiosis, 2,4-dinitrophenol, sodium azide, or iodoacetate on the absorption of digitoxin (GREENBERGER et al., 1969), digoxin (CALDWELL et al., 1969), and ouabain (CALDWELL et al., 1970).

2. Results Incompatible with Diffusion

On the other hand, inhibition of the absorption of one glycoside by another was clearly demonstrated under suitable conditions. Absorption of convallatoxol from tied loops of rat small intestine in vivo is impeded or even totally blocked by a 100-fold excess of convallatoxin, digoxin, or ouabain (Fig. 5). Specificity of this inhibition was underlined by demonstrating unimpaired absorption of sulfafurazol and 3-*O*-methylglucose from the same loops (SEIDENSTÜCKER and LAUTERBACH, 1976; SEIDENSTÜCKER, 1978). Metabolic inhibition was likewise demonstrated. In the mouse everted intestine, absorption of digitoxin – but not of digoxin and ouabain – was inhibited by anaerobiosis, 2,4-dinitrophenol, and probenecid (DAMM and WOERMANN, 1974).

Finally, inhibition by other drugs was reported. Neomycin retarded and decreased digoxin absorption (LINDENBAUM et al., 1976). Though neomycin is known

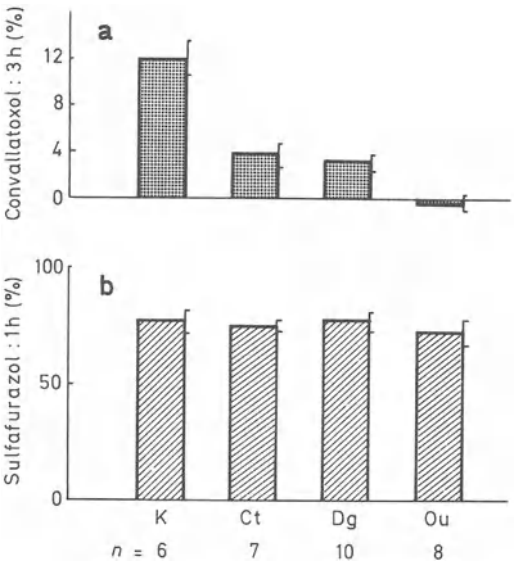

Fig. 5 a, b. Inhibition of the absorption of convallatoxol by other cardiac glycosides in tied-off jejunal loops of the rat. **a** instillation of 1 ml 10^{-6} *M* ^{3}H-convallatoxol either without a second glycoside (K) or with 10^{-4} *M* convallatoxin (Ct), digoxin (Dg), or ouabain (Ou). **b** constancy of 1 h absorption rate of 10^{-3}*M* sulfafurazol determined simultaneously in the same loops. (SEIDENSTÜCKER, 1978)

to cause mucosal damage (CAIN et al., 1968) it is interesting to note that absorption of digoxin was impaired at neomycin doses which left that of D-xylose unchanged. Sulfasalazine inhibited digoxin absorption by a mechanism not yet understood (JUHL et al., 1976).

IV. Dependence on Time

1. Results Compatible with Diffusion

So far, no results have been reported demonstrating, under strictly controlled conditions, the decay of an intestinal pool of a cardiac glycoside to follow pure first-order kinetics. For pharmacokinetic purposes absorption coefficients and absorption half-lives of certain glycosides have been calculated under the tacit assumption of the validity of Eq. (7). (For examples, see LARBIG et al., 1971; SCHAUMANN and WEGERLE, 1971; DENGLER et al., 1973 a).

2. Results Incompatible with Diffusion

While there is no clear-cut proof that the absorption coefficient is constant there are a number of reports indicating a decrease of absorption coefficient with time, i.e., the amount absorbed per unit time is diminished to a greater extent than can be ascribed to the diminution of the intestinal pool. In this sense, one can now interpret the results reported by KOHLI and VOHRA (1960). These authors observed,

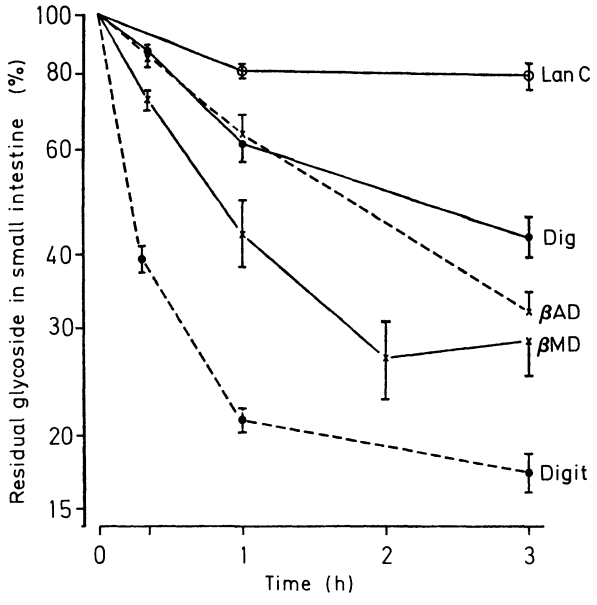

Fig. 6. Time course of glycoside absorption in the guinea pig. Injection of 0.8 μmole glycoside/kg body weight. Note that ordinate is logarithmic. Lan C lanatoside C; Dig digoxin; βAD β-acetyldigoxin; βMD β-methyldigoxin; Digit digitoxin. (SCHAUMANN et al., 1972)

in the cat, a 13% intestinal efficacy of 4 Hatcher doses of peruvoside within 15 min, but a further rise to only 19% within an additional 45 min period.

Also in the cat, the half-life of helveticosol absorption which resulted in electrocardiographic changes was only one half that which caused cardiac arrest (SCHAUMANN and WEGERLE, 1969). Helveticosol acetonide was absorbed with a half-life of 20 min during the first 8 min and with a half-life of 40 min afterwards in the guinea pig (SCHAUMANN et al., 1970). In a comparison of five glycosides none was absorbed by a first-order reaction (Fig. 6). With lanatoside C, revealing the most extreme deviation, no significant absorption was observed beyond the first h, though 80% of the dose remained unabsorbed in the intestine (SCHAUMANN et al., 1972).

At the same time RIETBROCK and co-workers (RIETBROCK et al., 1972; RIETBROCK, 1976) described a drastic decrease of the absorption coefficient of digoxin and β-methyldigoxin and to a lesser extent of β-acetyldigoxin (Fig. 7a). Expulsion of the glycoside solution to the lower parts of the intestinal tract cannot be the sole explanation for this phenomenon since a similar decline was also observed after intraileal glycoside administration.

NYBERG et al. (1974) calculated the absorption coefficient of digoxin in humans on the basis of AUC comparisons and found a decrease of 1 h^{-1} to approximately 0.05 h^{-1} within the first 2 h. After 3.5 h, absorption was practically finished despite an unabsorbed residue of $\gtrsim$ 30%. This result was later confirmed by determination of porto-arterial differences of digoxin concentrations (Fig. 7b) (ANDERSSON et al., 1975). No conclusive explanation for the striking decay of the absorption coefficient could be provided. Continuation of digoxin absorption even

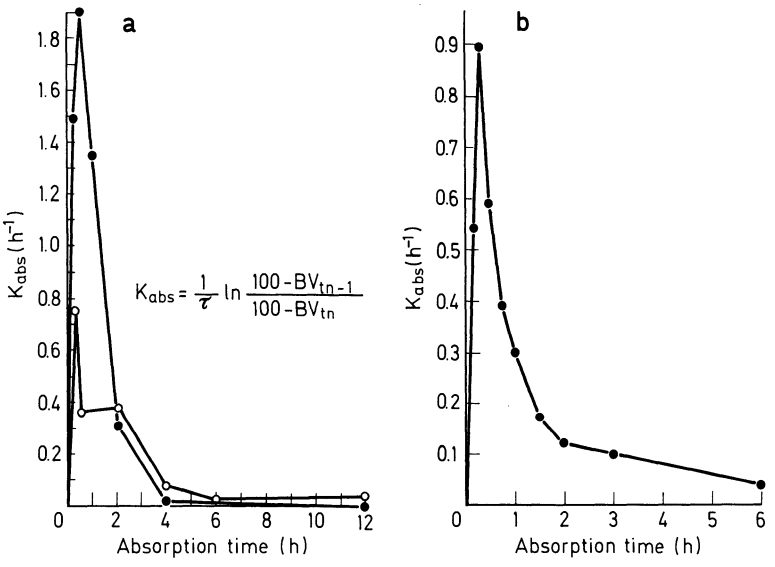

Fig. 7 a, b. Change of the absorption coefficient k_{abs} with absorption time. **a** rat. *solid circles* β-methyldigoxin; *open circles* digoxin. (RIETBROCK, 1976) **b** human. digoxin. (ANDERSSON et al., 1975)

in the distal parts of the intestine was confirmed by the authors by intrasigmoid administration (ANDERSSON et al., 1975) as well as by others (OCHS et al., 1975 b). A dilution of the glycoside solution during the course of absorption with a resulting reduction of the concentration gradient has been discussed. This effect, however, should be largely compensated by the accompanying increase in intestinal contact area.

V. Dependence on Blood Flow and Lymph Drainage

A pronounced dependence of glycoside absorption on portal blood flow was reported by HAASS et al. (1972) in the guinea pig. A rise of blood flow from 20 to 25 ml/min caused a 50% rise in digitoxin absorption. Similar effects were seen with digoxin and ouabain. Drainage of absorbed glycoside by the lymph seems negligible. Only minute amounts were found in the thoracic duct or intestinal lymph of the rat (BEERMANN and HELLSTRÖM, 1971), cat (FORTH et al., 1969 b), dog (OLIVER et al., 1971), and humans (BEERMANN et al., 1972 b).

C. Intestinal Secretion of Cardiac Glycosides

I. Secretion by the Isolated Mucosa of Guinea Pig Jejunum

The dose dependence of the absorption rate of convallatoxin suggested the participation of a transport mechanism in the absorption of cardiac glycosides (LAUTERBACH, 1964). However, experiments in vitro – though substantiating the

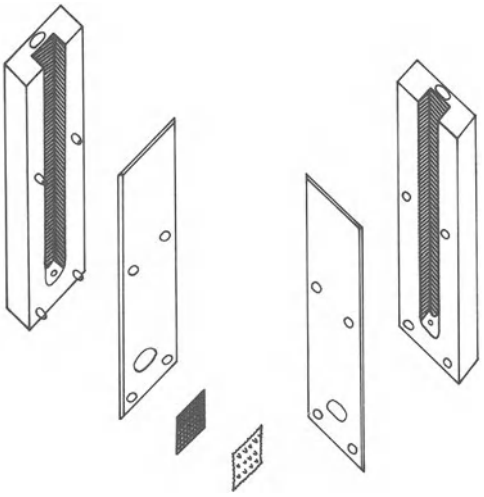

Fig. 8. Method of the isolated mucosa of guinea pig intestine. The isolated mucosa is captured on a nylon mesh and placed between two fenestrated polyvinyl chloride sheets, thus forming a separating membrane between two flux chambers. Window diameter 5 mm. The chambers are filled with 0.2 ml incubation solution and continuously aerated through drill holes in the chamber walls. (LAUTERBACH, 1977 b)

saturability of glycoside absorption – failed to demonstrate an *active* absorption mechanism concentrating the glycoside on the transluminal side (LAUTERBACH, 1967).

A clue to an understanding of the transport mechanisms involved in the intestinal permeation of cardiac glycosides was finally provided by the introduction of the method of the isolated mucosa of guinea pig intestine (LAUTERBACH, 1971 a, 1977 b). As compared with other in vitro methods, studies with the isolated mucosa mounted in a flux chamber are distinguished by a number of advantages, the most important of which are the determination of transepithelial fluxes in both directions under identical conditions and with equal accuracy, the simultaneous determination of drug uptake from the luminal as well as from the blood side of the mucosal tissue, and the suitability for large series of experiments needed for kinetic studies (Fig. 8). Investigations by this method revealed that the transport mechanism for cardiac glycosides suggested by previous experiments exists, but turns out to be a secretory system. Permeation of the three glycosides digoxin, dihydrodigoxin, and convallatoxol across the isolated mucosa in the lumen–blood direction was generally small, less than 0.3% was found in the solution on the blood side after 45 min. In contrast, after administration on the blood side up to 1.7% appeared in the countercompartment, i.e., permeation in the secretory direction proceeded up to 16 times faster than absorption[1] (Fig. 9) (LAUTERBACH, 1971 a, b, 1975, 1977 a). The secretion rate depended on the concentration administered in a characteristic manner; this is reproducible though not yet fully understood.

1 Faster permeation from the serosal to the the mucosal side than vice versa was also observed in perfused intestinal loops in vitro with convallatoxin in the rat (LAUTERBACH, 1968) and with digitoxin in the mouse (DAMM et al., 1975)

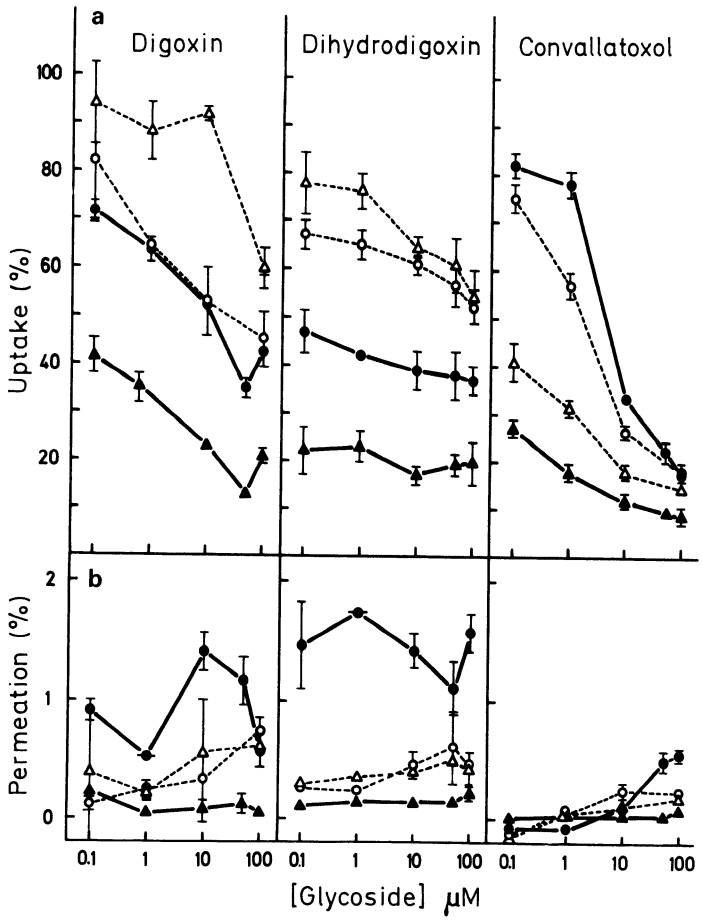

Fig. 9 a, b. Permeation and tissue content of digoxin, dihydrodigoxin, and convallatoxol in the isolated mucosa of guinea pig jejunum. The concentrations indicated on the abscissa were administered under aerobic conditions from the luminal *(solid triangles)* or the blood side *(solid circles)* as well as under anaerobic conditions from the luminal *(open triangles)* or the blood side *(open circles)*. Incubation time 45 min. Permeation has been corrected for fluxes across inulin-permeable shunt pathways. Ordinates: Glycoside concentration in the tissue, referred to the intracellular space (**a**) and glycoside concentration in the countercompartment (**b**) as percentages of the concentration administered. Means ± standard errors. (LAUTERBACH, 1977a)

The secretion was strictly dependent on aerobic energy. Under anaerobic conditions there was no longer any difference in the two opposing permeation rates; this was achieved by a decrease in the secretory as well as an increase in the absorptive flux (Fig. 9). Likewise reduction of the incubation temperature to 2 °C abolished the difference in the transepithelial fluxes (LAUTERBACH, 1971a).

The relative glycoside content of the tissue was higher after administration to the blood side than after luminal administration and indicated saturability by decreasing values with increasing concentrations used (Fig. 9). In contrast, for diffusing drugs the relative tissue content was found to be independent of the side of ad-

ministration and concentration of the drug whereas actively absorbed substrates were preferentially taken up from the luminal side (LAUTERBACH, 1971 a, 1977 a, b).

Identification of a secretory mechanism for cardiac glycosides poses the question of where in the enterocyte the transport mechanism is located. Though a transepithelial secretory net flux could be brought about by a transport mechanism in either the luminal or the basolateral membranes, at present a uniform interpretation of all findings is achieved only by the assumption of the existence of a transport mechanism in the basolateral membranes transferring the glycosides from the interstitium into the cell and a second one in the luminal membrane expelling them into the luminal solution. The effects of anaerobiosis which, in this model, have to be interpreted as the result of the inhibition of two transport mechanisms in series have been quoted as one argument for this assumption. While tissue content after luminal glycoside administration was, in some cases drastically, increased by anaerobiosis, tissue content originating from the blood side remained constant (digoxin), was increased (dihydrodigoxin), or diminished (convallatoxol) (Fig. 9) (LAUTERBACH, 1971 a, 1975, 1977 a). The concept of two transport mechanisms in series received further support by the demonstration of the inhibition by cyanide of glycoside efflux across the luminal border of preloaded mucosae (LAUTERBACH, 1975) as well as by analogous results obtained with quaternary ammonium bases (TURNHEIM and LAUTERBACH, 1977 a; TURNHEIM et al., 1977).

Neither tissue content nor secretion of digoxin and dihydrodigoxin were influenced by reduction of the Na^+ concentration of the incubation media to 10 m*M* or complete omission of Na^+ (LAUTERBACH, 1977 a).

II. Secretion by the Isolated Mucosa of Guinea Pig Ileum and Colon

Intestinal secretion of digoxin increased in the aboral direction. Permeation in the blood–lumen direction across guinea pig ileal mucosa was seven-fold higher than across jejunal mucosa (BRAUER and LAUTERBACH, unpublished). Secretion by colonic mucosa exceeded jejunal secretion ten-fold for digoxin and three-fold for dihydrodigoxin. The much higher secretory activity of the colon was accompanied by a doubled or even tripled tissue glycoside content (KILIAN et al., 1978). In contrast to the jejunum, guinea pig colon was able to maintain its secretory function, though at a reduced state, under anaerobic conditions. Presumably sufficient metabolic energy was provided by the much higher glycolysis of the guinea pig colon. Secretion was abolished by 10^{-3} *M* monoiodoacetate (KILIAN and LAUTERBACH, 1979).

As in the jejunum, permeation across paracellular shunts formed an important pathway for the *absorptive* transepithelial flux as indicated by the correlation between the permeation of glycoside and inulin. Normally, no correlation between the permeation of inulin and digoxin was observed in the blood–lumen direction; this was regarded as additional evidence that in the secretory direction the transepithelial flux is mainly transcellular. Stepwise reduction of the incubation temperature caused a corresponding decrease of digoxin secretion. At and below 7 °C, scattering of inulin and glycoside permeation around the same regression line with an ordinate intercept not deviating significantly from zero indicated interruption

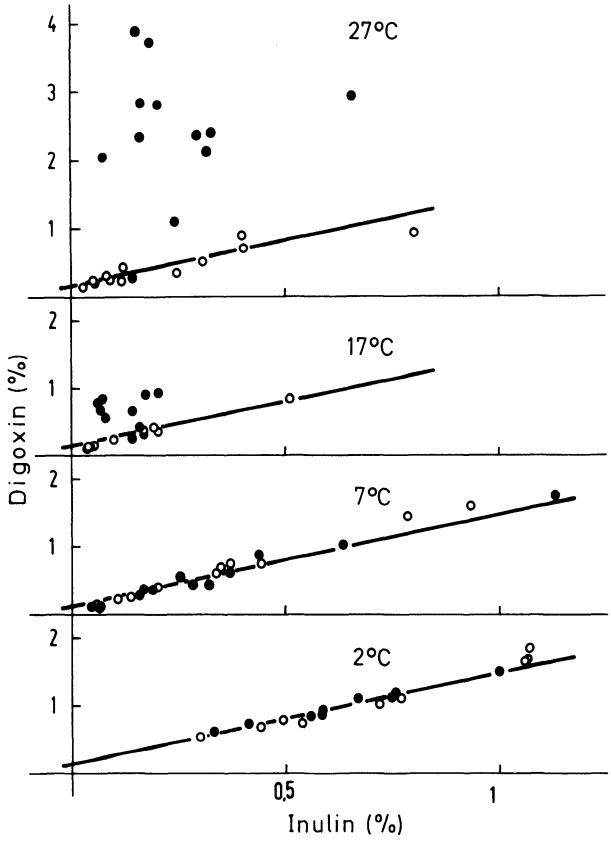

Fig. 10. Correlation between permeation of inulin and digoxin across the isolated mucosa of guinea pig colon at different temperatures. Administration of 10^{-6} *M* glycoside on the luminal *(open circles)* and blood side *(solid circles)*. Abscissa shows concentration of inulin, ordinate that of digoxin in the countercompartment as a percentage of the concentration administered. Incubation time 45 min. (KILIAN and LAUTERBACH, unpublished)

of secretion and restriction of transepithelial permeation more or less exclusively to paracellular shunts (Fig. 10) (KILIAN and LAUTERBACH, 1979).

III. Secretion by the Isolated Mucosa of Human Intestine

Preparations of isolated mucosae of human intestine were used to prove the existence of intestinal secretion of cardiac glycosides in humans. As in the guinea pig, permeation in the lumen–blood direction were strictly correlated with the simultaneous inulin permeations. The ordinate intercepts of the respective regression lines were close to zero indicating an extremely low permeability of the enterocytes in the absorptive direction. In the blood–lumen direction, permeation of digoxin as well as of dihydrodigoxin revealed much higher values, but no correlation with the simultaneous inulin permeation, indicating intestinal secretion in the ileum and, especially, in the colon (Fig. 11) (LAUTERBACH, 1979; LAUTERBACH and KILIAN, 1979).

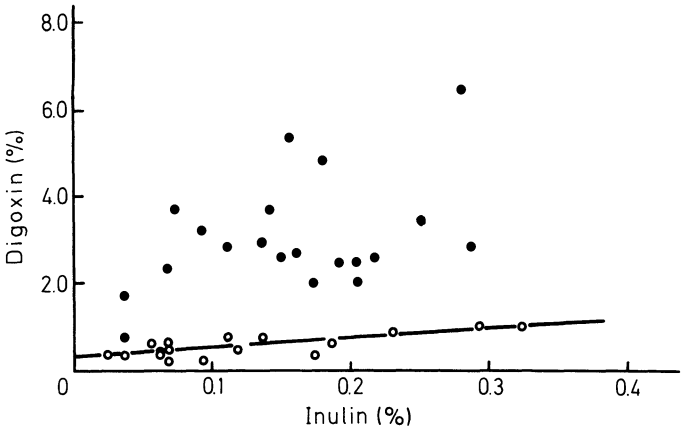

Fig. 11. Secretion of digoxin by the isolated mucosa of human colon. Administration of 10^{-5} *M* glycoside on the luminal *(open circles)* and blood side *(solid circles)*. Abscissa shows concentration of inulin, ordinate that of digoxin in the countercompartment as a percentage of the concentration administered. Incubation time 45 min. (KILIAN and LAUTERBACH, unpublished)

IV. Intestinal Secretion of Glycosides in Vivo

The possibility of extrabiliary excretion of cardiac glycosides into the intestine has been recognized for a long time. HATCHER and EGGLESTON (1919) discovered cardiotonic activity in the feces of rats with ligated bile ducts after i.v. digitoxin administration. ENGLER et al. (1958) were surprised to find that the glucose-free derivatives as well as further metabolites appeared in rat feces only 2 h after i.v. administration of strophanthoside K and thevetin B. In dogs with biliary fistulae, the intestinal excretion of digitoxin (GEILING et al., 1950; KATZUNG and MEYERS, 1965), digoxin (HARRISON et al., 1966), and ouabain (SELDEN et al., 1974) was observed. Also in human patients with biliary T tube drainage, small fecal excretions of digoxin (DOHERTY et al., 1970), ouabain (SELDEN et al., 1974), and peruvoside (FRÖLICH et al., 1972) have been observed, though the evidence for intestinal excretion here is questionable owing to the possibility of incomplete bile diversion. Anyway, even if cardiac glycosides cross the mucosal tissue merely by diffusion, permeation after i.v. administration down the existing concentration gradient into the intestinal fluid has to be expected. None of these investigations, however, has dealt with the possibility of secretion, i.e., excretion against a concentration gradient.

On the other hand, intestinal secretion of cardiac glycosides in vivo was demonstrated in appropriate experiments. After i.v. administration of digoxin the glycoside concentration in a solution circulating through an intestinal loop rose continuously and exceeded the blood level 3.5-fold after 3 h (Fig. 12). If the build-up of concentration gradients was favored by reduction of the intestinal volume to only 1 ml concentration gradients for digoxin and convallatoxol up to 26 and 5 in the guinea pig and 5 and 6 in the rat have been observed (LAUTERBACH, 1971 a, 1975).

Ouabain entered the rat small intestine after i.v. administration but no unequivocal uphill gradient was established. Thus, the apparent permeabilities calculated from the amounts entering and leaving the intestine and the plasma and intestinal

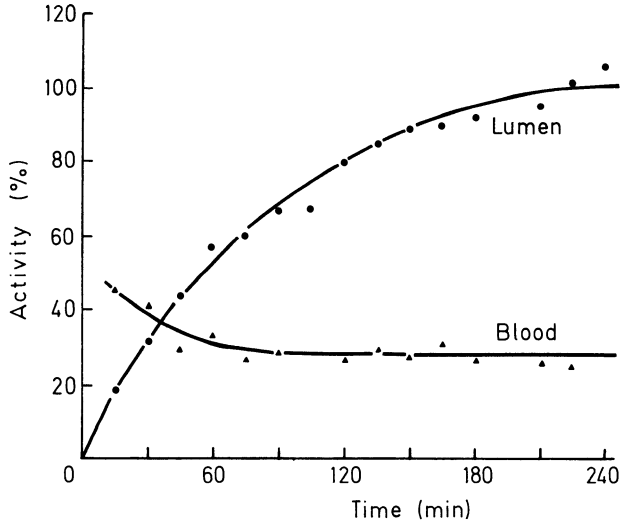

Fig. 12. Intestinal secretion of digoxin in the guinea pig in vivo. 0.13 nmol ^3H-digoxin/g body weight were administered i.v. after ligation of renal pedicles and bile duct. 10 ml isotonic saline was circulated through a 15 cm jejunal loop. Ordinate shows total radioactivity in one ml blood *(triangles)* and intestinal perfusion solution *(circles)* as percentages of the dose/g body weight. At the end of the experiments 31% of the total radioactivity in the blood and 66% in the perfusate were unchanged digoxin. (LAUTERBACH, 1975)

glycoside concentrations were compared. Depending on the concentrations and the duration of the experiments, the apparent permeability for blood–lumen permeation was at least three times higher than for the lumen–blood direction. It was concluded from this that ouabain is also a substrate of the secretory system for cardiac glycosides, but with a secretory rate too low to allow for the formation of an uphill gradient under experimentally realizable conditions (SEIDENSTÜCKER and LAUTERBACH, 1977; SEIDENSTÜCKER, 1978).

V. Comparative Aspects of Intestinal Glycoside Secretion

Cardiac glycosides were the class of drugs with which intestinal secretion was detected. They are not, however, the only such drugs. Intestinal secretion of quaternary ammonium compounds (LAUTERBACH, 1971 a; TURNHEIM and LAUTERBACH, 1977 a, b; TURNHEIM et al., 1977; PIEPER and LAUTERBACH, 1979) and of sulfonic acids (SUND and LAUTERBACH, 1978; LAUTERBACH, 1979) has been demonstrated as well. Obviously at least three separate systems for the excretion of drugs and other foreign compounds are localized in the mucosal epithelium of the intestine. It is interesting to note that the observations with organic cations and anions point (as deduced previously for cardiac glycosides) to an organization of the secretory system as two transport mechanisms in series in the basolateral and luminal membranes of enterocytes.

The intestine thus joins the kidney and the liver as another organ capable of clearing the body of foreign compounds by active secretion. At present, no com-

parison of capacities and substrate specificities can be given owing to the still limited amount of data. In general, the intestine deals with the same classes of compounds as do the kidney and the liver, for which secretion of cardiac glycosides (FALCH and TEIEN, 1973; LUKAS, 1973; STEINESS, 1974; SUMNER et al., 1976; LAUTERBACH, 1964; KUPFERBERG and SCHANKER, 1968), quaternary ammonium bases (for reviews see PETERS, 1960; SCHANKER, 1968; SMITH, 1971), and organic acids (WEINER, 1971, 1973; SCHANKER, 1968; SMITH, 1971) have been described. The exceptional position of the intestine in this context is its simultaneous function as an absorptive organ for just those compounds which are substrates of its secretory systems. Consequently, the interference of intestinal secretion must result in extremely complex absorption kinetics.

D. A Concept for the Intestinal Permeation of Cardiac Glycosides

We will now attempt to develop a concept to explain and unify the numerous, sometimes odd and contradictory results reviewed in Sect. B. This concept is based

1) On the demonstration of an active transport system for cardiac glycosides in the mucosal epithelium of the intestine;
2) On the fact, that all results at present available indicate a secretory nature of this transport system;
3) On the assumption that the intestinal transport mechanisms for cardiac glycosides are – like other biologic transport mechanisms – reversible, i.e., facilitate membrane permeation in both directions notwithstanding their preference for one.

If a glycoside crosses the enterocyte membrane not only by diffusion, a second term describing permeation by a transport mechanism has to be added to Eq. (3). Thus, the unidirectional permeation across one membrane is

$$\frac{dn}{dt} = -\left(J_{max} \times \frac{c}{c+K_m} + P \times c\right) A, \tag{10}$$

where J_{max} is the maximal transport capacity per unit area and unit time and K_m the half-saturation constant of the transport mechanism. (The other symbols have been defined in Sect. A).

In reality, intestinal glycoside permeation has to be described by at least a three-compartment model consisting of the luminal solution, the enterocyte, and the interstitial space. Owing to the conclusion that there are two transport mechanisms in series, probably both active, permeation of each of the two separating membranes involves a transport mechanism and a parallel, diffusive pathway. Preference of the transport mechanisms for one direction ("active transport") can be described by two different K_m values at the *cis* and *trans* side of the membrane. Hence, the total system is defined by at least eight, principally independent parameters[2]

2 This model is still a simplification. In reality, further factors would have to be taken into account, especially the permeability of the paracellular shunt pathways the importance of which has been stressed in chapter C

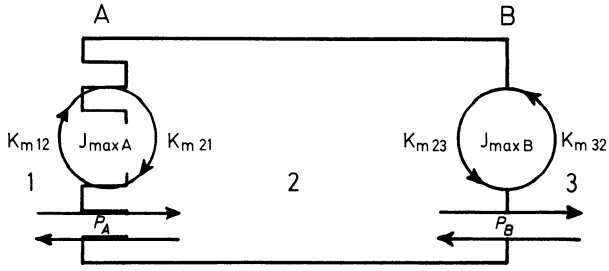

Fig. 13. Kinetic model for the description of the intestinal permeation of cardiac glycosides. The model consists of compartment 1 (intestinal lumen), compartment 2 (enterocyte), and compartment 3 (interstitial space on the blood side). Permeation of the luminal *A* and basolateral *B* membranes proceeds by transport mechanisms *(circles)* as well as by passive diffusion *(arrows)*. The transport mechanisms are characterized by their maximal velocities (J_{max}) and different half saturation constants (K_m) at the inner and the outer faces of the respective membrane. Diffusion is determined by the passive permeability *P*. (LAUTERBACH, 1975)

(Fig. 13). Given an appropriate setting of these parameters, a computer simulation of this system reveals a number of characteristic features (LAUTERBACH, 1975).

1) In both examples illustrated in Fig. 14 the model functions as a secretory system as is visualized by the higher value of the secretory than the absorptive rate and the accumulation of substrate in the gut lumen at low concentrations.

2) Irrespective of the parameter settings, there are two concentration ranges, where the permeation rate is independent of concentration:

a) At high concentrations, where $c \gg K_m$ and, hence, transport processes are saturated. Permeation proceeds by diffusion and, therefore, with an equal rate in both directions.

b) At low concentrations, where $c \ll K_m$ and, hence, transport processes are working in their range of proportionality. Within this range, participation of a transport process would not be detected by determination of the absorption rate at different concentrations, even if these are varied over several orders of magnitude. It is easily discovered, however, by comparison with the opposite flux, which is higher due to the secretory nature of the entire system.

3) Within the intermediate dose range, the secretory rate decreases. The absorption rate, however, may rise or fall. Reversion of the trend of the absorption characteristic from falling (Fig. 14a) to rising (Fig. 14b) is brought about by variation of only two half saturation constants, namely reducing K_{m21} and increasing K_{m23} which must result in an accelerated saturation of the luminal secretory mechanism.

Under suitable conditions, passage of the absorption rate through a maximum is clearly demonstrable (Fig. 15b).

Moreover, absorption rate is not only a function of dose, transport parameters and passive permeabilities, but also of the time of observation. These relations are most easily understood regarding a computer simulation of a secretory system performed in context with investigations on the absorption behavior of quaternary ammonium compounds showing comparable phenomena (Fig. 15a) (TURNHEIM and LAUTERBACH, 1980). The outstanding result is cessation of absorption in spite

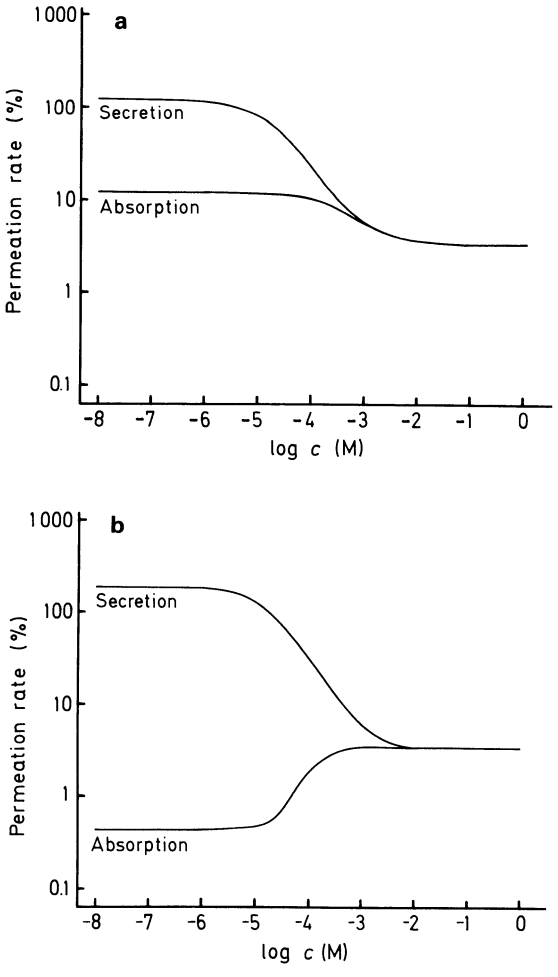

Fig. 14a, b. Computer simulation of the permeation kinetics in the three-compartment system depicted in Fig. 13. Abscissa shows the logarithm of the initial concentration on the side of administration. Ordinate shows permeation rate = amount disappearing from 1 ml luminal solution after luminal administration (absorption) or amount appearing in 1 ml luminal solution after administration on the blood side (secretion) as percentages of the amount contained initially in 1 ml solution at the side of administration.
In approximate accordance with a tied-loop experiment in a small animal, the volumes of compartments 1, 2, and 3 were assumed to be 1, 0.6, and 300 cm^3, respectively and the membrane area A = B = 20 cm^2. The kinetic parameters had the following values:

	a	b
$P_A = P_B$ (cm min^{-1})	2×10^{-4}	2×10^{-4}
$J_{maxA} = J_{maxB}$ (mole min^{-1} cm^{-2})	3.6×10^{-11}	3.6×10^{-11}
K_{m12} (M)	8×10^{-5}	8×10^{-5}
K_{m21} (M)	1×10^{-5}	1×10^{-6}
K_{m32} (M)	2.5×10^{-5}	2.5×10^{-5}
K_{m23} (M)	5×10^{-5}	2.5×10^{-3}

(LAUTERBACH, 1975)

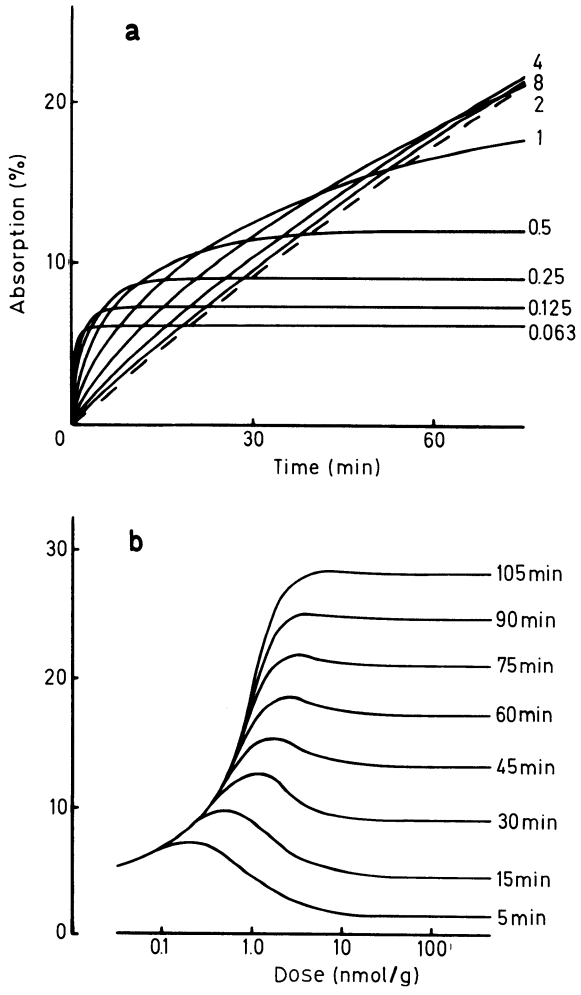

Fig. 15 a, b. Computer simulation of the absorption kinetics as a function of time and concentration in a two-compartment system. The substrate permeates the separating membrane by a secretory transport system and a parallel, diffusional pathway. Absorption is assumed to proceed in an animal of 350 g from the intestinal lumen (volume 1 cm^3) across an epithelium (area 30 cm^2) into an apparent distribution volume (800 cm^3). The parameters used for calculation have the following values: $P = 1 \times 10^{-4}$ cm min^{-1}, $J_{max} = 0.3$ nmol min^{-1} cm^{-2}, $k_{12} = 8 \times 10^{-5}$ M, $k_{21} = 1.2 \times 10^{-8}$ M. **a** time dependence of absorption at the indicated dose (nmol/g). The *broken line* represents the asymptote of the time course of absorption which is approached at very high intestinal substrate concentration. **b** dose dependence of absorption at the indicated absorption periods. (TURNHEIM and LAUTERBACH, 1980)

of huge amounts of unabsorbed drug owing to the establishment of an equilibrium between absorption and resecretion. However, this interruption is observed only within dose ranges sufficiently low to allow the secretory system to cope with the drug appearing at the transluminal side of the secretory system. If doses are increased and the secretory capacity is surpassed absorption tends to continue. The higher the dose, the later equilibrium is approached; it may never be reached.

Therefore, after sufficiently long periods of time, the absorption rate of high doses is superior to those of low ones. On be other hand, during the initial period of absorption low doses possess a kinetic advantage due to the relatively higher contribution of carrier-mediated absorptive flux to total absorption. The question now is: Which facts substantiate the concept and to which extent can it explain the diverging results reported?

To begin with, none of the results listed as compatible with diffusion exclude the possibility of the participation, or at least, the existence of a transport process. Definite proof for permeation by mere diffusion, e.g., by comparison of the transepithelial fluxes in both directions, has never been attained. Independence of the absorption rate on the intestinal concentration might just as well result from experimental conditions beyond the critical concentration range, or critical observation time, where nondiffuse kinetics can be observed. Failure of metabolic inhibitors to effect glycoside absorption might be due to predominant absorption of polar glycoside by paracellular routes (Fig. 10), only the disregarded secretory direction of the permeation process being dependent on metabolic energy or, again, missing the sensitive concentration range. DAMM et al. (1975) reported an effect of 2,4-dinitrophenol on digitoxin absorption in the mouse except at concentrations in the region of 5×10^{-7} *M*, where GREENBERGER et al. (1969) happened to perform their experiments in rats.

A decrease of the absorption rate with rising concentrations has been observed for a number of glycosides of pronounced or intermediate polarity, e.g., convallatoxin and convallatoxol. Likewise, specific inhibition of absorption by a second glycoside was demonstrated. An increase of absorption rate has been observed with glycosides of low polarity, e.g., digitoxin. It is tempting to speculate that just these glycosides caused a more rapid saturation of resecretion owing to the enhanced filling of the enterocyte by diffusion. Demonstrability of this phenomenon seems again to depend critically on the experimental conditions. Whereas the 60-min absorption rate of digitoxin from 4×10^{-5} to 3×10^{-4} *M* instillation solutions increased (LAUTERBACH, 1964), a constant absorption rate was observed, when the observation time was shortened to 20 min and concentration shifted to the lower range of $2 \times 10^{-6} - 5 \times 10^{-5}$ *M* (FORTH et al., 1969 a).

Passage of the absorption rate through a maximum with increasing intestinal concentration has indeed been observed for ouabain in the rat and has been interpreted as the subsequent saturation of secretory and absorptive sites of the transport mechanism (Fig. 16) (SEIDENSTÜCKER and LAUTERBACH, 1977; SEIDENSTÜCKER, 1978). Whereas absorption rate rose steeply between 10^{-6} and 10^{-5} *M*, no significant differences were observed in the region of the maximum between 10^{-5} and 10^{-4} *M*, the same concentration range within which CALDWELL et al. (1970) claimed a concentration-independent absorption rate in the rat in vivo. The strong dependence of absorption on the intestinal concentration might also explain contradictory results concerning the influence of polarity. Whereas HAASS et al. (1972) infused approximately 10^{-6} *M* ouabain solutions (where ouabain absorption is negligible in the rat, Fig. 16) and observed ouabain absorption in the guinea pig to be 1/14 of digoxin absorption, GREENBERGER et al. (1969) used approximately 2×10^{-5} *M* ouabain (where absorption is maximal, Fig. 16) and found ouabain absorption even slightly superior to digoxin.

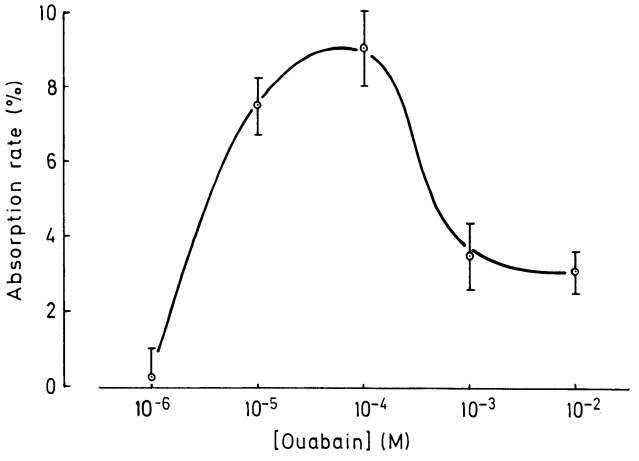

Fig. 16. Dose dependence of the absorption rate of ouabain in the rat. Instillation of 1 ml ^3H-ouabain into tied-off jejunal loops in situ. Abscissa shows intestinal ouabain concentration administered. Absorption period 3 h. Means ± standard errors. (SEIDENSTÜCKER, 1978)

Saturation of the secretory mechanism in the luminal membrane of the enterocyte by filling the small intermediate, intracellular compartment, has been discussed as the decisive step determining the shape of the ouabain absorption curve (SEIDENSTÜCKER, 1978). It might be worth investigating, whether the improved and accelerated absorption of digoxin from capsules, as compared with solutions (MALLIS et al., 1975; JOHNSON et al., 1976; LINDENBAUM, 1977) resulted from especially high local glycoside concentrations.

The characteristic pattern of absorption as a function of time and concentration (Fig. 15 a) has been demonstrated with digoxin in the guinea pig (Fig. 17). Absorption started rapidly after administration of 10^{-6} *M* digoxin but no further absorption was observed beyond 1 h. In contrast, no cessation was seen with 10^{-5} and 10^{-4} *M* solutions (SEIDENSTÜCKER and LAUTERBACH, 1976; SEIDENSTÜCKER, 1978). It is conceivable that the gradual decline of the absorption coefficient which has been observed with approximately 10^{-4} *M* glycoside solutions by SCHAUMANN et al. (1972) in the guinea pig (see Fig. 6) as well as by RIETBROCK (1976) and ANDERSSON et al. (1975) (see Fig. 7) has to be ascribed to an incomplete saturation of resecretion. The kinetic advantage of initial absorption from low concentration has been substantiated by direct determination of the porto-arterial differences after administration of 10^{-6} and 10^{-4} *M* digoxin (SEIDENSTÜCKER and LAUTERBACH, 1976; SEIDENSTÜCKER, 1978). The interpretation of the cessation of absorption as an equilibrium could be verified by perturbation experiments. Absorption was restarted by the transfer of the intestinal solution into a glycoside-free animal as well as by i.v. injection of an excess of unlabeled dihydrodigoxin (Fig. 18) (SEIDENSTÜCKER and LAUTERBACH, 1976; SEIDENSTÜCKER, 1978).

In summary, all theoretical predictions of the proposed concept of absorption by diffusion as well as *by* and *against* secretory transport mechanisms are verified by at least one example. The strong and complicated dependence of the absorption rate on the respective experimental conditions offers an explanation for many of

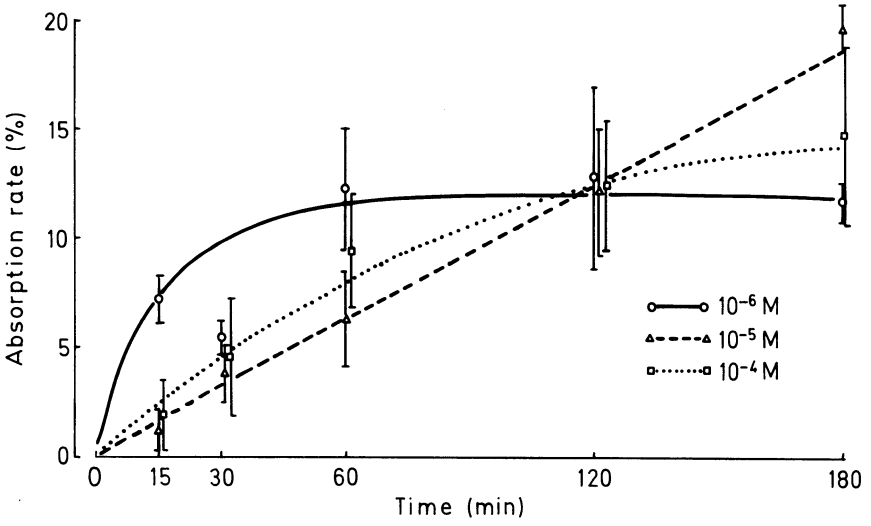

Fig. 17. Time course of the absorption of digoxin in the guinea pig at different concentrations. Instillation of 2 ml ^3H-digoxin solution into a tied-off jejunal loop in situ. Concentrations: *circles* 10^{-6} *M; triangles* 10^{-5} *M; squares* 10^{-4} *M*. Means ± standard errors. (SEIDENSTÜCKER, 1978)

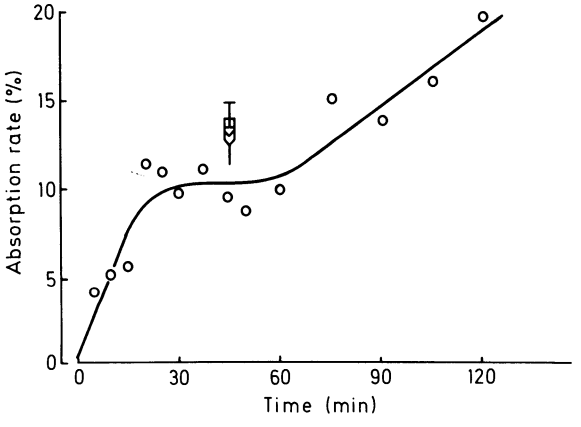

Fig. 18. Demonstration of an equilibrium between absorption and secretion of digoxin in the guinea pig. Instillation of 1 ml 10^{-6} *M* ^3H-digoxin into a tied-off jejunal loop in situ. I.v. injection of dihydrodigoxin 10^{-6} mol/kg body weight at the time indicated by a *syringe*. (SEIDENSTÜCKER, 1978)

the results so far not understood. These explanations should be subject to proof by properly designed experiments under strictly comparable conditions.

E. Conclusions

Contrary to widespread opinion, intestinal absorption of cardiac glycosides cannot be explained exclusively by diffusion. Numerous results incompatible with this theory have been published. Neither the inverse correlation between polarity and ab-

sorption rate, nor the independence of the absorption rate of the intestinal concentration, nor yet the constancy of the absorption coefficient are generally valid. The hypothesis, based on these incompatibilities, that a transport mechanism for cardiac glycosides must exist in the mucosal epithelium of the intestine has been substantiated. It turns out to be a secretory system transferring cardiac glycosides from the blood into the intestinal lumen against a concentration gradient.

A uniform explanation of all results hitherto known is achieved by assuming two parallel routes across the intestinal barrier: (i) a diffusive pathway and (ii) a mediated permeation by two transport mechanisms in series in the luminal and basolateral membranes of the enterocytes. Owing to the composite nature of the permeation process itself, absorption kinetics can be expected to become extremely complex – and have been experimentally observed to be so in several cases. Since absorption has to proceed *by* as well as *against* a transport mechanism, absorption rate and absorption coefficient are not only dependent on the respective glycoside and animal species but also (within critical dose ranges) on the concentrations administered and the absorption time. Hence, an absorption rate for a certain glycoside is valid only for strictly defined conditions concerning the species and method used as well as the dose or concentration administered and the observation time.

So far, experiments on the intestinal absorption of cardiac glycosides have been performed under rather static conditions in most cases. This holds true especially for studies in humans, where absorption rates have been determined with one dose at one time. It is to be expected that a wider variation of experimental conditions will reveal the participation of transport processes in the intestinal permeation of further cardiac glycosides in further species. Moreover, extension of the usual determination of the intestinal permeation of cardiac glycosides in the absorptive direction to measuring the secretory flux as well will most probably reveal that proportionality between intestinal dose and amount absorbed was no proof per se for permeation by diffusion and will demonstrate further examples of intestinal secretion. Finally, it is hoped that the very near future will bring a more detailed understanding of the influence of intestinal secretion on the absorption kinetics of drugs in general as well as of its quantitative role and clinical importance in drug excretion.

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CHAPTER 7

Cardiac Uptake and Binding of Cardiac Glycosides*

S. DUTTA

A. Introduction

In the classic studies of STRAUB (1910), as well as CLARK (1937), one finds the first attempts to show a relationship between the localization of cardiac glycosides by cardiac cells and their effect on the heart. Thus, STRAUB'S early experiments showed that excised frog heart contained certain receptive sites which bound a fatal concentration of digitalis from the perfusate. Furthermore, it was observed that the receptive capacity of heart muscle became saturated after one passage of digitalis through it, provided the perfusate concentration of this drug was sufficiently high. On the other hand, by meticulous calculations, CLARK showed that since it took 2 μg of ouabain to arrest a frog ventricle weighing 1 g and containing 3×10^8 cells, the lethal effect was due to the fixation of 10^7 ouabain molecules per cardiac cell. He also pointed out that the fixed ouabain molecules could not cover more than 3% of the surface of the cardiac cell.

Although CLARK'S calculation has provided the basis for the concept that the responses of digitalis in particular, and other drugs in general, are a consequence of their binding to a discrete area of cell surface, receptive area, or receptor, in no case could it be definitely proved at that time that cardiac glycosides actually interacted with any particular cellular component of cardiac cells. It is, therefore, logical that with the advent of a bioassay method for the measurement of minute amounts of cardiac glycoside in cardiac tissue in the 1950s, and radiometric methods in the 1960s, various workers began immediately to elucidate the uptake and binding kinetics of cardiac glycosides by cardiac cell components. These studies have progressed over the last fifteen years mainly along three different experimental lines and created a wealth of literature encompassing many different cardiac glycosides and all known mammalian cardiac preparations.

B. Experimental Approaches

The first approach utilized by LÜLLMANN'S group (KUSCHINSKY et al., 1967a, b; GODFRAIND and LESNE, 1967, 1972; BAKER and WILLIS, 1972; and BOARDMAN et al., 1972) involves essentially superfusing cardiac preparations, mostly thin-walled atrial or papillary tissue and in some cases, monolayered or suspended Girardi heart cells, with radioactive cardiac glycosides for various time periods. By measur-

* Dedicated to Professor BERNARD H. MARKS, on the occasion of his 60th birthday

ing the radioactivity of tissues which do not metabolize cardiac glycosides and subjecting these intact tissue levels of cardiac glycoside to kinetic analyses, it has been possible to obtain useful information in regard to the saturable versus nonsaturable uptake processes of cardiac glycoside by cardiac tissue.

The second approach, first applied by SJOERDSMA and FISCHER (1951) and later adopted by various workers utilizing different radiolabeled cardiac glycosides uses the techniques of perfusion of the isolated heart by the Langendorff method. The techniques as worked out by DUTTA et al. (1968 a) involved perfusion of radiolabeled cardiac glycoside such as ouabain at a constant flow for 60 min. At the end of the perfusion period, isolated heart is washed for 4–8 min to get rid of radioactivity in the extracellular space. The washed heart is then homogenized, subjected to subcellular fractionation and the various subcellular fractions analyzed for their content of radiolabeled cardiac glycosides by the conventional radiometric methods. Measurements of the cardiac glycoside content of inflowing and outflowing perfusate of the isolated heart or arterial and coronary sinus blood in the intact animal or patient have also enabled various workers (DUTTA et al., 1963; MARKS et al., 1964; SELDEN and NEILL, 1975) to determine cardiac uptake of cardiac glycosides by compartmental analyses.

The third approach involves an elaborate isolation of particulate fractions such as the Na^+, K^+-ATPase fraction (MATSUI and SCHWARTZ, 1968; ERDMANN and SCHONER, 1973 a, b), or the sarcoplasmic reticular membraneous fraction (DUTTA et al., 1968 b), or a simple preparation of homogenate (AKERA et al., 1973; ERDMANN, 1977) utilizing preferably bovine or canine cardiac tissue and measurement of their cardiac glycoside-binding capacity. Except for the time-consuming steps for the preparation of membraneous fractions with particular properties, such as the Na^+, K^+-ATPase-enriched preparation, the actual binding procedure by this approach is quite fast and allows one to obtain a good estimate of the concentration of the cardiac glycoside-binding sites per unit amount of cardiac tissue and their relative affinities for various cardiac glycosides in one batch of membraneous fraction.

Although the approaches were quite different in the binding and uptake studies for cardiac glycosides, the purpose was to identify clearly the specific binding site or sites for cardiac glycoside in cardiac cells. Therefore, the success of a particular approach in identifying cardiac binding sites, or a specific receptor for the pharmacologic effect of a given cardiac glycoside, has greatly dependent on obtaining a reasonable correlation between the binding characteristics as determined by that approach and the pharmacologic effect of the cardiac glycoside under study. Thus, in reviewing the results of uptake and binding of various cardiac glycosides as obtained from various experimental systems, an attempt will be made in this chapter to look for appropriate relationships between the rate at which a cardiac glycoside associates with its specific binding site and the rate of onset of pharmacologic effect, its rate of dissociation from the binding site and the rate of offset of pharmacologic effect, and lastly, its dissociation constant (K_D) as obtained from the uptake studies and the similar constants obtained from enzymatic and pharmacologic experiment.

C. Uptake of Radiolabeled Cardiac Glycosides by Superperfused Cardiac Preparations

I. General Characteristics and Kinetic Properties

A great deal of knowledge has been accumulated over the last ten years by LÜLLMANN and his co-workers on the uptake process of ^3H-digoxin (KUSCHINSKY et al., 1967a, b), ^3H-digitoxin, ^3H-ouabain (KUSCHINSKY et al., 1968a), ^3H-peruvoside (KUSCHINSKY et al., 1968b), and ^3H-digitoxigenin (KUSCHINSKY et al., 1968c). Basically, these studies have provided fundamental information in regard to the atrial concentrations of different cardiac glycosides at equilibrium when these compounds are presented to the atrial tissue by superperfusion in approximately equimolar concentration. Furthermore, these studies also revealed the rate at which the various cardiac glycosides reach equilibrium with binding sites in the superperfused tissue and the rate of their dissociation from the binding site upon washing with drug-free solution.

First of all, it has been learned that at equilibrium the concentrations of different cardiac glycosides in the superperfused tissue vary according to the lipid solubility of the compound used and therefore the tissue to medium radioactivity (T : M) ratio at equilibrium, as shown in Table 1, reaches as high as 10 in the case of digitoxin, the most lipid-soluble agent in this group of cardiac glycosides, and as low as 0.6 with the most polar agent, ouabain. Thus, it becomes evident that the ultimate tissue concentration of cardiac glycoside is irrelevant to its pharmacologic and toxic effects. It appears that in order of their respective lipid solubility, digitoxin > digoxin > ouabain (GREENBERGER et al., 1969) become accessible to the lipid cell membrane and nonspecifically enter into an extensive to negligible binding with various proteins present in the cell surface and cytosol. Thus, digitoxin, which is the most lipid soluble and which binds to the plasma protein with the highest affinity, shows the largest T : M ratio because of its readiness to penetrate the lipid membrane and its ability to remain bound to these various proteins. Conversely, ouabain manifests very poor accessibility through the lipid bilayer and goes into interaction with a very specific site. The importance of lipid solubility as the probable cause of nonspecific binding of digitoxin becomes more evident when it is observed that the T : M ratio goes down to as much as 4.84 when guinea-pig atrium is extracted with 50% aqueous glycerol, whereas the glycerol extraction has no such effect on the polar glycoside, ouabain (KUSCHINSKY et al., 1968d). The influence of lipid solubility in the differential uptake of digitalis is also noted in atria superperfused with blood as shown in Table 1. However, in blood, as much as 90%–95% of the plasma digitoxin is albumin bound whereas ouabain is 100% free and therefore, if appropriate correction is not made for the plasma binding as reported by LÜLLMANN et al. (1969), the apparent T : M ratio as such would not reflect the true distribution in regard to lipid solubility of these agents in the blood-perfused or intact system.

It has been observed that isolated atrial preparations (LÜLLMANN and VAN ZWIETEN, 1969) and Girardi heart cells (BAKER and WILLIS, 1972) take up radiolabeled cardiac glycosides from the medium slowly. The uptake process has been shown (KUSCHINSKY et al., 1968a) to reach equilibrium exponentially and there-

Table 1. Comparison of uptake parameters of various cardiac glycosides in atrial tissue superperfused with Tyrode solution or blood

Cardiac glycosides	Tyrode-perfused[a]				Blood-perfused[c]			
	Concentration in medium (μM)	Tissue: medium ratio	$t_{1/2}$ of uptake (min)	$t_{1/2}$ maximal effect (min)[b]	Concentration in blood (μM)	Corrected Tissue: plasma ratio	$t_{1/2}$ of uptake (min)	$t_{1/2}$ of maximal effect (min)[d]
Ouabain	0.17	0.5–0.7	4	15	0.099	0.45	6.5	5.8
Digoxin	0.32	2.8	32	21.5	0.32	1.6	16	23
Digitoxin	0.13	10	8	12	0.65	8.8	21	56
Digitoxigenin	0.27	8	20	20	2.2	8.4	5.5	

[a] (KUSCHINSKY et al., 1967a, 1968a, c)
[b] 0.12 μM ouabain in atria (PETERS et al., 1974); 0.3 μM digoxin in papillary muscle (LÜLLMAN and RAVENS, 1973); 0.1 μM digitoxin and digitoxingenin in isolated heart (DUTTA et al., 1976)
[c] (LÜLLMANN et al., 1969) [d] (FORESTER et al., 1974)

Table 2. Comparison of binding parameters of ouabain in superperfused cardiac tissue in relation to pharmacologic effect in guinea-pig

Tissue	Physiologic salt solution used (mM)	Maximum binding number of sites (pmol/g wet wt.)	Binding sites avogadro's No. ouabain molecules/g wet wt.	Conc. of half-maximal binding K_D (nM)	Conc. of half-maximal effect ED_{50} (nM)	Reference
Papillary muscle	Tyrode (K^+ 2.7)	1,900	1.1×10^{15}	600	340[a] 150[b]	LÜLLMANN et al. (1975)
Atrial muscle	Tyrode (K^+ 6.0)	372	2.2×10^{14}	389	150[c]	GODFRAIND and GHYSEL-BURTON (1977)
Atrial muscle	Tyrode (K^+ 2.7)	219	1.31×10^{14}	193	110[d]	GODFRAIND and GHYSEL-BURTON (1977)
Atrial muscle	Tyrode (K^+ 2.7)	158	0.95×10^{14}	312		GODFRAIND and LESNE (1972)

[a] Papillary muscle (REITER, 1967)
[b] Papillary muscle (FLASCH and HEINZ, 1978)
[c] Left atrial strip in Krebs–Henseleit, K^+ 5.8 (KU et al., 1976)
[d] Atria in Tyrode, K^+ 2.7 (PETERS et al., 1974)

fore one may characterize individual uptake by the superperfused preparation on the basis of the time taken by a given cardiac glycoside to reach half-maximal concentration, i.e., the $t_{1/2}$ of the uptake process. Comparison of these values as obtained for four well-known cardiac glycosides reveals a wide difference in this parameter (Table 1), and this variation of $t_{1/2}$ values among these four cardiac glycosides does not seem to have any relationship with the lipid solubility of these agents nor with the published values of the $t_{1/2}$ of onset of positive inotropic effect. From the standpoint of lipid solubility, it appears that, at least in Tyrode solution, the agents which are remarkably polar, such as ouabain and digoxin, vary greatly in their $t_{1/2}$ value; ouabain shows the highest and digoxin the lowest $t_{1/2}$ values. Similarly, there is also no particular accord in the $t_{1/2}$ values between digitoxin and digitoxigenin, which are almost equal in their lipid solubility. However, in the blood-perfused system, there seems to emerge a pattern among ouabain, digoxin, and digitoxin in that a direct relationship is observed between the lipid solubility and the $t_{1/2}$ of uptake of these agents.

Now, in regard to the relationship between the $t_{1/2}$ of uptake and the $t_{1/2}$ of onset of positive inotropic effect, once again it appears that there is lack of agreement between these two parameters as obtained in various cardiac preparations of guinea-pig for four cardiac glycosides, as shown in Table 1. For example, it is noted that although the $t_{1/2}$ values for the positive inotropic effects of digoxin and digitoxigenin are quite similar, there exist wide difference in their $t_{1/2}$ values to reach the maximal concentration. A similar discrepancy is noted between ouabain and digitoxin. However, in the blood-perfused system, the $t_{1/2}$ values of atrial uptake for ouabain, digoxin, and digitoxin seem to follow the trend, at least qualitatively, of the $t_{1/2}$ values of the positive inotropic effect, as observed by measuring the shortening of the systolic time intervals in normal subjects (FORESTER et al., 1974). Thus, it is noted in Table 1 that the fast $t_{1/2}$ of ouabain effect parallels its rapid uptake in the blood-perfused system and conversely the slow equilibration of digitoxin matches its slow $t_{1/2}$ of positive inotropic effect, only in a very qualitative sense. There is no clear explanation as to why there exists a better accord between the $t_{1/2}$ values of uptake and the $t_{1/2}$ of maximal positive inotropic effect in the blood-perfused system than the atrial tissue perfused with Tyrode solution. It is possible that the physiologic conditions in the intact or the blood-perfused system are supportive of better drug uptake and concurrent manifestation of pharmacologic effects.

The rate of dissociation of bound cardiac glycoside has been examined in various superperfused preparations such as atrial tissue (KUSCHINSKY et al., 1968 a, b), Girardi cells (BAKER and WILLIS, 1972) and papillary muscle (LÜLLMANN et al., 1975). It appears that in comparison with the uptake of cardiac glycosides by these tissue preparations, the washout of these agents is much more complex and shows certain characteristics. For example, the washout of ouabain from ^3H-ouabain-loaded papillary muscle by drug-free solution for 3 h revealed two distinct washout curves for the disappearance of radiolabeled ouabain of half-lives of 16 and 135 min. Since positive inotropic activity of ouabain in the guinea-pig washes out much more rapidly from cardiac tissue (AKERA et al., 1978; PETERS et al., 1974), these long half-lives must be interpreted as the washout of ouabain from sites irrelevant to the pharmacologic effect. Similarly, from studies with atrial preparations and the washout of bound digitoxigenin, digoxin, and digitoxin (KUSCHINSKY

et al., 1967a, b, 1968a, b, c), no correlation was noted between the $t_{1/2}$ of washout and the $t_{1/2}$ of disappearance of the positive inotropic effect.

Further analyses of the uptake processes of cardiac glycosides by guinea-pig atrial tissue (GODFRAIND and LESNE, 1972) and papillary muscle (LÜLLMANN et al., 1975) have provided more information in regard to the number of sites and affinity values of cardiac glycoside uptake by these tissues. Thus, GODFRAIND and LESNE (1972) utilized the modified Langmuir equation in order to fit their uptake values, and provided a set of kinetic parameters (Table 2) such as the maximal number of cardiac glycoside binding sites and the concentration of cardiac glycoside at which half of the saturable binding sites remain occupied (dissociation constant, K_D). On the other hand, having subjected their data on ^3H-ouabain uptake by the papillary muscle to the double reciprocal plot, LÜLLMANN et al. (1975) provided relevant values of the maximal binding sites and K_D of ^3H-ouabain accumulation for the ventricular tissue. Comparison of these values reveals that the ventricular tissue seems to have more binding sites for ouabain than the atrial tissue. In fact, by treating the respective data on the maximal binding sites in atrial and ventricular tissue by Avogadro's number (6×10^{23}), it has been possible to estimate that approximately 10^{14} and 10^{15} cardiac glycoside molecules can bind to 1 g atrial and ventricular tissue respectively. Further calculations have led to the conclusion that the calculated amounts of ouabain can occupy about 0.03% and 0.30% of the cell surface of atrial and ventricular tissue respectively. In the case of digitoxin, the value turns out to be 0.13% of arterial plasma membrane. It is interesting that CLARK's (1937) estimated value, as described earlier for ouabain, is not that much different from these newly found estimates.

Not only does ventricular tissue contain more binding sites for ouabain than atrial tissue, it appears that the K_D value for guinea-pig papillary muscle as reported by LÜLLMANN et al. (1975) is two- to three-fold higher than the reported K_D value for atrial tissue in this species (GODFRAIND and LESNE, 1972; GODFRAIND and GHYSEL-BURTON, 1977), obtained at similar concentrations of potassium ion (Table 2). This difference in the K_D values between these two sites would then suggest that the papillary muscle of guinea-pig relative to its atria is much less sensitive in its response to ouabain. The ED_{50} values, as reported in the literature, seem to indicate such a difference in sensitivity between these two cardiac sites (REITER, 1967; PETERS et al., 1974). Furthermore, good agreement between the K_D values for ouabain as obtained in superperfused atrial and papillary muscles of the guinea-pig and the ED_{50} values for ouabain in these preparations perhaps indicate a common binding site for this cardiac glycoside. Comparison of similar values for digitoxin also indicates a good agreement and common site of binding for this cardiac glycoside (GODFRAIND, 1975). It may be concluded from consideration of the studies of superperfused papillary muscle (LÜLLMANN et al., 1975) and atrial tissue (GODFRAIND and GHYSEL-BURTON, 1977) that the method of binding and the kinetic treatment of the data used in these studies appear to work quite well in identifying the specific binding sites in intact tissue.

II. Characteristics of Uptake in Relation to Rate of Stimulation

Because of the observation that a specific number of myocardial contractions, rather than an exposure time of a certain duration, determines the positive inotro-

Table 3. Uptake of ^3H-digoxin by guinea-pig atrial preparation resting and under stimulation followed by washout and subsequent stimulation of both preparations. (Adapted from ROTH-SCHECHTER et al., 1970)

Functional activity	Bath concentration (pmol/ml)	Tissue concentration (pmol/g)			Inotropic activity (% of control)
		30 min exposure to digoxin	Followed by washout	Subsequent stimulation to produce effect	
Nonstimulated	1,280	909 ± 64	794	294 ± 25	65
Stimulated	1,280	1,114 ± 115	781 ± 89	269 ± 12	85
Nonstimulated	128	143 ± 8	134 ± 23	34 ± 5	
Stimulated	128	149 ± 1	129 ± 16	31 ± 9	

pic effect of cardiac glycosides, it has been postulated that there exists a relationship between frequency of contractions and uptake of cardiac glycosides by the pharmacologically relevant site (MORAN, 1967). In order to test this postulate, KUSCHINSKY et al. (1967a) investigated the effect of rate of stimulation on atrial tissue uptake of ^3H-digoxin and showed that resting auricles took up the label more slowly than auricles that were stimulated either at 30 or 180 beats/min. The $t_{1/2}$ value for resting atria in the presence of 0.128 nmol/ml digoxin was found to be 52 min while auricles beating at either 30 or 180 beats/min reached their half-maximal value at 28 min. Although these results in a way substantiated the importance of frequency of contraction in the uptake of cardiac glycoside by atrial tissue, it was not really known from this study whether this stimulation-induced uptake was relevant to the pharmacologic effect or not. In order to investigate this aspect further, ROTH-SCHECHTER et al. (1970) measured digoxin content in relation to contractile force in a protocol where atrial tissue was kept either in the resting state or stimulated in the presence of 1 μg/ml (1.28 nmol/ml) ^3H-digoxin for 30 min and then both preparations were washed and kept under stimulation for an additional 30 min for production of positive inotropic effect. It is noted in Table 3 that the study of ROTH-SCHECHTER et al. (1970) confirmed the results of KUSCHINSKY et al. (1967a) in that there was a significant difference of ^3H-digoxin uptake in resting compared with contracting atrial preparations immediately after exposure for 30 min. However, following washout and subsequent stimulation of resting and nonresting preparations for 30 min, which resulted in 65% and 85% increases in inotropic activity respectively, there was no significant difference in the ^3H-digoxin content of these preparations. This would indicate that the difference in digoxin uptake at the receptor sites was so minute that the low specific activity (0.56 Ci/mmol) used in this study might have failed to reveal the difference by the available techniques for measuring radioactivity.

Although the results obtained by ROTH-SCHECHTER et al. and the subsequent study of LÜLLMANN et al. (1975) have been negative in establishing a relationship between uptake of cardiac glycoside and rate of stimulation, the relatively recent studies of LÜLLMANN and his co-workers (BENTFELD et al., 1977; BUSSE et al., 1979) have shown a clear dependence of the rate of ouabain binding to the atrial stimulation rate of 0.1 and 2 Hz and failure of this dependence when the frequency of

stimulation is increased to higher rates of 4 and 5 Hz. It appears that as the frequency of stimulation increases from 0 to 5 Hz, the availability of binding sites for ouabain goes through biphasic changes owing to an interplay of Na pump, conformational changes of Na^+, K^+-ATPase, and intracellular accumulation of sodium. Further work will be needed with different cardiac preparations utilizing various other cardiac glycosides to understand the complex relationship between frequency of contractions and the availability of binding sites in the cardiac cell surface.

D. Uptake of Cardiac Glycosides by Perfused Cardiac Preparations

SJOERDSMA and FISCHER (1951) were the first to perfuse ^{14}C-digitoxin through heart isolated from rat, guinea-pig, rabbit, and cat. Because of this study, these workers could demonstrate for the first time that isolated heart avidly bound digitoxin from the perfusate showing a tissue to medium ratio between 3 and 9. Later, HARVEY and PIEPER (1955) determined subcellular localization of ^{14}C-digitoxin by isolated guinea-pig heart and noted the highest digitoxin level in the post-mitochondrial aqueous fraction. Although it was postulated at that time that certain light membraneous elements in the aqueous fraction of guinea-pig heart specifically bound ^{14}C-digitoxin, experimental evidence in support of such a contention could not be obtained until the early 1960s because of the lack of available techniques. With the development of improved counting techniques by the liquid scintillation method and the availability of tritium-labeled cardiac glycosides of high specific activity by the Wilzbach procedure, work was immediately initiated by various investigators (DUTTA et al., 1963, 1968a; GERBER et al., 1968) to determine the localization of cardiac glycosides at the cellular and subcellular elements of intact, as well as in isolated heart. Mostly, the results of these studies indicated a link between subcellular cardiac accumulation of cardiac glycosides and their pharmacologic effects on the heart.

I. Gross Cardiac Uptake of Cardiac Glycosides in Relation to their Effects

The idea that the pharmacologic, as well as toxic, effect of cardiac glycosides results when a discrete amount of these agents gets "fixed" on, or in the myocardial cell surface, perhaps prompted various workers to determine myocardial uptake of cardiac glycosides in relation to pharmacologic effects. These studies were conducted mostly in intact dogs utilizing primarily radiolabeled ouabain and digoxin. Following administration of these agents intravenously and measurements of their respective content in the ventricle and plasma in these studies, attempts have been made to obtain relationships among the cardiac tissue and plasma levels of cardiac glycosides and positive inotropic, dysrhythmic, and lethal effects. Tables 4 and 5 compare the results of myocardial and plasma content of ouabain and digoxin respectively as reported by various workers in relation to the dosage used and the observed effects. It will be noted in these tables that the cardiac content of both ouabain and digoxin appears to increase in parallel with the manifestation of ef-

Table 4. Comparison of myocardial ouabain content in relation to inotropic, arrhythmic, and lethal effects

Dose	Effect	Termination time (min)	Left ventricle (pmol/g wet wt.)	Plasma (pmol/ml)	Tissue/Plasma ratio	Reference
8 μg/kg; 2 μg kg^{-1} min^{-1}	Lethal	46±4	685	307	2	RHEE (1973)
3 μg/kg; 1 μg kg^{-1} min^{-1}	Lethal	84±7	725	123	6	DUTTA et al. (1974)
3 μg/kg; 1 μg kg^{-1} min^{-1}	Arrhythmia	40	488	121	4	DUTTA et al. (1974)
3 μg/kg; 1 μg kg^{-1} min^{-1}	Arrhythmia	60	432	119	4	DUTTA et al. (1977)
40 μg/kg; 72 ng kg^{-1} min^{-1}	Arrhythmia	300	669	28	24	RHEE et al. (1976)
21 μg/kg	Not measured	10	327	25	13	SELDEN and NEILL (1975)
30 μg/kg; 36 ng kg^{-1} min^{-1}	(+) Inotropy (29%)	60 (Biopsy)	288	10	28	HOUGEN and SMITH (1978)
30 μg/kg; 36 ng kg^{-1} min^{-1}	(+) Inotropy (46%)	120 (Biopsy)	296	8	37	HOUGEN and SMITH (1978)
20 μg/kg; 36 ng kg^{-1} min^{-1}	(+) Inotropy (22%)	120	230	8	29	RHEE et al. (1976)
20 ng/kg; 36 μg kg^{-1} min^{-1}	(+) Inotropy (24%)	300	294	9	32	RHEE et al. (1976)
46 μg/kg	(+) Inotropy (24%)	10 (Biopsy)	102			LUCHI et al. (1971)
46 μg/kg	(+) Inotropy (35%)	40 (Biopsy)	120			LUCHI et al. (1971)
3 μg/kg; 1 μg kg^{-1} min^{-1}	(+) Inotropy	15	262	116	2	RHEE (1973)

Table 5. Comparison of myocardial digoxin content in relation to inotropic, arrhythmic, and lethal effects

Dose	Effect	Termination time (min)	Left ventricle (pmol/g wet wt.)	Plasma (pmol/ml)	Tissue/Plasma ratio	Reference
140 μg/kg	Arrhythmia	120	656	50	13	TAUBERT and SHAPIRO (1975)
2.5 μg kg^{-1} min^{-1}	Arrhythmia	55	610	231	3	HALL et al. (1977)
50 μg/kg; followed by 50 μg/kg; every 30 min	Arrhythmia	Biopsy sample (at peak effect)	1116	144	8	HOUGEN et al. (1979)
80 μg/kg	(+) Inotropy (32%)	60	261	10	26	DEUTSCHER et al. (1972)
80 μg/kg	(+) Inotropy (22%)	120	243	6	40	DEUTSCHER et al. (1972)
80 μg/kg	(+) Inotropy (53%)	60	405			GOLDMAN et al. (1973)
50 μg/kg	(+) Inotropy (intensity not indicated)	Biopsy sample (at peak effect)	348	34	10	HOUGEN et al. (1979)
50 μg/kg	(+) Inotropy (35%)	Biopsy sample 10	306	50	6	STEINESS and VALENTIN (1976)
50 μg/kg	Not measured	5	166	57	3	HOPKINS et al. (1974)
	Not measured	60	170	24	7	HOPKINS et al. (1974)
30 μg/kg	(+) Inotropy (18%)	120	156	5	31	TAUBERT and SHAPIRO (1975)

fects such as positive inotropic (therapeutic), onset of dysrhythmic (toxic), and lethal effects. Thus, in spite of individual variations of results from one laboratory to another, it is noted that in the case of ouabain the lethal, dysrhythmic, and positive inotropic effects are associated with the accumulation of 705, 530, and 240 pmol of the drug respectively per g of ventricular tissue. A similar trend is observed in the case of digoxin (Table 5), in that apparently it takes an accumulation of 794 pmol of digoxin per g of ventricle tissue to induce a dysrhythmic effect whereas the positive inotropic effect is noted at the much lower concentration of 257 pmol of this drug per g wet weight.

It has also been noted (DUTTA et al., 1974; RHEE, 1973) that when ouabain is slowly infused at a constant rate of 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ following a loading dose of 3 μg/kg, there is a gradual increase in accumulation of ouabain in ventricular tissue in parallel with the manifestation of positive inotropic, dysrhythmic, and lethal effects at 15, 40, and 84 ± 7 min respectively. At these time intervals, ventricular tissue seems to accumulate 262, 488, and 725 pmol ouabain per g wet weight respectively. Furthermore, DUTTA et al. (1974) and RHEE (1973) have also demonstrated that if the rate of infusion of ouabain is raised so that 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ is infused following a loading dose of 8 μg/kg, it is possible to induce a lethal effect at 46 ± 4 min (Table 4), at which time the ventricle accumulates 685 pmol ouabain per g wet weight, a value statistically similar to the one obtained following administration of the lower dose. Thus, it becomes evident that irrespective of dosage used, at the time of lethal effect, a discrete amount of ouabain seems to accumulate in order to induce this fatal effect.

In the light of this study on the increased accumulation of cardiac glycosides by ventricular tissue in relation to the development of positive inotropic, toxic, and lethal effects, the question arises as to whether there also exist observable increases in the accumulation of cardiac glycosides in relation to the graded positive inotropic response. Although this question is yet to be answered by direct experimentation, it appears from the various studies, where ouabain has been infused continuously in order to sustain at least quasi-steady-state levels of positive inotropic effect, that there does not seem to be any particular relationship between the myocardial ouabain content and the levels of ultimate positive inotropic effects. Thus, HOUGEN and SMITH (1978) observed no appreciable change in the ouabain concentration in biopsy samples taken at 60 and 120 min in spite of the fact that between these two times, positive inotropic activity increased from 29% to 46% over control (Table 4). Similarly, LUCHI et al. (1971) also reported no change in myocardial ouabain concentration between 10 and 40 min while a substantial increase in positive inotropic activity took place during this time interval. On the other hand, RHEE et al. (1976) observed a slight increase in total ventricular concentration of ouabain following administration of 20 ng/kg as loading and 36 $ng \cdot kg^{-1} \cdot min^{-1}$ as infusion doses of this drug to dogs; this produced a steady-state positive inotropic effect (22%–24%) for 300 min. Thus, from these studies (LUCHI et al., 1971; RHEE et al., 1976; HOUGEN and SMITH, 1978), it appears that the ventricular cardiac glycoside content as such does not seem to have any particular relationship to the gradual increase in the positive inotropic effect. As explained in Sects. D.II and D.III, this lack of relationship between the ventricular accumulation of cardiac gly-

cosides and positive inotropism might be due to a time-dependent process that translocates these agents from an initial binding site to a specific site for initiation of the pharmacologic effect.

II. Kinetic Properties of Cardiac Glycoside Extraction by Cardiac Preparations

Working with sheep and measuring ^3H-ouabain concentration in arterial and coronary sinus blood following intravenous injection, DUTTA et al. (1963) first observed that it took only 8–16 min for the disappearance of arteriovenous difference in this species. Later, MARKS et al. (1964) also noted in patients undergoing cardiac surgery that it took only 4 min for the arteriovenous difference to disappear following administration of ouabain. A recent study of SELDEN and NEILL (1975), however, reported a slightly longer time (9–12 min) for the extraction of ouabain

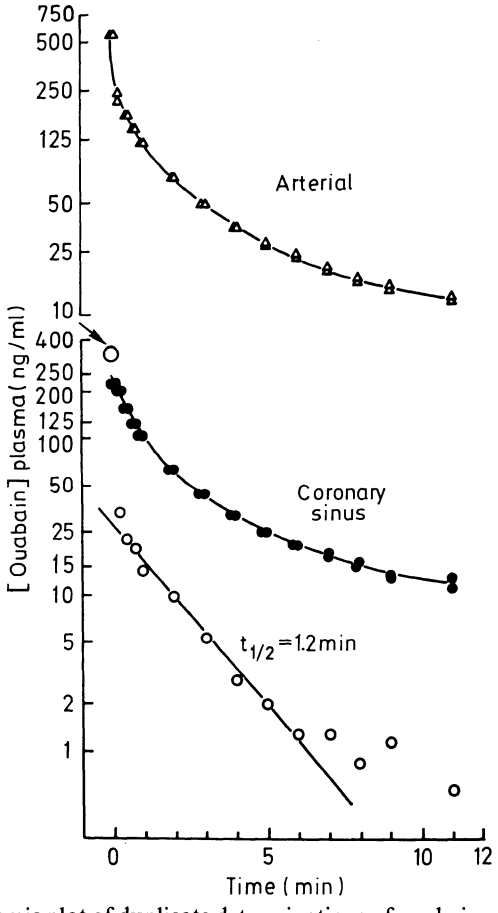

Fig. 1. Semilogarithmic plot of duplicate determinations of ouabain concentration in arterial (△–△) and coronary sinus (●–●) plasma against time after i.v. ouabain in a dog. The difference in ouabain concentration (○–○) between arterial and coronary sinus plasma, initially 330 ng/ml *(arrow)* declined exponentially between 0.5 and 6 min with a half-life of 1.2 min. (Adapted from SELDON and NEILL, 1975)

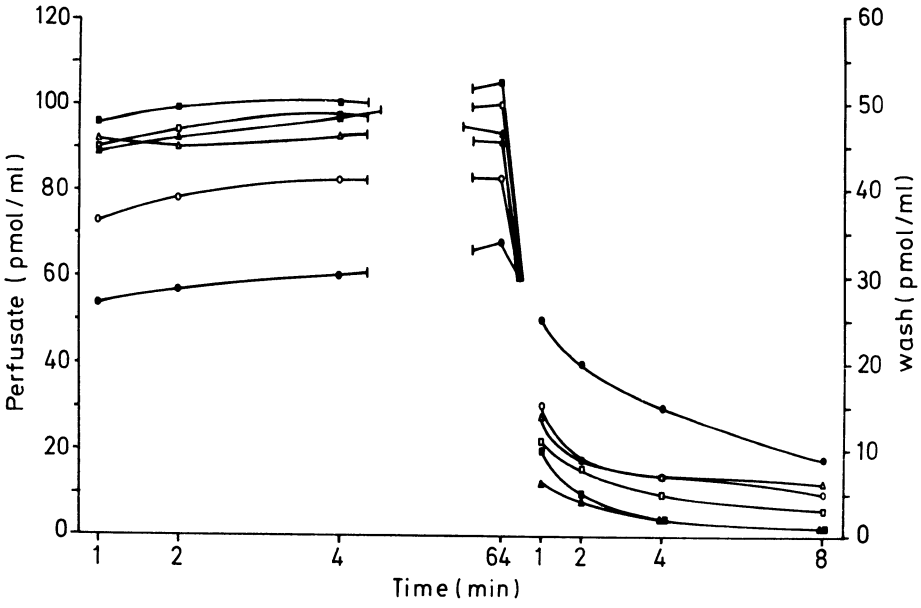

Fig. 2. Comparison of uptake and washout of cardiac glycosides by isolated guinea-pig heart. Cardiac glycosides, 0.1 nmol/ml in medium, were perfused for 64 min. Following drug perfusion, heart was perfused for 8 min with drug-free medium. Right and left ordinates refer to cardiac glycoside concentration in medium during uptake and washout periods, respectively. ●, digitoxin; ○, proscillaridin; △, convallatoxol; ▲, ouabain; □, digoxin; ■, dihydroouabain. (Adapted from DUTTA et al., 1968 b)

in their patients. They also indicated that in dogs arteriovenous difference disappeared within 10 min of ouabain administration in most of their experiments. By semilogarithmic plotting of arteriovenous differences as a function of time, SELDEN and NEILL (1975) also showed that the difference narrowed in an exponential manner with $t_{1/2}$ of 1–2 min for dogs (Fig. 1) and 2–6 min for patients.

On the other hand, by measuring the cardiac glycoside content of the inflowing perfusate versus outflowing perfusate in isolated guinea pig heart perfused at a constant flow, it was learned that the time necessary for the extraction of cardiac glycoside was different for polar and nonpolar glycosides (DUTTA et al., 1968 b). Figure 2 shows that equilibrium was reached between the heart and the perfusing medium in 4 min with ouabain whereas digitoxin continued to be extracted by the heart tissue from the perfusate throughout the entire 64 min perfusion. With digitoxin, the steady state was one in which the heart was extracting approximately on average 30% of the perfusing drug over this 64 min period while with ouabain, less than 10% was extracted during this period. Digoxin, dihydroouabain and convallatoxol behaved like ouabain. Recently, MARZO and GHIRARDI (1974) and MARZO et al. (1976) showed that the extraction of strophanthoside K and deslanatoside C by the isolated guinea pig-heart, like that of ouabain, was completed within 4 min.

Since with agents such as inulin and sucrose arteriovenous difference disappears within 1 min of administration of these agents to dogs (SELDEN and NEILL, 1975), it suggests that a short but discrete time is utilized by ouabain to move from

the extracellular space to its initial site of interaction in or on the outer surface of the cardiac plasma membrane. Furthermore, since there is no manifestation of any pharmacologic effect during this period of ouabain removal from the extracellular space in the isolated heart (DUTTA et al., 1968 a) nor in intact animals (SELDEN and NEILL, 1975; DUTTA et al., 1963), and patients (MARKS et al., 1964; SELDEN and NEILL, 1975), these studies imply that this initial site of interaction of ouabain is a step proximal to the putative pharmacologic site of interaction. In other words, the uptake process of cardiac glycosides probably involves multiple steps, initially binding to the cell surface and then translocating to other subsurface sites for initiation of the pharmacologic effect as proposed initially by DUTTA et al. (1968 b) and recently by means of an illustrative model by FRICKE (1976).

III. Translocation of Cardiac Glycosides from their Initial Site of Interaction

A test of the translocation hypothesis for cardiac glycosides was made by DUTTA et al. (1968 a) by studying in detail the time-dependent uptake process for one of the polar cardiac glycosides, digoxin, by isolated guinea-pig heart. In this study, by measuring the amount of digoxin actually present in the whole homogenate prepared from isolated guinea-pig heart at 4, 16, 32, and 64 min of digoxin perfusion and 8 min of drug-free wash, it was possible to show (Fig. 3) that there was a continual but slow accumulation of digoxin by isolated guinea-pig heart during the entire perfusion period, as if the amount of digoxin that was extracted during the first 4 min of drug perfusion (Fig. 2) was being bound firmly during the next 64 min period. Support for this line of speculation was found when homogenates that were prepared from heart after various times of perfusion and subsequent washout of the extracellular digoxin, were separated into soluble supernatant and total particulate fractions by a single high speed centrifugation and the resultant values of supernatant to pellet (S:P) ratio were plotted as a function of time as shown in Fig. 3. It will be noted that the similar time dependence as observed for the time-dependent change of digoxin contents in the whole homogenate is noted also in S:P ratio in that the ratio shifts from a high to a much lower value as a function of time. These shifts are due probably to a process which causes digoxin to change from being present mostly in the supernatant of heart cells to being bound in the particulate components. The delay in the accumulation of digoxin by the heart, as well as the delay in the onset of pharmacologic effect, therefore, appears to be due to the time required for possible translocation and subsequent binding to the specific cellular elements that are responsible for the pharmacologic effect. Alternatively, it suggests that initially digoxin enters into a transitional binding with a specific conformation of its putative receptor and with time tends to bind more firmly to another conformation.

This time-consuming process of digoxin uptake by the isolated guinea-pig heart has also been observed by MARZO and GHIRARDI (1974) utilizing strophanthoside K. These workers, as well as DUTTA et al. (1968 a) have demonstrated that the uptake into the particulate fraction seen in perfused heart could not be accounted for by simple physiochemical interaction of cardiac glycosides with particulate elements (Table 6). A comparison of S:P ratios as observed in the isolated heart

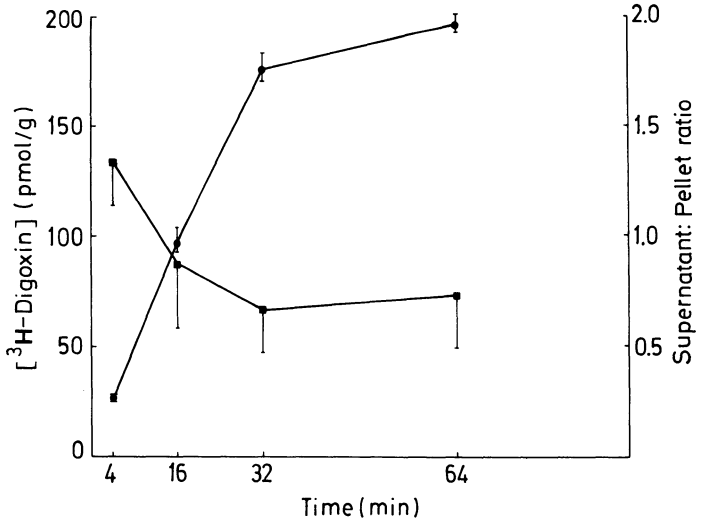

Fig. 3. Left ordinate is ^3H-digoxin concentration (pmol/g) in guinea-pig heart homogenate after various times of perfusion with 1×10^{-7} *M* digoxin *(circles)*. Right ordinate is ratio of ^3H-digoxin concentration is supernatant to that in pellet fraction *(squares)*. Homogenates, as obtained from digoxin-treated heart for various time periods, were subjected to centrifugation at 166,000 *g* for 1 h to prepare the supernatant and pellet fractions. (Adapted from DUTTA et al., 1968a)

Table 6. Comparison of digoxin and strophanthoside K in control and perfused guinea-pig heart homogenate[a]

	Homogenate 100%	Supernatant (%)	Supernatant: pellet ratio
10% Homogenate in Krebs–Henseleit (K–H); incubation for 64 min at 28 °C in the presence of cardiac glycoside:			
Digoxin	100 (2)	88.0	7.5
Strophanthoside K	100 (4)	93.8±0.2	15.2±0.8
10% Homogenate after perfusion with K–H for 64 min at 28 °C in the presence of cardiac glycoside:			
Digoxin	100 (12)	39.0±4.0	0.7±0.1
Strophantoside K	100 (8)	33.0±2.0	0.5±0.04

[a] Values are given as percentages of the cardiac glycoside present in the homogenate and expressed as a mean ± standard error of number of experiments shown in parentheses. (Adapted from DUTTA et al., 1968a; MARZO and GHIRARDI, 1974)

versus homogenate alone makes it clear that the uptake of these agents is greatly dependent on the functional integrity of the cardiac cell.

Furthermore, this time-dependent process of digoxin uptake appears to have an interesting pharmacologic implication. For example, when individual values of homogenate content of digoxin and their respective S : P ratios are expressed as per-

centages of their steady-state values and plotted in a semilogarithmic graph paper, it is noted that their respective half-lives are very similar (approximately 11 min). This half-life of uptake of digoxin by isolated heart compares very well with the time course of development of positive inotropic effect ($t_{½}$=15 min), as reported by LÜLLMANN and RAVENS (1973) in papillary muscle exposed to 1.0 μM digoxin. It is of further interest that FRICKE et al. (1975) showed that the time courses of the positive inotropic action of ouabain, digoxin, and digitoxin in isolated rat heart are extremely short, in the range of 48–54 ss. Correspondingly, in this species, the time course of cardiac accumulation is also very fast with all three cardiac glycosides and varies between 2 and 4 min. These studies, then, provide tentative evidence that the time dependence of the onset of positive inotropic effect of a cardiac glycoside may be related to a time-consuming process for binding and/or translocation of this agent to subcellular components.

Since in these studies the pellet fraction of guinea-pig heart has been prepared by sedimentation of whole homogenates by the use of sucrose solution of single density (0.33 M), this fraction is bound to contain a heterogenous mixture of various cell components of different cytologic origin. In order to identify further the subcellular site or sites with which cardiac glycosides enter into a specialized relationship for their translocation and/or ultimate interactions for the manifestation of positive inotropic effect, DUTTA et al. (1968 a) subjected the pellet fraction to differential centrifugation to prepare nuclear, mitochondrial, and microsomal fractions and obtained evidence that the microsomal fraction contained most of the digoxin present in the pellet fraction while the mitochondrial and nuclear content of digoxin was significantly lower. A similar observation was made about the same time by GERBER et al. (1968) by exposing the isolated guinea-pig heart to ^{3}H-digitoxin and observing preferential localization of this agent to the light particulate fraction. Thus, these studies lead to the conclusion that within the microsomal or light particulate fraction of heart tissue there are primary and secondary binding sites for the pharmacologic effect of cardiac glycosides.

IV. Characteristics of Microsomal Cardiac Glycoside-Binding Sites

The nature of microsomal elements that bind cardiac glycosides have been vigorously investigated by various workers in isolated heart. In order to test the hypothesis regarding a correlation between uptake and pharmacologic effect, this work concerned itself with: (1) the structure–activity relationship of microsomal binding, comparing pharmacologically inactive glycosides with the potent ones; (2) studies to determine the kinetic parameters of microsomal binding in relation to pharmacologic effect; (3) species variations, comparing cardiac glycoside sensitive animals with digitalis-insensitive species; and (4) the effect of ions such as potassium and other cardiac glycoside-antagonizing agents.

1. Microsomal Content in Relation to Pharmacologic Effect

One important indication of the pharmacologic significance of the microsomal fraction as the site of positive inotropic effects becomes evident in the experiments conducted by various investigators (DUTTA et al., 1968 a, b; GERBER et al., 1968;

Table 7. Concentration of various cardiac glycosides in the subcellular fraction (pmol/mg protein) of guinea-pig heart

Drug	Nuclei	Mitochondria	Microsomes	Reference
Proscillaridin	4.38±0.37	6.61±0.39	13.50±1.35	DUTTA et al. (1968b)
			13.90±1.54	DUTTA and MARKS (1972)
Digitoxin	1.72±0.15	1.86±0.16	2.97±0.35	DUTTA et al. (1968b)
			5.05±0.69	DUTTA and MARKS (1972)
Strophanthoside K	1.71±0.15	0.82±0.05	2.75±0.275	MARZO and GHIRARDI (1974)
Deslanatoside	0.84±0.2	0.53±0.15	2.86±1.00	MARZO et al. (1976)
Convallatoxol	0.93±0.13	1.04±0.18	1.72±0.17	DUTTA et al. (1968b)
Ouabain	0.84±0.04	0.94±0.09	2.20±0.12	DUTTA et al. (1968b)
	1.21±0.11	1.18±0.15	6.46±0.90	FRICKE (1978)
Dihydroouabain	0.05±0.01	0.05±0.01	0.11±0.02	DUTTA et al. (1968b)
Digoxin	0.78±0.08	0.94±0.12	1.79±0.28	DUTTA et al. (1968b)
	3.64±0.52	3.39±0.17	7.84±0.29	KIM et al. (1972)
			2.51±0.08[a]	KIM et al. (1972)
			1.49±0.1	DUTTA and MARKS (1972)

[a] Loosely bound

FRICKE et al., 1969; KIM et al., 1972; MARZO and GHIRARDI, 1974; MARZO et al., 1976) on isolated guinea-pig heart. In these studies, following perfusion of the heart with a number of different cardiac glycosides and subsequent preparation of subcellular fractions, it was noted that without exception the microsomal fraction contained the highest concentration of these agents (Table 7) while concentrations in the mitochondrial and nuclear fractions were significantly lower. Furthermore, by measuring the force of contraction in relation to accumulation of ^{3} *H*-digoxin, KIM et al. (1972) were able to demonstrate a significant correlation ($\gamma = 0.84$) between the loosely bound digoxin fraction of the microsomes and the positive inotropic effect. Although it is not clear to what component of the microsomal fraction digoxin binds so loosely in order to cause a positive inotropic effect, it is interesting that the amount of digoxin released by shaking and allowing to stand in the cold (KIM et al., 1972) seems to compare well with the amount of digoxin and other polar glycosides such as ouabain, strophanthoside K, which are retained specifically at the microsomal level (DUTTA et al., 1968b).

Another indication of the pharmacologic significance of microsomal binding comes from the observation that an approximately 20-fold difference exists between the microsomal concentrations of ouabain and its relatively inactive derivative, dihydroouabain (DUTTA et al., 1968b). At the same time, however, these studies have also revealed that among the active cardiac glycosides which show a similar positive inotropic dose–response relationship such as digoxin and proscillaridin, there is a wide difference in the microsomal concentrations of these agents. This difference between polar cardiac glycosides such as ouabain and digoxin on the one hand, and lipid-soluble agents such as digitoxin and proscillaridin on the other, arises primarily because the latter glycosides, in addition to their binding to the specific sites, possess high affinity to bind nonspecifically to various membraneous proteins. However, among structurally similar cardiac glycosides of approximately equal lipid solubility (ZAVECZ, 1974), there appears to be partial agree-

Table 8. Comparison of inotropic effect and microsomal content of cardiac glycosides

Digitaloids concentration (1×10^{-7} M)	(+) Inotropy at 64 min (% of control)	Microsomal binding (pmol/mg protein)
Digitoxigenin-bis-digitoxoside	41	1.4±0.2
Digitoxigenin-mono-digitoxoside	38	1.5±0.2
Digitoxigenin	14	1.4±0.1
Digoxigenin-bis-digitoxoside	26	1.6±0.1
Digoxigenin-mono-digitoxoside	59	4.1±0.3
Digoxigenin	54	2.4±0.1

Adapted from DUTTA et al., 1976; STEPHEN et al., 1976

ment between the microsomal content of these cardiac glycosides and positive inotropic effect (STEPHEN et al., 1976; DUTTA et al., 1976). For example, as shown in Table 8, the results of the microsomal content of digoxigenin and digitoxigenin and their respective bis- and mono-glycosides in relation to the steady-state positive inotropic effects demonstrate that the highest microsomal concentration is seen with digoxigenin-mono-digitoxoside which is the most potent positive inotropic agent in this series. The next highest microsomal content is seen with digoxigenin which is second in the order of inotropic magnitude. However, the microsomal content for the rest of the cardiac glycosides is very similar and ranges between 1.4 and 1.6 even though they demonstrate wide differences in positive inotropic response. It is possible that this lack of agreement between microsomal content and positive inotropic effects may have been caused by the differences in the removal of these agents during 8 min washout of the extracellular space.

2. General Kinetic Considerations

Analyses of microsomal binding sites, particularly in relation to the possible designation of one of these sites as a cardiac glycoside receptor, have been conducted mainly by studying the saturability of the microsome prepared from isolated guinea-pig heart. In order to eliminate as far as possible the involvement of non-specific binding, these studies primarily utilized the relatively polar cardiac glycoside, ouabain. Having exposed isolated guinea-pig heart to various concentrations of ouabain for 64 min, followed by 8 min washout, DUTTA and MARKS (1969) observed that, over the concentration range tested, which included concentrations producing responses varying from threshold inotropic effect to toxicity, the ouabain-binding capacity of the microsomal fraction became saturable. The double reciprocal plot of these data (Fig. 4) is approximately linear. From the intercept on the ordinate and on the negative abcissa, the microsomal ouabain binding shows a calculated maximum of 16 pmol/mg protein and a dissociation constant (K_D) of 0.6 μM. For comparison, the data for ^3H-ouabain binding in the mitochondrial fraction is also shown in Fig. 4 and makes it clear that because of coprecipitation of microsomal membraneous elements, the maximum binding capacity for mitochondria was less than half (40%) that of the microsomes, while the K_D of the mitochondrial site is the same as that of microsomal binding. It is interesting to note that the K_D value obtained in the microsomal fraction prepared from

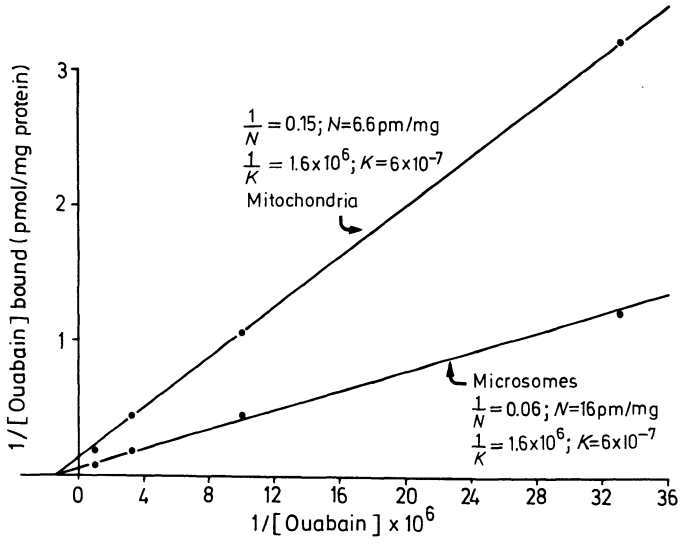

Fig. 4. Double reciprocal plot of ouabain concentration (pmol/mg protein) for guinea-pig heart microsomal and mitochondrial fractions, prepared from heart perfused with ouabain at various concentrations. *K*, Concentration of half-maximal binding; similar to K_D as used in Table 2. *N*, Maximum number of binding sites

ouabain-perfused guinea-pig heart compares very well with the similar value obtained in papillary muscle by LÜLLMANN et al. (1975) as reported earlier (Table 2), even though the K_D obtained in the guinea-pig microsomal fraction from perfused heart excluded any extracellular ouabain.

3. Species Differences

With respect to the microsomal binding of perfused heart, it has also been learned that different species show considerable differences in their ability to bind cardiac glycosides in the microsomal fraction. Importantly, this difference in the microsomal content parallels their respective species susceptibility in demonstrating a positive inotropic effect. Thus, after 64 min perfusion of isolated rat and guinea-pig heart with similar concentrations of digoxin, DUTTA et al. (1968 a) demonstrated substantial differences in the microsomal concentration of digoxin in these two species, well known for their differences in digitalis sensitivity (Table 9). Similarly, the literature also shows significant differences in the microsomal ouabain content obtained from isolated heart under similar protocols between guinea-pig and cat (KAWAGISHI, 1971; FUJINO et al., 1971) and between dog and cat (SCHWARTZ et al., 1974; ALLEN et al., 1975) in line with their respective species differences in sensitivity.

Although it is difficult to relate the microsomal ouabain content to specific membraneous fragments, the results obtained by SCHWARTZ and co-workers appear to indicate higher localization of ^3H-ouabain to fragments containing Na^+, K^+-ATPase activity, and to a lesser but significant extent, to sarcoplasmic reticular membraneous elements. Japanese workers (KAWAGISHI, 1971; FUJINO et al., 1971),

Table 9. Species differences in microsomal uptake of cardiac glycoside following perfusion of isolated heart

Species	Cardiac glycoside	Perfusate concentration (nmol/ml)	Duration of perfusion (min)	Microsomal content (pmol/mg protein)	Reference
Rat	Digoxin	0.1	64	0.41	DUTTA et al. (1968a)
Guinea-pig	Digoxin	0.1	64	1.69	DUTTA et al. (1968a)
Cat	Ouabain	0.1	30	2.56	KAWAGISHI (1971)
	Ouabain	0.1	30	5.71[b]	FUJINO et al. (1971)
Dog	Ouabain	0.25	15	16.10[a]	SCHWARTZ et al. (1974)
	Ouabain	0.50	Variable	32[a] 12[b]	ALLEN et al. (1975)

[a] Membraneous fraction shown to have NaK, ATPase activity
[b] Supposedly mostly sarcoplasmic reticular membraneous fraction with no Na^+, K^+-ATPase activity

on the other hand, seem to have found evidence that ^3H-ouabain localizes specifically with sarcoplasmic reticular fragments isolated from ouabain-treated guinea-pig heart and shows, in contrast, no particular affinity for Na^+, K^+-ATPase-containing membraneous fragments.

4. Agents that Reduce the Microsomal Content of Cardiac Glycosides

Both clinical and experimental studies have provided evidence that changes in sodium, potassium, or calcium concentrations in the external medium influence the positive inotropic or toxic effects of cardiac glycosides. These studies led to various investigations to determine the effects of Na^+, K^+, and Ca^{2+} respectively on the microsomal binding of cardiac glycosides by isolated guinea-pig heart. Thus, DUTTA and MARKS (1969, 1972) first observed the uptake of ouabain, digoxin, digitoxin, and proscillaridin by isolated guinea-pig heart to be directly related to sodium when the concentration of this ion was varied over a very wide range in the perfusing medium. Additionally, it was observed that the uptake of these four cardiac glycosides was inversely related to the potassium concentration in the perfusate (DUTTA and MARKS, 1969, 1972; BASKIN et al., 1973). Comparison of microsomal content among these four cardiac glycosides also revealed that these effects of sodium and potassium ions were relatively less marked with nonpolar glycosides such as digitoxin and proscillaridin in comparison with ouabain and digoxin. From a recent study (HALL et al., 1977) on the effect of hypokalemia produced by glucose and insulin infusion of anesthetized dogs, it was also learned that hypokalemia enhanced the rate of microsomal digoxin uptake which paralleled the time course by which hypokalemic dogs demonstrated toxicity. However, the left ventricular microsomal digoxin concentration did not differ significantly between normokalemic and hypokalemic animals at toxicity, whereas there was statistically significant inhibition of Na^+, K^+-ATPase in the hypokalemic groups than the normal animals, indicating localization of digoxin to microsomal sites other than Na^+, K^+-ATPase.

The absence of calcium from the perfusion medium seemed to reduce the digoxin and digitoxin content of the cardiac microsomal fraction of guinea-pig heart to approximately half the control value. The absence of magnesium, on the other hand, had no significant effect on either glycoside (DUTTA and MARKS, 1972). In regard to the effect of divalent ions, it is important to point out that FRICKE and KLAUS (1975) observed, in contrast to the study of DUTTA and MARKS (1972), a marked reduction of the myocardial uptake of both digoxin and digitoxin with an increase in the calcium concentrations of the perfusate that varied from 0.45 to 7.2 m*M*. Although superficially these studies may indicate a certain influence of extracellular calcium in the regulation of cardiac glycoside concentration at the microsomal binding sites, such an inference must be drawn with caution, particularly in view of the fact that the absolute increase in positive inotropic activity was noted to be different by the previously mentioned digitaloids in the presence of changing concentrations of calcium (FRICKE and KLAUS, 1975).

Finally, KIM et al. (1972), after critical examination of the effect of drugs such as aldosterone and chloropromazine, were able to demonstrate that both drugs decreased the positive inotropic effect of ^3H-digoxin and reduced, in parallel, the microsomal content of this glycoside. This study thus indicated an important relationship between the microsomal binding of cardiac glycoside and the positive inotropic effect. Similarly, in the context of cardiac dysrhythmia, attempts have been made to test the hypothesis that drugs that combat digitalis-induced cardiotoxicity may do so by reducing the concentration of digitalis at the microsomal binding site. BASKIN et al. (1973) and ERDMANN (1977) provided tentative support for this line of reasoning in that phenytoin was found to reduce the microsomal content of ouabain in parallel to its therapeutic effect against ouabain-induced dysrhythmia. However, GODFRAIND et al. (1971), as well as BINNION and DAS GUPTA (1975), were unable to demonstrate decreased accumulation of cardiac glycosides by isolated guinea-pig atria and intact dog heart respectively, during the suppressant action of diphenylhydantoin against ouabain-induced dysrhythmia. Thus, it appears that some workers who have studied the antidysrhythmic effects of diphenylhydantoin in relation to cardiac uptake of cardiac glycoside seem to find no appreciable effect of this agent on the uptake by intact cardiac tissue, though it is not known from these studies (GODFRAIND et al., 1971; BINNION and DAS GUPTA, 1975) if diphenylhydantoin affects, by contrast to the cardiac uptake, cardiac glycoside binding only at the microsomal level.

E. Binding of Cardiac Glycosides to Fragmented Cardiac Membranes

Ever since REPKE and PORTIUS (1963) introduced the classic hypothesis proposing Na^+, K^+-ATPase as "the long-sought molecular point of attack of cardiac glycosides, i.e., the digitalis receptor" (REPKE et al., 1974), there has been an unending search for the characterization of this receptor at the subcellular level. Initially, the research on the characterization of the digitalis receptor was conducted mainly by measuring kinetically the sensitivities of Na^+, K^+-ATPase to digitalis in the presence of various ions (MATSUI and SCHWARTZ, 1968). With the availability of highly specific active tritium-labeled cardiac glycosides, it became possible in the late

1960s to develop an in vitro cardiac glycoside-binding system and to identify by direct radioligand binding the major site or sites within the isolated membraneous fraction.

The first outstanding contribution in this line of endeavor came from SCHWARTZ's laboratory (MATSUI and SCHWARTZ, 1968) in the demonstration that a partially purified Na^+, K^+-ATPase fraction of beef heart was able to bind digoxin when the incubation medium contained certain ligands such as ATP, Mg^{2+} or Pi, Mg^{2+}. Furthermore, it was also observed that: (1) the in vitro binding process was saturable with a dissociation constant (K_D), well within the dose range that was required to produce threshold positive inotropic effect and cardiotoxicity; (2) a tentative relationship existed between the inhibition of the Na^+, K^+-ATPase and the amount of digoxin bound by this fraction; and (3) potassium inhibited the binding. At the same time the study of DUTTA et al. (1968 b) with freshly isolated beef sarcoplasmic reticular fraction (SRF) showed that of the six cardiac glycosides studied in vitro in the presence of either ATP, Mg^{2+}, Na^+ or ATP, Mg^{2+}, the magnitude of binding of the cardiac glycosides was similar for ouabain, dihydroouabain, digoxin, and convallatoxol, while the lipid-soluble glycosides, digitoxin, and proscillaridine showed distinctly greater binding. Since in this study particularly, ouabain and its relatively inactive derivative dihydroouabain, an agent known to have poor affinity for Na^+, K^+-ATPase (WILSON et al., 1970), bound identically with the SRF. This observation probably meant that the cardiac glycosides–SRF binding system must be of different characteristics from that of the Na^+, K^+-ATPase system. However, the fact that various studies on Ca^{2+} uptake and/or release have provided no evidence for the direct effect of cardiac glycosides on the SRF system, it has been generally assumed that the cardiac glycoside–SRF binding has no pharmacologic significance, and no further follow-up studies have been conducted on the SRF system.

On the other hand, there exist a good number of in-depth studies characterizing the binding properties of various cardiac glycosides with the cardiac membraneous fraction containing high Na^+, K^+-ATPase activity. Since these studies have been adequately reviewed (ERDMANN and SCHONER, 1974; SCHWARTZ et al., 1975; AKERA, 1977; see Vol. 56/I, Chaps. 14 and 15), it is not appropriate at this time to engage in lengthy discussion of the voluminous literature. Instead, in this section, an attempt is made to compare the dissociation constants (K_D) of cardiac glycoside–receptor complexes as worked out by the binding method with the equivalent constants worked out by pharmacologic and enzymatic studies.

Table 10 shows the K_D values of nine well-known cardiac glycosides as determined by the displacement of ^3H-ouabain from Na^+, K^+-ATPase-enriched membraneous fragments from beef heart (BOSSALLER and SCHMOLDT, 1979) compared with the IC_{50} values of beef as well as guinea-pig heart Na^+, K^+-ATPase activity. This table also shows the ED_{50} values of these agents as measured by the cumulative increase in inotropic effect of these agents as noted in guinea-pig papillary muscle. As pointed out by FLASCH and HEINZ (1978), such a comparison of the K_D, I_{50}, and ED_{50} values of these cardiac glycosides reveal that in both the digitoxin and digoxin series, aglycone-mono-digitoxosides show the highest activity in regard to binding, enzyme inhibition, and positive inotropic effect, followed by bis-digitoxosides, tri-digitoxosides, and aglycones. Thus, this parallelism among the

Table 10. Comparison of dissociation constants K_D with other biochemical and pharmacologic constants of nine cardiac glycosides

Cardiac glycoside	K_D (nM)[a]	I_{50} (nM) Beef heart[b]	I_{50} (nM) Guina-pig heart[c]	ED_{50} (nM) Guinea-pig papillary muscle[d]
Digitoxigenin-mono-digitoxoside	0.64±0.08	4.85±0.15	140	49
Digitoxigenin-bis-digitoxoside	0.98±0.04	6.79±0.31	170	41
Digitoxin	1.43±0.06	7.73±0.46	220	130
Digoxigenin-mono-digitoxoside	2.87±0.31	8.06±0.48	640	120
Digoxigenin-bis-digitoxoside	3.16±0.14	8.19±0.57	480	300
Ouabain	3.50±0.17	9.70±0.21	1,060	150
Digoxin	5.30±0.19	10.0 ±0.13	600	100
Digitoxigenin	6.14±0.51	65.0 ±11	1,750	620
Digoxigenin	41.0 ±4.51	406.0 ±75	10,170	5,600

[a] K_D determined by displacement of ^3H-Ouabain from beef heart membraneous fraction; BOSSALLER and SCHMOLDT (1979)
[b] (BOSSALLER and SCHMOLDT, 1979)
[c] (FLASCH and HEINZ, 1978)
[d] (FLASCH and HEINZ, 1978)

order of the K_D, I_{50}, and ED_{50} values of these cardiac glycosides results, as observed by FLASCH and HEINZ (1978), in a very high correlation coefficient (0.92–0.99) among these constants, and indicates a cause and effect relationship between the cardiac glycoside binding and the resultant Na^+, K^+-ATPase inhibition which in turn leads to a positive inotropic effect. However, the existence of such a cascade for cardiac glycoside-induced positive inotropy remains questionable since for a given cardiac glycoside there exists a wide disagreement among the K_D, I_{50}, and ED_{50} values. It is noted that the I_{50} values for digitoxin-mono-digitoxoside and digitoxigenin are approximately one order of magnitude higher than the corresponding K_D values. A similar difference between the I_{50} and K_D values has been observed with ouabain by other workers (ERDMANN and SCHONER, 1973b), although in the study of BOSSALLER and SCHMOLDT (1979) the difference between the K_D and I_{50} values of ouabain is quite small. Recently, THOMAS et al. (1979) have observed a nearly 50-fold difference between the I_{50} and ED_{50} values of digitoxigenin in the cat. According to them, the differences between these constants may reflect differences in factors involved in drug distribution between the intact tissue and the isolated enzyme preparation or this could mean "that the ‚contractility' receptor is distinct from the receptor which binds digitalis to Na^+, K^+-ATPase."

More to the point, the recent studies of SHARMA and BANERJEE (1978) show a three-fold decrease in the K_D value in the rat following thyroidectomy, without any alteration in the Na^+, K^+-ATPase activity of the cardiac membranes. These workers (SHARMA and BANERJEE, 1977) also showed that the selective destruction of sympathetic nerve endings alters considerably the specific ouabain binding to the cat heart membraneous fragments. It appears from the latter study that more than

80% of the total number of ouabain-binding sites in the cat heart are lost owing to sympathectomy and the remaining 20% of the total binding sites, supposedly associated with the contractile cells, show very high affinity for ouabain binding. These findings, along with the recent observations of FRICKE and KLAUS 1977, 1978) and WELLSMITH et al. (1979), are consistent with the idea that in the cardiac membraneous fragments there exist two or more specific binding sites for cardiac glycosides of different affinity.

F. Summary

Applying the radioligand binding procedure in superperfused tissue preparations, isolated perfused heart, and isolated fragmented membraneous particulates, attempts have been made to identify the mechanism by which cardiac glycosides are taken up by cardiac cells from the extracellular space and the molecular site or sites with which they interact in order to initiate their pharmacologic effect. These studies have revealed, as predicted by GOLDSTEIN (1949) in his classic review, that cardiac glycosides do "enter into definite specialized relationship with particular tissue proteins." Furthermore, from each of these systems, much has been learned about the rate at which cardiac glycosides associate with their putative receptor, and the rate of dissociation of cardiac glycosides from their binding sites. Most importantly, these studies have also provided significant information regarding the maximal number of binding sites for cardiac glycosides that are present in cardiac tissue and their respective affinity for various cardiac glycosides. Because of the availability of these kinetic data with regard to cardiac glycoside binding to the heart, it is now possible to characterize the digitalis receptor with some objectivity. It has become evident that relative to the in vitro system, the affinity values (K_D), as obtained from superperfused atrial preparation for the polar cardiac glycoside, ouabain, correlate quite well with the concentration of ouabain required to produce half-maximal contraction of this tissue (ED_{50}), there being only a two-fold difference between these two parameters. Consideration of various kinetic data, as obtained in vitro, makes it apparent that there are possibly two classes of specific receptors for cardiac glycosides in the incubation mixture. Because of this complexity, and in contrast to the identification of the adrenergic receptor in the cardiac membraneous fraction (ALEXANDER et al., 1975), the task of identifying the digitalis receptor by the radioligand procedure in vitro will be much more difficult.

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CHAPTER 8

Bioavailability of Cardiac Glycosides

T. R. D. SHAW

A. General Aspects

The concept of bioavailability of drugs was developed by pharmacists. They introduced it to reflect the fact that the way in which a drug is formulated can determine its absorption and therefore the onset and magnitude of its action. To define a drug formulation's bioavailability is to define both the rate and the extent of that drug's entry into the circulation and tissues of the body from the site of administration. In brief, it describes how fast and how fully the drug gets to the tissues. Bioavailability is determined by the drug's chemical characteristics, by the method of formulation and also by physiological factors acting on the administration route. It can be calculated from blood levels of the drug or from the amounts excreted in the urine. The techniques are described below.

The term "bioavailability" (also sometimes called "biological availability," "physiological availability" or "systemic availability") is not synonymous with intestinal absorption. A drug might be completely absorbed from the gut but be metabolised in the gut mucosa or liver before it reaches the circulation – the first-pass effect. Also, availability to the systemic circulation can be applied to other modes of administration, such as intramuscular injection.

Since the amount of drug in the tissues determines the response, bioavailability is of relevance for every medicine. However, the importance of any given degree of variation in bioavailability depends on what type of drug is involved. For some, such as antibiotics, small differences in absorption are not of therapeutic importance. For those like cardiac glycosides, which have a narrow toxic : therapeutic ratio, even small variations would be of clinical significance. The wider aspects of bioavailability have been reviewed by WAGNER (1971), BRODIE and HELLER (1972), and KOCH-WESER (1974).

The same drug is often formulated by different manufacturers, each with its own production technique. Every company makes its product, not continuously, but in a series of separate batches. To ensure equivalent and consistent bioavailability the pharmacopoeiae, until recently, relied on the disintegration test to ensure adequate release of a drug after ingestion. This test sets time limits in which tablets must break up into fragments small enough to pass through a specified size of wire mesh. The first official disintegration test was included in the Pharmacopoeia Helvetica in 1934 and was introduced to the United States Pharmacopeia in 1950. However OSER et al. (1945), in a study which has been taken to be the origin of bioavailability measurement, showed that the urinary excretion of vitamins did not always correlate with such disintegration tests. LEVY and NELSON (1961) con-

cluded that if a drug was poorly soluble in the gastrointestinal fluids then fragments passing through the mesh could still have a surface area which was insufficient to allow rapid enough release of the drug for absorption to occur within the small intestine. They pointed out that this would be of particular importance if the drug also had low lipid solubility which would further retard its absorption across the intestinal mucosa. At this time there was insufficient evidence that these considerations were of practical therapeutic importance. The discovery of the bioavailability problem of digoxin demonstrated the validity of these theories and was crucial in the acceptance of the need for a test of tablet dissolution rate for some medicines.

B. Methods of Measurement

An estimate of bioavailability can be made by observation of the drug response when this can be detected accurately. This was employed to measure the absorption of cardiac glycosides using the effect on ventricular rate in atrial fibrillation (WAYNE, 1933; GOLD et al., 1953) and systolic time intervals (WEISSLER et al., 1966). These experiments showed that oral doses of digoxin and digitoxin could be well absorbed. However for accurate quantification an assay is needed which is able to measure the drug concentration in the body fluids after therapeutic doses. A variety of assays for cardiac glycosides became available in the late 1960s (BUTLER, 1972). The radioimmunoassay and the ^{86}Rb assay had suitable sensitivity, accuracy and simplicity for bioavailability studies, in which many samples have to be analysed, and unlike radioisotopes they could be used in conjunction with the normal pharmaceutical formulations of the glycosides. They were the key which opened up the digitalis bioavailability problem.

The use of blood and urine levels to measure drug absorption into the whole body is based on mathematical models. In these the body tissues are simplified into a small number of compartments. In the two-compartment model the blood is part of the central compartment into which all absorption occurs and from which all elimination takes place. Diffusion occurs bidirectionally between the central compartment and a peripheral compartment representing the tissues which are less highly perfused but which may have a large drug-binding capacity. From the mathematical equations which describe a drug's absorption and excretion in this type of model, simpler indices of blood and urine drug levels can be derived to measure bioavailability after either single or multiple doses. The compartmental theory and bioavailability equations have been clearly described by NOTARI (1975), WAGNER (1975), GREENBLATT and KOCH-WESER (1975), GREENBLATT et al., (1976) and AZARNOFF and HUFFMAN (1976).

For single doses the area under the time plot of plasma concentration, when extrapolated to infinity, is proportional to the amount absorbed. The total cumulative amount excreted in the urine is also a direct index of percentage absorbed. The rate of absorption is reflected in the time to peak blood level: more precise figures for rate of absorption can be obtained from computer analysis (WAGNER, 1975). With repeated dosing, blood levels eventually reach a steady state at which drug input balances the drug elimination during the dose interval. The rise to the

steady state is exponential and the time to reach it depends only on the elimination rate, being 94% complete after four half-lives. At steady state, the area under the plasma level curve, and the urinary excretion during the interval between doses are all directly proportional to the amount of drug absorbed; the predose plasma level is a simple estimate of the amount absorbed, although it can be altered by very prolonged absorption.

The *relative* bioavailability of a formulation is defined by comparison with a standard liquid preparation of the drug given orally. The *absolute* bioavailability is defined by comparison with an intravenous injection. Whether relative or absolute bioavailability is needed depends on the objectives of the study. These indices of bioavailability apply to all drugs which have first-order pharmacokinetics, but the close scrutiny which has been given to the bioavailability of the cardiac glycosides has highlighted some practical problems.

There is no single "gold standard" oral formulation with which to make comparisons of relative bioavailability. The volume and type of solvent and the position and feeding of the subject can influence the rate of absorption; the nature of the solution and the circumstances of administration should always be stated (American Pharmaceutical Association, 1972). Even with intravenous doses, the speed of infusion has been shown to affect the measurement of bioavailability. Infusion over 1 h gives a higher cumulative urinary excretion of digoxin than a bolus injection (Greenblatt et al., 1974a) and a 3-h infusion produces more excretion than a 1-h infusion (Marcus et al., 1976). It has been suggested that this apparent anomaly is due to a change in digoxin metabolism with different infusion rates (Stoll and Wagner, 1975). It would seem best therefore to use an infusion rate which approximates the rate of oral absorption.

Single-dose studies have been extensively used in the investigation of digoxin bioavailability, but there is some controversy about the accuracy of this method. In theory the blood level measurements should be extrapolated to infinity for a precise measure of the absorption. Truncated curves give a bias in favour of the more rapidly absorbed preparation (Sorby and Tozer, 1973; Sanchez et al., 1973; Beveridge et al., 1975; Keller and Rietbrock, 1977). However, the elimination half-life of digoxin is about 1.5 days and blood levels are low compared with tissue levels. A prolonged absorption curve can be difficult to obtain since later plasma levels are below the sensitivity of the unmodified digoxin radioimmunoassay. Many studies of digoxin bioavailability have had plasma level curves limited to 8–24 h. In practice these have been useful to detect differences in digoxin bioavailability, although often overestimating the magnitude of difference. Kramer and Reuning (1978) calculated that 24-h and 72-h curves underestimated the bioavailability of 0.5 mg doses of FDA reference digoxin tablets by 26% and 9% respectively. Wagner and Ayres (1977) recommend that when blood level curves are used to quantify the extent of absorption they should be prolonged to include four equally spaced blood levels in the log-linear elimination phase and calculations be made to extrapolate to infinity.

In the urine the digoxin concentration is higher and collection time is not restricted by assay sensitivity, but completeness of collection over many days requires very conscientious subjects. Beveridge et al. (1975) found that as urinary digoxin collection periods increased from 4 to 8, 24, 48, and 72 h the correlation

with cumulative excretion as extrapolated to infinity became progressively better. Good agreement has been found between 24-h (GREENBLATT et al., 1974b) and 48-h (HUFFMAN et al., 1974) periods and the 6-day cumulative excretion, but no consensus has been reached regarding the shortest acceptable time for urine collection.

Steady-state measurements in blood and urine avoid the problem of extrapolation to infinity but involve greater exposure of the subject to the drug effect and require strict compliance to the doses. Pharmacokinetically they resemble more closely the clinical use of the drug (LEVY, 1974) and will take account of slowly equilibrating tissues which have been shown to exist for digoxin (RIETBROCK and KUHLMANN, 1977). PREIBISZ et al. (1974) found that differences in the bioavailability of digoxin tablet brands at steady state conditions were consistently less than estimates made from single doses in the same subject. LLOYD et al. (1978) also recorded smaller differences at steady state but 24-h measurements of blood and urinary digoxin levels were good predictors of the presence or absence of a difference in absorption. Steady-state measurements appear to be the most accurate and convincing way to obtain the relative bioavailability of cardiac glycoside preparations. Single doses are useful to predict any difference in bioavailability. They have been the preferred way to establish absolute bioavailability with an intravenous dose; multiple intravenous doses are difficult to give but have been used in an increasing number of studies. The more recent experiments on glycoside bioavailability have tended to include both steady-state comparisons of oral formulations and single-dose studies which incorporate an intravenous infusion.

C. Digoxin Tablets

The bioavailability of digoxin tablets suddenly came into prominence in 1971. Following their clinical observation that some patients did not give the expected response to digitalisation, LINDENBAUM et al. (1971) in New York studied three brands of digoxin tablets and showed very marked differences in serum digoxin levels during 5 h after administration. MANNINEN et al. (1971) noted a 44% change in the steady-state serum digoxin levels with two brands of tablet marketed in Finland. In the United Kingdom, it was found that fine-grinding of some digoxin tablets could considerably increase their absorption (SHAW et al., 1972).

Subsequently steady-state studies and also single-dose studies which included urinary excretion data for 24 h or more demonstrated that: (a) many commercially available brands of digoxin tablets, which met *USP* standards of content and disintegration, had a much lower rate and extent of absorption than a digoxin solution; (b) there were considerable differences between brands in terms of their rate and extent of absorption; (c) batches of tablets from the same manufacturer could show wide variation in bioavailability (HUFFMAN and AZARNOFF, 1972; LINDENBAUM, 1973, 1975; LINDENBAUM et al., 1973a, b; JOHNSON et al., 1973a, b; GREENBLATT et al., 1973; WAGNER et al., 1973; SHAW et al., 1973, 1974a; PREIBISZ et al., 1974; FLECKENSTEIN et al., 1974; IISALO and RUIKKA, 1974; REISSELL et al., 1974, 1977; KARJALAINEN et al., 1974; HUFFMAN et al., 1975; NYBERG et al., 1977; LLOYD et al., 1978). These differences in bioavailability were supported by a number of

other studies based on 4–8 h plasma concentration curves after single doses. In the steady-state experiments, brands of tablets in clinical use were shown to have a full spectrum of bioavailability with tablets of poorest bioavailability giving digoxin levels which were half or less those obtained with brands of highest bioavailability.

One would anticipate that with differences of this degree a change of tablet brand could provoke digoxin toxicity during maintenance therapy. In general it appears that underdigitalisation with tablets of low bioavailability was a commoner problem since physicians tended to restrict doses to the traditional levels which had been established with well-absorbed preparations (SHAW, 1974). Those investigating digoxin tablet absorption tried to ensure that they did not cause toxicity, but instances were observed when toxicity did result from bioavailability changes. In Scandanavia, a move from low to high bioavailability tablets at the same mean dosage of 0.27 mg/day led to toxicity in 7% of patients and a suggestion of improved digitalis effect in about twice that numer (REDFORS et al., 1973). An outbreak of digoxin toxicity was noted at an Israeli hospital when a local pharmaceutical supplier increased the bioavailability of his tablets without notice (DANON et al., 1977). Individual cases seen in clinical practice were reported (SHAW, 1974). The differences in blood levels were also found to parallel changes in the control of atrial fibrillation (REDFORS et al., 1973; SHAW et al., 1973) and in systolic time intervals (FLECKENSTEIN et al., 1974).

The variation in brand bioavailability has been found to correlate with the rate at which the digoxin went into solution when the tablets were immersed in water or hydrochloric acid in vitro. By 1975, 21 studies had shown a relationship between in vitro dissolution rate and digoxin bioavailability (GREENBLATT et al., 1976). There was found to be a strong correlation between steady state digoxin levels and dissolution rate (LINDENBAUM et al., 1973 b; JOHNSON et al., 1973 a; SHAW et al., 1973; PREIBISZ et al., 1974). There are a few reports in which bioavailability appeared not to have the usual relationship with dissolution rate. KLINK et al. (1974) concluded that two digoxin brands, one slow dissolving and one very slow dissolving (rate calculated to be 8% in solution at 1 h), were as well absorbed over 48 h as an elixir. YLITALO et al. (1975) noted similar steady-state levels with a 58% 1-h tablet and a solution. One brand studied by REISSELL et al. (1977) appeared to have a fast dissolution rate and poor absorption. However the great weight of published and unpublished evidence pointed to a useful correlation between bioavailability and dissolution rate and to the in vitro tests being able to ensure batch-to-batch consistency.

The main factor underlying the variations in dissolution rate and absorption appears to have been the size of the particles of digoxin in the tablets. Preparations with digoxin of small particle size give much better absorption than similar preparations with a large size of particle. (SHAW and CARLESS, 1974; JOUNELA et al., 1975; BEVERIDGE et al., 1975; JOHNSON et al., 1978 a). The three pure digoxin powders used by many European digoxin tablet manufacturers all had a relatively large particle size which gave poor absorption but when these powders underwent the tabletting process they were associated with different degrees of bioavailability up to maximum levels. It is likely that differences in the mixing of digoxin with the tablet excipients produced varying degrees of reduction of particle size. It is possible that, in addition, changes tock place in the amorphous/crystalline structure

characteristics of the particles (FLORENCE et al., 1974). Poor mixing during tabletting may also lead to unsatisfactory variation in drug content from tablet to tablet. Marked deviation from the nominal dose has been seen with some digoxin tablets (BANES, 1971; VAN OUDTSHOORN, 1972; MANNINEN and KORHONEN, 1973).

The magnitude of each country's digoxin bioavailability problem depended on the extent of use of digoxin as a cardiac glycoside and on the number of pharmaceutical companies involved. In the United Kingdom a particularly difficult situation existed. Over 20 brands of digoxin tablet were marketed and they showed a wide range of dissolution rates, with some brands even altering alarmingly from batch to batch (BECKETT and COWAN, 1973; FRASER et al., 1974). In addition the Lanoxin brand used by half of all patients on digoxin underwent a halving of its bioavailability throughout the period 1969–1972 owing to a minor transient alteration in manufacturing technique (JOHNSON et al., 1973 b; SHAW et al., 1974 a). Detection of the re-enhanced bioavailability of Lanoxin in 1972 coincided with distribution of the new formulation and as an interim measure pharmacists for a time were asked to dispense Lanoxin only when it had been prescribed by its brand name (Lancet, 1972). In the United States, Lanoxin is made by a separate process and did not undergo this fluctuation. However about 30 firms in the United States manufactured digoxin tablets and there was a wide band of dissolution rates, ranging from 3.8% to 93.6% in solution at 1 h (HARTER et al., 1974).

The discovery of the digoxin bioavailability problem produced a dilemma for the drug-regulating agencies since it emerged before there was data to support new pharmacopoeia standards. They came to adopt a three-part solution. (1) Manufacturers in the United Kingdom and United States had to submit information about the manufacturing technique and bioavailability of their brand. (Pharmaceutical Journal, 1973; Federal Register, 1974). (2) Dissolution rate tests were adopted and standards set by the national pharmacopoeiae (see Chap. 9). (3) Greater attention was paid to tablet content control. Criteria for digoxin tablet content were introduced to the British Pharmacopoeia in 1972.

However the response of the national agencies was not uniform. The minimum allowable dissolution rate became 90% at 1 h in Holland, 75% in the United Kingdom and 55% in the United States: the last was later increased to 65%. The U.S. Food and Drug Administration was anxious both to eliminate very poorly absorbed brands and to delay the introduction of very rapidly dissolving tablets until it was known whether or not the higher peak levels would induce toxicity. Accordingly they also set upper limits of dissolution rate of 90% at 15 min and 95% at 1 h (HARTER et al., 1974; HARTER, 1975). In addition they have required in vivo bioavailability data of each brand with the requirement that in 12 subjects the area under the digoxin plasma concentration curves for 0–5 h be at least 75% that of the mean of areas for a digoxin solution and a reference tablet (dissolution rate 75% in 1 h). KRAMER et al. (1977) have pointed out that intersubject variability is such that a formulation with 65% relative bioavailability would have a 10% probability of passing this 75% limit.

The studies which relate steady-state digoxin levels to dissolution rate would suggest that a modest amount of variation in bioavailability could still exist between tablets of dissolution rates between 65% and 90% h. Six-day urinary-excretion of digoxin was 24% higher with American Lanoxin tablets of dissolution rate

85%–90%/h than with the same make of tablets of dissolution rate 64% and 65% at 1 h (GREENBLATT et al., 1974c). The American Lanoxin tablet has been found to be significantly less well absorbed than a solution in the studies of GREENBLATT et al. (1973) – 55% compared with 65%; HUFFMAN et al. (1974) – 62% compared with 77%; and LLOYD et al. (1978) – 41% compared with 62%. However MARCUS et al. (1976) got equivalent bioavailability with solution and Lanoxin of rate 75%/h. The British Lanoxin tablet (98% dissolution at 60 min) was just as well absorbed as a solution (JOHNSON and LADER, 1974; MANNINEN et al., 1976a). Equal absorption was obtained from very rapidly dissolving formulations of different structure (JOHNSON and LADER, 1974; SHAW et al., 1974b). NYBERG (1977) has reviewed the data associating digoxin absorption with in vitro dissolution rate. He concluded that rates above 90% in 2 h did not increase bioavailability.

LEVY and GIBALDI (1974) have argued that American digoxin tablets should be made of equivalent bioavailability to solution since this would be within the capability of biopharmaceutical technology and would reduce inter- and intrapatient variation. The other view is that remaining differences in digoxin bioavailability are overshadowed by unpredictable individual variations in absorption capacity and clinical response (GREENBLATT et al., 1976). One uncontrolled study suggested that higher peak levels could induce transient arrhythmias (MANNINEN et al., 1976b) but this was not found by a number of other groups and is not the general clinical experience.

D. Other Digoxin Formulations

The bioavailability studies have confirmed that even a solution of digoxin does not give complete absorption of the dose. This had already been appreciated by the early clinical work and from the comparisons of oral and intravenous doses of radioisotopic digoxin (DOHERTY et al., 1961; DOHERTY and PERKINS, 1962). Digoxin in solution has been compared with intravenous doses in several studies. The methodology details have varied and a range of figures for the total percentage availability has resulted (HUFFMANN and AZARNOFF, 1972; HUFFMAN et al., 1974, 1975; WAGNER et al., 1973; WAGNER and AYRES, 1977; GREENBLATT et al., 1973; MARCUS et al., 1976; BINNION, 1976; LLOYD et al., 1978). The average value for these studies approximates 80%. This figure is a guide to the reduction in dosage needed when a patient has to receive intravenous digoxin therapy.

The incomplete absorption of digoxin has stimulated attempts to augment its bioavailability. Digoxin has been prepared dissolved in polyethylene glycol 400 (90%), ethanol (6%), propylene glycol (3%), and water (1%) and then encased in a soft gelatin capsule. Experiments which contrast the bioavailability of capsules and solution are shown in Table 1. The capsule formulation used by LLOYD et al. was produced by Arnar Stone Laboratories, the others by Burroughs Wellcome. Overall, there was improved, although not complete, absorption from the capsule. Digoxin capsules have also been compared with tablets. In steady-state cross-over experiments they had a bioavailability of 111% (JOHNSON et al., 1977) and 104% (RODGERS et al., 1977) relative to British Lanoxin, and 127% relative to American Lanoxin (LLOYD et al., 1978). O'GRADY et al. (1978) compared the administration

Table 1. Bioavailability of digoxin capsules compared with oral solution and/or intravenous infusion. Infusion is over 1 h except when stated

Reference (n = number of subjects)	Method of assessment	Relative bioavailability of capsule (%) (solution = 100%)		Absolute bioavailability of capsule (%) (intravenous = 100%)	
		AUC	CUE	AUC	CUE
Mallis et al. (1975) $n=10$	AUC 0– 6 h CUE 0– 1 days	132	114		
Johnson et al. (1976a) $n=7$ $n=12$ (45 i.v. infusion)	AUC 0–24 h CUE 0– 6 days CUE 0–10 days	120	112		97
Binnion (1976) $n=6$	AUC 0– 6 h CUE 0– 6 days	117	111	75	95
Marcus et al. (1976)	AUC 0– 6 h CUE 0– 6 days	112	117	75	99
(3-h infusion)	(CUE 0– 6 days)			(60)	(86)
Lindenbaum (1977) $n=12$	AUC 0– 6 h CUE 0– 6 days	118	114		
Lloyd et al. (1978) $n=12$	AUC 0–24 h CUE 0– 1 days Steady state: AUC during dose interval CUE during dose interval	104 (101)	101 (106)	79	63
Mean values[a]		117	112	76	89

AUC = area under curve plasma digoxin concentration–time measurements; CUE = cumulative urinary excretion of digoxin

[a] Values in parentheses excluded from calculation of mean values

of the polyethylene glycol solution with and without intact capsules and concluded that the higher absorption was due to the solvents rather than to the soft gelatin encapsulation. The principal aim of achieving greater absorption was to reduce intersubject variability. Johnson and Bye (1975) had previously found that individual subject absorption at single doses had a good correlation with their steady state levels. Their studies with capsules (Johnson et al., 1976a, 1977) suggested that intersubject variance was reduced but this has not been confirmed by the other investigators. Whether the increased cost of a capsule formulation would be justified therefore remains questionable. Other complex formulations to give ultrafast dissolution have been developed including digoxin/inert carrier coprecipitates, (Reddy et al., 1976) digoxin–hydroquinone complex (Bochner et al., 1976) and silica matrices (Flasch et al., 1978) but no clear advantage in absorption has been shown.

Intramuscular digoxin does not have the same bioavailability as an intravenous dose. Six-day urinary recovery after intramuscular injection was 83% that of the same intravenous dose in the single-dose comparisons of Greenblatt et al. (1973),

and the rate of rise of blood levels was no faster than with oral doses. Intramuscular injections of digoxin are painful and result in large areas of muscle necrosis at the site of injection (STEINESS et al., 1974), reflected by elevated serum creatinine phosphokinase values (GREENBLATT et al., 1973).

E. Other Cardiac Glycosides

I. Digitoxin

Unlike digoxin, reports on the bioavailability of digitoxin are few. However it is a more lipid-soluble drug and appears to be very well absorbed. The studies on clinical effect by GOLD et al. (1953), and WEISSLER et al. (1966) suggested that it was completely absorbed and the same conclusion was reached by BEERMANN et al. (1971) who measured urinary excretion of radioisotopic digitoxin up to 21 days after an oral dose (with polyethylene glycol marker) and an intravenous injection. STORSTEIN (1974) found similar plasma levels of digitoxin after intravenous and oral doses. Two brands of digitoxin tablet were assessed by STOLL et al. (1973). The dissolution rates were 95% at 15 min and approximately 65% at 1 h. They found no difference in the areas under the plasma level curves at 360 h and after extrapolation to infinity.

VOHRINGER et al. (1977) compared the blood and urine levels of digitoxin after 0.5 mg single doses given intravenously, as dragees, as oral solution and as tablets of dissolution rate 100% at 10 min. All the oral doses were well absorbed. When correction was made for the fact that an ampoule contains a slight excess volume in addition to the stated dose and volume (VOHRINGER et al., 1978), the absolute bioavailability of the dragee was between 92 and 98%. GREEFF et al. (1979) studied the absorption of digitoxin and digoxin tablets in the same subjects using intravenous doses of each glycoside to establish absolute bioavailability. The digitoxin tablet (100% dissolution at 20 min) gave complete absorption of its dose while the digoxin tablet (78% dissolution at 60 min) was 50% absorbed. The bioavailability of a range of commercial digitoxin tablets was said by WOOD et al. (1975) to show "a clear dependency" upon dissolution rate but no data were included in this communication.

A report by the U.S. Food and Drug Administration (FDA Drug Bulletin, 1976) stated that studies conducted for that agency had shown a bioavailability problem to exist with digitoxin. The brand-to-brand variation differences were much less than with digoxin, with the worst digitoxin brand having "absorption as low as 60%." In 1977 the FDA increased the dissolution test digitoxin standard which they provisionally set in 1974. Half of the American brands of digitoxin tablet had then to be reformulated.

II. Lanatoside C

Even a solution of lanatoside C is rather poorly absorbed, with about half the dose reaching the tissues (BEERMANN, 1972a). No effect on its absorption was encountered from antacid, anticholinergics or food (ALDOUS and THOMAS, 1977).

III. Methyldigoxin and Acetyldigoxin

Methyldigoxin is a semisynthetic derivative of digoxin which was developed in Germany and is marketed for clinical use. It has a methyl group attached to the terminal sugar of the digoxin molecule. This change gives it a water solubility more than 10 times that of digoxin (Schaumann unpublished, quoted in Hinderling et al., 1977) and it is also much more lipid soluble (Wirth et al., 1972). Initial studies indicated that it was a particularly well-absorbed preparation (Konig and Ohly, 1970; Storz, 1970; Larbig et al., 1971; Beermann, 1972 a, b). Its pharmacokinetics differ from those of digoxin with a greater degree of hepatic metabolism with conversion principally to digoxin, and there is a first-pass effect (Beermann, 1972 b; Rietbrock and Abshagen, 1973; Rietbrock et al., 1975; Hinderling et al., 1977). Methyldigoxin and digoxin have similar cardioactivity and similar binding to digoxin assay antibody and to plasma proteins.

In singe-dose studies of absolute bioavailability the systemic absorption of methyldigoxin was estimated to be 80% by Boerner et al. (1976) and 75% by Rietbrock et al. (1976). In steady-state studies of absolute bioavailability the former authors found almost identical blood levels after maintenance intravenous and oral doses, but in the latter study oral doses gave levels only 75% of those obtained by the intravenous route. Similar single-dose bioavailability was reported by Hinderling et al. (1977).

A comparative study with digoxin bioavailability must reflect its different pharmacokinetics. In a group of bioavailability studies using 7-day urinary collections and with intravenous doses of both digoxin and methyldigoxin as standards, Greeff et al. (1977) found only slightly greater absorption of methyldigoxin. Johnson et al. (1976 b) calculated the absolute bioavailability of methyldigoxin to be 87%. This was less than the absorption from digoxin capsules (97%) and greater than that from American Lanoxin tablets (75%). The two glycosides were each assessed by comparing the 10-day urinary excretion after an oral dose with the urinary excretion after an intravenous injection of the same glycoside. In the steady state methyldigoxin (0.4 mg/day) gave slightly higher levels than the digoxin tablets at a dose of 0.5 mg/day and methyldigoxin's intersubject variance was less. A preference for a digoxin or methyldigoxin formulation for clinical use must be based on the whole pharmacokinetic pattern rather than on absorption alone, since they differ in several respects.

Acetyldigoxin is another derivative of digoxin which is well absorbed. (Ruiz-Torres and Burmeister, 1972; Bodem et al., 1974). The absorption relative to intravenous doses was 68% for a solution (Klotz et al., 1976) and 70% for tablets (Greeff et al., 1977). The cross-over studies of Flasch (1975) suggested that tablets and solution of acetyldigoxin were better absorbed than digoxin preparations.

F. Effect of Nonbiopharmaceutical Factors

I. Impairment by Drug Interaction

An increasing number of drugs have been found to affect the bioavailability of digoxin and digitoxin.

1. Neomycin

The co-administration of neomycin (2–4 g/day) reduced steady-state digoxin levels by 28% (LINDENBAUM et al., 1976). There was also an immediate effect on bioavailability when neomycin and digoxin were given simultaneously as single doses. No binding between the drugs was observed in vitro and no change occurred in the digoxin elimination half-life. The mechanism of the effect is unknown.

2. Sulphasalazine

Sulphasalazine treatment was found to reduce digoxin absorption by 18% (JUHL et al., 1976): with this drug also the mechanism of interaction is obscure.

3. Diphenylhydantoin

Diphenylhydantoin has been reported to reduce digoxin levels by a third; this was considered due to altered absorption rather than to liver enzyme induction (LAHIRI and ERTEL, 1974).

4. *p*-Aminosalicylic Acid

BROWN et al. (1978) found that *p*-aminosalicylic acid impaired both D-xylose and digoxin absorption. It caused a decrease of 20% in the 6-day urinary excretion after a single dose of digoxin at the end of a 2-week course of PAS.

5. Antacids

Certain antacids were reported to bind strongly to digoxin and digitoxin in vitro (THOMPSON, 1973; KHALIL, 1974). In a bioavailability study using 6-day urinary excretion data, BROWN and JUHL (1976) found that a mean reduction of 28% in digoxin absorption was caused by magnesium hydroxide, magnesium trisilicate and aluminium hydroxide liquid formulations. The effect on bioavailability was similar for each antacid and a surprising finding was that it did not correlate with the binding affinity of the different antacids. A similar decrease was noted with kaolin–pection, as originally suspected by BINNION (1973). However only a small and not significant decrease in steady-state digoxin blood levels was found when magnesium and aluminium silicates were given as tablets (VOHRINGER et al., 1976). Simultaneous administration with Gelusil (aluminium hydroxide plus magnesium trisilicate) solution did not impair digoxin absorption in dogs (LOO et al., 1975).

6. Anion-Exchange Resins

Cholestyramine binds both digoxin and digitoxin (CALDWELL and GREENBERGER, 1971), and in humans it nearly doubled the elimination rate of digitoxin, presumably by interrupting enterohepatic circulation (CALDWELL et al., 1971). For digoxin, cholestyramine was noted to reduce blood levels during the absorption phase in three subjects (BINNION, 1973) and at steady state in two subjects (SMITH, 1973). Subsequently HALL et al. (1977) found a slight increase in faecal excretion

of digoxin with cholestyramine but no consistent effect on blood levels or urinary excretion levels. However BROWN et al. (1978) demonstrated that cholestyramine could cause up to 31% reduction of 6-day urinary recovery of digoxin after single doses of American Lanoxin tablets – steady-state digoxin levels fell from 0.78 to 0.52 mg/ml when their subjects took 4 g of cholestyramine four times daily. The decrease in absorption was related to the size and timing of the cholestyramine dose. They reasoned that this interaction could be minimised by separating the digoxin and cholestyramine administration times. With an 8 h interval between digoxin and cholestyramine (8 g twice daily) the mean steady-state levels in the subjects was virtually unaffected at 0.72 ng/ml.

Another anion exchanges resin, colestipol, also binds digitoxin in vitro (BAZZANO and BAZZANO, 1972), but its effects on elimination rate was not confirmed by BEVER et al. (1976) who compared serum digitoxin half-lives in a randomised controlled trial of colestipol therapy for patients who had high digoxin levels and, in some cases, toxicity.

7. Activated Charcoal

Activated charcoal is a powerful binding agent and when given at the same time as digoxin it greatly reduced its absorption (HARTEL et al., 1973).

These drug interactions have tended to involve relatively modest changes in digoxin bioavailability but in some individuals the effects appear to have been more prominent. The possibility of such interaction should be looked for when there is an unexpectedly poor response to an adequate digitalis dose. The binding agents may also be helpful in cases of overdosage, particularly if they can be given very soon after a single large excessive intake, as with a dosage error in hospital.

II. Gastrointestinal Disease

A recent meal slows digoxin absorption but does not diminish it (WHITE et al., 1971; SANCHEZ et al., 1973; GREENBLATT et al., 1974 d; JOHNSON et al., 1978 b). Gastric acid may cleave the sugar components from the digoxin molecule even to the cardioinactive digoxigenin (BEERMANN et al., 1972; GAULT et al., 1977). The extent to which this occurs depends on the pH and time of exposure to the acid. Only a small percentage of the digoxin is degraded when the stomach pH is within the normal range, but degradation may be pronounced if there is severe hyperacidity. Partial gastrectomy had no discernible effect on digoxin absorption (BEERMANN et al., 1973).

The influence of drugs which alter the gastrointestinal transit time were at one stage thought to provide important alterations in digoxin bioavailability. MANNINEN et al. (1973 a) measured a 40% rise in steady-state plasma digoxin levels with propantheline, which slows transit time, and a 36% decrease with metoclopramide, which increases gut mobility. However this effect is seen only with slowly dissolving tablets which have limited absorption in normal circumstances and the current

tablet formulations are not affected (MANNINEN et al., 1973b; JOHNSON et al., 1978a).

The effect of diet has been but little investigated. A high fibre meal (5 g crude fibre) lowered digoxin absorption by 18% after a single dose (BROWN et al., 1978). TURNER et al. (1977) found that when dietary histories of cardiac patients were compared with digoxin levels and dosage requirements, only fat content showed a significant but minor correlation. Since postprandial digoxin absorption is not impaired they hypothesised that fat intake might influence the biliary excretion.

The effect of diseases which produce clinical malabsorption has been studied by several groups. The initial report by HEIZER et al. (1971) indicated that maintenance digoxin levels in patients with malabsorption were one-third of those in a control group of cardiac patients. Others have not found this magnitude of effect. HALL and DOHERTY (1974) found only small changes in the serum and faeces of 12 patients with a variety of malabsorption states. The 7-day urinary excretion was moderately reduced but this was thought to reflect poorer renal function in the patients with malabsorption.

Patients studied before and after jejuno-ileal bypass surgery which produced moderate to severe malabsorption of fat and D-xylose did not show any significant reduction of their digoxin absorption (MARCUS et al., 1977). BRACHTEL and GILFRICH (1977) recorded low serum and urine levels during maintenance digoxin therapy in half their patients with systemic sclerosis. One patient who fortuitously developed a transient episode of severe diarrhoea during a digoxin bioavailability study was re-studied and found to have had very impaired absorption during his illness (KOLIBASH et al., 1977). JUSKO et al. (1974) reported one patient with radiation-induced malabsorption who apparently had poor absorption with American Lanoxin tablets but not with a digoxin elixir.

It is clear that the presence of gastrointestinal malabsorption does not necessarily imply impairment of digoxin absorption although this may occur in a few individuals. The retention of adequate digoxin bioavailability in gastrointestinal malabsorption, despite most digoxin absorption normally taking place in the small intestine (BEERMANN et al., 1973), may be helped by the fact that absorption from the colon is more effective than was previously imagined (OCHS et al., 1975; ANDERSSON et al., 1975).

G. Conclusions

New assay techniques have opened up the field of bioavailability of cardiac glycosides. Digoxin and, to a lesser extent, digitoxin were found to have a biopharmaceutical problem, with many brands of tablet dissolving slowly and being poorly absorbed. New drug-agency and pharmacopoeia standards have together almost eliminated these differences in bioavailability. Digoxin capsules and digoxin derivatives have been introduced to improve bioavailability but whether the degree of improvement is worthwhile in clinical terms and in cost-effectiveness is still uncertain. A number of drug interactions which reduce bioavailability have been identified and the effects of gastrointestinal function on digoxin absorption has been studied.

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CHAPTER 9

Pharmaceutical Quality Control Standards for Cardiac Glycosides

G. A. STEWART

A. Introduction

The pharmaceutical quality control of cardiac glycosides described in pharmacopeias includes analytic standards for the drug substances as herbal plants or cardiac glycosides and for their formulated products. These standards include criteria that enable the nature and quality of the drug substance to be defined: a description of the substance, solubility, tests for identity, assay, foreign substances, related cardiac glycosides, specific optical rotation, loss on drying, and ash. Tests for absence of microbial pathogens are carried out where appropriate.

Pharmacopeia formulations comprise injections, elixirs, tinctures, tablets, and capsules. Standards for these include a description of the product and tests of identification, assay, and for foreign substances and related glycosides as appropriate. For injections, tests for acidity of solution, alcohol content, and sterility are required. For tablets and capsules, there are tests to determine uniformity of drug content and to demonstrate satisfactory dissolution of the drug substance in aqueous media.

B. Cardiac Glycoside Preparations in Clinical Use

Table 1 lists the herbal plants and cardiac glycosides described in most pharmacopeias as well as some in clinical use that are not. The cardiac glycosides of the *Digitalis* species are most widely used, particularly digitoxin and digoxin. The Russian pharmacopeia [1] gives the greatest coverage for cardiac glycosides outside the *Digitalis* species.

C. Quality Control Standards and Test Procedures

I. Bulk Drug

1. Description and Solubility

Herbal plants are described in terms of color, taste, and smell. Histologic examinations of the widely used *D. purpurea* distinguish it from other plants. Highly purified glycosides are mostly white odorless powders or crystalline substances insoluble in water and soluble to varying degrees in organic solvents.

1 Pharmacopeia references are to current pharmacopeias unless stated otherwise

Table 1. Herbal plants and their cardiac glycosides used clinically

Plant species	Plant/cardiac glycoside(s)	Pharmacopeia reference
Adonis vernalis L.	Adoniside	Rus. P.
Crataegus oxyacantha L.	Liquid extract (berries) [flower]	9 including (Rus. P.) [Braz. P.]
Crataegus sanguinea Pall.	Liquid extract (berries) [flower]	Rus. P.
Convallaria majalis L.	Convallatoxin	Rus. P., Ger. P.
Digitalis ferruginea L.	Digalen-neo	Rus. P.
Digitalis Lanata Ehrh.	Leaf/powder	BPC, Austrian Pharmacopeia (Aust. P.), Argentinian Pharmacopeia (Arg. P.), Brazilian Pharmacopeia (Braz. P.), Polish Pharmacopeia (Pol. P.), Indian Pharmacopeia (Ind. P.)
	Lanatoside C	Many – 14
	Deslanoside	*BP*, Arg. P., Czechoslovakian Pharmacopeia (Cz. P.), Jap. P., Nord. P., USP
	Digoxin	Many – 16
	Acetyldigoxin (α and β)	None
	β-methyldigoxin (semi-synthetic)	
Digitalis purpurea L.	Leaf/powder	At least 21
	Gitalin (digitoxin/gitaloxin/gitoxin)	None
	Gitaloxin (16-formylgitoxin)	None
	Digitoxin	At least 20
	Acetyldigitoxin	Cz. P., Hung. P., USNF XIV, Pol. P.
	Penta-0-acetylgitoxin	None
Erysimum canescens Roth.	Erysimin	Rus. P.
Nerium oleander L.	Oleandrin	Rus. P.
Scilla maritima var. alba	Proscillaridin	None
	Methylproscillaridin	None
Strophanthus gratus, Wall and Book	Ouabain (Strophanthin G)	Many – 21
Strophanthus Kombé Oliv.	Strophanthin K	Arg. P., Aust. P., Chilean Pharmacopeia (Chil. P.), It. P., Portuguese Pharmacopeia (Port. P.), Rus. P., Spanish Pharmacopeia (Span. P.)
	Cymarin	Ger. P.
Thevetia neriifolia Juss.	Thevetin	None
	Peruvoside	None

2. Identity Tests

Tests for identity are based on colorimetric or fluorescence tests for the genins or sugars. They are carried out directly on solutions of the cardiac glycosides or after location of the glycosides by thin-layer chromatography (TLC). The $\alpha\beta$-unsaturated lactone ring of the genin reacts with 3,5 dinitrobenzoic acid or 3,5 dinitrobenzene in the presence of alkali to produce a violet color (digoxin, digitoxin, deslanoside, lanatoside C) or intense blue color (ouabain). Digitoxose reacts with xanthydrol to give a red color or with ferric chloride in the presence of acid to yield a brown ring at the interface, the upper layer being green and changing to blue. Rhamnose is detected by the red color produced with Fehling's solution (ouabain). The $\alpha\beta$-unsaturated lactone ring also reacts with sodium nitroferricyanide in the presence of alkali to yield a brownish yellow color changing to yellow (adoniside) or a red color that fades (convallatoxin and erysimin). After TLC or paper chromatography, the location and identity of the cardiac glycoside in terms of a reference substance are revealed by spraying with a strong acid in the presence of an oxidising agent to induce fluorescence within the steroid nucleus (ouabain, deslanoside, digitoxin, digoxin, lanatoside C). IR and UV scans of the cardiac glycoside also provide evidence of identity.

3. Specific Optical Rotation

Specific optical rotation is indicative of the identity and purity of a cardiac glycoside. High glycoside concentrations are used, but the introduction of sensitive electronic polarimeters would permit the use of smaller quantities of drug. The range of specific optical rotations for each cardiac glycoside differs slightly between pharmacopeias reflecting the purity of the standards and the precision of each method.

4. Assay Methods

a) Biologic

Biologic methods have long been used to standardize herbal plant products, notably those derived from *Digitalis* species.

α) *Frog Method.* All preparations containing digitalis leaf have been assayed in terms of the first international standard of *D. purpurea* (1926) or of subsequent standards related by assay to it. The frog quantal method of assay, historically the first to be described, is still retained in the Russian pharmacopeia. Frogs of the same sex from one of three recommended species are balanced by body weight in groups of five and maintained at low environmental temperature. The heart of each frog is exposed, and injections of herbal tinctures of cardiac glycosides are made subcutaneously into the femoral lymph sacs, the cardiac ventricle, or the large cutaneous vein. The number of frogs, ideally three or four in each group of five, in which cardiac arrest occurs is recorded. Potency is determined in frog units of activity related to the sensitivity of the frogs to the injection of one frog unit of activity of the appropriate standard reference preparation.

β) Cat Method. HATCHER and BRODY (1910) described a direct assay method in cats that became the bioassay method of choice and is still retained in the Russian pharmacopeia (Rus. P.). Healthy cats of both sexes weighing 2.0–3.5 kg, fasted for 16–20 h are anesthetized lightly with ether or urethane.

Doses of the test preparation as a tincture diluted with saline, warmed to body temperature, are injected into a femoral vein at a constant rate of 1 ml/min until cardiac arrest occurs, the duration of the test being not less than 30 and not more than 55 min. At least six cats receive the standard preparation and six the test preparation. The assay may be conducted over a period of 15 days. The mean lethal dose for each group is calculated. The potency of the test preparation relative to the standard and the standard deviation of the assay is determined by standard statistical procedures. Potency may be expressed in cat units of activity using the expression $(K \times M/A)$ where K = the dilution of the preparation, M the weight of the cat in kg, and A the dose of the diluted preparation in ml.

γ) Pigeon Methods. A quantal assay method based on the emetic action of digitalis in pigeons was first described by HANZLIK (1929) but was not accepted as a pharmacopeia test. BRAUN and LUSKY (1948) developed a direct assay by intravenous infusion of digitalis in the pigeon, similar to the cat method, and this has found general pharmacopeial acceptance; most are based on the method described in the United States Pharmacopeia (*USP*, XVIII). Adult healthy pigeons similar in breed and of no more than twofold range in weight are assigned at random into two groups with not less than six in each. Food but not water is withheld for a period of 16–28 h. The pigeons are lightly anesthetized with ether, immobilized, and the alar vein exposed into which a cannula is placed. The digitalis reference standard *(USP)* and the test sample are extracted with 80% ethanol for about 24 h at about 25 °C. The standard tincture is diluted with saline such that the estimated fatal dose per kg body weight of pigeon will be 15 ml. The test tincture is similarly diluted. Pigeons in one group each receive an intravenous injection of 1 ml of the diluted standard tincture per kg of body weight every 5 min until they die in cardiac arrest. Similarly, pigeons in the other group are injected with the diluted tincture of the test preparation. The average number of doses required to produce death in each group must be between 13 and 19 and the larger number must not exceed the smaller by more than four, otherwise the data are regarded as preliminary. The potency of the test preparation is calculated in terms of the standard preparation by standard statistical procedures. The assay is continued until the confidence interval is 0.30 *USP* digitalis units or less. The Russian pharmacopeia permits the expression of activity in terms of pigeon units derived from an expression similar to that for cats.

δ) Guinea Pig Method. KNAFFL-LENZ (1926) introduced a direct assay method using guinea pigs similar to the cat method. It was widely used and is described in the British Pharmacopeia *(BP)* for the assay of prepared digitalis leaf powder. The estimated potency must be not less than 90% and not more than 111% of the stated potency with fiducial limits of error of not less than 80% but not more than 125% of the stated potency.

ε) Comparison of Biologic Methods of Assay. The found potency of a sample of powdered digitalis may vary according to the biologic method used. This can be attributed to differences in the nature and amounts of cardioactive substances

in the standard and test preparations, and the difference in the sensitivity and rapidity of response of the various animal species to them. Six different batches of *D. purpurea* incorporated into the third international digitalis standard varied markedly in potency between animal species relative to the second international standard. Potencies in the frog were up to 1.75 times higher than in the cat (MILES and PERRY, 1950). Biologic assay of herbal products and of crude glycoside preparations derived from them provides an estimate of activity within broad limits preventing the use of adulterated plants or plants of low activity. However, with the ever increasing availability of purified cardiac glycosides that enable dosing to be precisely determined, there is less justification for the continued use of herbal products.

b) Chemical Assays

Pharmacopeial methods for chemical assay of cardiac glycosides are mostly based on the colored radical ion produced when an aromatic nitrocompound is reacted with the $\alpha\beta$-unsaturated lactone ring of a cardiac glycoside. The reagents include dinitrobenzoic acid/sodium hydroxide, dinitrobenzene/sodium hydroxide, dinitrobenzene/tetraethylammonium hydroxide, and picric acid (trinitrophenol)/sodium hydroxide. Of these; alkaline sodium picrate is the most widely used because the yellow color generated is more stable. The color generated with alkaline dinitrobenzoate is transient, and its measurement must be made at a precise time after development. The methods are not specific for a single glycoside so that the presence of any contaminating glycosides must be demonstrated by chromatographic techniques. However, they are simple, rapid, precise, and reproducible. The cardiac glycoside and its corresponding pharmacopeial reference preparation are dissolved in an appropriate solvent and each diluted with solvent to an appropriate concentration. The reagent is added to a fixed volume of the glycoside solutions, the reactions monitored at constant temperature, and the absorbance of the solutions measured after a set time at about the maximum wavelength in a spectrophotometer. The "purity" of the cardiac glycoside is calculated from the ratio of absorbances and the concentration of the solutions used. The assay values must lie within certain narrow limits that differ between pharmacopeias (e.g., digitoxin, European Pharmacopeia [Eur. P.] 95%–105%, *USP* 90%–101% of molecular formula). The differences may be ascribed to differences in the quality of the reference substances and to laboratory and operator variance.

For the less pure glycosides (digitoxin and ouabain), the *USP* requires each glycoside to be separated from related impurities using column chromatography before assay. This preparative procedure is of value where the amount of related glycoside impurities is significant. The lower limit of assay for digitoxin of 90% (*USP*, Japanese Pharmacopeia [Jap. P]) would indicate that the purity of digitoxin available for clinical use is lower than indicated by the Eur. P. where the chromatographic separation of the digitoxin is not carried out before assay and where the assay limits are 95% and 105%.

c) Presence of Foreign Substances

α) *Other Cardiac Glycoside Impurities.* The B and D series of *Digitalis* cardenolides may be present as trace impurities in samples of acetyldigitoxin, digitox-

in, and digoxin. Fluorescence induced by acid in glycol solutions forms the basis of their assay in terms of a gitoxin reference standard. The amount of such impurities in terms of gitoxin should not exceed 8% USNF, 14th edn. (acetyldigitoxin), 1% Eur. P. (digitoxin), 4% Eur. P. (digoxin), 3% *USP* (Digoxin), and 4% Jap. P. (digoxin).

β) Other Glycoside Impurities. TLC procedures are used to detect other cardenolide impurities in acetyldigitoxin (USNF XIV, Hungarian Pharmacopeia [Hung. P]), deslanoside (*BP*, Jap. P.), lanatoside C (*BP*, Jap. P.), and convallatoxin and cymarin (German Pharmacopeia [Ger. P.]). Solutions of a standard reference substance and of the cardiac glycoside under investigation are applied as spots of several concentrations of each solution on to a TLC plate. The plates are developed with a solvent system, dried, and sprayed with a reagent to detect the spots. The location and size of spots obtained with the sample under test are compared with those obtained with the reference substance, and an order of the amounts of impurities present is estimated.

γ) Digitonin. The absence of digitonin must be demonstrated by showing that no precipitate is obtained when a solution of cholesterol is added to a solution of digitoxin in an appropriate solvent (*USP*, Jap. P.).

δ) Alkaloids. The absence of alkaloids must be demonstrated by showing that no precipitate is obtained when a solution of tannic acid or iodine is added to a solution of ouabain (*USP* and Jap. P.).

ε) Aglycones and Other Glycosides in Ouabain. Not more than 5% should be present when estimated against the standard reference substance for ouabain using anthrone as chromogen *(USP)*.

ζ) Foreign Matter. The proportion of stems, brown leaves, flowers, and other foreign organic matter should not exceed 2% (*BP* 1973, USP, Jap. P., British Pharmaceutical Codex [BPC]) or 1% (Hung. P.).

d) Loss on Drying

All pharmacopeias limit the loss in weight (sometimes referred to as water content) of substances when dried under defined conditions. The limits are almost the same between pharmacopeias for a given glycoside or for *D. purpurea* although drying conditions may vary. Limits are set in relation to the equilibrium moisture content and compliance with them ensures adequate stability of a glycoside during storage.

e) Ash

For *D. purpurea* the hydrochloric acid insoluble ash should not exceed 5% (Eur. P., USP, Jap. P., Ger. P.) or 6% (Hung. P.). The sulfated ash content of deslanoside, digitoxin, lanatoside C, and digoxin must not exceed 0.1% *(BP)* and not more than 0.1% for acetyldigitoxin (USNF XIV) and 0.5% for deslanoside (USNF, Jap. P.), digitoxin, lanatoside C, ouabain, and digoxin (Jap. P.); the limit for digoxin in Hung. P. is not more than 0.2%. With 100 mg samples of digitoxin, ouabain, and digoxin, the amounts of sulfated ash should be negligible *(USP)*.

f) Microbial Tests

A 1-g sample of *D. purpurea* must be free from *E. coli* and a 10-g sample free from *Salmonella (BP)* and a 25-g sample free from *Salmonella* (USP).

II. Pharmaceutical Preparations

Formulated products containing cardiac glycosides comprise injections, tablets, capsules, elixirs, tinctures, and solutions for oral administration and sometimes suppositories.

1. Injections

The following injections are described in pharmacopeias: digoxin 250 mcg/ml *(BP)*, 100 mcg/ml and 250 mcg/ml *(USP)*, unspecified concentrations (Jap. P. and Hung. P.), digoxin pediatric 25 mcg/0.25 ml *(BP)*, deslanoside 200 mcg/ml *(BP)*, 400 mcg/2 ml and 800 mcg/4 ml (USNF XIV), ouabain 250 mcg/ml *(USP)*, unspecified concentrations (Jap. P. and Nordica Pharmacopeia [Nord. P.]), digitoxin 200 mcg/ml *(USP XIX)* digitalis, unspecified concentration (Nederlands Pharmacopeia [Neth. P.]), strophanthin K 0.5% w/v (Rus. P.), convallatoxin 0.3% w/v (Rus. P.), and erysimin 0.033% w/v (Rus. P.).

Tests used for identity of the cardiac glycosides in the injections are mainly those described for the drug substances, and assay methods are based on either the acid ferric chloride or alkaline picrate reactions, apart from those injections in Rus. P. and digitalis (Neth. P.) that are assayed biologically. Assay limits are within $\pm 10\%$ of labeled amount apart from those for digoxin given in the *USP* ($\pm 8\%$) and Nord. P (86%–110%). The alcohol content of digitoxin and digoxin injections determined by distillation should lie between 9% and 11% *(USP)*.

2. Elixirs/Tinctures/Solutions

a) Elixirs

Digoxin elixir (50 mcg/ml *USP*, BPC 1973) is identified using alkaline dinitrobenzene or ascending chromatography *(USP)* or by the acid ferric chloride reaction (BPC 1973). It is assayed by the acid ferric chloride reaction [limits: $\pm 8\%$ *(USP)* or $\pm 10\%$ (BPC1973) of labeled amount]. Fluorescent impurities as gitoxin must not exceed 3% *(USP)*. The alcohol content is 9.0%–11.5% v/v (USP) and 9.2%–10.8% v/v (BPC 1973).

b) Tinctures

Tinctures of *D. Purpurea* are relatively unstable but are still described (Neth. P., Mexican Pharmacopeia [Mex. P.]). They are assayed biologically. Tincture of *Strophanthus* (Neth. P.) contains 0.5% glycosides and is assayed in terms of strophanthoside by the alkaline dinitrobenzoic acid reaction.

c) Solutions

Digitalis solution (Neth. P. 1966) is identified using the acid ferric chloride reagent and assayed biologically. Lanatoside C solution (Hung. P.) is identified using the acid ferric chloride reagent and assayed by the alkaline picrate method. Oleandrin solution (0.022%) and digalen-neo (activity in animal units per ml) are identified using acid ferric chloride and adoniside (23–27 frog units per ml) using alkaline nitroferricyanide. All are assayed biologically (Rus. P.).

3. Tablets and Capsules

a) Tests for Identity and Assay

Powdered digitalis (10 u/g) formulated as tablets [*BP* (60 mg), USNF XIV (30–100 mg), Nord. P. (50 mg)] or capsules 60 and 100 mg (USNF XIV) is identified morphologically and assayed biologically using pigeons (*BP*, USNF XIV, Nord. P.) or guinea pigs *(BP)*. The estimated potency in terms of stated potency must lie between 85%–117% *(BP)*, between 85%–120% (USNF XIV), or 80%–120% (Nord. P.). The fiducial limits of error shall lie between 80%–125% of the stated potency *(BP)*. Tablets of cardiac glycosides described in pharmacopeias are: digoxin (BP) 62.5 mg and (*BP*, *USP XIX*, Jap. P., Hung. P., Nord. P.) 125, 250, and 500 mcg, digitoxin (*BP*, *USP XIX*, Jap. P., Hung. P., Rus. P.) 50, 100, 150, and 200 mcg, Acetyldigitoxin (USNF XIX, Hung. P.) 100 and 200 mcg and lanatoside C (Jap. P., Hung. P.) content not stated. The glycosides are identified using acid ferric chloride or alkaline dinitrobenzene or by chromatography and assayed mainly by the alkaline picrate method. Other assay procedures are used for digoxin (*BP*, xanthydrol reaction, *USP*, Jap. P. acid ferric chloride reaction) and digitoxin (Rus. P., bioassay). Assay limits are within $\pm 10\%$ of labeled amount apart from those for digoxin tablets in *USP* ($\pm 8\%$) and Nord. P. (86%–110%) and for digitoxin tablets in Nord. P. (85%–110%) and Rus. P. (0.85–1.2 frog units or 0.17–0.19 pigeon units).

b) Physicochemical Test Requirements for Solid Dosage Products

In addition to tests for identity and assay, there is a need to ensure that a cardiac glycoside that is administered in very small doses is evenly distributed throughout the batch of the formulated solid dosage product (usually a tablet) and uniformly and readily available from the product when administered to the patient.

α) *Tablet Weight Variation.* Twenty tablets are weighed individually and the weights of not more than two tablets are allowed to deviate from the mean weight by more than a set value and not more than one tablet by more than twice the permitted tolerance. The tolerances are dependent on the average weight of the tablet and differ between pharmacopeias:

Deviation (%)	Average weight (mg)		
	Eur. P.	*USP*	Jap. P.
10	< 80	< 130	< 120
7.5	80–250	130–324	120–300
5	> 250	> 324	> 300

More complex criteria of assessment of weight variation obtain with capsules (USP).

β) *Tablet Disintegration.* Six tablets are placed individually in glass tubes closed at the bottom with a stainless steel 10 mesh wire. A plastic disc is placed on top of each tablet, and the assembly of six tubes is oscillated within the disintegra-

tion fluid (usually water) maintained at 37 °C at a rate of 29–32 cycles/min such that at the highest point on the upward stroke the wire mesh remains at least 2.5 cm below the surface of the water. All tablets must have disintegrated and pass through the mesh within 30 min (*USP*, USNF, Jap. P.), within 15 min (Eur. P), or within 10 min (Italian Pharmacopeia [It. P.] supplement 1978, digoxin).

γ) *Content Uniformity*. In the absence of good manufacturing procedures, wide variations in cardiac glycoside content can occur. With generic digoxin tablets, FAULKNER (1971) found a between tablet variation of 59%–108% of the stated dose and MANNINEN and KORHONEN (1973) a variation of 39%–189%.

Highly sensitive, precise methods are required for studies on drug content uniformity so that the individual tablet variation observed is attributable to the manufacturing process and not the assay procedure. In 1965 the *USP* directed that the bulk assay method be modified to permit analysis of single tablets. Insufficient sensitivity was often a problem, and a special fluorimetric assay was recommended (*USP* XVIII) for the assay of the content uniformity of digitoxin tablets.

Automated procedures using the Technicon Antoanalyzer are more suitable for content uniformity assay. Two main types of methods have been studied. The acid-induced fluorescence method described by KHOURY (1966) and CULLEN et al. (1970) has been widely used. The fluorimetric method of WELLS et al. (1961) has been automated to provide a sensitive (5 μg/ml) and precise measure of content uniformity of digoxin tablets (coefficient of variation ±0.7%). MYRICK (1969) described a method for the content uniformity of digoxin tablets in which the digitoxose moiety is oxidized by periodic acid and the oxidation product (malonic dialdehyde) condensed with thiobarbituric acid to yield a red color, the absorbance of which is in proportion to glycoside concentration. With both types of methods, coefficients of variation of below 1% for repeated analysis of a standard solution can be achieved. The methods can also demonstrate a similar precision in uniformity of content in batches of tablets manufactured to high standards of good manufacturing practice (FAULKNER, unpublished data). The limits for content uniformity differ between the *USP* and *BP*. The *USP* specifies that the content of each of ten tablets is within 85%–115% of the average of the limits specified in the potency definition of the individual monograph (usually 100%). If the glycoside content of not more than one of the tablets falls outside this range, provided this value is not below 75% or above 125%, a further 20 additional tablets must be tested. The cardiac glycoside content of the additional 20 tablets must fall within 85%–115% of the average of the assay limits specified. The content of nine of ten tablets should lie between 80% and 120% *(BP)* and between 85% and 115% of the mean (Nederland Ministry of Health, 1973, It. P. supplement 1978), and all ten must lie between 75% and 125% (*BP*, Nederland Ministry of Health, 1973) and between 80% and 125% (It. P. supplement 1978). No retest is permitted. The mean is the average of the values obtained in the content uniformity test. The essential difference between these requirements and that of the *USP* is that the latter requires the uniformity to be measured in relation to deviation from the stated dose whereas the former consider uniformity in terms of the average potency of the batch. The potency of the batch is covered by the assay limits in the monograph but the limits on content uniformity in the *BP* permit a greater tolerance than those of the *USP*.

δ) Dissolution. Dissolution testing has been applied both to predict bioavailability and to demonstrate uniformity of manufacture. The three major types of apparatus advocated for the measurement of dissolution of a drug from a solid dose product, usually from a tablet, are the rotating basket (*BP* and *USP*), the stirred beaker (LEVY and HAYES, 1960), and various column flow systems (LANGENBUCHER, 1969). Devices aimed at predicting bioavailability are more complex because attempts have been made to simulate gastric conditions by inclusion of synthetic lipid barriers (Sartorius apparatus) and by careful control of fluid flow patterns.

Perhaps because of the complexity, no such device has appeared in any pharmacopeia. Earlier methods such as the USNF XIV rotating bottle apparatus and use of the *USP* XIX tablet disintegrator as a dissolution tester are not now commonly employed. The rotating basket apparatus of USNF (XIII) and *USP* (XVIII) is a modification of one described by PERNAROWSKI et al. (1968). Another modification of this apparatus was adopted in the *BP* 1973 (Addendum, 1975) using a cylindric flask instead of the tapered resin flask of the *USP* (XVIII). The basket and shaft assembly of the *USP* and *BP* are essentially similar. The stirred paddle method has been added to the *USP* for the examination of certain products. The dissolution standards for digoxin tablets *(BP, USP)* and digitoxin tablets *(USP)* are now widely accepted. In the case of digoxin, dissolution rate measured by the rotating basket apparatus has been shown to correlate with bioavailability (JOHNSON et al., 1973). In order that results from the dissolution test may be meaningful, the concentration of the cardiac glycoside in solution should not approach saturated solubility. Methods of analysis for the determination of cardiac glycosides in aqueous media need to be very sensitive because of the very low solubility of glycosides in such media. Although methods such as radioimmunoassay (LADER et al., 1972) have been applied, the most practicable method of suitable sensitivity is the fluorimetric procedure of WELLS et al. (1961). In the presence of methanol, ascorbic acid, hydrochloric acid, and peroxide catalyst, digoxin and digitoxin are converted to fluorescent species. Care must be taken to ensure cleanliness of apparatus and addition of reagents at accurately timed intervals to achieve reproducible results. The reproducibility of the dissolution method described by JOHNSON et al. (1973) was studied using a number of instruments and operators on several occasions.

A coefficient of variation of 3.7% for a single determination was obtained (MCCRERIE, unpublished data). A major variable was assay of the dissolution fluid itself. The fluorimetric assay can be automated using a Technicon Autoanalyzer. Instead of hydrogen peroxide specified by WELLS et al. (1961), benzoyl peroxide is used because its concentration has a far less critical effect on fluorescence yield. Samples can be assayed at a rate of 50/h with a coefficient of variation of below 1% (FAULKNER, unpublished data).

Studies with the *USP* flask, the *BP* flask, and a spherical flask showed that vessel shape had no significant effect on the dissolution of Lanoxin (digoxin) tablets. The *USP* employs a dilute hydrochloric acid solution for dissolution of digoxin tablets whereas the *BP* specifies water. KHALIL and EL-MASRY (1978) have observed substantial decomposition of digoxin in acidic dissolution fluid but without a significant effect on the dissolution result because the fluorimetric assay responds only to the genin moiety.

ε) *Pharmacopeia Requirements for Dissolution.* Of six tablets of digoxin tested together, the amount of digoxin dissolved after 1 h must be not less than 75% *(BP)* or 90% (Nederland Ministry of Health, 1973) of the stated dose. No retests are permitted. In the *USP* six tablets of digoxin are tested individually, with permission to test a further six tablets if necessary, and the amount of digoxin dissolved after 1 h in 11/12ths of the tablets must be not less than 65% and none less than 55% *(USP)*. A further variation is described in It. P. (supplement 1978) where of five tablets tested individually the amount of digoxin dissolved from each tablet after 30 min shall be not less than 70%. With digitoxin tablets the specification given in the *USP* differs from that for digoxin tablets in that the quantity of digitoxin dissolved after 30 min for each of six tablets shall be not less than 60% and not more than 85% after 1 h. Pharmacopeia methods to predict the absorption of cardiac glycosides from formulated solutions intended for oral administration must also be devised.

III. General Pharmacopeial Tests Applied for Formulated Products

There are a number of general pharmacopeial tests that apply to all pharmaceutical products. Excipients such as water, alcohol, glycerol, preservatives for injections, starches, sugars, binding agents, and lubricants used in tabletting must comply with an analytic specification before use.

All injections must satisfy tests for sterility and raw materials; particularly those of natural origin must be shown to be free from pathogenic organisms. Ampuls should be tested for freedom from alkalinity, and containers for solid dosage products should have tight fitting closures to prevent ingress of moisture. After filling, liquid products must be checked for uniformity of fill and dropper bottles for uniformity of drops delivered.

IV. Product Stability

Stability data should be obtained by examining samples at the time of batch manufacture and thereafter at regular intervals following storage at several temperatures and humidities and by exposure to light in the intended containers so that shelf life and storage conditions can be defined. These should include chemical stability using a validated stability-indicating method and physical stability particularly in relation to the dissolution rate of tablets since some solid dosage formulations may undergo a change in dissolution properties on storage.

V. The Future

Nonpharmacopeial Methods for Identity, Purity; Assay, and Stability

The pharmacopeial methods that characterize the quality of a cardiac glycoside are generally applicable to all cardenolides and are not specific for an individual glycoside. By comparison with the standard reference cardiac glycosides, in some cases by the application of TLC procedures in identity tests, and by the application of measurements of specific optical rotation, the identity and quality of individual

cardiac glycosides can be determined. Only some of these tests may be applicable to the formulated products.

The pharmacopeial tests, however, do not describe the identity of very closely related glycosides that may be present or may arise as degradation products during storage of the formulated product. For example, they do not permit the detection and quantification of digoxigenin bisdigitoxoside or of digoxigenin monodigitoxoside in digoxin, both of which will be estimated as digoxin.

The application of classic column chromatography and high performance liquid chromatography (HPLC), particularly the latter, will now permit identification and assay of the prime glycoside and other glycosidic impurities, including those closely related to the prime glycoside, within a single test system rapidly and precisely.

In recent years HPLC methods have been published for cardiac glycosides employing ion exchange, normal phase, and reversed phase mechanisms. An early separation of *Digitalis* "A" series cardenolides was achieved on a strong cation exchange column (EVANS, 1974), but subsequent work, mostly with *Digitalis* cardenolides, has been carried out on more efficient silica gel and bonded reversed phase columns (CASTLE, 1975; ERNI and FREI, 1977). Work also has been reported on reversed phase HPLC of *Strophanthus* glycosides and bufadienolides (DAVYDOV et al., 1978).

Both reversed phase and normal phase HPLC show high selectivity and are suitable for the identification of cardiac glycosides. An empirical relationship between chemical structure and retention time for *Digitalis* cardenolides on a silica gel column has been demonstrated (COBB, 1976), and similar relationships obtain for cardenolides and bufadienolides on a bonded (octadecylsilane), reversed phase column (SHIMADA et al., 1976). Normal phase and reversed phase HPLC are complementary technique. Normal phase HPLC may give greater selectivity, but reversed phase HPLC can give more rapid analyses, particularly when highly polar compounds are being examined.

Detection of cardiac glycosides on HPLC can cause problems due to their low UV absorbance, since wavelengths of maximum absorbance are, generally in the range of 200–220 nm and it is necessary to use a variable wavelength UV detector to obtain maximum sensitivity. The number of solvents that have a good UV transparency at 220 nm is limited, which restricts the choice of the mobile phase. This is more serious for normal phase chromatography than for reversed phase, and most reported mobile phases for use on silica gel columns are not transparent below 230 nm. Hence, normal phase chromatography is only applicable to the more concentrated solutions of cardiac glycosides where sensitivity is not a problem.

An example of the use of normal phase HPLC is the determination of digoxin in *D. lanata* leaf (COBB, 1976). Digoxin in an extract of *D. lanata* leaf can be resolved from the numerous related cardenolides on a silica gel column using a mixture of cyclohexane, ethanol, and acetic acid as the mobile phase. Digoxin elution is monitored at a wavelength of 234 nm, the lowest usable wavelength with the mobile phase, and the low maximum absorbance wavelength of the cardenolides is used to advantage by incorporation of a novel internal standard that can be monitored at 256 nm free from interference from underlying cardenolides in the complex mixture. A high degree of precision is obtainable. With a mean value of

0.150% digoxin, a single assay determination carried out on the same occasion is within the range 0.145%–0.155% ($P=0.95$).

Reversed phase HPLC allows detection at the maximum absorbance wavelength of cardiac glycosides and has advantages over normal phase HPLC in the analysis of pharmaceutical formulations in that sample preparation is simplified because the active constituent can be determined at low concentrations. Liquid formulations can be injected directly into the chromatograph and tablets pulverized, slurried with mobile phase, and the clear supernatant solution obtained on centrifugation can be used. Loop injectors allow a quantitative reproducibility of 1% relative standard deviation, and because of this good reproducibility external standardization can be used (ERNI and FREI, 1977). Detection limits vary with the capacity factor (k^1) because strongly retained compounds are more highly diluted with mobile phase when they are eluted from the column. Many cardiac glycosides can be detected at the level of 10 ng in a 25 µl injection.

Higher sensitivities can be achieved by derivitization of cardiac glycosides to enhance their UV absorbance. *Digitalis* cardenolides esterified with 4-nitrobenzoyl chloride before injection on to a silica gel column can be detected at the level of 50 ng/ml for a 25 µl injection (NACHTMANN et al., 1976). The method has been applied to pharmaceutical formulations, and reasonable quantitative precision has been obtained. Another possibility for the enhancement of detection response of cardiac glycosides is the use of a post-column derivitization procedure whereby the eluant stream from the column is mixed with a reagent before entering the detector. This would allow fluorimetric detection to be used for cardiac glycosides. Such methods will eventually be introduced into pharmacopeias to replace tests for identity, purity, and assay.

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Clinical Pharmacology

CHAPTER 10

Effects of Cardiac Glycosides on the Failing and Nonfailing Heart

D. T. MASON and G. LEE

A. Introduction

The digitalis glycosides have been employed widely in clinical medicine for nearly two centuries as the principal drug in the treatment of congestive heart failure. Many important advances in the past few years have considerably improved understanding of the physiologic, subcellular, and pharmacodynamic properties of the glycosides and their application in patients (MASON and AWAN, 1979). This chapter focuses attention on the cardiac action of digitalis on the failing and nonfailing heart. Finally, these observations are integrated to provide a unified concept of the actions of the glycosides on the cardiocirculation.

B. Fundamental Positive Inotropic Action

I. Failing Ventricle

The beneficial effects of digitalis in patients with congestive heart failure result from its direct stimulation of the depressed contractile state (BLOOMFIELD et al., 1948; FERRER et al., 1960; MASON and BRAUNWALD, 1968; MASON et al., 1969). Digitalis augments the low cardiac output and reduces the elevated left ventricular end-diastolic pressure of the dysfunctioning heart, shown by the series of ventricular function curves in Fig. 1, relating cardiac output to ventricular filling pressure. The steepest curve represents normal cardiac performance. With decline of contractility, which is the fundamental disturbance of the failing myocardium (MASON, 1973), ventricular function becomes depressed as shown by the lowest curve. At normal end-diastolic pressure (point A), the failing heart is unable to deliver an adequate cardiac output; therefore the ventricle must dilate to operate at an abnormally high filling pressure (point B) to pump a normal cardiac output, the level of which is indicated by the horizontal broken line. With the administration of digitalis, the positive inotropic action of the drug raises ventricular function toward normal, shown by the intermediate curve. Thus, the glycoside improves the fundamental physiologic defect causing ventricular failure, depressed contractility (MASON et al., 1970), and this increase in contractile state allows a lesser need for Frank-Starling preload compensation, so that a normal cardiac output can be delivered as a substantially lower ventricular filling pressure (point D).

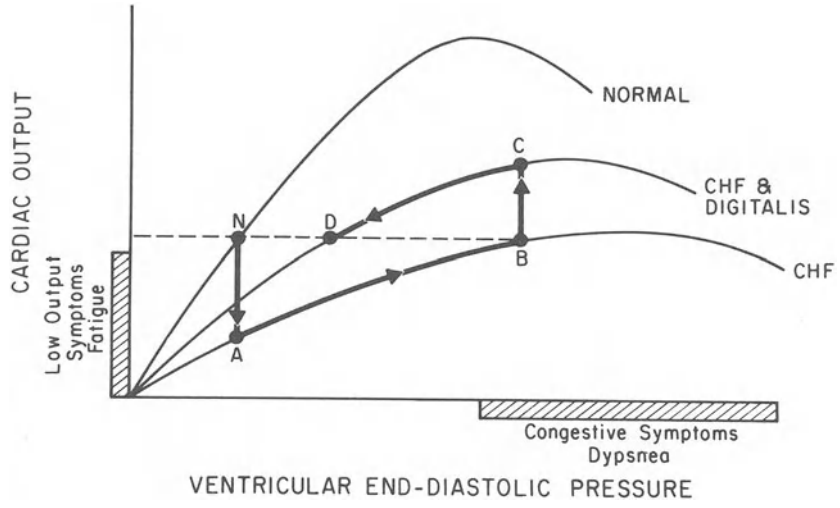

Fig. 1. Representative left ventricular function curves in a normal heart, in congestive heart failure (CHF), and in heart failure after treatment with digitalis. (MASON, 1973)

II. Normal Ventricle

Although there is now general agreement that digitalis stimulates the force of contraction of the failing myocardium, there has been considerable confusion concerning its effects on the nonfailing heart. This problem has been resolved by the demonstration that the glycoside directly elevates the contractile state of the normal ventricle (BRAUNWALD et al., 1961; MASON and BRAUNWALD, 1963; SONNENBLICK et al., 1966), although the increased contractile state is not translated into an increase in cardiac output. In Fig. 2, high fidelity recordings of left ventricular pressure and its simultaneous rate of change (dp/dt) are shown before and after administration of ouabain in a patient with normal cardiac performance. Ouabain increased left ventricular peak dp/dt, signifying that digitalis augments contractility of normal heart muscle since the loading variables influencing peak dp/dt of end-diastolic pressure and arterial diastolic pressure remained constant (MASON, 1969). This finding that ouabain increases the contractility of the nonfailing heart has also been obtained from the right ventricle of normal subjects (Fig. 3; MASON and BRAUNWALD, 1963).

III. Diseased Nonfailing Ventricle

In patients with heart disease without failure, the cardiac reserve mechanisms of ventricular dilation, hypertrophy, and adrenergic stimulation maintain cardiac output at a normal level (MASON, 1973; MASON et al., 1970). Further, experimental and clinical work has shown that contractile state is diminished in chronic hemodynamic overload and in cardiomyopathies even before the onset of overt congestive heart failure (MASON, 1973). Thus, as shown in Fig. 4, the diseased but compensated heart is characterized by the moderately depressed ventricular function

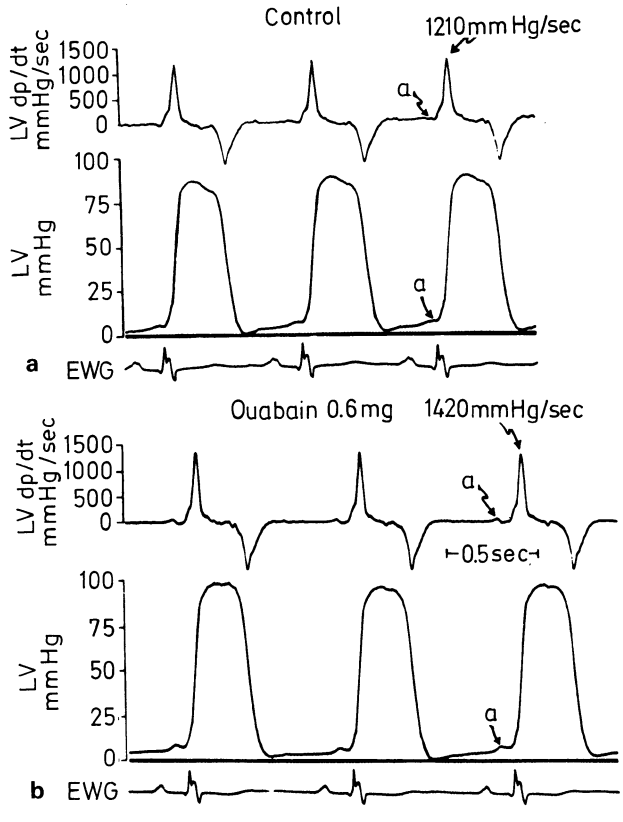

Fig. 2a, b. Simultaneous high fidelity recordings of left ventricular (LV) pressure and its rate of change (dp/dt) during the control period **a** and 30 min after the administration of 6.0 mg ouabain **b** in a patient with normal left heart function; A = accentuation of LV late-diastolic pressure by left atrial contraction. After ouabain administration, the A deflection was increased, thereby suggesting that ouabain also improved the strength of left atrial contraction. (MASON and BRAUNWALD, 1963)

curve (3), intermediate to the severely flattened performance curve (4) of the failing heart and the steep curve (1) of the normal heart. Digitalis in patients with ventricular dysfunction without failure does not elevate the cardiac output above normal (horizontal broken line); instead, the upward shifted ventricular function curve (2) provided by the positive inotropic effect of digitalis allows normal cardiac output to be maintained at a substantially lower ventricular filling pressure (point B in contrast to point C). The drugs thus allows less encroachment on cardiac compensatory mechanisms, reduces ventricular dilation and improves effort dyspnea even in the absence of overt pulmonary congestion.

IV. Atrial Myocardium

In addition to stimulation of ventricular contractility, digitalis also directly increases the force of contraction of the atrial myocardium (CAPONE et al., 1972). Augmentation of left atrial contractility by ouabain is shown in Fig. 5 in a patient

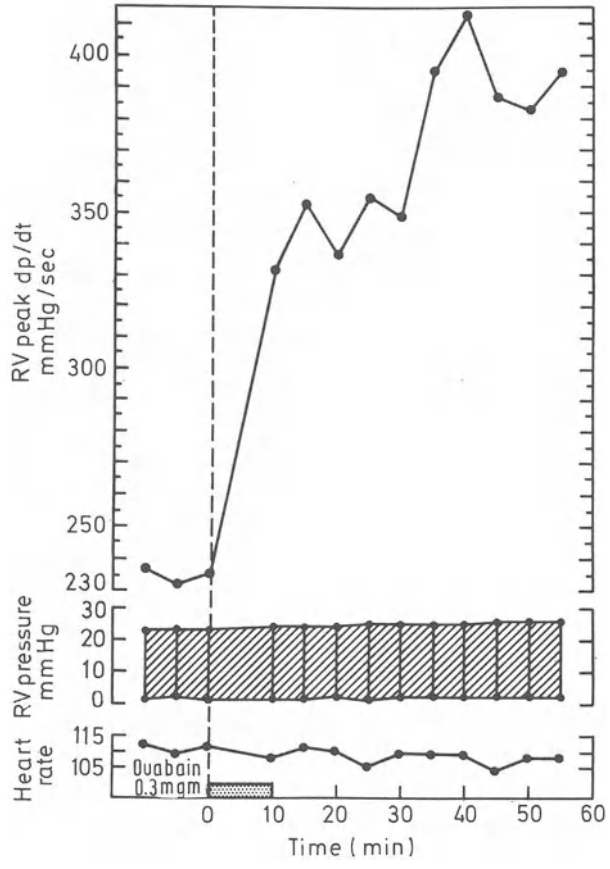

Fig. 3. Sequential measurements of the peak rate of change (d*p*/d*t*) of right ventricular (RV) pressure in a patient with a normal cardiovascular system. (MASON and BRAUNWALD, 1963)

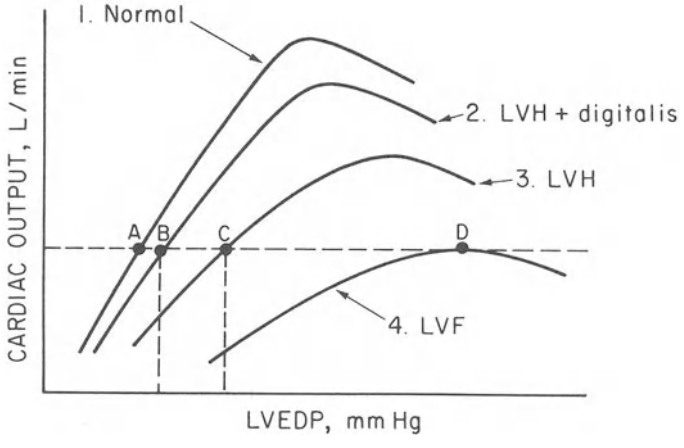

Fig. 4. Ventricular function curves relating cardiac output to left ventricular end-diastolic pressure (LVEDP) in the normal heart *1*, left ventricular hyperthrophy (LVH) with *2* and without *3* digitalis, and in left ventricular failure (LVF) *4*. (MASON, 1973)

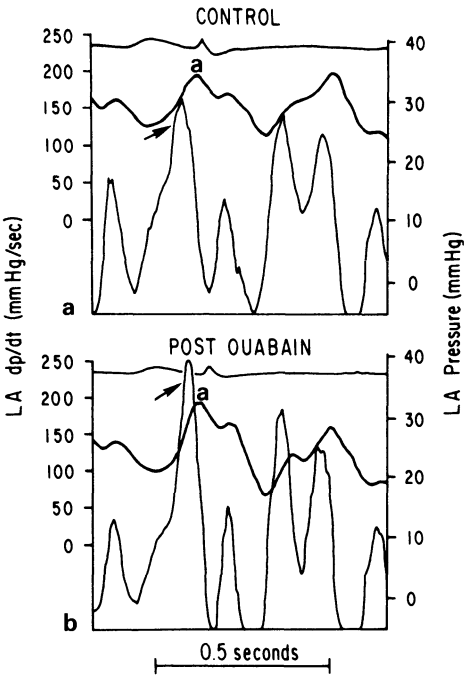

Fig. 5a, b. Simultaneous high fidelity recordings of left atrial (LA) pressure and its pressure change (dp/dt) during the control period **a** and after intravenous administration of digitalis **b** in a patient with mitral stenosis and normal sinus rhythm. In both **a** and **b**, phasic LA pressure is the top tracing and LA dp/dt the bottom recording

with pure mitral stenosis in normal sinus rhythm. Left atrial pressure and dp/dt are illustrated before and after administration of the drug. After ouabain, the height, amplitude, and rate of rise of the atrial contraction wave (A) and corresponding dp/dt (arrow) are augmented, indicating that the agent stimulates the inotropic state of the atrium.

From these observations of the direct stimulating action of digitalis on normal and diseased ventricular and atrial myocardium, it is apparent that the glycosides exert the same fundamental positive contractile action on both normal and failing hearts. Therefore, the notion held previously that the drug has a harmful inotropic effect on the nonfailing ventricle can no longer be maintained.

C. Cardiac Energetics

As elaborated by BRAUNWALD (1971) the oxygen consumption of the heart is largely regulated by the interplay among three major hemodynamic related determinants (Fig. 6). Most important is intramyocardial tension (left ventricular afterload), which is governed by ventricular systolic pressure and the radius of the ventricle (MASON, 1978). The other two major variables controlling cardiac oxygen requirements are the heart rate and contractile state.

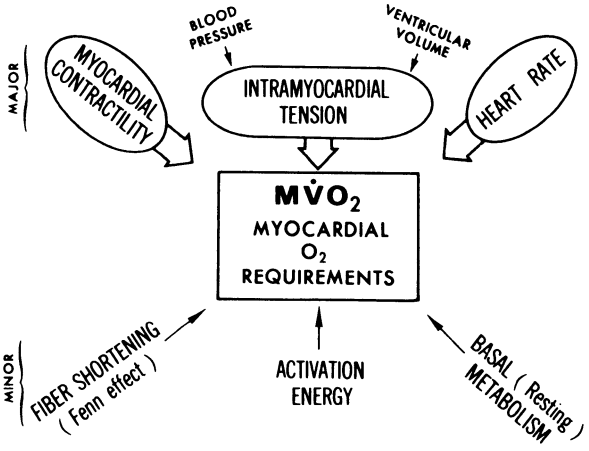

Fig. 6. Diagram showing the three major and three minor determinants of myocardial oxygen consumption ($M\dot{V}O_2$). (MASON, 1973)

I. Normal Ventricle

The positive inotropic effect of digitalis elevates myocardial oxygen consumption (COLEMAN, 1967; COVELL et al., 1966). In the normally functioning heart, the predominant effect of digitalis on myocardial energetics is an increase in cardiac oxygen demands.

II. Failing Ventricle

Conversely, digitalis reduces overall myocardial oxygen consumption and increases cardiac efficiency in the failing heart (COVELL et al., 1966). Thus, in the enlarged dysfunctioning heart, ventricular tension is diminished by the reduction in heart size resulting from the inotropic action of the glycoside. In terms of cardiac oxygen needs, this indirect decline in wall tension is greater than is the direct increase in contractility produced by digitalis. Thus, in chronic ischemic heart disease with ventricular dysfunction, digitalis may exert an antianginal effect. This improvement in myocardial energetics in heart failure brought about by digitalis is not a fundamental property of the agent; rather, in this state, it overrides the energy-wasting effect normally associated with the direct positive inotropic action of the glycoside.

III. Coronary Artery Disease

Studies in experimental animals have shown that raising myocardial oxygen requirements in the normal ventricle by stimulating contractility with digitalis increases the extent of ventricular ischemia and necrosis after subsequent coronary occlusion (MAROKO, et al., 1971). In contrast, in the presence of ventricular dysfunction and cardiomegaly, treatment with the glycoside prior to coronary obstruction reduces the area of myocardial ischemia and infarction (WATANABE et al., 1972).

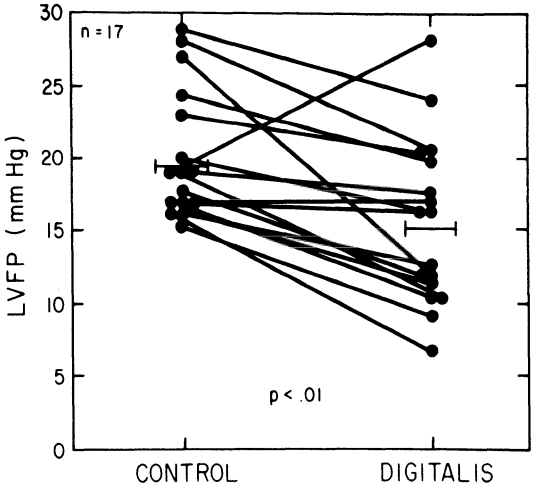

Fig. 7. Effects of intravenous digitalis on left ventricular end-diastolic pressure (LVFP) in patients with acute myocardial infarction in whom the preglycoside LVFP was above normal (greater than 12 mm Hg). All patients had congestive heart failure without cardiogenic shock

D. Acute Myocardial Infarction

There has been considerable debate concerning the role of digitalis therapy in acute myocardial infarction. In cardiogenic shock when the ventricle is largely destroyed, it is of little value, as is the case with other cardiotonic agents.

I. Failing Ventricle

Conversely, in acute congestive heart failure without shock in the immediate period after infarction, studies in our coronary care unit have shown that intravenous ouabain or digoxin produced substantial improvement in ventricular function in the majority of patients (Fig. 7), although the results were somewhat inconsistent (AMSTERDAM et al., 1972 b). Elevated left ventricular end-diastolic pressure was reduced while low cardiac output rose or normal cardiac output was restored in most patients.

II. Diuretics and Nitrates

In the management of congestive heart failure due to acute myocardial infarction, it is our current practice to use intravenous furosemide or oral nitrates to relieve pulmonary congestion when the left ventricular filling pressure, measured by Swan–Ganz catheterization of the pulmonary artery, is greater than 18–20 mm Hg. Mild to moderate elevations of left ventricular filling pressure (12–18 mm Hg) usually do not require therapy and return to normal levels in about 5 days with improvement of diminished compliance after infarction. In patients with filling pressures exceeding 18–20 mm Hg in whom sufficient reduction of this variable is not achieved with furosemide and nitrates, intravenous digoxin is given in approximately two-thirds of its standard parenteral digitalizing dose.

III. Digitalis Mechanisms in Infarcted Ventricle

To evaluate some of the factors contributing to the inconsistent cardiotonic response to digitalis in acute myocardial infarction, the inotropic effect of ouabain was evaluated in a preparation of isolated right ventricular papillary muscle from cats in which the myocardium was made hypoxic (AMSTERDAM et al., 1972 a). It was found that the increase in peak tension caused by ouabain during normal oxygenation was markedly attenuated during hypoxia, whereas isoproterenol retained its full positive inotropic action at the level of hypoxia. Therefore, the ability to respond to inotropic stimulation by digitalis is impaired in the hypoxic myocardium. This inherent impaired response to inotropic stimulation by digitalis in hypoxic heart muscle is additive to reduced contractile response resulting from decreased bonding of the agent to ischemic myocardium induced by coronary occlusion (HOPKINS et al., 1972).

Nevertheless, at least some enhancement of depressed contractility is achieved in acutely ischemic myocardium surrounding the necrotic area after experimental coronary occlusion in the intact canine heart (AMSTERDAM et al., 1974). That dyssynergy of the infarcted ventricular segment might be worsened as a result of inotropic stimulation by glycosides remains speculative clinically. Among all the factors responsible for the reduced hemodynamic response to digitalis in the infarcted heart, the most important appears to be that the normal portion of the ventricle is already operating at the near-peak level of increased contractile state owing to cardiac sympathetic stimulation and elevated blood-borne levels of endogenous catecholamines, coupled with the fact that the necrotic muscle zone is incapable of physiologically meaningful responses to cardiovascular drugs.

E. Dose–Contractile Response Relationship

The traditional concept of the relation between the dose and the positive contractile action of digitalis has been that little contractile benefit is achieved until a certain digitalizing dose is reached, and the positive contractile action of the glycosides then diminishes as higher doses are administered and toxicity is approached. However, recent evidence indicates a linear relation between therapeutic dose and contractile response (KLEIN et al., 1971; LEE et al., 1972; WILLIAMS et al., 1966). This linear dose–response relation has been demonstrated in isolated ventricular papillary muscles from cats for digoxin, ouabain, and acetylstrophanthidin (MASON et al., 1972). Thus, small or large quantities of the glycosides have the same qualitative contractile action, the extent of which is proportional to the dose employed. Therefore, a patient need not receive a maximally tolerated dose of digitalis to achieve some salutary effect. Even small amounts of the glycoside provide some therapeutic action, a point to be kept in mind when the agent is used in patients who may be prone to toxicity.

F. Time Course of Contractile Action

The time to maximal inotropic effect in patients differs widely among the various glycosides, even after intravenous administration. To investigate the time course

of contractile action of the glycosides, LEE et al. (1972) determined the temporal inotropic responses of papillary muscle to doses producing a 50% increase in peak tension of acetylstrophanthidin, ouabain, and digoxin in oxygenated Krebs solution for periods of 2 h. It was found that the contractile force responses to equivalent doses of the three agents increased similarly to a plateau after 2 h. Apparently the differences in onset and peak action among these glycosides administered parenterally are largely the result of their different serum protein bindings or metabolism and not due to differential effects of the preparations on heart muscle.

G. Unified Concept of Digitalis Cardiocirculatory Effects

I. Failing Versus Normal Heart

In contrast to its effects on the normal heart (MASON and BRAUNWALD, 1964), digitalis reduces peripheral vasoconstriction (both arterial and venous) in congestive heart failure (MASON and BRAUNWALD, 1964). The elevated systemic vascular resistance and venous tone seen prior to the administration of the glycoside represent characteristic compensatory mechanisms in heart failure that are largely produced by increased activity of the sympathetic nervous system (MASON et al., 1970). With the administration of digitalis, its direct positive inotropic action results in augmentation of low cardiac output with decline in cardiac enlargement. The large rise in cardiac output allows reflex withdrawal of increased sympathetic activity in the

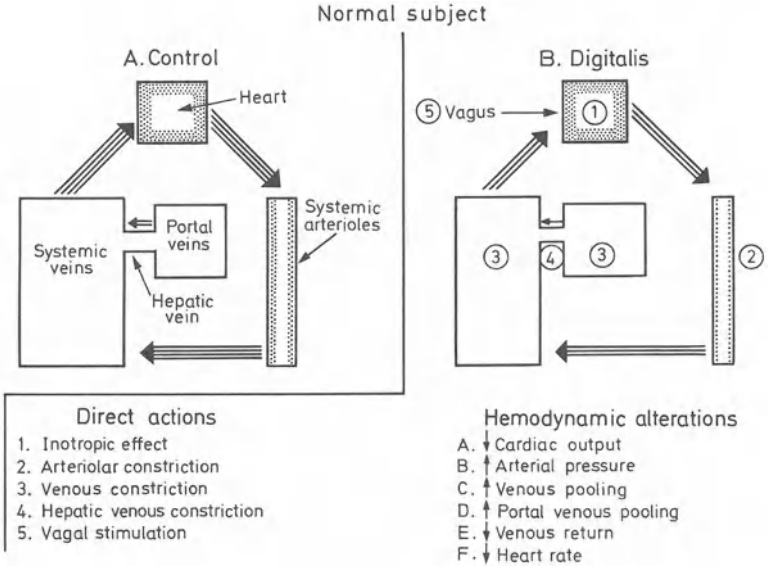

Fig. 8. Diagrammatic representation of the hemodynamic effects of digitalis in a normal subject. The cardiocirculation is shown in the control state and after digitalis administration. Direct actions of the glycoside on the heart, peripheral vessels, and the vagus, as well as hemodynamic alterations resulting from the direct actions of the agent are shown. *Circled numbers* refer to direct actions of digitalis. Width of *arrows* indicates degree of blood flow. (MASON, 1974)

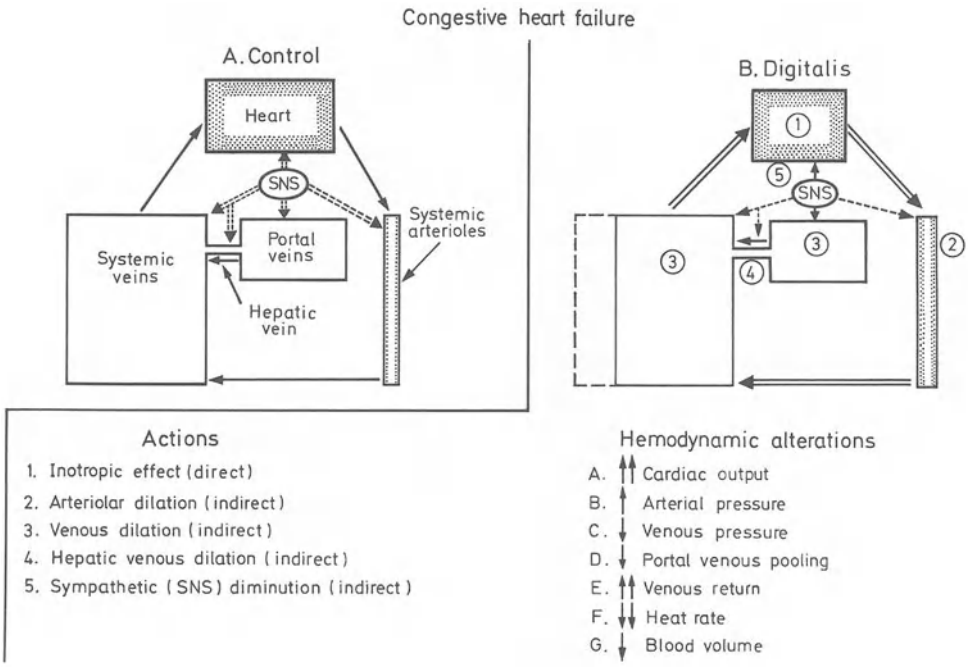

Fig. 9. Diagrammatic representation of the hemodynamic effects of digitalis in a control subject and a patient with congestive heart failure. *Broken arrows* represent activity of the sympathetic nervous system (SNS). *Circled numbers* refer to direct and indirect actions of digitalis. *Broken line* indicating expansion of the systemic venous reservoir in represents an initial increase in blood volume in this compartment; later the size of this compartment is reduced after diuresis with decline in total blood volume. Width of *arrows* indicates degree of blood flow. (MASON, 1974)

peripheral vascular beds, thereby leading to systemic arterial and venous dilation (MASON and BRAUNWALD, 1968). This indirect vasodilation is greater in magnitude than the glycoside stimulation of vascular smooth muscle and thereby overrides the smaller, direct vasoconstrictor action of digitalis.

Evidently, digitalis possesses both direct and indirect cardiac and peripheral vascular effects, and the overall alterations of hemodynamics depend on the integration of these effects and the cardiac functional status of the patient before administration of the agent. Direct vasoconstriction occurs in consort with increased contractility in the normal heart, resulting in no change in cardiac output (Fig. 8). In contrast, in the failing heart, digitalis-induced increase in contractility improves cardiac output, so that indirect dilation predominates in the peripheral vascular beds (Fig. 9).

II. Digitalis Effectiveness Relative to Type of Heart Disease

The glycosides are of limited value in heart failure that is mainly due to mechanical disturbances, such as constrictive pericarditis, pure valvular aortic stenosis, and

isolated mitral stenosis in normal sinus rhythm (BEISER et al., 1968; CAPONE et al., 1972; MASON and BRAUNWALD, 1968). The glycosides are of most clinical benefit in improving cardiac function when heart failure results from chronic primary and secondary depression of ventricular contractility, such as in nonobstructive cardiomyopathies, coronary artery disease, systemic hypertension, acquired valvular regurgitation, and types of congenital heart disease with chronic ventricular volume overload (MASON, 1974).

H. Conclusions

The fundamental therapeutic hemodynamic action of digitalis is clearly the stimulation of ventricular contractile state. The expression of this increase in contractility is dependent on the integrity of cardiocirculatory status at the time the glycoside is administered. Thus, as a result of interplay between direct and indirect cardiac and vascular effects, variable and even opposite effects on cardiac output, peripheral circulatory dynamics, and myocardial oxygen consumption can take place. In congestive heart failure, stimulation by digitalis of depressed contractile state raises lowered cardiac output. Thereby the direct vasoconstrictor action of digitalis is overriden by an indirect decrease in systemic vascular resistance resulting from sympathetic withdrawal accompanying use of the agent in congestive heart failure. The positive inotropic action of digitalis directly elevates myocardial oxygen consumption. This, in turn, is overriden by indirect decreases in ventricular tension and heart rate which reduce cardiac oxygen needs with digitalis therapy in heart failure.

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CHAPTER 11

The Effect of Disease on Cardiac Glycoside Pharmacokinetics*

G. BODEM, H. R. OCHS, and H. J. DENGLER

Abstract/Summary

The effect of diseases involving kidney, gastrointestinal tract, thyroid, and cardiovascular system on the disposition of digitalis glycosides is reviewed in this article.

The glycosides ouabain and digoxin are cleared predominantly by renal excretion of the intact drugs. Not surprisingly, total clearance of these two compounds is dependent on renal function. Elimination half-life is prolonged and total clearance reduced in patients with reduced creatinine clearance. Likewise, steady-state serum concentrations of digoxin at any given dose become higher as renal function declines. However, individual variability is considerable, thereby limiting the utility of dosage schemes based on nomograms or equations. A further complication is that tissue distribution of digoxin is altered in renal insufficiency, such that serum concentrations take on a different meaning in patients with renal failure. In the case of digitoxin, renal clearance accounts only for part of total clearance. A clear relationship of creatinine clearance to total digitoxin clearance has not been established. However, digitoxin protein binding is reduced in renal insufficiency, requiring a change in the clinical interpretation of total (free plus bound) digitoxin serum concentrations in such patients.

Although digoxin absorption may be impaired in patients with serious malabsorption syndromes, most studies demonstrate normal or near normal absorption despite gastrointestinal disease or extensive ablative surgery. Hepatic cirrhosis does not alter the kinetics of digoxin, but is associated with impaired demethylation of β-methyldigoxin. Although hepatic biotransformation accounts for a substantial fraction of total clearance of digitoxin, elimination of digitoxin is if anything more rapid in patients with liver disease as opposed to healthy controls.

Clearance of both digitoxin and digoxin varies directly with thyroid function. Clearance is increased and half-life shortened in thyrotoxicosis, and the reverse is true for hypothyroidism. This may be explained by parallel changes in creatinine clearance, but further study is needed to define the mechanism of alterations in glycoside clearance in relation to thyroid function.

Continuing refinement of analytic and pharmacokinetic techniques will lead to rapid progress in research in this area, and a need for continuing reevaluation of the state of the art.

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Introduction

The pharmacokinetics of digitalis glycosides have been the subject of extensive review (Iisalo, 1977; Aronson, 1980; Perrier et al., 1977). Research on glycoside kinetics has progressed at a rapid pace, requiring continuing reevaluation of the state of our understanding of this problem. The present article focuses on the effect of disease states (renal, gastrointestinal, thyroid, and cardiac) on the absorption, distribution, and clearance of a number of digitalis glycosides. Evidence is critically reviewed, and interpreted with respect to possible clinical implications.

A. Renal Insufficiency

I. Strophanthin

Strophanthin disposition in renal failure has been evaluated in only two studies. Kramer et al. (1970) determined an elimination half-life of 14 h in normals as compared to 60 h in anuric patients. Similar results were reported by Brass and Philipps (1970) using tritiated strophanthin. They found a half-life value of 18 h in healthy individuals as compared to 68 h in anuric patients. The findings clearly indicate that the elimination half-life of strophanthin is prolonged in renal failure.

II. Ouabain

Selden et al. (1974) studied ouabain plasma levels and its removal by dialysis in 14 dialysis-dependent patients. Each patient received 400 μg unlabeled ouabain at the beginning of a routine 4-h hemodialysis. Nine patients also received 83 μCi tritiated ouabain in this study. After drug distribution was complete, plasma ouabain concentrations (determined by radioimmunoassay) declined exponentially with a mean ($\pm$SD) half-life of 50 ± 19 h in patients. This was significantly longer than the mean of 21 h in normal controls (Selden and Smith, 1972).

The extent of ouabain removal by hemodialysis, measured both by radioimmunoassay of unlabeled ouabain and using tritium count recovery in the dialysis bath, averaged $11.5 \pm 2.2\%$ in the first 2 h and 3.4% in the following 2 h. Thus, ouabain appears to be poorly dialyzable, possibly due to extensive tissue distribution (Marks et al., 1964; Selden and Smith, 1972; Selden et al., 1974). Protein binding cannot explain its poor dialyzability, since the drug is only 5%–10% bound (Kramer et al., 1970).

III. Digoxin

Several authors have reported excessively high serum digoxin levels in patients with renal impairment receiving apparently normal maintenance doses (Beller et al., 1971; Doherty et al., 1975; Marcus et al., 1966). Tables 1 a and 1 b compare steady-state serum levels of digoxin, β-methyldigoxin and β-acetyldigoxin measured 12–18 h after the most recent dose in a series of digitalized patients. At any given dose, mean levels increase with higher serum creatinine concentrations (Table 1 a). More importantly, clinicians were not completely aware of this rela-

Table 1a. Relationship of steady-state serum digoxin levels to serum creatinine concentration among patients receiving a fixed daily dose. Adapted from BODEM et al. (1979)

Glycoside preparation	Dose (mg/day)	No. of patients	Mean (±SEM) steady-state serum digoxin levels (ng/ml)
β-Acetyldigoxin	0.4		
Creatinine < 1.3		103	1.28±0.1
Creatinine > 1.3		32	1.89±0.2
β-Methyldigoxin	0.2		
Creatinine < 1.3		67	1.04±0.1
Creatinine > 1.3		17	1.78±0.3
Digoxin	0.5		
Creatinine < 1.3		26	1.53±0.2
Creatinine > 1.3		10	1.78±0.3

Table 1b. Steady-state serum digoxin concentration in relation to renal function and dose. Adapted from BODEM et al. (1979)

Glycoside preparation	Range of creatinine concentration[a]	Mean (±SEM) serum creatinine concentration (ng/ml)	N	Mean (±SEM) daily dose (mg/day)	Mean (±SEM) steady-state level (ng/ml)
Digoxin	Normal	1.0 (±0.03)	68	0.46 (±0.02)	1.5 (±0.14)
	High	2.3 (±0.30)	21	0.46 (±0.04)	2.7 (±0.74)
β-Methyldigoxin	Normal	1.0 (±0.02)	104	0.23 (±0.01)	1.3 (±0.09)
	High	3.8 (±1.43)	27	0.22 (±0.01)	2.0 (±0.35)
β-Acetyldigoxin	Normal	1.0 (±0.02)	163	0.40 (±0.01)	1.3 (±0.09)
	High	2.2 (±0.24)	46	0.41 (±0.01)	1.7 (±0.21)

[a] Normal: < 1.3 mg/100 ml
High: > 1.3 mg/100 ml

tionship, and tended to administer similar daily doses to patients with normal as well as high creatinine concentrations, yielding higher serum digoxin levels in the latter group (Table 1 b). Figure 1 also illustrates the increase in digoxin serum concentrations with increasing age (OCHS et al., 1978 a). The age-related increase in serum levels probably reflects the decline in renal function in the elderly. This may occur despite normal serum creatinine concentrations.

A high proportion of patients with digitalis intoxication have impaired renal function (BELLER et al., 1971; BODEM et al., 1979). The incidence of adverse effects during clinical use of digoxin is reported to be as high as 18% in the general medical patient population (SHAPIRO et al., 1969) and as high as 40% in patients with severe renal impairment (OGILVIE and RUEDY, 1972).

Increased steady-state serum digoxin concentrations, and more frequent clinical toxicity, is explained by the close relationship between renal function and digoxin clearance. Depending on the injection or infusion time, 60%–80% of an in-

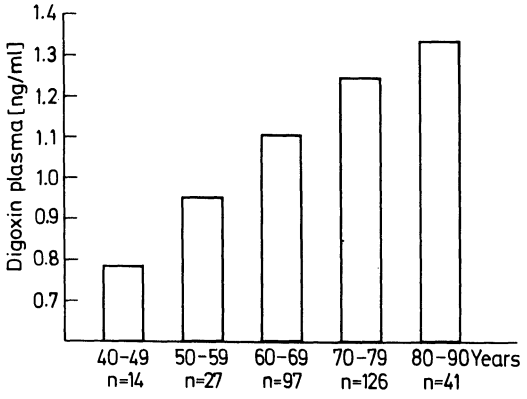

Fig. 1. Mean serum digoxin concentrations in relation to age among a series of patients receiving a fixed dose of β-acetyldigoxin (0.4 mg/day). (Adapted from OCHS et al., 1978a)

travenous dose of digoxin is normally excreted intact by the kidneys (BODEM and OCHS, 1979; GREENBLATT et al., 1974). Since total urinary recovery of digoxin after intravenous administration is less than the infused dose, nonrenal routes of digoxin elimination must contribute to its total clearance. However, renal clearance of the intact drug accounts for the majority of total digoxin clearance.

Both tubular reabsorption and secretion of digoxin have been demonstrated (BLOOM et al., 1966; DOHERTY et al., 1969; MARCUS, 1972; STEINESS, 1974; ROMAN and KAUKER, 1976; WALDORFF et al., 1978). STEINESS (1974) has shown that digoxin serum levels may rise when tubular secretion is blocked by concurrent administration of spironolactone. In patients with hypokalemia, active renal tubular secretion of digoxin is reduced (STEINESS, 1978). This also occurs during combined treatment with digoxin and furosemide. Tubular secretion is enhanced by potassium supplementation.

Numerous reports indicate a linear relationship between creatinine clearance and digoxin clearance (BLOOM et al., 1966; EWY et al., 1969; OHNHAUS et al., 1974; GAULT et al., 1976; JELIFFE, 1967; JELIFFE and BROOKER, 1974). From these relationships several authors constructed nomograms to predict digoxin half-life or maintenance dose (JUSKO et al., 1974a). However, MARCUS (1978) was not able to predict digoxin half-life from creatinine clearance. HALKIN et al., (1975) found that variation in digoxin clearance was more closely related to urine flow and urea clearance than to creatinine clearance. Therefore, the utility of the nomogram approach based upon creatinine clearance remains controversial. Similarly conflicting results have been reported on the relationship between digoxin steady state blood levels and creatinine clearance. Significant correlations were reported by FALCH (1973), GROSSE-BROCKHOFF (1976) and OKADA et al. (1978), but BAYLIS et al. (1972) found no such relationship. Even if digitalis tolerance is increased in uremic patients due to altered myocardial uptake (KRAMER et al., 1978), it seems prudent to anticipate a need for reduced maintenance doses of digoxin in patients with renal insufficiency.

Using radioimmunoassay, JUSKO and WEINTRAUB (1974) studied the relationship of myocardial digoxin distribution at autopsy to estimated ante mortem renal

function. The myocardium-serum concentration ratio became progressively smaller in patients with decreasing creatinine clearance. These altered tissue-serum concentration ratios probably explain the changes in volume of distribution as discussed below (ARONSON and GRAHAME-SMITH, 1976; BRASS, 1970).

DOHERTY et al. (1967) examined the distribution of tritiated digoxin in human tissues in patients who received the labeled drug prior to death. The highest tissue/serum ratios were seen for heart, kidney, and liver, and the lowest for brain. Anephric patients and those with renal failure showed highest tissue/serum concentration ratios in heart, muscle, and liver. In patients with normal renal function, the highest ratio was found in the kidney.

Studies by DOHERTY et al. (1964) showed a 7-day stool excretion of 31% of the dose after oral administration of tritiated digoxin in anephric patients as compared to 12% in normals. After kidney transplantation in these patients, 21% of the radioactivity was detected in the stool within 7 days. Urinary excretion in the same time period was 42% of the dose after transplantation. The serum half-life of digoxin is prolonged from 1.5–2 days in individuals with normal renal function to 4–10 days in dialysis-dependent patients (DOHERTY and PERKINS, 1962; DOHERTY et al., 1967). The results are similar with β-methyldigoxin (KRAMER et al., 1970) and β-acetyldigoxin (BRASS and PHILIPPS, 1970). It must be emphasized that disappearance of digoxin from serum following single doses in humans is best explained by a sum of 2 or more exponential terms, consistent with 2- or 3-compartment pharmacokinetic models (DENGLER et al., 1973; JUSKO and GIBALDI, 1973). Reliable estimation of elimination half-life requires a sufficient duration of sampling after a single dose to ensure that the beta or elimination phase has been reached. Variations in study design and duration of sampling may account for part of the variability in results between different studies. Differences in volume of distribution (Vd) may also account for changes in half-life since half-life depends on both clearance and Vd. KOUP et al. (1975) found Vd values ranging from a mean of 330 liters in patients with severe renal impairment to 530 liters in young, healthy adults. REUNING et al. (1973) estimated an average Vd of 510 liters in normal subjects as compared to 230–380 liters for patients with renal disease.

DENGLER et al. (1973) determined a mean Vd of 375 liters in patients suffering from heart failure but with serum creatinine concentrations in the normal range. Data in young subjects show mean Vd values of 4.9–5.9 liters/kg (OCHS et al., 1978 b). OHNHAUS et al. (1974) also observed considerable variability in Vd (range 3.0–1.7 liters/kg) in patients with differing degrees of renal function. The reduction of Vd in azotemia appears to be unpredictable and not necessarily related to the degree of renal insufficiency. OHNHAUS et al. (1979 a) also reported that absolute bioavailability of oral digoxin was not altered in patients with renal failure despite the decrease in Vd. A decreased Vd in patients with severe renal impairment may be of clinical importance since it implies a need for reduced loading doses. However, the reduced Vd in renal failure is attributable to reduced uptake into tissues, including myocardium. In nine patients with end stage renal disease, an intravenous dose of 0.7 mg resulted in a mean serum digoxin level of 1.43 ng/ml (range: 1.0–2.1) 24 h later, with none of the patients showing evidence of toxicity (GAULT et al., 1976). Although a given loading dose will lead to a higher serum level in a renal failure patient, this does not imply a proportionately higher myocardial level.

Patients with end stage renal disease who weigh 40 kg can tolerate a maintenance dose of 0.0625 mg daily, while the 90 kg patient usually requires 0.1 mg/day (0.125 mg 5 days a week) to maintain a serum digoxin level in the therapeutic range. Digoxin is not removed to any significant extent by hemodialysis, since most of the drug is distributed to tissues. Less than 3% of an intravenously administered dose of digoxin remains in the blood after 1 h (MARCUS et al., 1964). The average amount of digoxin removed during a single 6–10 h hemodialysis has been estimated as being approximately 4% of body stores (IISALO and FORSSTROM, 1974; ACKERMANN et al., 1967). Thus, it is not necessary to adjust the dose of digoxin on dialysis days. Peritoneal dialysis also seems ineffective in removing digoxin (ACKERMANN et al., 1967). In 14 patients who underwent peritoneal dialysis after receiving radioactive digoxin, only 3.9% of the dose was recovered from the dialysate.

IV. Digitoxin

In contrast to the disposition of digoxin, the pharmacokinetic behavior of digitoxin in renal failure is incompletely understood. Since extrarenal routes of clearance are of much greater importance for digitoxin than for digoxin, the relationship of total digitoxin clearance to renal function is complicated.

1. Absorption and Excretion

The rate of digitoxin absorption seems not to be influenced in hemodialysis patients (FINKELSTEIN et al., 1975). However, urinary excretion of intact digitoxin is decreased in renal insufficiency. VÖHRINGER et al. (1976) recovered an average of 5.9% of an oral dose of tritiated digitoxin during 9 days of urine collection in six patients with renal impairment. This compares with 12.7% of the dose in six normal subjects. Although urine collections were imcomplete and therefore underestimated the actual urinary elimination of digitoxin, the findings have been confirmed in other studies (STORSTEIN, 1974a, b; PETERS et al., 1977).

STORSTEIN found that an average of 13.6% of the daily digitoxin dose was excreted at steady-state by 12 patients with reduced renal function (creatinine clearance: 6.6 ml/min) in contrast to 11 patients with mild renal impairment (creatinine clearance: 64 ml/min) who excreted 35.7% of the daily dose. STORSTEIN used the rubidium assay which quantities not only digitoxin but also metabolites which interfere with rubidium uptake. Thus, the excretion of intact digitoxin may be overestimated. LUKAS and PETERSON (1966), using the double isotope dilution derivative assay, recovered an average of 18.7% of the daily dose in the urine of healthy subjects, as opposed to two patients with renal insufficiency who excreted 0.3% and 7.7%.

JELIFFE (1967) reported a relationship between fractional urinary excretion of digitoxin and creatinine clearance. Based on these data he predicted that the half-life of digitoxin would increase from 5.6 days in patients with normal creatinine clearance to 8.7 days in anuric patients. The recommended dosing adjustments in renal failure, however, have not been confirmed by studies during the last 10 years.

Fecal excretion of digitoxin in two patients with kidney disease was studied by LUKAS and PETERSON (1966). Of the administered dose 4.3% and 4.4% was excreted in the stool in contrast to healthy persons who excreted a mean of 13.4±4.2%. VÖHRINGER et al. (1976), however, demonstrated fecal excretion of 8% of the dose in patients with renal impairment, which was higher but not significantly different from the 5.4% value in healthy individuals.

Disappearance of digitoxin from serum has been studied by several authors (LAHRTZ et al., 1969; KRAMER et al., 1970; STORSTEIN, 1974a, b; VÖHRINGER et al., 1974, 1976). LAHRTZ et al. (1969) used radioactive digitoxin which was administered to five patients requiring hemodialysis. Serum levels of radioactivity were higher than in control patients, but half-life could not be reliably calculated due to an insufficient duration of sampling (72 h). KRAMER et al. (1970) found no difference in the half-life of radioactivity in patients undergoing dialysis as opposed to individuals with normal renal function. STORSTEIN (1974a, b) observed a shorter half-life of digitoxin and cardioactive metabolites (3.9 days in patients with renal failure in contrast to 8.1 days in control subject). PETERS et al. (1977) also observed a shortening of the half-life in uremia.

RASMUSSEN et al. (1973) and VÖHRINGER et al. (1976), however, found no significant difference in digitoxin elimination half-life in uremia in comparison to individuals with normal renal function. STORSTEIN (1974a, b) demonstrated an increased transformation of digitoxin to hydroxylated metabolites. VÖHRINGER et al. (1976) did not detect significantly higher digoxin levels by radioimmunoassay in patients with renal failure receiving digitoxin maintenance therapy.

BODEM and VON UNRUH (1979), using a specific GC-MS method, did find higher levels of dihydrodigitoxin in patients with renal failure receiving normal maintenance doses of digitoxin in contrast to patients with cardiac failure and normal renal function.

Thus the effect of renal disease on digitoxin kinetics remains incompletely understood. Small numbers of patients, variations in the specificity of assay techniques, and the long elimination half-life of digitoxin necessitating a prolonged duration of sampling, have all made differences in the results of published studies difficult to reconcile (KRAMER, 1977).

2. Protein Binding

The effect of renal failure on protein binding of digitoxin also is not clearly established. STORSTEIN (1976a, b) reported that protein binding was not changed in patients with renal failure (97.5±4% bound) in comparison to healthy subjects (97.3±0.5%). On the other hand, KRAMER et al. (1974), SHOEMAN and AZARNOFF (1972) and GRABENSEE et al. (1978) all found a significant reduction in the extent of protein binding among patients with severe renal impairment.

STORSTEIN (1976a, b) observed a reduction of protein binding in patients undergoing hemodialysis from approximately 97% prior to dialysis to 95% immediately after the procedure. This was attributed to a heparin-induced release of free fatty acids causing displacement of digitoxin from its binding sites on albumin. However, the alterations in binding were not observed to be associated with any changes in clinical effects.

3. Nephrotic Syndrome

STORSTEIN (1976b) described a significantly reduced half-life of digitoxin in five nephrotic patients (4.9 ± 2.2 days) in contrast to five normals (8.2 days). In severe nephrotic syndrome, hypoalbuminemia caused by the renal protein loss is accompanied by a slight diminution of digitoxin protein binding. STORSTEIN determined a mean value of $96.2 \pm 1.4\%$ bound digitoxin which appears to be only slightly smaller than the $97.3 \pm 0.5\%$ observed in controls. However, a change from 3% to 4% unbound drug means approximately a 30% relative increase. Nephrotic patients had a larger volume of distribution (1.0 ± 0.3 liter/kg vs. 0.6 ± 0.1 liter/kg) and increased clearance of digitoxin (0.1 ml/min per kg vs. 0.035 ml/min per kg) based on total (free plus bound) digitoxin concentrations. Since $t_{1/2}$, Vd and clearance are based on total rather than umbound drug, the actual unbound Vd and intrinsic clearance differ much less between groups.

B. Gastrointestinal Disease

The mechanisms regulating the absorption of digoxin from the gastrointestinal tract in humans are not known. In most studies passive diffusion accounts for glycoside absorption (DAMM et al., 1975). Only for the more polar glycosides (oubain, digoxin, convallatoxin) is there any suggestion of active transport (DAMM et al., 1975).

The following section describes the extent to which disease states and surgical reconstruction of the gastrointestinal tract can alter the pharmacokinetics of cardiac glycosides.

I. Effect of Surgical Intervention on Digoxin Absorption and Excretion

The biologic availability of digoxin tablets was evaluated during maintenance therapy in ten hospitalized patients who had had a Billroth II gastric resection at least 2 years previously (OCHS et al., 1975a). Urinary excretion and serum levels over a 10-day period were compared with those of control persons without gastrointestinal disease. The mean daily urinary excretion of digoxin was $38\% \pm 0.7\%$ of the administered dose in gastrectomized patients, and the serum digoxin level 24 h after the last dose (0.5 mg) was 1.3 ± 0.04 ng/ml. These values did not significantly differ from those in the control group, nor were there differences in digoxin elimination in urine or the digoxin/creatinine excretion ratio (Fig. 2).

These results were supported in a study using radioactive digoxin in five patients with partial gastrectomy, one patient with vagotomy and pyloroplasty, and one patient with jejeunocolostomy (BEERMANN et al., 1973). Blood levels and cumulative excretion of radioactivity in all patients were within the limits previously encountered in healthy persons (BEERMANN et al., 1972). KRAUSZ et al. (1979) found that digoxin absorption was not impaired in a patient with complete small bowel resection except for the proximal 15 cm of jejeunum. Also, MARCUS et al. (1977) found no differences in digoxin absorption in seven patients before and after jejeunoileal bypass. Thus, these surgical interventions have little if any effect on the extent of digoxin absorption.

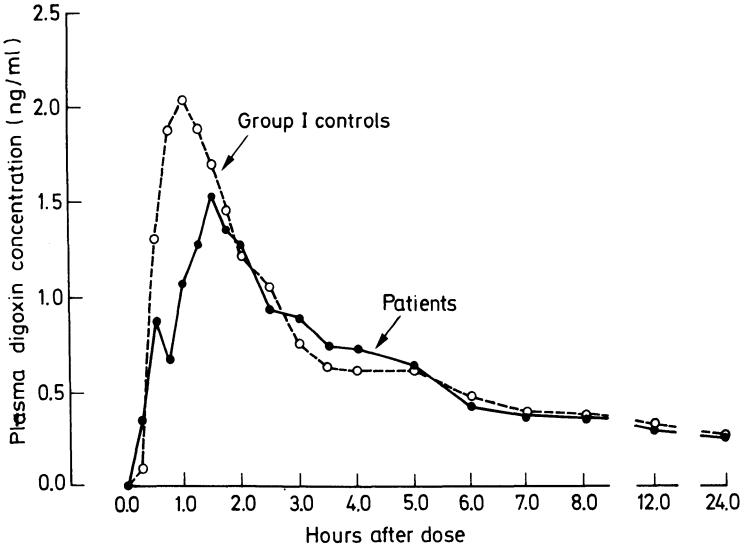

Fig. 2. Serum digoxin levels in controls and radiated patients. (SOKOL et al., 1978)

II. Effect of Abdominal Radiation Therapy on Digoxin Absorption and Excretion

Two studies have demonstrated that radiation therapy of the abdomen (or less probably, the underlying disease) may be associated with malabsorption of digoxin (SOKOL et al., 1978; JUSKO et al., 1974b). SOKOL et al. (1978) found that 24-h urinary excretion of digoxin in radiated patients was significantly less than in controls. Neither age, sex, nor renal function explained the difference. Plasma digoxin concentration in radiated patients, however, were significantly lower than in controls only at 0.75 h and 1 h after a single 0.5 mg oral dose (Fig. 3). JUSKO et al. (1974b) found that a patient with radiation-induced malabsorption syndrome absorbed digoxin poorly from a tablet preparation. Substitution of digoxin elixir overcame the bioavailability problem. The findings suggest that radiation therapy of the abdomen might lead to reduced bioavailability of digoxin tablets.

III. Malabsorption Syndrome

Absorption of digoxin has been evaluated in patients with malabsorption and maldigestion under steady state conditions (HEIZER et al., 1971) and after a single radiolabeled dose (HALL and DOHERTY, 1974). At steady state, mean serum digoxin levels for nine patients with malabsorption were significantly less than those in the control group, whereas levels for two patients with pancreatic insufficiency did not differ from controls. The authors concluded that digoxin is poorly and erratically absorbed by patients with malabsorption because of mucosal defects or hypermotility, but may be more normally absorbed by patients with pancreatic insufficiency (HEIZER et al., 1971). HALL and DOHERTY (1974) did not confirm these results after administration of a single dose of tritiated digoxin in liquid formulation. KOLIBASH et al. (1977) observed, in a single patient, a marked decline in serum digoxin concentrations during an episode of severe diarrhea.

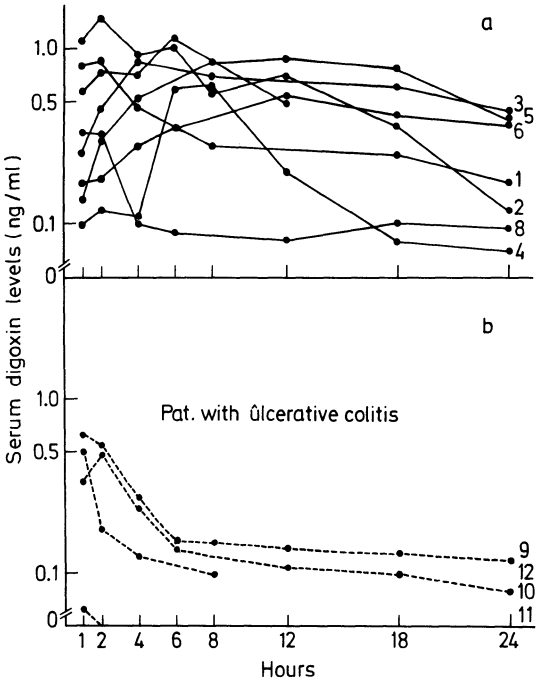

Fig. 3 a, b. Serum concentrations of digoxin in two groups of patients: **a** normal colon mucosa, **b** ulcerative colitis. (OCHS et al., 1975 b)

Absorption of digitoxin in patients with chronic diarrhea due to hyperthyroidism, cirrhosis, cancer of the pancreas and other diseases was studied by TAKANASHI et al. (1978). Lower serum digitoxin concentration during 6 h after a dose was found in patients as opposed to healthy controls. However, the findings are difficult to interpret due to the short observation time. MYHRE et al. (to be published) studied the kinetics of digitoxin in patients with malabsorption due to celiac disease, or with rapid intestinal transit due to postgastrectomy dumping syndrome in comparison to healthy control subjects. Absorption of digitoxin was rapid in both groups of patients, but the extent of absorption was reduced in comparison to controls. Biologic availability based on 24 h of sampling was only 40.6% in celiac disease and 50.4% in gastrectomized patients with dumping syndrome. However, when digitoxin was also given intravenously to three patients with celiac disease, serum digitoxin levels again were lower than those in controls. Since protein binding was identical among groups, malabsorption as such cannot be the only factor responsible for reduced bioavailability. Drug distribution or clearance may also be changed.

IV. Absorption of Digoxin from the Colon in Normal Subjects and Patients with Colitis

In 12 patients undergoing coloscopy, 0.5 mg digoxin in solution was injected into the transverse colon (OCHS et al., 1975 b). The 24-h urinary excretion of 17% $\pm$ 3.4% of the dose in eight patients with normal colonic mucosa is consistent with

extensive absorption from the distal bowel. In four patients with ulcerative colitis, only 1.7% ± 0.6% of the dose was excreted in 24 h. Similar findings were reported by ANDERSSON et al. (1975) who injected digoxin solution into the sigmoid colon and measured glycoside concentrations simultaneously in portal and peripheral venous blood. They also demonstrated considerable absorption of digoxin from the sigmoid colon.

Contrary to earlier suggestions (WAGNER, 1974), colonic absorption of digoxin is possible.

V. Kinetics of Digoxin and β-Methyldigoxin in Patients with Acute Hepatitis and Cirrhosis

In normal subjects, digoxin and β-methyldigoxin are metabolized in the liver to a small but significant degree (DOHERTY et al., 1970; RIETBROCK and ABSHAGEN, 1973; DOHERTY et al., 1961). MARCUS and KAPADIA (1964) demonstrated that the quantitative excretion and metabolite profile following administration of tritiated digoxin to patients with severely impaired liver function due to cirrhosis was similar to that in healthy controls. ZILLY et al. (1975) elucidated the pharmacokinetics and metabolism of intravenously administered tritiated digoxin and β-methyldigoxin in patients with acute hepatitis. Digoxin plasma levels in patients were comparable to those in controls, and the recovery of the label in urine was identical in both groups. However, plasma glycoside concentrations were higher in patients with acute hepatitis than in control subjects after administration of β-methyldigoxin. This increase may be attributable to decreased demethylation in hepatitis. SOMOGYI et al. (1978) measured serum levels of digoxin and β-methyldigoxin during and after recovery from acute hepatitis. They found no difference in serum concentrations for either glycoside during and after hepatitis.

VI. Pharmacokinetics and Metabolism of Digitoxin in Patients with Chronic Active Hepatitis

STORSTEIN and AMLIE (1979) reported that protein binding of digitoxin in hepatitis patients is significantly reduced (96.2% in comparison to 97.3% in healthy controls, $P < 0.0025$), probably due to the lowered serum albumin levels. Digitoxin was eliminated significantly more rapidly, with a mean $t_{1/2}$ of 4.4 days in this group of patients compared to 8.2 days in healthy persons (Fig. 4). The shortened half-life cannot be explained by an increase in renal excretion of digitoxin or cardioactive metabolites, since the cumulative excretion during 8 days was nearly the same in normals and hepatitis patients. Total body clearance of digitoxin and cardioactive metabolites was more than twice as high in patients with liver disease (4.77 ml/min) as in control subjects (2.22 ml/min) ($P < 0.0005$). Renal clearance of digitoxin, however, did not differ between both groups. The results of STORSTEIN and AMLIE (1979) indicate that the shortened half-life in chronic active hepatitis is attributable to increased biotransformation of digitoxin to its metabolites. This assumption was supported by thin layer chromatographic studies of digitoxin and seven of its cardioactive metabolites; patients with chronic hepatitis had less unchanged digitoxin in serum ($P < 0.0025$) and urine ($P < 0.0025$).

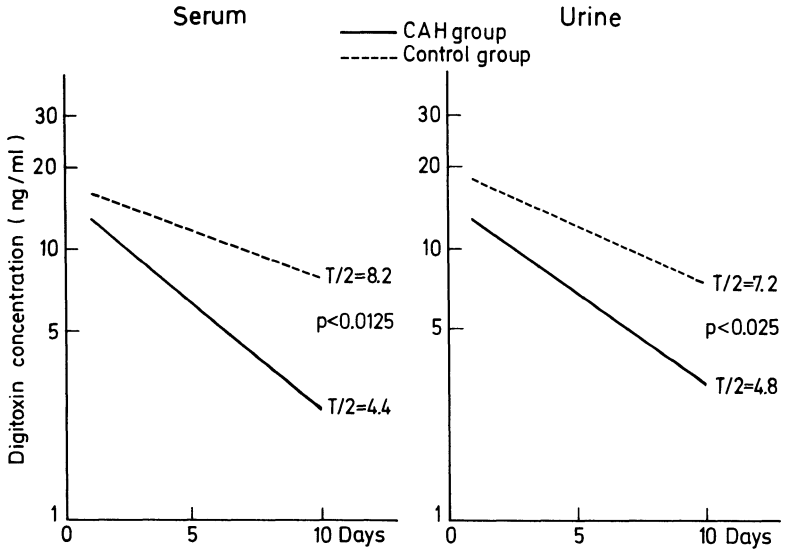

Fig. 4. Serum elimination $t_{1/2}$ and urinary excretion of digitoxin in patients with chronic active hepatitis compared with control subjects. (STORSTEIN and AMLIE, 1979)

VII. Kinetics of Digitoxin in Patients with Acute and Chronic Hepatic Insufficiency

ZILLY (1979) investigated the kinetics and metabolic changes after a single dose of tritiated digitoxin in patients with acute hepatitis ($n=4$), compensated ($n=3$) and decompensated ($n=1$) portal cirrhosis. Six healthy persons served as controls. In addition, steady-state levels of digitoxin were determined by radioimmunoassay in ten patients with acute hepatitis, four with compensated and four with decompensated cirrhosis. After a single dose of digitoxin, total clearance, elimination half-life and volume of distribution were within normal range in patients with acute hepatitis. In those with decompensated cirrhosis, the elimination of digitoxin was enhanced, due to increased renal excretion of the glycoside and its metabolites. The chloroform-extractable urinary metabolites were not different from those found in normals. Plasma protein binding of digitoxin in these patients was diminished. Under steady state conditions acute hepatitis and cirrhosis did not lead to excessive accumulation of digitoxin.

LAHRTZ et al. (1969) studied the elimination of tritiated digitoxin in patients with biliary obstruction and other liver disease. Excretion of radioactivity in stool and urine was similar to that in controls. However, the decay of radioactivity was only measured over 72 h, making it impossible to estimate half-life reliably.

C. Thyroid Disease

Hyperthyroid patients appear to require larger doses of digitalis (WATTERS and TOMKIN, 1975; KLASSEN et al., 1977). Unusually high doses may be needed to control ventricular rate in atrial fibrillation due to thyrotoxicosis (WENCKEBACH and WINTERBERG, 1927; BOAS, 1931; BARKER et al., 1932, FRYE and BRAUNWALD, 1961;

MORROW et al., 1963). However, hypothyroid patients need smaller doses of cardiac glycosides to treat congestive heart failure (MORROW et al., 1963).

The altered sensitivity to digitalis in patients with thyroid disease has been attributed to a change in intrinsic myocardial function (BUCCINO et al., 1967; PEACOCK and MORAY, 1963; MORROW et al., 1963). The "resistance" to digoxin in thyrotoxicosis might be due to similar actions of thyroxine and digoxin on sodium- and potassium-dependent ATPase (LINDSAY and MARKER, 1976). However, alterations in glycoside kinetics are another possible explanation, and have been evaluated by several investigators using either radioactive glycosides (DOHERTY and PERKINS, 1966; EICKENBUSCH et al., 1970) or radioimmunoassay (CROXSON and IBBERTSON, 1975; GILFRICH and MEINERTZ, 1978; SHENFIELD et al., 1977). Interpretation of results obtained with radioactive glycosides is difficult because methods differ in specificity (EICHELBAUM, 1976).

DOHERTY and PERKINS (1966) were the first to examine the disposition of tritiated digoxin in relation to altered thyroid function. Doses of 0.75–1.5 mg tritiated digoxin were administered intravenously or orally to 13 hyperthyroid, 10 hypothyroid and 12 euthyroid patients. Levels of radioactivity were lowest in hyperthyroid, intermediate in euthyroid and highest in hypothyroid patients, suggesting that these pharmacokinetic changes might explain altered sensitivity. They found an inverse relationship between ^{131}iodine thyroid uptake and serum digoxin levels 5 h after intravenous administration. The serum half-lives in the three groups were not significantly different, nor were the half-lives of urinary excretion. In two hyperthyroid patients who became hypothyroid following treatment with radioiodine, no significant changes in the plasma half-live or cumulative urinary excretion of radioactivity in urine or stool were observed. The same was true for a hypothyroid patient who was treated with thyroid hormone and became euthyroid.

To explain the lower or higher plasma digoxin levels in thyrotoxicosis and myxedema, respectively, DOHERTY and PERKINS (1966) proposed an altered volume of distribution of digoxin in these conditions. ISMAIL-BEIGI and EDELMAN (1971) showed higher tissue levels of sodium-potassium ATPase activity in thyrotoxic animals. Therefore, the suggestion of changes in tissue concentrations or distribution of digoxin in thyroid dysfunction may be valid. Experiments with euthyroid, hyperthyroid and hypothyroid dogs, however, failed to demonstrate significant changes in tissue digoxin in relation to thyroid function, while the serum level changes were similar to those seen in patients (DOHERTY and PERKINS, 1966).

CROXSON and IBBERTSON (1975) using radioimmunoassay studied steady-state serum digoxin concentrations in 17 hyperthyroid and 16 hypothyroid patients 24 h after the final oral dose. These patients had been treated with 0.5 mg digoxin daily for 7 days. The mean serum digoxin levels were 0.67 ng/ml (range 0.3–1.7 ng/ml) in the hyperthyroid patients and 1.46 ng/ml (range 1.0–2.0 ng/ml) in the hypothyroid patients. Serum concentrations and plasma half-lives of digoxin were significantly different between the two groups. In two hyperthyroid patients, cumulative urinary digoxin excretion over the 7-day period was 41% and 39%, and in one hypothyroid patient 23% of the given dose.

Since creatinine clearance is elevated in hyperthyroidism and decreased in hypothyroidism, alterations in digoxin kinetics in thyroid disease may be attributable to changes in renal function (BRADLEY et al., 1974). These assumptions were con-

firmed by LAWRENCE et al. (1977), who administered tritiated digoxin to nine hyperthyroid and four hypothyroid patients. Mean plasma radioactivity did not differ between the two groups, but digoxin clearance and glomerular filtration rate were significantly correlated.

Reduced intestinal absorption in thyrotoxicosis is also a possibility. HUFFMAN et al. (1977) showed an increase in serum digoxin concentration from 0.3–0.4 to 1.4 ng/ml, and a similar increase in daily urinary excretion of digoxin, 3 months after treatment with ^{131}I in a patient receiving 0.25 mg daily. Steady-state levels of digoxin as well as daily urinary excretion were similar with 0.3 mg per day intravenously and with 0.75 mg per day orally. This suggests that only 35%–40% of the dose was absorbed by this thyrotoxic patient, compared to the usual value of 65%–70%. Lower levels of digoxin measured by radioimmunoassay were also observed by SHENFIELD et al. (1977) in four hyperthyroid patients after oral administration of a 0.5 mg dose. Four hypothyroid patients were also studied. In only one patient was the plasma digoxin level significantly higher before than after several months of thyroid treatment; digoxin values reached in the other patients were either the same as, or lower than, before treatment. GILFRICH and MEINERTZ (1978) administered 1 mg digoxin intravenously to eight patients with thyrotoxicosis. The same patients were studied after they became euthyroid with treatment. Plasma levels of digoxin seemed to decline more rapidly during thyrotoxicosis, but precise pharmacokinetic calculations were not possible. Therefore, urinary excretion rates of digoxin were used to calculate the half-live of digoxin. Excretion half-live was approximately 24 h in the thyrotoxic state as compared with 40 h in the same patients after thyroid function had returned to normal. Cumulative urinary excretion of digoxin was appreciably lower during thyrotoxicosis (51% of the dose) than after normalization of thyroid function (78%). Since the drug was given intravenously, a decrease in the extent of absorption during hyperthyroidism cannot account for these differences.

Similar changes in plasma levels of tritiated ouabain and digitoxin have been observed by EICKENBUSCH et al. (1970) in patients with thyrotoxicosis or myxedema. Following the intravenous administration of tritiated ouabain to hypothyroid patients, higher plasma levels of radioactivity were observed, whereas hyperthyroid patients had lower levels than controls with normal thyroid function. In one hypo- and one hyperthyroid patient who had been treated successfully, the rate of disappearance of tritiated ouabain from plasma was almost identical to that in euthyroid patients. The cumulative urinary excretion of tritiated ouabain over 48 h was 52% in euthyroid, 64% in hyperthyroid and 37% in hypothyroid patients. The same plasma level pattern (lower levels in hyperthyroid and higher levels of radioactivity in hypothyroid patients) was observed after the intravenous administration of tritiated digitoxin. No significant changes in cumulative urinary excretion of tritiated digitoxin during the 72-h collection period were seen between the three groups.

The authors conclude that lower plasma levels of both glycosides in thyrotoxic patients result from increased urinary excretion. Conversely, the lower urinary excretion of ouabain and digitoxin during hypothyroidism would explain the higher plasma levels under these conditions. However, interpretation of the data is complicated, since plasma levels and urinary excretion of tritiated digitoxin were mea-

sured only for 72 h after the dose. Furthermore, possible interference by tritiated water was not considered.

D. Cardiovascular Disease

Although the clinical use of digitalis glycosides is almost entirely confined to patients with cardiovascular disease, there is minimal information on the effect of such a disease on glycoside pharmacokinetics. The absorption of oral digoxin was not impaired in a series of patients with right-sided congestive failure (OHNHAUS et al., 1979 b). In 12 patients with left-sided failure due to acute myocardial infarction, the rate of digoxin absorption was slowed in comparison with healthy controls (KORHONEN et al., 1979). The effect was probably due to coadministration of other drugs that altered gastrointestinal motility. The completeness of digoxin absorption was not different between patients and controls. Thus, cardiovascular disease as such does not alter digoxin clearance based on currently available data. However, reduced clearance should be anticipated when the disease is severe enough to reduce creatinine clearance.

E. Conclusion

The findings discussed in this paper raise more questions than they answer. Few conclusions or recommendations can be made with certainty. Impairment of renal function impairs glycoside clearance for those drugs whose major route of clearance is renal excretion of the intact compound. However, renal insufficiency also alters tissue uptake of glycosides, such that serum concentrations as such require a different approach to interpretation. Glycoside absorption is surprisingly resistant to interference from extensive gastrointestinal disease or major gastrointestinal surgery. The effects of liver disease on glycoside disposition are conflicting and difficult to understand. Sensitivity to digitalis glycosides is suspected to vary inversely with thyroid function. This may be explained by increased glycoside clearance in thyrotoxicosis and, conversely, reduced clearance in hypothyroidism. However, the mechanism of these changes is not clearly defined.

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CHAPTER 12

Clinical Indications and Choice of Cardiac Glycosides, Clinical Conditions Influencing Glycoside Effects

F. GROSSE-BROCKHOFF and U. PETERS

A. Indications for Glycoside Therapy

I. General Considerations

The therapeutic use of digitalis glycosides and strophanthin is governed by their fundamental effects, i.e., positive inotropic (increase in contractile force), negative chronotropic (reduction of heart rate), negative dromotropic (slowing of conduction), and bathmotropic (lowering of stimulus threshold). The fact that cardiac glycosides in therapeutic doses, besides increasing the contractile force of the heart in cardiac failure, also have the same effect on the nonfailing heart, has only recently been recognized (BRAUNWALD et al., 1961; MASON and BRAUNWALD, 1963; SONNENBLICK et al., 1966). Among the consequences of this new thinking is a change in attitudes to the indications for glycosides, as will be explained later.

The indications, contraindications, and contingent indications for digitalis therapy are set out below:

1. *Indications for digitalis therapy:*
   a) All forms of myocardial insufficiency
   b) Supraventricular arrhythmias with tachycardia.
      Absolute tachyarrhythmia in cases of atrial fibrillation or flutter.

2. *Contraindications to digitalis therapy:*
   Absolute: Obstructive cardiomyopathy (IHSS, HOCM)
   Relative: Bradyarrhythmias; When cardioversion is planned; Pronounced hypokalemia; Pronounced hypercalcemia.

3. *Contingent indications for digitalis therapy:*
   Mitral stenosis; Chronic cor pulmonale; Angina pectoris; Myocardial infarction; Myocarditis; Hypertension.

The property of greatest importance from the therapeutic viewpoint is the augmentation of contractile force (positive inotropy). For this reason acute or chronic myocardial failure constitutes the principal and most rewarding field of indications for cardiac glycosides. In addition, the heart benefits from the reduction in sinus rate, which makes for greater economy in cardiac work. In higher dosage cardiac glycosides impede the conduction of the cardiac impulse. This attribute is utilized to reduce ventricular frequency in the treatment of absolute tachyarrhythmia associated with atrial fibrillation or flutter, and also in the treatment of paroxysmal tachyarrhythmia. On the other side of the coin is the fact that slowing of conduction in the atrium-AV node region and the Purkinje system entails the danger of provoking certain forms of heart block and heterotopic arrhythmias, in particular

extrasystoles. On the other hand, those arrhythmias which arise as a result of severe cardiac dilatation in cases of coronary insufficiency frequently disappear when digitalis is administered.

II. The Pathogenesis and Severity of Myocardial Insufficiency as Factors Governing the Indications for the Management of Digitalis Therapy

In addition to manifest myocardial failure, there are two other conditions which must be distinguished. The first is latent myocardial failure, which presents as myocardial insufficiency during exercise, and the second is compensated heart failure. By proper use of digitalis glycosides or diuretics with or without vasodilators such patients' compensation can be maintained. In keeping with these views, in 1945 the New York Heart Association has classified myocardial insufficiency into grades 1–4 as judged by functional criteria. One of the factors governing the decision whether or not to give digitalis and, if so, in what dose, is the grade of myocardial insufficiency. For example, though grade 4 demands moderately rapid or even rapid digitalization, in grade 2 it is enough to start treatment with the maintenance dose, and indeed it may even be possible to achieve compensation by other measures, e.g., modifying the patient's mode of living. Cases of heart failure due to myocardial insufficiency must be carefully distinguished from cases where extracardiac factors are responsible. Among such conditions are thyrotoxicosis, hypertensive crises, and hypervolemia due to fluid retention in acute renal failure. In such cases the first line of treatment must be antithyroid drugs, antihypertensive agents, or elimination of fluid by hemofiltration. Cardiac glycosides constitute the second line of treatment, and may not even be required at all.

Several conditions figure in the pathogenesis of myocardial failure. It may be due to patchy rarefaction of functioning myocardium resulting from disseminated myocardial fibrosis in patients with coronary heart disease or it can be the result of a large scar in the heart wall (cardiac aneurysm), the sequel of a healed myocardial infarct. Alternatively, it may be the outcome of chronic hypertensive or high output overloading as in cases of congenital or acquired cardiac defects, arterial or pulmonary hypertension, or hypertrophic obstructive cardiomyopathy. Among other primary cardiac causes, mention should be made of congestive cardiomyopathy, the secondary cardiomyopathies associated with alcoholism and vitamin B_1 deficiency, and various forms of myocardial inflammation. Among other causes are conditions such as endocarditis fibroplastica (Löffler) and other forms of endocardial fibrosis which restrict the compliance of the myocardium. Lastly, there is the state of bradycardiac heart failure resulting excessive slowing of the heart and ineffective hemodynamic action. In such cases the possibility of overdigitalization must always be remembered.

The commonest causes of myocardial failure are coronary heart disease, arterial hypertension, and valve lesions. In most such patients treatment with cardiac glycosides is highly successful. However, myocardial failure due to the primary cardiomyopathies or to conditions which restrict the movement of the heart calls for different treatment, which will be discussed later. Before starting glycoside therapy it is essential to exclude the diseases set out in the list below, because the admin-

istration of cardiac glycosides to such patients will be unavailing unless the way has previously been prepared by appropriate treatment with steroids, immunosuppressive agents, or antidiabetic drugs:

1. Myocarditis associated with:
   a) Rheumatic disease; b) Lupus erythematosus; c) Dermatomyositis; d) Scleroderma; e) Polyarteritis nodosa; f) Eosinophilic myocarditis (Löffler); g) Wegener's granulomatosis; h) Fiedler's giant-cell myocarditis.
2. Metabolic disorders:
   a) Diabetic coma; b) Uremia; c) Hormonal disorders, especially hyperthyroidism and hypothyroidism.

III. Contraindications

Muscular subvalvar aortic stenosis (IHSS or obstructive cardiomyopathy) is an absolute contraindication to digitalis therapy, because the inotropic action of digitalis may worsen the muscular stenosis. However, it should be borne in mind that the onset of myocardial failure with dilatation of the ventricles may modify the hemodynamic conditions and that the danger of worsening the obstruction by giving digitalis may diminish or disappear altogether. In such circumstances it is possible to use beta-blockers and digitalis in combination and by cautious regulation of dosage and by careful attention to their advantages and dangers to distinguish between their respective effects. Among the relative contraindications to digitalis are the various forms of SA and AV block, which invariably call for caution in dosage. The use of pacemakers has largely overcome the former difficulties of recompensation with digitalis in such arrhythmias. Digitalis therapy should not be started before planned cardioversion; if the patient is already digitalized, treatment should if possible be discontinued a few days beforehand because of the danger of provoking arrhythmias. A watch must be kept for hypokalemia or hypercalcemia, either of which calls for careful observation and cautious regulation of dosage.

IV. Special Factors Governing the Indications for Glycoside Therapy in Various Heart Diseases

The diseases listed later as contingent indications for digitalis therapy present certain special features which influence the use of digitalis glycosides.

1. Mitral Stenosis

Patients with mitral stenosis can be expected to derive benefit from digitalis only if there are signs of right ventricular failure or if there is atrial fibrillation with a rapid heart rate. No benefit from digitalis has been observed in patients with mitral stenosis who are in sinus rhythm and have no signs of right heart failure (Beiser et al., 1968).

2. Chronic Cor Pulmonale

The physician who wishes to use digitalis in patients with chronic cor pulmonale is confronted by two main problems. First, the customary dosage of digitalis gly-

cosides is less effective than would normally be expected, because although the contractile force of the right ventricle is enhanced, the pressure overload remains unchanged. Second, clinical experience has shown that patients with cor pulmonale tend to be excessively sensitive to digitalis (FERRER, 1975; SCHÜREN et al., 1974).

It has been proved that there is invariably an abnormal end-diastolic pressure rise in the right ventricle during exercise in patients with chronic cor pulmonale. This is independent of the grade of heart failure and is not significantly influenced by digitalis. Cases of chronic cor pulmonale have been divided into two types: type A or the "pink puffer" in which emphysema predominates and type B or the "blue bloater" in which bronchitis is the main feature. Investigations of the response of these two types to digitalis gave the following results (JEZEK and SCHRIJEN, 1973; SCHÜREN and HÜTTEMANN, 1974): If right ventricular function is depicted by a curve (Fig. 1) constructed by plotting end-diastolic right ventricular pressure against right ventricular work under conditions of rest and exercise, it is found that digoxin, given to patients of group B, displaces the curve upwards and to the left, while given to patients belonging to the mixed group it causes displacement to the left alone. In patients of group A, the hemodynamic determinants of right ventricular function remain practically unaltered. These investigations indicate that the positive inotropic action of glycosides will produce an improvement in right ventricular function only in those patients who belong to type B (the bronchitic form) or to the mixed type of chronic obstructive airways disease and who display the clinical symptoms and signs of previous or currently existing right ventricular failure.

The heightened sensitivity to digitalis observed in patients with chronic cor pulmonale must be ascribed to hypoxemia, hypercapnia, and increased endogenous catecholamine stimulation of the heart, in addition to the hypokalemia which is commonly present (FERRER, 1975; SCHÜREN et al., 1974). However, signs of digitalis excess in chronic cor pulmonale are not infrequently due to actual overdosage, which arises from the commonly held view that digitalis glycosides are poorly absorbed from the intestine in patients with right heart failure. It has nevertheless been demonstrated that in patients with chronic cor pulmonale the bioavailability of digoxin remains unchanged irrespective of whether they are in a state of myocardial compensation or myocardial decompensation (OHNHAUS, 1978).

3. Angina Pectoris

Cardiologists have long recognized that in patients with angina pectoris due to coronary heart disease without myocardial failure treatment with cardiac glycosides may worsen the symptoms, while giving digitalis glycosides may provoke an acute attack of angina pectoris. This fact can now be understood in terms of the action of cardiac glycosides on myocardial oxygen consumption (BRAUNWALD and MAROKO, 1974; GROSSE-BROCKHOFF et al., 1977; KREBS, 1976; MASON, 1974; SMITH and HABER, 1973; SONNENBLICK et al., 1968). It is tend that digitalis augments contractile force without increasing cardiac oxygen consumption, but this applies only to the failing heart. In patients with manifest myocardial failure cardiac glycosides reduce oxygen consumption, because the decreases in heart rate, in end-diastolic ventricular pressure, in heart size, and therefore in wall tension all tend to diminish

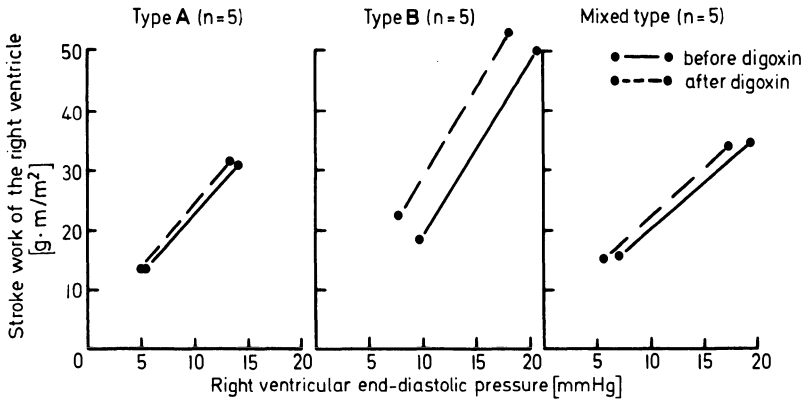

Fig. 1. Effect of digoxin (1.25 mg i.v.) on the parameters of the right ventricular function curve at rest and during ergometer exercise in five patients with chronic cor pulmonale and chronic obstructive airways disease of the predominantly emphysematous type A, five patients with chronic cor pulmonale and chronic obstructive airways disease of the predominantly bronchitic type B, and five patients with the "mixed type" (SCHÜREN and HÜTTEMANN, 1974)

oxygen consumption, whereas any increase in oxygen consumption caused by the speeding up of myocardial contraction is of relatively small significance. When angina pectoris is accompanied by myocardial insufficiency, treatment with cardiac glycosides may reduce the frequency of anginal attacks. However, when the myocardium is not failing, the sole effect of the positive inotropic action of the glycosides is to accelerate contraction rate and hence to increase oxygen consumption. This means that in patients with coronary insufficiency without myocardial failure digitalis glycosides may provoke or accentuate attacks of angina. Digitalis therapy for patients with coronary heart disease and angina pectoris is therefore indicated only when there are signs of manifest or latent myocardial failure. In doubtful cases a cautious trial of digitalis therapy must be carried out.

4. Myocardial Infarction

From numerous studies in the last few years it has become clear that there is no single, universally valid answer to the question of digitalis treatment in acute myocardial infarction (BLEIFELD and HANRATH, 1975; BRAUNWALD and MAROKO, 1974; GROSSE-BROCKHOFF et al., 1977; KARLINER and BRAUNWALD, 1972; LOEB et al., 1973; SCHRÖDER et al., 1972). Clinical studies have shown that the danger of inducing dangerous arrhythmias by administering digitalis to patients with myocardial infarcts is negligibly small. Among the arrhythmias associated with acute myocardial infarction there is one which constitutes a generally accepted indication for digitalis, namely the acute onset of absolute tachyarrhythmia due to atrial fibrillation. Digitalis can be given to patients with acute myocardial infarction for the sake of its inotropic action, but the decision must depend on circulatory indices. Digitalis therapy is clearly indicated in patients with acute myocardial infarction and left ventricular failure (characterized by a left ventricular filling pressure or mean pulmonary artery pressure in excess of 22 mm Hg).

The thoughts on myocardial oxygen consumption set out in Sect. A.IV.3 are of equal relevance to acute myocardial infarction. In other words, measures to improve the pump function of the heart are not necessary in patients with acute myocardial infarction provided the pressure and output values are normal. Such cases make up approximately 50% of all infarct patients. In such circumstances digitalis therapy is accompanied by the disadvantages of increased oxygen consumption and entails some risk of causing extension of the infarct (BRAUNWALD and MAROKO, 1974).

5. Myocarditis

In the various forms of myocarditis, administration of cardiac glycosides cannot be expected to confer benefit unless the underlying disease (e.g., rheumatic or diphtheritic myocarditis) has been effectively controlled by appropriate therapeutic measures.

6. Hypertension

Longstanding hypertension is one of the commonest causes of myocardial failure. However, the question whether and when digitalis glycosides should be used in patients who have no manifest clinical signs of heart failure is one which has occasioned some disagreement. Hemodynamic studies in patients with compensated essential hypertension (STRAUER, 1978) have shown that a single dose of 0.01 mg/kg digoxin causes a significant increase in inotropy (dp/dt_{max}), much as in normal hearts (MASON and BRAUNWALD, 1963), though there is a decrease in cardiac index and stroke volume. At the same time, coronary blood flow decreases and coronary vascular resistance rises. On the other hand, there is no significant change in left ventricular end-diastolic pressure (mean LVED = 15.9 mm Hg before digoxin and 15.7 mm Hg after digoxin). The oxygen consumption of the heart remains constant, a fact which is explained mainly by a decrease in external cardiac work (STRAUER, 1978). These investigations show that no hemodynamic benefit can be expected from digitalis in patients with compensated hypertension. The main aim of our therapeutic endeavours must be to reduce the afterload. However, in patients who have residual signs of heart failure on exercise or indeed clinically manifest heart failure (grades 2–4) despite satisfactory control of hypertension, digitalis glycosides are indicated and will improve cardiac function.

B. Criteria of Adequate Glycoside Treatment

I. Experimental Studies Under Clinical Conditions

Cardiac catheter studies have convincingly shown that digitalis glycosides and strophanthin restore normal cardiac output and stroke volume in patients in whom these indices have been depressed by myocardial failure. They also bring down the elevated end-diastolic ventricular pressure and reduce the increased heart volume (ALTSHULE, 1954; COURNAND et al., 1952; MASON et al., 1969; SMITH and HABER, 1973). In concordance with the results of STARLING and VISSCHER (1927) in heart-

lung preparations, BING (1965) reached the conclusion – based on their comparative measurements of oxygen consumption by healthy and failing human hearts – that in cardiac failure there is no demonstrable evidence of any disorder of the oxybiotic metabolic process itself. There is, however, an abnormality of energy utilization, i.e., of the conversion into mechanical energy, which necessarily entails some deterioration in the efficiency of the work of the heart. These authors demonstrated a significant improvement in efficiency in response to glycoside treatment. This improvement in the efficiency of the work of the heart, or in other words the so-called utilization effect produced by digitalis glycosides, can be explained by assuming that the relief of the cardiac overloading (diminution in the size of the ventricles, reduction in diastolic ventricular pressure and in diastolic fiber tension) brings with it a decrease in the oxygen consumption of the heart which is greater than the increase on oxygen consumption due to the augmentation of cardiac output.

II. Clinical Criteria

Various methods for controlling digitalis therapy based on the indications are listed below:

1. General methods:
   a) Questioning and examination of the patient; b) Electrocardiography; c) Radiologic examination.
2. Special methods:
   a) Quantitative measurement of serum glycoside level; b) Serum and saliva electrolytes ($Ca^{2+} \times K^{+}$).

These simple and inexpensive methods are usually sufficient to ensure adequate control. Despite modern advances, doctors rely mainly on clinical criteria to assess the success of treatment, to detect nonresponders, and to monitor the occurrence of side effects. The success of treatment is measured in terms of cardiac recompensation. As cardiac performance improves, the signs of myocardial failure regress; among the best and simplest criteria for following the response to treatment are the improvement in at rest dyspnea with a decrease in respiratory rate and the elimination of edema fluid with a decrease in body weight. A full list of clinical indices that show improvement in response to digitalis therapy is given below:

1. In patients with manifest heart failure:
   a) Relief of or definite improvement in dyspnoea at rest; b) Relief of or definite improvement in cyanosis; c) Increased urine output (negative fluid balance) with loss of weight and disappearance of edema, ascites, or pleural effusions; d) Relief of venous engorgement; e) Shrinkage of the congested liver; f) Relief of arrhythmias (e.g., tachycardia, extrasystoles, disappearance of any pulse deficit).
2. In patients with signs of heart failure on exercise:
   a) Relief of undue exertional dyspnoea.

The electrocardiogram (ECG) is of limited value for the control of digitalis therapy, except insofar as it can be used to assess absolute tachyarrhythmia. A summary of possible ECG changes is listed below:

1. Normal ECG changes during digitalis therapy:
   a) Prolongation of atrioventricular conduction; b) Trough-shaped ST-segment depression with flattening or inversion of T waves; c) Shortening of ventricular excitation time (QT interval); d) In patients with tachyarrhythmias:
   Decrease in heart rate (established by proper records) and correction of any arrhythmia.
2. ECG changes suggestive of digitalis overdosage:
   a) 1 st-degree AV block; b) Extrasystoles; c) Pronounced bradycardia (< 50 beats/min); d) Supraventricular tachycardia with block; e) Atrial fibrillation; f) Any other form of arrhythmia.

Prolongation of AV conduction time beyond 0.2 s, trough-shaped ST segment depression with T wave flattening or preterminal T wave inversion, and shortening of the QT interval are indeed signs of excessive digitalization, but they are by no means always present. Furthermore, there is no parallelism between the degree of these changes and the intensity of the glycoside effect. In no circumstances should these ECG changes, viewed in isolation, be regarded as signs of glycoside overdosage or taken as a reason for reducing the dose. More importance must be attached to arrhythmias induced by digitalis: they must be regarded as evidence of digitalis overdosage or of reduced glycoside tolerance. The ECG is of crucial significance in revealing arrhythmias of this kind. In principle it is wise to proceed from the assumption that any arrhythmia arising during digitalis therapy should be taken as a warning of possible overdosage or diminished glycoside tolerance.

Objective evidence of the improvement in the signs of myocardial failure brought about by digitalis therapy can also be obtained by radiologic examination. Though it is true that seriously damaged hearts often fail to show any radiologic evidence of reduction in size, the relief of pulmonary congestion can be demonstrated by radiography.

III. Interpretation of Serum Glycoside Measurements

Quantitative determination of serum glycoside levels provides a valuable addition to the methods for controlling digitalis therapy. However, when interpreting a serum glycoside value it must be borne in mind that the concentration of glycosides in the serum is not a wholly reliable guide to the potency of the digitalis effect, because the sensitivity of the myocardium to digitalis is affected by a variety of other factors. Nevertheless, under controlled conditions it has proved possible to demonstrate by echocardiography a significant relationship between steady-state serum digoxin levels and the speed of movement of the posterior wall of the heart (DENGLER et al., 1978; GILFRICH, 1974).

The serum digitalis level is determined by the amount absorbed from the alimentary tract, the interval between doses, the distribution volume, and the serum elimination half-life. In one uniform and homogeneous group of patients the serum levels of digoxin and digitoxin differed by 30%–50% between individuals, though the dose was constant. Part of this variability can be eliminated by matching dosage to body weight. As adipose tissue stores considerably less digitalis than muscle (BENTHE, 1975; KUHLMANN et al., 1978), the distribution volume corresponds most closely to the fat-free body weight. Apart from the dose and kinetics of the drug, one important reason for the individual differences in serum digitalis level seems

to lie in the genetically determined rate of metabolic breakdown (BUTLER and LINDENBAUM, 1975; CLARK and KALMAN, 1974; PECK et al., 1973; PETERS et al., 1977a, 1978a; VESELL, 1974).

The therapeutic range of serum glycoside levels is stated to be 10–35 ng/ml for digitoxin and 0.6–2.0 ng/ml for digoxin, the subtherapeutic range being correspondingly lower (CARRUTHERS et al., 1974; DOHERTY, 1973; FLECKENSTEIN et al., 1977; PETERS et al., 1974; RIETBROCK et al., 1977b, 1978; SHAPIRO et al., 1972; SMITH and HABER, 1970).

In hospital, the physician is frequently presented with a patient who, despite having allegedly taken a normally adequate maintenance dose at regular intervals, displays manifest signs of heart failure on admission. If the serum glycoside level is within the therapeutic range and if it can be confirmed that the patient has in fact been taking adequate doses at regular intervals, there are still many factors which can account for this discrepancy: excessive physical exertion, decompensated hypertension, an acute flare-up of rheumatism in a patient with endomyocarditis, any collagen disease, loss of stabilization in diabetes mellitus, cardiac arrhythmias, myocardial infarction, and severe valve defects. Treatment must be modified in accordance with the findings (see Sect. A, IV).

Measurement of the serum digitalis level not infrequently yields extremely low values (e.g., below 0.5 ng/ml for digoxin or below 10.0 ng/ml for digitoxin). The commonest causes of this phenomenon are as follows:

1. Irregular medication with digitalis or underdosage (CARRUTHERS et al., 1974; WEINTRAUB et al., 1973),
2. Diminished biological availability,
3. Interference with intestinal absorption (binding of digitalis to charcoal, anion exchange resins, or adsorbents; malabsorption syndrome),
4. Accelerated metabolic breakdown (genetic or induced by so-called enzyme inductors),
5. Enlargement of the distribution volume,
6. Displacement from protein binding by other drugs.

Several workers have issued warnings against overrating the value of digitalis estimations (CHAMBERLAIN et al., 1971; FOGELMAN et al., 1971; GROSSE-BROCKHOFF et al., 1977; HAUSAMEN and PETERS, 1976; INGELFINGER and GOLDMAN, 1976; LASAGNA, 1976; SMITH and HABER, 1970). In hospital practice there have been cases in which low serum digitalis levels have been found despite clear signs of intoxication, or in which there have been elevated digitalis levels without any evidence of poisoning. However, in many cases measurement of serum digitalis levels is of more value in making a tentative diagnosis of digitalis intoxication than the information obtained from the patient or the drug history. There are no pathognomonic or specific ECG signs of digitalis intoxication (HAUSAMEN and PETERS, 1976; RISLER et al., 1975). When obvious evidence of poisoning, e.g., colored vision, is lacking, and serum digitalis estimations are not available, the only way of confirming the diagnosis of digitalis intoxication is to suspend treatment and observe the response (HAUSAMEN and PETERS, 1976).

The main objections to overrating the value of digitalis estimations can be summarized as follows:

1. Serum levels give little information regarding the concentration of the drug bound to digitalis receptors or acting upon them.

2. There is some overlap between toxic and nontoxic serum levels, so that sharp separation between these two ranges is impracticable.
3. The subtherapeutic range cannot be clearly defined.
4. Though pharmacodynamically important, the unbound digitalis fraction cannot be measured in isolation.
5. Various factors which interfere with the technique of determination (digitalis mixtures, radioisotopes, intrinsic fluorescence of serum) are not taken into account.
6. If a mixture of metabolites or glycosides is present, measurement of the pure glycoside will not give a true picture of the glycoside concentration acting upon the heart.
7. Digitalis-induced arrhythmias cannot be correlated with the serum level.

Against these criticisms and objections must be set certain advantages which are of value for assessing patients in hospital:

1. The serum digitalis level can be quickly and accurately measured, so that therapy based on serum levels is feasible.
2. In most cases, signs of digitalis intoxication are accompanied by elevated serum levels.
3. In many cases, knowledge of the serum digitalis level enables the physician to discriminate between digitalis-induced and other forms of arrhythmia such as bigeminy (coupled beats), delayed AV conduction, and second- or third-degree AV block.
4. Depending on the specificity of the assay, serum digitalis levels correspond to the cardioactive glycosides in the serum.
5. By measuring the serum digitalis level, the physician can check whether or not the patient has been taking digitalis.
6. Serum digitalis determinations can be used to check the biological availability of digitalis preparations.
7. Pharmacokinetic studies can be carried out under steady-state conditions.
8. In patients who are vomiting or who have abnormalities of central nervous system origin, the serum digitalis level will show whether the patient has been overdosed.
9. Measurements of serum digitalis levels have proved of great value in patients with impaired renal function (see Sect. B, II).

The wide variations in the therapeutic and toxic ranges and the overlap between them make it clear that the management of the glycoside therapy must remain a matter of empirical clinical judgement. As in Withering's day, the physician must be guided by the following maxim: the success or failure of treatment rests upon detailed observation of the individual patient and of the symptoms and signs which he presents after the initiation of glycoside therapy.

C. Guidelines for the Therapeutic Use of Glycosides

I. Significance of the Pharmacological Data

The introduction of the concepts latency, effective dose, therapeutic saturation dose, decay rate, persistence rate, and maintenance dose represents a substantial advance in the management of treatment with cardiac glycosides. Great credit is

due to AUGSBERGER (1951, 1954) for having calculated precise dosage recommendations for the various digitalis glycosides, these calculations being based on data previously published in the literature (GOLD, 1946; DE GRAFF, 1950; SPANG and OBRECHT, 1949). AUGSBERGER introduced the concept of the "effective level" – we prefer to call it the "effective dose" – which represents the quantity of glycoside in milligrams present in the body at any given time, the decay rate having been taken into account. With the aid of the figures which he gives, the physician can ascertain adequate initial and maintenance doses for intravenous or oral administration and can make a comparison between the various digitalis glycosides in terms of their dose-effect relationship. In the same way it is possible to arrive at reliable figures for absorption and elimination and to estimate the appropriate dose when testing new glycosides (e.g., β-acetyldigoxin and β-methyldigoxin) (STORZ, 1966, 1968, 1972). The figures in question are measurements which do not correspond to the values as directly determined, but which are ascertained by clinical observations of the effect of the drug. It is even more remarkable that comparative studies of glycoside concentrations in serum and urine, carried out since AUGSBERGER's work was published, are broadly in agreement with the stated pharmacological data. However, recent research (for review see: GROSSE-BROCKHOFF and HAUSAMEN, 1975) has revealed certain facts of importance in practical therapy and certain necessary corrections of the pharmacological data, in particular the total body dose, the maintenance dose, the absorption ratio, and the decay or elimination rate (JELLIFFE et al., 1972; LUKAS and PETERSON, 1966; LUKAS, 1972; MARCUS et al., 1966; MARCUS, 1975; MOE and FARAH, 1975; PETERS et al., 1974); this is discussed in more detail below.

II. Misuse of the Pharmacological Data

Criticism has been directed mainly against unduly rigid therapeutic schedules based on the pharmacological data for cardiac glycosides. The well known dosage tables, from which the effective dose can be easily read off, may have contributed to this regidity. It must be clearly understood that the pharmacological data can provide no more than a basic framework for rational glycoside therapy. In every individual the therapeutic plan and the dosage must of course be adapted to the factors which modify glycoside requirements. These factors have already been discussed. Metabolic breakdown of glycosides by the body deserves emphasis. Deviations from normal metabolism are important, especially in the case of digitoxin. Changes in metabolic state may affect the dosage of glycosides, e.g., the increased conversion of digitoxin into dihydrodigitoxin and other metabolites in renal failure, or the increased formation of metabolites with little or no cardiac activity as a result of enzyme induction (e.g., by barbiturates). These findings have uncovered new problems which call for further research. Recent investigations (CLARK and KALMAN, 1974; GREENWOOD et al., 1975; PETERS et al., 1977, 1978a) show that hydrogenation of the lactone ring can assume major proportions (up to 50%), even in the case of digoxin. This means that patients with a high rate of metabolic turnover of dihydrodigoxin may in certain circumstances require considerably larger doses of digoxin than are usually needed. It is not yet known such patients are becoming more frequent. This work opens new vistas in pharmacokinetics. One point thrown into prominence by these findings is that the crucial

Table 1. Pharmacological characteristics of digitalis glycosides

Glycoside	Therapeutic body pool (mg)	Oral maintenance dose mg/day	Absorption ratio %	Elimination rate %
Digoxin	0.6–1.2	0.375–0.5	60– 70	20
β-Acetyldigoxin	0.6–1.2	0.3 –0.4	80	20
β-Methyldigoxin	0.6–1.2	0.2 –0.3	90	15–20
Lanatoside C	0.6–1.2	0.75 –1.0	40	20
Digitoxin	0.6–1.2	0.07 –0.1	90–100	9

criterion which determines the dosage to be given is not the blood level but the observed effect or lack of effect in the individual patient.

1. Therapeutic Saturation Dose (Therapeutic Body Pool)

Until a few years ago it was still accepted – here in Germany at least – that for digitalis glycosides the total body load (therapeutic saturation dose) was 2 mg (GILLMANN and GROSSE-BROCKHOFF, 1963). In the light of recent experience (DENGLER et al., 1978; GROSSE-BROCKHOFF et al., 1977; KAUFMANN, 1975; LUKAS, 1973a; MARCUS et al., 1966; MARCUS, 1975) a lower estimate is certainly correct. The total body load of 2 mg was worked out by using tachyarrhythmia as the model (AUGSBERGER, 1951). In such patients particularly high doses are necessary to achieve the optimum therapeutic effect. Reduction of the total body load to 1 mg, as has been suggested from various quarters (KAUFMANN, 1975; LUKAS, 1972; MARCUS et al., 1966), would appear adequate. This latter figure holds good for intravenous and oral administration alike, though in the latter instance only that proportion of the therapeutic saturation dose which is actually absorbed should be counted. The therapeutic saturation dose is approximately equivalent to the "therapeutic body pool," which was stated by MARCUS et al. (1966) to amount to 1 mg for digoxin in patients receiving an oral dose of 0.5 mg/day, while LUKAS (1973a) gave a figure of 0.87 mg for digitoxin in patients on an oral dose of 0.1 mg/day. DENGLER et al. (1978) estimated that the body pool amounted to 0.88 mg in patients receiving a daily oral dose of 0.5 mg digoxin; for an oral dose of 0.25 mg the body pool was 0.44 mg, and for a dose of 1.0 mg it was 1.75 mg.

2. Absorption

As regards the absorption ratio, it has proved possible to confirm or in some instances to correct the pharmacological data by measurements of digitalis concentrations in blood and urine. The absorption ratios given in Table 1 represent the means of the data published in the literature (review: CLASEN et al., 1979; FLASCH, 1975; FLASCH et al., 1978; GILLMANN and GROSSE-BROCKHOFF, 1963; GOLD, 1946; KRAMER et al., 1979; LARBIG, 1975; MOE and FARAH, 1975). The absorption ratios obtained in this way are not necessarily identical with the absorption ratios calculated from the pharmacological data. In accordance with KAUFMANN's (1975) suggestion it might be better to refer to the latter as "efficacy ratios."

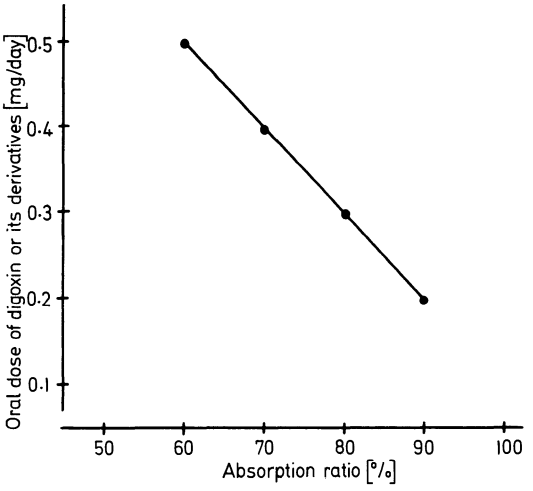

Fig. 2. This graph shows how the average daily oral maintenance dose of digoxin and its derivatives depends on the absorption ratio

Recent studies of the biological availability of digoxin tablets have demonstrated that it can be enhanced by improvements in pharmaceutical formulation (CLASEN et al., 1979; FLASCH et al., 1978; KRAMER et al., 1979). Though in itself commendable, the development of improved pharmaceutical formulations for digoxin tablets carries with it an increased risk of digitalis poisoning (DANON et al., 1977). It is therefore imperative that the manufacturers of digoxin tablets should state the absorption ratio or biological availability, so that the physician, by appropriate adjustment of dosage, can ensure a safe changeover from one digoxin preparation to another. As the elimination rates of digoxin, β-acetyldigoxin, and β-methyldigoxin do not differ substantially in patients with normal renal function, there is a straight line relationship, as shown in Fig. 2, between the absorption ratio and the daily oral dose.

3. Elimination

For practical purposes the elimination ratio is of particular significance. Though it was previously assumed that the half-life – as a measure of elimination rate – was a constant quantity for each of the various glycosides, recent pharmacokinetic investigations have revealed that the half-life of any given glycoside may vary substantially, these variations being dependent on numerous factors such as impairment of renal function, the size of the distribution volume, changes in metabolic transformation, hormonal disorders, interactions with other drugs, and biological scatter (BODEM and VON UNRUH, 1978; CLARK and KALMAN, 1974; DENGLER et al., 1973; GRABENSEE et al., 1978; GROSSE-BROCKHOFF et al., 1973; LARBIG, 1975; LUKAS, 1973a; OHNHAUS et al., 1974; PETERS et al., 1974, 1977b, 1978a; SCHNEIDER and RUIZ-TORRES, 1977; SMITH and HABER, 1973; SOLOMON et al., 1972; VESELL, 1974).

When considering data for excretion in the feces, it is essential to bear in mind that different glycosides vary in their behavior in the enterohepatic circulation.

Less than 15% of a dose of digoxin is excreted in the feces (BINNION, 1978; DOHERTY and PERKINS, 1966; RIETBROCK et al., 1977 b), but the fecal excretion of digitoxin and its metabolites is estimated to be substantially higher (approximately 40%). Digitoxin is the only digitalis glycoside in which the enterohepatic circulation is of any practical importance. Approximately one-quarter of a dose of digitoxin is handled in this way (OKITA, 1957, 1967).

III. Dosage and Body Weight

In pediatrics it is customary to prescribe the dose of digitalis in accordance with the patient's body weight and surface area (VON BERNUTH and LANG, 1978; VON HARNACK and JANSSEN, 1977; O'MALLEY and STEVENSON, 1973; ROGERS et al., 1971; WETTRELL and ANDERSON, 1977). In adult medicine this practice has not gained general acceptance, although recent studies (LARBIG, 1975; LUKAS and PETERSON, 1966; PETERS et al., 1974; SCHNEIDER and RUIZ-TORRES, 1977) have shown that body weight is a by no means negligible determinant of the distribution volume for digitalis glycosides. As adipose tissue stores only minimal quantities of digitalis (KUHLMANN et al., 1978), the main factor which influences the serum digitalis level is the fat-free body mass, which consists principally of the skeletal musculature. Especially in old age, the latter plays a highly significant role (HAGER et al., 1979).

Investigations of this subject have shown significant correlations, though with differing correlation coefficients, between the dose of digitoxin or digoxin per unit body weight and the serum digitalis levels (LUKAS and PETERSON, 1966; PETERS et al., 1974; SCHNEIDER and RUIZ-TORRES, 1977). Variance analyses in ambulant patients have proved that only about 7.6% of the total variation in digoxin level is explicable in terms of dose (PECK et al., 1973). This is more or less valid for all groups of patients with various diseases, and within each patient group there are definite overlaps between digitalis levels. Apart from the dose of digitalis, the main factors which determine the cumulation of each digitalis glycoside are its overall elimination constant and the interval between doses. In the practical management of digitalization it is inconvenient to alter the interval between doses, and the usual procedure is to adjust the dose while keeping the interval constant. As is clear from the pediatric example, the serum digoxin level varies widely (variation coefficient: 30.8%–78.6%), even when body weight or surface area is taken into account in prescribing the dose (O'MALLEY and STEVENSON, 1973; ROGERS et al., 1971).

It is therefore questionable whether adjustment of digitalis dosage in keeping with body weight or surface area would ensure a higher degree of therapeutic safety in adult medicine, though this procedure has recently been advocated by the author of a nomogram in which the digitalis dose is plotted as a variable dependent upon body weight (SCHNEIDER and RUIZ-TORRES, 1977). Nevertheless, in certain cases it is advisable to take body weight into account when selecting dosage.

IV. Choice of Digitalis Glycoside

When choosing a glycoside, the physician must bear in mind that the higher the absorption ratio the lower are the fluctuations in absorption. The glycosides can

Table 2. Onset of action and time of maximum effect of digitalis glycosides and strophanthin

Glycoside	Onset of action	Time of maximum effect
	(minutes after i.v. administration)	
Strophanthin	3–10	60
β-Methyldigoxin	3–10	60
Digoxin	10–20	120–240
Lanatoside C	10–20	120–240
Digitoxin	30	120–240

be arranged in the following sequence: digitoxin, β-methyldigoxin, β-acetyldigoxin, digoxin, lanatoside C. Time will tell whether improvements in pharmaceutical formulation together with closer limits on the variation between batches will raise the absorption ratio of digoxin to levels comparable with those of β-acetyldigoxin or β-methyldigoxin. Personal experience with digitoxin, extending over many years, has proved that it is entirely feasible to manage all kinds of cases with one glycoside alone. Glycosides having absorption ratios no better than 50% should no longer be used.

V. Technique of Glycoside Administration

Now that digitalis glycosides with an absorption ratio of 80%–90% are available, the need for intravenous administration is felt much less frequently than before, especially as the peak concentration after an oral dose is reached in as short a time as 60–90 min. Apart from malabsorption states, impaired consciousness, and postoperative cases, there are now very few situations in which intravenous strophanthin or digitalis therapy is necessary.

Glycosides in alcoholic solution give the best absorption (Flasch, 1975). However, if only for practical reasons, the tablet form is usually preferred. Studies of the ingestion of digoxin in alcoholic solution in capsules have clearly demonstrated higher absorption (Binnion, 1978; Grosse-Brockhoff et al., 1977; Lindenbaum, 1976). It remains to be seen whether this form of medication will find acceptance. Suppositories, not infrequently used at one time, are now obsolete. There have been no studies of the absorption ratio of digitalis glycosides given per rectum.

The physician has to decide between rapid, moderately rapid, and slow digitalization, the last being effected by starting treatment with the maintenance dose alone. The choice will depend on the degree of myocardial failure. Rapid digitalization need be resorted to only in exceptionally severe and life-threatening heart failure. For such situations digoxin and its derivatives, in particular β-methyldigoxin (Döring et al., 1973), are appropriate, as they have a short latent period of action, like that of strophanthin (Table 2). Another finding of recent research is the fact that there is no difference between digoxin and digitoxin in the time which they require to reach their maximum effect (Peters, 1978; Peters et al., 1978c). In emergencies, the first drugs to be given are rapidly acting nitrate preparations and

diuretics, while digitalis glycosides take second place. For this reason much of the debate about latent periods and times of maximum effect and many of the arguments for and against one preparation or another are now irrelevant.

VI. Alterations in Dosage Consequent on Changes in Glycoside Requirements

Hyperthyroidism and malabsorption syndrome may cause increased glycoside requirement, while impaired renal function (which does not affect digitoxin), hypothyroidism, hypokalemia, hypercalcemia, hypomagnesemia, hypoxia, and disorders of acid-base balance may decrease the glycoside requirement (BUTLER and LINDENBAUM, 1975). Some of these conditions have already been discussed under other headings. However, special mention must be made of renal diseases, hepatic diseases, and hormonal diseases, in particular hyperthyroidism and hypothyroidism.

When using digoxin the physician should bear in mind that increased glycoside requirements may be due to the accumulation of an excess of dihydrogenated metabolites (CLARK and KALMAN, 1974; GREENWOOD et al., 1975; LUCHI and GRUBER, 1968; PETERS et al., 1977a; WATSON et al., 1973), which are believed to be devoid of effect on the heart (BACH and REITER, 1964; LAGE and SPRATT, 1966; OKARMA et al., 1972). According to recent research, this group of metabolites can be detected in over 90% of patients receiving digoxin (CLARK and KALMAN, 1974; PETERS et al., 1978a). In 53% of the patients the dihydrogenated digoxin metabolites in the urine accounted for over 10% of the dichlormethane-soluble metabolites, while in 7% of the patients the proportion was over 35%. In the blood, concentrations of up to 40% were found in a few cases (PETERS et al., 1978a). It should be noted that the dose of digoxin, the presence of impaired renal function, and an increased digoxin body pool had no influence on the rate of formation of dihydrogenated digoxin metabolites (PETERS et al., 1978a).

1. Dosage for Patients with Impaired Renal Function

Approximately 80% of the lanata glycoside digoxin and β-acetyldigoxin is excreted via the kidneys, the corresponding figure for β-methyldigoxin being 70% (DOHERTY, 1973; RIETBROCK et al., 1977a, b). The elimination rates are governed by the glomerular filtration rate. A close correlation can accordingly be demonstrated between the digoxin level or digoxin clearance and the inulin clearance or endogenous creatinine clearance (GROSSE-BROCKHOFF et al., 1973; RISLER et al., 1974a; STEINESS, 1973). The decrease in glomerular filtration rate which accompanies advancing age is one of the main reasons for the high incidence of intoxication by digoxin and its derivatives in elderly people (BELLER et al., 1971; GROSSE-BROCKHOFF et al., 1973; HAUSAMEN and PETERS, 1976).

Various workers (DETTLI, 1976; GAULT et al., 1976; JELLIFFE and BROOKER, 1974; OHNHAUS et al., 1974) have constructed nomograms for determining the dosage of digoxin. These are based on the fact that there is a linear relationship between the global elimination constant of digoxin and the endogenous creatinine clearance. The nomogram devised by OHNHAUS et al. (1974), which assumes a glo-

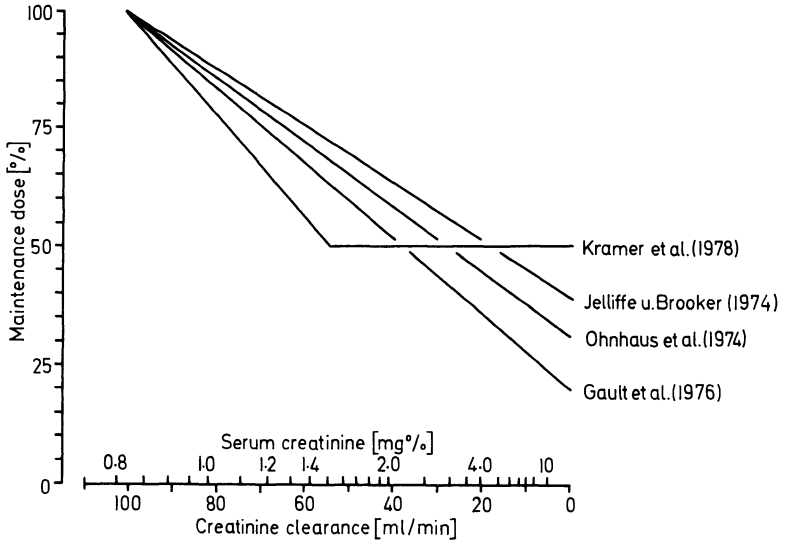

Fig. 3. This graph shows how the dose of digoxin has to be reduced as the degree of renal functional impairment increases (KRAMER et al., 1978)

bal elimination constant for digoxin of 0.144 $days^{-1}$ for patients with anuria, or 0.456 $days^{-1}$ for patients with normal renal function, has proved of value in choosing the dosage of digoxin (RISLER et al., 1974 b), but in view of the wide individual variations attempts to achieve great accuracy may be misleading.

The dosage nomogram devised by GAULT et al. (1976) does not differ in any essential respect from the one just described (Fig. 3). For the sake of simplicity in practice, the creatinine clearance can be calculated from the serum creatinine concentration by the use of the following formula:

$$\text{Creatinine clearance} = \frac{(140 - \text{age} \times \text{weight kg})}{72 \times \text{serum creatinine (mg/100 ml)}}$$

(COCKCROFT and GAULT, 1976).

The dosage guidelines in Table 3 appear to be satisfactory in practice: if endogenous creatinine clearance falls to 50% then the dose of digoxin should be reduced to 50% of the normal oral maintenance dose of 0.5 mg/day. If the endogenous creatinine clearance is below 20 ml/min the recommended maintenance dose of digoxin is 0.125–0.17 mg/day, and for β-acetyldigoxin or β-methyldigoxin 0.1 mg/day. In patients with advanced chronic renal failure (< 30 ml/min) the saturation or total body dose of digoxin or its derivatives should be reduced to 0.6–1.0 mg, because in such cases the distribution volume of digoxin is diminished (WAGNER, 1975).

Digitoxin and its metabolites are mainly eliminated via the kidney, 60%–70% being excreted by this route (OKITA et al., 1953; VÖHRINGER and RIETBROCK, 1978). Because of their high degree of protein binding and pronounced tubular reabsorption they are excreted much more slowly than digoxin and its derivatives (GRABENSEE et al., 1978; LUKAS and PETERSON, 1966; PETERS et al., 1977 b; RIETBROCK et al., 1977 b). There is no toxic cumulation of digitoxin in patients with impaired renal

Table 3. Dosage of digoxin and its derivatives[b],[c] in relation to the degree of renal failure. The absorption ratios (tablets) are assumed to be 60% for digoxin, 80% for β-acetyldigoxin, and 90% for β-methyldigoxin

	Dose for initial digitalization (mg)		Maintenance dose (mg)	
	Intravenous	Oral	Intravenous	Oral
Cl_{Cr}[a] = 100 ml/min	0.6–1.2	1.0 –2.0	0.2 –0.3	0.375–0.5
		[b]0.8 –1.5	[b]0.2 –0.3	[b]0.3 –0.4
		[c]0.7 –1.3	[c]0.2 –0.3	[c]0.2 –0.3
Cl_{Cr}[a] = 50 ml/min	0.6–1.2	1.0 –2.0	0.1 –0.15	0.2 –0.25
		[b]0.8 –1.5	[b]0.1 –0.15	[b]0.15 –0.2
		[c]0.7 –1.3	[c]0.1 –0.15	[c]0.1 –0.15
Cl_{Cr}[a] = <20 ml/min	0.6–1.0	1.0 –1.7	0.075–0.1	0.125–0.17
		[b]0.8 –1.3	[b]0.075–0.1	[b]0.1 –0.15
		[c]0.7 –1.1	[c]0.075–0.1	[c]0.075–0.1

[a] endogenous creatinine clearance
[b] β-acetyldigoxin
[c] β-methyldigoxin

function, as it has an alternative excretion pathway via the bile and the feces. VÖHRINGER, investigating uremic patients, found that overall excretion was unaffected, a 60% reduction in the renal elimination of digitoxin and its metabolites being made good by a doubling of the amount excreted in the feces (VÖHRINGER, 1978). The serum elimination half-life is, if anything, shortened (PETERS et al., 1977b; STORSTEIN, 1973, 1978). The dosage nomograms devised for digitoxin (DETTLI, 1976; JELLIFFE et al., 1970) are based on the assumption that in patients with impaired renal function the overall elimination constant is diminished (as is the case with digoxin) and they are therefore invalid. A clinical trial by BELLER et al. (1971) showed that abnormal renal function does not increase the risk of digitalis poisoning in patients receiving digitoxin. There is hence no need to reduce the dose of digitoxin in patients with chronic renal failure (PETERS et al., 1974; RASMUSSEN et al., 1972; RIETBROCK et al., 1977b). Recent findings suggest that in some patients with uremia there may be some decrease in the sensitivity of the myocardium to digitalis (KRAMER et al., 1978; PETERS et al., 1978c). However, it is still too soon to draw therapeutic conclusions from this work.

In protein deficiency states, e.g., the nephrotic syndrome, or in hemodialysis patients receiving heparin, in whom the degree of protein binding of digitoxin is known to be reduced, there is some possibility of a toxic increase in the nonprotein bound digitoxin fraction (STORSTEIN and JANSSEN, 1976; STORSTEIN, 1978). In such cases, if compensatory mechanisms fail to increase elimination, the dosage will have to be lowered. However, in the nephrotic syndrome the serum digitoxin level is often so low (10–15 ng/ml) that despite the reduced protein binding of digitoxin there is no cumulation of the nonprotein bound fraction (PETERS et al., 1974; STORSTEIN, 1978). In such cases the conventional average maintenance dose of 0.1 mg digitoxin a day can be administered as usual.

2. Dosage for Patients with Impaired Hepatic Function

Investigations by OKITA et al. (1955), LUKAS (1973b), MARCUS et al. (1966), and MARCUS (1973) have shown that in man the liver is the principal site for metabolic transformation of the digitalis glycosides digitoxin and digoxin. However, the significant effects of impaired hepatic function and liver disease on the pharmacokinetics of digitalis glycosides (total body clearance, protein binding, metabolism, and excretion) have been revealed only by more recent research. The liver is an important accessory organ for the excretion of digitoxin and β-methyldigoxin: in subjects with normal hepatic and renal function 37% of digitoxin and 27% of β-methyldigoxin are excreted in the feces, though for digoxin the corresponding proportion is only 10%–18% (KLOTZ, 1978; RIETBROCK et al., 1977c; VÖHRINGER and RIETBROCK, 1978). In the case of digitoxin, which is 97% bound to albumin, the liver also affects protein binding because as the site of albumin synthesis it controls the serum albumin concentration. There is a significant correlation between the albumin concentration and the proportion of digitoxin bound to protein (LUKAS, 1973c).

For digoxin and β-methyldigoxin the protein-bound fraction is only about 20%, while for strophanthin it is less than 10% (BENTHE, 1975; LUKAS, 1973c). This means that changes in protein binding do not affect the pharmacokinetics of these glycosides.

Pharmacokinetics studies have shown that the kinetics of digoxin are unaffected by liver diseases (LAHRTZ et al., 1969; MARCUS et al., 1966; MARCUS, 1973; ZILLY et al., 1975). In acute hepatitis the O-demethylation of β-methyldigoxin in the liver is retarded (ZILLY et al., 1975), and extra caution is therefore advisable when using it. Other digoxin preparations can be prescribed without reduction of dosage for patients with acute or chronic hepatitis or with cirrhosis.

The excretion of digitoxin is not delayed in cirrhosis, although there are changes in certain important pharmacokinetic data (LAHRTZ et al., 1969; PETERS et al., 1978b; PETERS, 1978; ZILLY et al., 1976).

Decreased protein binding of digitoxin, which is most commonly due to hypoalbuminemia, has the effect of increasing total body clearance and the distribution volume (PETERS et al., 1978b; PETERS, 1978; ZILLY et al., 1976). For this reason there is little danger of toxic cumulation and normal doses are usually well tolerated (PETERS, 1978; ZILLY et al., 1976). However, in certain cases' where an appropriate increase in the elimination mechanisms is not possible, the normal average oral maintenance dose of 0.1 mg digitoxin/day may lead to toxic cumulation of the protein-unbound digitoxin fraction (PETERS et al., 1978b). When treating patients with cirrhosis it is therefore advisable to reduce the average oral maintenance dose of digitoxin to 0.08 mg/day. The serum digitoxin level should not be allowed to exceed 20.0 ng/ml.

In chronic aggressive hepatitis the elimination of digitoxin is speeded up and there is hence no need to reduce the dose (STORSTEIN and AMLIE, 1975).

3. Hormonal Factors

Digitalis glycosides resemble the sex, suprarenal hormones and the bile acids in possessing a cyclopentanoperhydrophenanthrene structure. As impotence and gynecomastia have been noted in some patients receiving digitalis glycosides, and

as these effects have proved reversible on stopping treatment, it has been thought that the glycosides have an intrinsic estrogenic action (NAVAB et al., 1965; LE WINN, 1953). Recent studies in experimental animals indicate that digitalis glycosides cause abnormalities in estrogen degradation (RICKEN, 1975). It is still uncertain whether the steroid hormones estrogen, androgen, cortisone, and aldosterone interfere with the metabolic transformation of digitalis glycosides, and the clinical relevance of any such interactions, insofar as they affect therapy with digitalis glycosides, is still obscure. There is some evidence from experimental work in rats and mice that spironolactone, an aldosterone antagonist with a steroid molecule, accelerates the metabolic transformation and excretion of digitoxin, digoxin, and β-methyldigoxin (ABSHAGEN, 1973; CASTLE and LAGE, 1973). In man the changes in the metabolism and elimination of digitalis glycosides caused by concurrent treatment with spironolactone are quite small (WIRTH et al., 1976) and are probably of no clinical significance (ABSHAGEN, 1972; KRÄMER et al., 1973).

Heightened sensitivity to digitalis has been described in aldosteronism and in hyperparathyroidism, but as yet there have not been any controlled trials designed to distinguish between the hormonal effects and the associated electrolyte abnormalities such as hypokalemia or hypercalcemia. Because of the additive effects of digitalis and calcium, digitalis glycosides should be administered with special caution in patients with hyperparathyroidism and concurrent hypercalcemia.

Hypothyroidism and hyperthyroidism are the best known clinical examples of states which alter digitalis tolerance. In the former it is decreased and in the latter increased. Numerous investigations have shown that changes in the pharmacokinetics of the glycosides are the main factors responsible for the alterations in tolerance. In patients with hypothyroidism DOHERTY and PERKINS (1966) found significantly higher serum levels of ^{3}H-digoxin than in hyperthyroid patients, dosage being identical. Similar findings have been reported for ^{3}H-ouabain and ^{3}H-digitoxin (EICKENBUSCH et al., 1970). In a recent study performed under steady state conditions hyperthyroid patients receiving 0.5 mg digoxin daily by mouth had a mean serum digoxin level of 0.8 ± 0.4 ng/ml as against 1.4 ± 0.4 ng/ml in the control group (KOKENGE et al., 1978). In hypothyroid patients, on the other hand, the serum digoxin level was 3.2 ± 1.1 ng/ml – more than twice as high. Further investigations in which digitoxin was given showed no significant differences in serum digitoxin level between the hyperthyroid and hypothyroid groups (KOKENGE et al., 1978). Changes in distribution volume (DOHERTY and PERKINS, 1966; GILFRICH and MEINERTZ, 1978), in the renal excretion or clearance of digoxin (CROXSON and IBBERTSON, 1975; EICKENBUSCH et al., 1970; GILFRICH and MEINERTZ, 1978), and in absorption are major factors in these differences.

Every clinician is aware that an increase in the dose of digitalis will often fail to slow the supraventricular tachycardia of hyperthyroidism, a fact which seems to point to some pharmacodynamic interaction between the thyroid hormone and the glycoside in the myocardial cell. In such cases β-receptor blockers, which act by damping adrenergic stimulation, have proved effective in slowing the heart rate.

D. Interactions

Of the interactions between drugs and digitalis glycosides, only those which are of clinical relevance will be discussed here. They are:

1. *Pharmacokinetic interactions:*
   a) Change in intestinal absorption or in the enterohepatic circulation
   (Digoxin: propantheline, metoclopramide, charcoal, neomycin, cholestyramine)
   (Digitoxin: cholestyramine, charcoal)
   b) Displacement from protein-binding
   (Digitoxin: phenylbutazone, tolbutamide, clofibrate, heparin)
   c) Alteration of metabolic transformation
   (Digitoxin: phenobarbital, diphenylhydantoin, phenylbutazone, rifampicin, spironolactone)
   d) Change in excretion
   (Digoxin: quinidine, *l*-dopa)

2. *Pharmacodynamic interactions:*
   a) Interaction on the digitalis receptor
   (diphenylhydantoin, potassium)
   b) Interaction in intracellular metabolism
   (β-stimulators, β-receptor blockers, antiarrhythmic substances)

Changes in the absorption ratio have more effect on digoxin, which is hydrophilic, than on digitoxin, which is lipophilic. In subjects receiving digoxin tablets of low bioavailability, metoclopramide, which stimulates bowel motility, was found to lower the serum digoxin level, while propantheline, which reduces bowel motility, raised it (MANNINEN et al., 1973). However, these differences were not found in subjects receiving tablets of high bioavailability or alcoholic digoxin solution, and their therapeutic relevance is questionable. Even in therapeutic doses, neomycin (3 g/day), cholestyramine, and animal charcoal have been reported to lower the bioavailability of digoxin (LINDENBAUM et al., 1976; SHAW, 1978). The synthetic resins cholestyramine and cholestipol bind digoxin and digitoxin in the intestine and may indeed be useful in the management of recent digitalis poisoning for removing residual digitalis left in the bowel. In the case of digitoxin these anion exchangers also bind metabolites arising from the enterohepatic circulation, the latter being a major factor in the slow elimination of that glycoside (CALDWELL and GREENBERGER, 1971; OKITA, 1957, 1967; STORSTEIN and MJOLNEROD, 1973). The enterohepatic circulation is of little significance in the handling of digoxin (KLOTZ, 1978). Interactions in protein binding are of importance only in connexion with a glycoside such as digitoxin, a high proportion of which is protein bound. SOLOMON et al. (1972) investigated phenylbutazone, sulfadimethoxine, warfarin, clofibrate, and phenobarbital in vitro, using the drugs in concentrations of 20–100 µg/ml, and found that they had no effects on the protein binding of digitoxin. Only tolbutamide and clofibrate – and they only in concentrations of 150–250 µg/ml – caused any fall in the degree of protein binding. Tolbutamide depressed the percentage of digitoxin bound to protein from 92.6% to 88.9%, the corresponding figure for clofibrate being 87.2%. However, it should be noted that both drugs were present at roughly twice their therapeutic concentrations. STORSTEIN (1973) investigated patients receiving heparin during hemodialysis and found that the proportion of digitoxin bound to protein dropped from 96.8% to 92.2%. The change in protein binding is ascribed to release of free fatty acids in response to

heparin (STORSTEIN and JANSSEN, 1976). This finding may be of some significance in connexion with the arrhythmias which occur during hemodialysis.

A phenomenon of considerable clinical importance in connexion with digitoxin is the metabolic interaction with certain drugs such as diphenylhydantoin (SOLOMON et al., 1972), rifampicin (PETERS et al., 1975), phenobarbital (JELLIFFE and BLANKENHORN, 1966; SOLOMON et al., 1971), phenylbutazone (SOLOMON et al., 1972), and spironolactone (WIRTH et al., 1976), which behave as enzyme inductors of mixed-function oxidases. All of these except spironolactone are capable of depressing the serum digitoxin concentration to subtherapeutic levels. They do this mainly by augmenting the so-called cardio-inactive, water soluble metabolite fraction.

Raised serum digoxin concentrations – in some cases reaching toxic levels – have recently been noted during antiarrhythmic treatment with quinidine (DÖRING and KÖNIG, 1978; EJVINSSON, 1978; HOOYMANS and MERKUS, 1978; LEAHEY et al., 1978; RISLER et al., 1979). They are attributed to diminished renal excretion, renal clearance, and volume of distribution of digoxin (HAGER et al., 1979; LEAHAY et al., 1978; RISLER et al., 1979). Elevated serum digitoxin levels have also been reported during concomitant treatment with quinidine (PETERS et al., 1980; SCHENCK-GUSTAFSSOHN et al., 1978), due to an increased half-life of digitoxin (PETERS et al., 1980). The clinical significance and risk of digitoxin toxicity seem to be less (PETERS et al., 1980; STORSTEIN et al., 1979) because of a minor increase of the serum digitoxin concentrations.

E. Prophylactic Digitalization?

Animal experiments seem to indicate that the cardiac hypertrophy which follows experimentally produced pulmonary or aortic stenosis is less pronounced in animals which are given cardiac glycosides before and during the experiments (WILLIAMS and BRAUNWALD, 1965). However, these results have not yet been confirmed, and clinical observations have provided no concrete support for them. When considering prophylactic digitalization it must be remembered that the inotropic action of the glycoside raises the oxygen consumption of the undilated ventricle, and that glycoside treatment may therefore be harmful rather than beneficial (GROSSE-BROCKHOFF and GRABENSEE, 1976; KREBS, 1976; MASON, 1974; SONNENBLICK et al., 1968). There is no doubt that myocardial failure, manifest or latent, in the senile heart calls for digitalis therapy. Furthermore, it is well known that elderly patients require and tolerate glycosides in small doses only. The reasons are reduced glomerular filtration rate and the lessened tolerance associated with coronary heart disease. The proposal that, as a matter of principle, patients over a certain age (60 years or more) should receive prophylactic digitalization is quite unjustified. Indeed, it is by no means uncommon to meet patients over the age of 70 years with fully efficient hearts.

The question of prophylactic digitalization before operations is one that calls for special discussion. Besides the adverse synergistic interactions between cardiac glycosides and certain agents used in anesthetic practice (succinyl chloride, cyclopropane), there are other factors such as postoperative disorders of acid-base bal-

ance, hypokalemia, and impairment of renal excretory function which increase the risk of toxic side effects from cardiac glycosides. As digitalis can provoke almost any kind of arrhythmia, it is often difficult to decide whether an arrhythmia arising in a previously digitalized patient is due to a toxic effect of the glycoside or to postoperative factors. In the writer's opinion there is no justification for routine preoperative or postoperative digitalization in patients without signs of myocardial failure. The work of JULER et al. (1969) has demonstrated a raised morbidity rate in digitalized patients and even an increased mortality rate from arrhythmias among patients on digitalis undergoing surgery. They put forward the view that the incidence of side effects from routine digitalization is numerically more significant than the decrease in morbidity and mortality from cardiac failure, a decrease which they found to be of no significance. The decision whether to digitalize a patient – either before the operation or afterwards, on account of the onset of heart failure or an arrhythmia – is one that must be based on individual circumstances. Rule-of-thumb prescribing is totally inappropriate.

F. Do Digitalis Glycosides Differ in Their Mode of Action?

It has already become clear that different glycosides differ considerably in their pharmacokinetics. The question whether they also differ in their mode of action is one which is raised from time to time. Pharmacological research has not so far revealed any evidence of such differences in mode of action between the various digitalis glycosides. On the clinical side it has been suggested that there are differences in effect between various digitalis glycosides and strophanthin (BELZ and ERBEL, 1978; BELZ et al., 1978; STORZ, 1966, 1968). These suggestions are based on measurements of cardiac function by indirect techniques, as for example by BLUMBERGER (1940) or WEISSLER et al. (1972), and are not convincing enough to call for any changes in therapeutic practice.

G. Digitalis Treatment in Infancy and Childhood

Digoxin and its derivatives are almost the only cardiac glycosides used for the treatment of heart failure in children (VON BERNUTH and LANG, 1978; IISALO and DAHL, 1974; IISALO, 1977; WETTRELL et al., 1974; WETTRELL and ANDERSON, 1977). They are administered by mouth in alcoholic solution or by the intravenous route. The dosage of the glycoside is governed by body weight or surface area. As regards dosage guidelines, there are no universally accepted rules [for reviews see (VON BERNUTH and LANG, 1978; WETTRELL and ANDERSON, 1977)]. In the past it was customary to give children, especially in the saturation phase, considerably higher doses of digitalis than adults. This practice arose from the clinical observation that children tolerate higher serum digitalis concentrations than adults (ROGERS et al., 1971; WAGNER, 1975; WETTRELL et al., 1974; WETTRELL and ANDERSON, 1977). However, recent research based on dose-effect parameters (LEVY et al., 1972) has shown that oral doses of digoxin as low as 30 μg/kg body weight will produce an inotropic effect of the same intensity as doses of 80 μg/kg body weight. Current dosage guidelines for premature babies, neonates, young infants, and small chil-

Table 4. Dosage[a] of digoxin for premature and newborn babies, infants, and children

	Dose for initial digitalization mg/kg body weight		Maintenance dose (mg/day) mg/kg body weight		Authors
	p. o.	i. v.	p. o.	i. v.	
Premature and newborn babies <1 month	0.03 −0.04	0.02 −0.03	0.01	0.007	IISALO and DAHL (1974) v. BERNUTH and LANG (1978)
Infants					
1–12 months	0.068	0.05	0.017	0.012	ROGERS et al. (1971)
3–12 months	0.078–0.065	0.053–0.043	0.015–0.013	0.011–0.0087	VON HARNACK and JANSSEN (1977)
Children					
1–10 years	0.05	0.04	0.012	0.008	WETTRELL and ANDERSSON (1977)
1–12 years	0.065–0.046	0.043–0.03	0.013–0.009	0.0087–0.0092	VON HARNACK and JANSSEN (1977)

[a] When administering the derivatives β-methyldigoxin or β-acetyldigoxin by mouth the physician must remember their higher absorption ratios of 90% and 80% respectively

dren are based on these pharmacodynamic findings and also make allowances for age-specific differences in elimination (Tables 4 and 5). The absorption ratio of digoxin is the same in newborn babies and in older children. According to recent research (WETTRELL and ANDERSON, 1975) it amounts to 72%, and is hence much the same as in adults. Because of the immaturity of the kidneys and the correspondingly lower renal clearance, the serum elimination half-life of digoxin in neonates is prolonged to 44 h, as compared with the time of 19 h in babies over 1 month old and in older children (WETTRELL and ANDERSON, 1977). If given the same dose per kilogram body weight, premature and newborn babies will reach considerably higher serum digoxin levels than older infants. The half-life of digoxin is influenced by changes in body clearance and also by the distribution volume of digoxin, which in neonates is roughly 1.5–2.0 times larger than in adults.

$$t_{1/2} = \frac{V_d}{Cl_{tot}} \times \ln 2$$

($t_{1/2}$ = half-life, V_d = distribution volume, Cl_{tot} = total body clearance).

WETTRELL and ANDERSON (1977) advocate the use of serum digoxin measurements for control of treatment in children belonging to the following classes:

1. Newborn and premature infants – because of the slow excretion of digoxin,
2. Patients with impaired renal function,
3. Patients in the early postoperative phase,
4. Patients showing signs of overdosage or underdosage.

Table 5. Dosage of digitoxin for infants and children (VON HARNACK and JANSSEN, 1977)

	Dose for initial digitalization mg/kg body weight		Maintenance dose (mg/day) mg/kg body weight	
	p. o.	i. v.	p. o.	i. v.
3–12 months	0.038	0.032	0.0038	0.0032
1–12 years	0.032	0.0226	0.0032	0.0022

Research in America and Europe on the relation between serum digoxin levels and the occurrence of toxic manifestations has shown that in children – unlike adults – the toxic range does not begin until a concentration of 3.5 ng/ml is reached (VON BERNUTH and LANG, 1978; KRASULA et al., 1974).

The therapeutic range is below 3.0 ng/ml, and digoxin poisoning is hence unlikely if the serum digoxin is below this level (VON BERNUTH and LANG, 1978; ROGERS et al., 1971; WETTRELL and ANDERSON, 1977). It is of course necessary to exclude any factor which may lower the patient's digitalis tolerance. Although in these investigations different maintenance doses were given to children of different age groups, the serum digoxin levels as actually measured were not far above or below 2.0 ng/ml (WETTRELL and ANDERSON, 1977). Because of the very small quantities of digoxin required by neonates, it is advisable to dilute the digoxin solution in the proportion 1:10 with physiologic saline (VON BERNUTH and LANG, 1978).

H. Other Drugs Used in Conjunction with Digitalis for the Treatment of Heart Failure

Diuretics and vasodilators are used as adjuvants to digitalis glycosides in the treatment of myocardial failure. The need for these agents depends largely on the severity of the cardiac condition. By giving drugs which lower the preload and afterload on the heart, the physician can achieve economy in cardiac work and can raise the efficiency of the heart (BRAUNWALD, 1977; MATHEY, 1979). Diuretics (benzothiadiazine, furosemide, ethacrynic acid) lower ventricular filling pressure, pulmonary artery pressure, and pulmonary venous pressure, besides correcting pulmonary congestion. They reduce ventricular predistension. The patient's symptoms, and dyspnoea in particular, are relieved, even though the stroke volume index may drop (STAMPFER et al., 1968). In certain cases of heart failure of grade II diuretics even seem to effect an increase in cardiac output (RADER et al., 1964).

Potassium-conserving diuretics such as the aldosterone antagonist spironolactone or agents such as amiloride and triamterene are frequently used in conjunction with benzothiadiazines, because they lessen the risk of hypokalemia induced by the latter.

Other drugs used in cardiac patients are nitrites, sodium nitroprusside, hydralazine, and prazosin (AWAN et al., 1977; BRAUNWALD, 1977; FRANCIOSA et al., 1978; GRAY et al., 1975; MATHEY, 1979; PIERPONT et al., 1978; SCHRÖDER, 1977).

Table 6. Action and dosage of vasodilators (MATHEY 1979)

Drug	Peripheral arterial resistance	Peripheral venous resistance	Stroke volume	Ventricular filling	Dosage
Sodium nitroprusside[a] (Nipride, Nipruss)	↓	↓	↑	↓	16–400 μg/min
Nitroglycerin[a]	(↓)	↓	(↑)	↓	50–150 μg/min
Nitrates[b] (Isoket, ISO-Mack, Maycor, Corovliss)	–	↓	–	↓	4 × 20–80 mg
Hydralazine[b] (Nepresol)	↓	–	↑	–	4 × 50–75 mg
Prazosin[b] (Minipress)	↓	↓	↑	↑	4 × 1–5 mg

[a] Intravenous administration
[b] Oral administration

Acting in different ways, they gradually reduce the preload and afterload on the heart (Table 6). Nitroglycerine exerts its vasodilator effect mainly on the venous system. It lowers right and left ventricular filling pressures, but stroke volume generally remains unaltered. Nitrites are among the drugs of first choice in the management of acute left ventricular failure and pulmonary edema.

Sodium nitroprusside is one of the drugs which relieves the load on the heart mainly by lowering peripheral arterial resistance. It is given by the intravenous route only and requires regular monitoring of blood pressure. Hydralazine and prazosin can be given by mouth and are hence more suitable for long-term therapy. Their effect is particularly useful in patients with coexisting hypertension. Hydralazine acts exclusively by lowering peripheral arterial resistance, and stroke volume rises by an average of 50%. Prazosin reduces both arterial and venous resistance, and as a result there is a rise in stroke volume together with a fall in ventricular filling pressure (MATHEY, 1979).

J. Strophanthin

Strophanthin glycosides are used in the form of *k*-strophanthin and *g*-strophanthin (ouabain). Though they were of considerable therapeutic value in their day, as rightly emphasized by FRÄNKEL (1933) and EDENS (1948), they were largely supplanted by the introduction of pure glycosides isolated from *Digitalis purpurea* and *Digitalis lanata*. As compared with the older digitalis preparation, these glycosides have considerably higher absorption ratios when given by mouth, and the only re-

maining role for strophanthin is intravenous therapy (see below). The *k*-strophanthins are subdivided into α-, β-, and γ-forms, depending on the nature of the sugar residue. According to recent research, the more lipophilic *k*-strophanthin α (cymarin) is absorbed in the intestine up to an extent of approximately 35% (GREEFF et al., (see Chap. 3); KRÄMER et al., 1972; STORZ, 1969). The serum half-life of the *g*-strophanthins is 20 h (KRAMER et al., 1970; SELDEN and SMITH, 1973; SELDEN et al., 1974) and the decay ratio for *k*-strophanthin α is 40% (STORZ, 1969). They are excreted largely (70%) via the kidneys and tend to cumulate in patients with impaired renal function, so that adjustment of dosage is called for in such circumstances (BRASS and PHILIPPS, 1970; KRAMER et al., 1970). In patients with terminal renal failure the cumulative amount recovered from the urine was only 12% of the dose (BRASS and PHILIPPS, 1970), and strophanthin should as a rule not be used in such cases. The therapeutic body pool for all strophanthins is around 0.6 mg (BRASS and PHILIPPS, 1970; GILLMANN and GROSSE-BROCKHOFF, 1963). The maintenance dose by intravenous administration is 0.25 mg a day. Because of the low absorption ratio of the commercially available *k*-strophanthins it is extremely doubtful whether they should be given by mouth. The pharmacokinetic properties of *g*-strophanthin are in general similar, but the proportion excreted by the kidneys is even larger than in the case of *k*-strophanthin (GREEFF et al., unpublished work). The *g*-strophanthin preparations offered for administration by mouth have an extremely low absorption ratio of less than 10% (SELDEN et al., 1975; GREEFF, 1977) and are hence quite unsuitable for oral glycoside therapy.

K. Meproscillarin

Meproscillarin, one of the glycosides of *Scilla maritima*, has recently acquired a considerable therapeutic reputation. This drug is a semisynthetic derivative of proscillaridin. Its biological availability is 60%–70%, as compared to 30%–35% for proscillaridin (BELZ and BELZ, 1978; GROSSE-BROCKHOFF et al., 1977). The efficacy of meproscillarin on the human heart has been demonstrated by studies of cardiac contraction (TURINA and KRAYENBÜHL, 1978). In clinical trials, one in general practice (647 patients) and one in hospital, the drug was shown to have definite beneficial effects on various parameters of cardiac function in approximately 80% of patients with myocardial failure (ECKARDT et al., 1978; KLEIN and PAVEK, 1978). The oral maintenance dose is between 0.5 and 1 mg a day, and the intravenous therapeutic saturation dose is stated to be 2.5 mg (HERKEN and BRANDES, 1978). In healthy subjects the mean serum half-life was 49.3 h, while in patients with chronic renal failure it varied between 31.4 and 80.8 h (BECKMANN et al., 1978). The serum level ranged from 0.2 to 1.0 ng/ml both in healthy subjects and in patients with chronic renal failure, but lower and higher levels were also found. Even in subjects receiving identical doses, the scatter of serum levels was as wide as 10–20 times. Up to 90% of meproscillarin is excreted in the bile (RIETBROCK, 1978).

The fact that there is no tendency to cumulation in patients with chronic renal failure makes meproscillarin a suitable alternative to digitalis glycosides in such cases. Side effects involving the central nervous system, the heart, and the gastrointestinal tract were encountered in approximately 9% of patients receiving long-

term therapy. The most prominent symptom was diarrhea, which occurred in 4%–5% of the patients (TWITTENHOFF et al., 1978). Further clinical trials are needed to discover feasible alternatives to digitalis glycosides, to define the range of indications for their use, and to work out exact dosage guidelines.

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CHAPTER 13

Side Effects and Intoxication of Cardiac Glycosides: Manifestations and Treatment

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A. Introduction

Although the digitalis glycosides are generally the most valuable drugs available for the treatment of heart failure, a relatively high incidence of toxic manifestations has accompanied the widespread employment and beneficial positive contractile action of these agents. Thus, digitalis intoxication appears to be among the most common adverse drug reactions and has been reported to occur in as many as 20% of patients receiving the glycosides (STONE and FISCH, 1969; GOTSMAN and SCHRIRE, 1966; GILFRICH and SCHÖLMERICH, 1975; DOERING et al., 1977a, b; LARBIG et al., 1978). Although the most common and earliest side effects are related to the gastrointestinal tract owing to glycoside action on the central nervous system rather than local gastric irritation, disorders of cardiac rhythm are the first manifestations in one-third of patients. It has been estimated that arrhythmias and conduction disturbances are provoked in up to 80% of patients in whom toxic effects are observed (CHOU, 1969; FISCH and KNOEBEL, 1970). Both retrospective and prospective clinical studies of digitalis overdose have demonstrated considerable variability of cardiac and extracardiac manifestations without predictability concerning the rhythm disorder produced, even with administration of the identical glycoside preparation in the same patient (CHURCH et al., 1962; DREIFUS et al., 1963). Hence, each of the digitalis glycosides is equally capable of producing the signs and symptoms of digitalis toxicity, and no special advantage is obtained with some preparations in regard to earlier onset of less hazardous extracardiac reactions or reduced incidence of arrhythmias.

In this chapter, the electrophysiologic properties of the digitalis glycosides are examined and the possible subcellular mechanisms underlying these effects are considered, in order to establish a fundamental framework for understanding the basis of clinical arrhythmias induced by the drugs. Next, certain problems in the clinical recognition of digitalis intoxication are taken into account. Then, the various types of disorders of cardiac rhythm and conduction induced by digitalis are reviewed with attention focused upon special features of electrical toxicity important in patient care. The factors that modify and predispose to the development of adverse glycoside reactions are described, and the interrelations between digitalis and potassium are evaluated. Finally, the current status of the management of toxic electrophysiologic manifestations of the glycosides is considered, and new aspects in the treatment of digitalis-induced tachyarrhythmias and heart block are discussed.

B. Electrophysiologic Properties

I. Automaticity, Conduction, and Responsiveness

The influence of digitalis on the electrophysiologic properties of heart muscle is complex (MASON et al., 1971). The effects vary considerably because of the dose, the type of cardiac tissue involved, and the state of autonomic innervation of the heart. The excitability of atrial and ventricular automatic tissue is raised through a reduction in resting potential. A glycoside-induced increase in automaticity is mediated by the greater slope of diastolic depolarization, and thereby subsidiary pacemaker activity is enhanced. The rate of rise of the action potential is reduced; therefore, depolarization is prolonged and the velocity of conduction is depressed (MOE and MENDEZ, 1951; SWAIN and WEIDNER, 1957). On the scalar electrocardiogram this may be observed as a lengthening of the PR interval and sometimes more advanced degrees of atrioventricular (AV) block. The speed of depolarization is partly dependent on the level of resting membrane potential, the direct relation of which is termed membrane responsiveness; this property is depressed by digitalis whereas excitability is increased (WEIDMANN, 1955; SINGER and TEN EICK, 1969).

II. Refractoriness

The glycosides also exert important effects on the duration of the action potential during repolarization and thereby influence the refractoriness of cardiac tissues, defined as the period after depolarization during which a suitable stimulus cannot evoke a propagated action potential. This property of digitalis is of major significance in slowing rapid ventricular rate in patients with atrial fibrillation, and this action in itself is useful in reducing congestive heart failure (BEISER et al., 1968).

Digitalis can prolong the functional refractory period of the AV node, which has the longest refractory of any type of cardiac tissue, and in this manner the rate at which stimuli from supraventricular pacemakers are transmitted to the ventricles is determined. In addition, the refractory period of the AV node is greatly prolonged by vagal activity and shortened by sympathetic activity. The action of digitalis in increasing the refractory period of this specialized structure is complex and involves vagal stimulation, inhibition of adrenergic influences, and the direct effect of the drug (MENDEZ and MENDEZ, 1953; MORROW et al., 1963 b). In contrast to its effects on the AV node, digitalis shortens the refractory period of atrial and ventricular myocardium (MENDEZ and MENDEZ, 1953), a property that leads to a decrease in the QT interval of the electrocardiogram. Digitalis also produces other effects on the electrocardiogram which take place during the course of repolarization. The ST segment and often the T wave are altered in a fashion so that their vectors are opposite in direction to the major QRS forces, actions that are direct and not influenced by anti-autonomic drugs.

III. Disorders of Impulse Formation

Although the mechanisms responsible for the genesis of digitalis-induced arrhythmias are not completely defined, it is generally acknowledged that two basic processes are responsible, as also is the case in disorders of cardiac rhythm unrelated

to digitalis (SCHAMROTH and YOSHONIS, 1969). Certain arrhythmias produced by digitalis are attributable to ectopic pacemaker activity discharging irregularly or rhythmically in specialized conductive tissue other than the sinoatrial node, and perhaps in other tissue as well; these are considered disorders of impulse formation. This property of digitalis which increases spontaneous diastolic depolarization in subsidiary pacemakers, leads to the development of premature extrasystoles, especially in the ventricles when the supraventricular pacemaker is not accelerated.

IV. Disorders of Impulse Conduction

Other arrhythmias produced by digitalis are considered disorders of impulse conduction, the persistence of which results from circular movements of electrical wavelets of the re-entry or reciprocal excitation type initiated by a premature beat. The conduction velocity and the duration of the refractory period are central to the perpetuation of re-entrant arrhythmias. It is likely that many digitalis-induced tachyarrhythmias result from disorders of either impulse formation or conduction, or both. Indeed, the combination of increased automaticity and depressed conduction rate in the ventricles may initiate the re-entry of abnormal impulses, which then may lead to the development of ventricular tachycardia and fibrillation. On occasion, multiple effects of digitalis are seen simultaneously in an individual patient, for example, ventricular tachycardia, complete atrioventricular block, and atrial asystole (for further details see Chap. 12).

V. Subcellular Basis of Toxicity

The tachyarrhythmic action of digitalis related to increased automaticity appears to be due to the inhibitory action of the drug on the activity of the Na^+, K^+-pump during diastole (GLYNN, 1957; MASON et al., 1969; PALMER and NECHAY, 1964; see Chap. 14). It is postulated that through this action the return of potassium into the cell is impeded after repolarization and the cation leaks from the cell during diastole, thereby leading to a reduction of intracellular potassium and an increase in extracellular potassium. This toxic pump-inhibiting action of digitalis is opposed by the addition of potassium salts (PALMER and NECHAY, 1964) and magnesium salts (SELLER et al., 1970). As a result of the altered activity of the membrane pump, there is postulated an imbalance of transmembrane flux of cations in quantitative favor of entrance of sodium into the cell, thus resulting in increased diastolic depolarization and ectopic pacemaker activity.

Potassium loss does not appear to be related to the other cardiac toxic actions of digitalis. Depression of conduction velocity in the development of AV nodal block is actually aggravated by administration of potassium (FISCH et al., 1964). Perhaps the action of digitalis on the cell membrane to diminish sodium entry during depolarization is responsible for delayed conduction; this explanation is consistent with the action of digitalis in promoting calcium ingress at the expense of sodium at the time of depolarization. Digitalis-induced decrease of conduction velocity in the AV node enhances decremental conduction with slowing of the wavefront in this structure and thereby diminishes the responsiveness and increases the functional refractory period of the AV junction. The drug actually reduces the

duration of the action potential in single fibers of the AV node and in atrial and ventricular conductive tissue. This influence of digitalis on the refractory period of single cardiac conductive fibers can perhaps be explained by the effect of the glycoside on the ease of potassium efflux during repolarization. Thus, it would be anticipated that digitalis enhances the egress of potassium from these cells, shortening the duration of the action potential and decreasing their refractory period.

C. Recognition of Toxicity

Since no specific cardiac arrhythmia is absolutely pathognomonic of digitalis excess, the diagnosis of glycoside toxicity is not based entirely on electrocardiographic evidence (MASON et al., 1971). Thus, a number of factors must be taken into account, such as the onset and duration of action, metabolism, and excretion of the digitalis preparation employed, and the predisposing background to glycoside sensitivity, as well as the electrocardiographic features and disappearance of the arrhythmia when the drug is not being administered.

I. Digoxin Pharmacodynamics

Pharmacodynamic studies using tritiated digoxin have shown that oral digoxin is absorbed considerably better than was previously appreciated (DOHERTY, 1973; DOHERTY et al., 1970).

Digoxin is water soluble and removed from the body in the unaltered state by the kidneys (DOHERTY and PERKINS, 1962). Thus, patients with renal failure are highly susceptible to the development of digoxin toxicity (BELLER et al., 1971; JELLIFFE et al., 1972; SMITH and HABER, 1970; GILFRICH and SCHÖLMERLICH, 1975; see Chap. 12). Urinary digoxin clearance correlates closely with glomerular filtration rate as estimated by creatinine clearance, and digoxin clearance may be depressed even before elevation of blood urea nitrogen levels (BLOOM and NELP, 1966). Digoxin dosage should be determined on the basis of lean body weight; obese patients should not be administered large doses based on total body weight (EWY et al., 1971). In contrast, digitoxin is lipid soluble, and its dosage is related to total body weight in obesity. Elderly patients generally have an increased susceptibility to digoxin toxicity because of their relative impairment of renal excretion and smaller body size (EWY et al., 1969).

II. Digitoxin Pharmacodynamics

Most of the glycosides are bound to plasma albumin in some degree. About 90% of digitoxin in the serum is protein bound (LUCAS and PETERSON, 1964). The agents with an intermediate onset of action are bound to a lesser extent, and ouabain is apparently not bound at all. The half-life of radiodigitoxin in the serum is 9 days, and that of radiodigoxin is 1–2 days (DOHERTY et al., 1961). Digitoxin is metabolized primarily by the liver (VÖHRINGER et al., 1976), but most of its metabolites ultimately appear in the urine (CALDWELL and GREENBERGER, 1971; DOHERTY, 1973). The more rapid rate of excretion of digoxin as compared with that of digitoxin ap-

pears to be related to the greater hydroxylation and solubility of digoxin (DOHERTY, 1973). Only a small fraction of digoxin is excreted in the biliary tract (CALDWELL and CLINE, 1976). The active forms of digitalis leaf and digitoxin are eliminated slowly from the body over the course of 2–3 weeks, whereas the effects of digoxin and deslanoside are dissipated in 2–6 days; ouabain is eliminated in 2–3 days.

III. Digitalis Radioimmunoassay

One of the most important advances in digitalis pharmacology has been the development of the radioimmunoassay technique for the accurate quantification of glycoside concentrations. Since the original development of the immunologic method for digoxin (BUTLER, 1970; SMITH et al., 1969) and digitoxin (OLIVER et al., 1968), radioimmunoassay has become widely available for routine clinical use in medical centers. Recently, a radioimmunoassay for ouabain also has been developed (SMITH, 1972).

Patients receiving daily maintenance doses of 0.25–0.50 mg oral digoxin without toxicity usually have therapeutic concentrations between 1 and 2 ng/ml of serum, whereas approximately 90% of patients with electrical toxicity have serum levels above 2 ng/ml (BELLER et al., 1971; SMITH and HABER, 1970). Patients with atrial fibrillation who require more than 0.50 mg of digoxin daily for rate control appear relatively less susceptible to toxicity, and they may have levels above 2 ng/ml. Although the range of overlap between nontoxicity and toxicity is 1.5–3.0 ng/ml, in the presence of levels above 2 ng/ml, it is generally prudent to discontinue digoxin until the serum level falls below this concentration (DOERING et al., 1977a, b). Diminished renal function appears to account fairly satisfactory for elevated concentrations of digoxin in patients intoxicated with this glycoside (SMITH and HABER, 1970). However, no significant difference in renal function has been observed in patients with toxicity from digitoxin, which is excreted in the bile.

IV. Acetylstrophanthidin Tolerance Test

Certain indirect tests have been advocated to determine the adequacy of digitalization (STONE and FISCH, 1969). However, the acetylstrophanthidin tolerance test has been largely abandoned since a number of deaths from ventricular fibrillation have occurred with its use. On rare occasions, the administration of 0.1 mg ouabain intravenously at 1 h intervals, with continuous electrocardiographic monitoring, has been employed in judging the degree of digitalization in patients with refractory heart failure.

V. Electrical and Vagal Stimulation Tests

Digitalis lowers the threshold for spontaneous repetitive ventricular extrasystoles in response to electrical stimuli, and this mechanism has been suggested as an electrical test for digitalis toxicity (CASTELLANOS et al., 1967). Thus, the concealed toxic effect of increased excitability has been demonstrated by the induction of ventricular tachycardia after single artificial pacemaker impulses of small energy delivered

in diastole. Since these impulses often fall late in diastole, the mechanism was defined as increased excitability rather than enhanced vulnerability. Vagal stimulation produced by carotid sinus massage, neostigmine, and edrophonium has been employed with variable success in atrial tachyarrhythmias to reduce transmission of impulses through the AV node, and thereby allow expression of digitalis-induced ectopic ventricular foci, the automaticity of which previously had been concealed in the presence of the more rapid supraventricular rate (FISCH and KNOEBEL, 1970). It has been postulated that in atrial fibrillation, an elevated resting heart rate or excessive increase in rate during exercise indicates inadequacy of digitalization. However, in patients with increased levels of sympathetic nervous activity in the postoperative period, with fever, or with advanced heart failure, the quantity of digitalis required to suppress the effect of increased adrenergic stimulation in shortening the refractory period of the AV node might approach toxic levels of the drug.

D. Conditions Affecting Toxicity

I. Hypokalemia, Hypomagnesemia, and Alkalosis

Digitalis intoxication may be precipitated by agents that promote potassium loss from the body, such as diuretics, adrenocorticoids, cathartics, and cation-exchange resins (MASON et al., 1971). Considerable caution must therefore attend the treatment of pulmonary edema with the concomitant use of large doses of diuretics and digitalis. The potassium loss in these instances is largely from the intracellular pool, and the level of serum potassium may be normal in the presence of increased susceptibility to digitalis intoxication. Also, the redistribution of potassium that accompanies rapid correction of acid-base abnormalities may alter the sensitivity to toxicity with digitalis. The infusion of glucose and insulin which lowers the concentration of extracellular potassium potentiates digitalis toxicity. In addition, hypomagnesemia (SELLER et al., 1970; LOEB et al., 1968), a condition frequently observed in alcoholism, and alkalosis (WARREN et al., 1968) can also cause digitalis toxicity.

II. Hypercalcemia

Although hypercalcemia enhances digitalis toxicity, this influence appears to occur only with high levels of serum calcium (NOLA et al., 1970). It is possible that calcium interferes with the Na^+, K^+-pump or that calcium increases the binding of digitalis to the myocardium.

III. Hypoxemia, Stroke, and Renal Disease

The high incidence of digitalis intoxication in patients with chronic cor pulmonale is most likely related to arterial hypoxia (BELLER et al., 1971). Perhaps the apparent increased susceptibility to digitalis-induced arrhythmias in strokes is also due to hypoxia or to increased sympathetic stimulation. Renal insufficiency impairs digitalis excretion and thereby increases glycoside toxicity with digoxin (MARCUS et al., 1966; SMITH and HABER, 1970).

IV. Hormone and Related Influences

It has been reported that sensitivity to digitalis is reduced by halothane anesthesia, hypothermia, and by cardiopulmonary bypass with loss of glycoside into the pump circuit (BEYDA et al., 1961; EBERT et al., 1963; MAGINN et al., 1961). Both the inotropic and toxic actions of digitalis are inversely related to the level of circulating thyroid hormone (BUCCINO et al., 1967; FRYE and BRAUNWALD, 1961; MORROW et al., 1963a). Thus, digitalis toxicity is provoked in hypothyroidism and diminished in hyperthyroidism. The extent of adrenergic activity appears to influence the toxic actions of digitalis (MORROW et al., 1963a, b; WILLIAMS and SEKIYA, 1963). Adrenergic stimuli appear to increase digitalis-induced arrhythmias, whereas depletion of myocardial catecholamine stores and antiadrenergic drugs exert a somewhat protective effect. In contrast, it has been postulated that reserpine promotes the development of digitalis-induced arrhythmias in patients with atrial fibrillation (LOWN et al., 1961), an unexpected finding in view of the protective effect of reserpine administration in certain instances upon the dose of the glycoside required to produce fatal ventricular fibrillation in experimental animals in normal sinus rhythm (ROBERTS et al., 1963).

V. Heart Disease

The toxic effects of digitalis also appear to be related to the presence or absence of heart disease. In myocardial infarction, the dose of glycoside required to produce ectopic, ventricular arrhythmias is reduced by one-third (SMITH and HABER, 1970); these digitalis-induced arrhythmias are more refractory to therapy and their duration is prolonged (LEVINE and SOMLYO, 1962; MARCUS, 1969; MORRIS et al., 1969). Furthermore, these arrhythmias may occur before evidence of toxic gastrointestinal effects. The dose of digitalis utilized in treating cardiac pump dysfunction in acute myocardial infarction should be reduced to approximately one-half to two-thirds of the level usually given. Reduced tolerance to digitalis probably also occurs in other patients with heart disease, although to a lesser extent than in myocardial infarction.

VI. Patient Age

Normal adult subjects who have accidentally taken a digitalis overdose do not always exhibit electrical toxicity, even with very large amounts of the drug. Children are less prone to the development of digitalis toxicity, and in children with heart disease the dose per unit body weight is often up to 50% higher than that for adults with heart disease (ROBINSON, 1960). Electrical toxic effects in children and healthy adults are usually manifested as the vagal action of the glycoside with sinus bradycardia, sinus arrhythmia, or AV conduction disturbances (LEVINE and SOMLYO, 1962), in contrast to the usual development of ventricular tachyarrhythmias observed in adults with heart disease. It is important to remember that the dose of digitalis in elderly patients should be less than in younger persons because of the smaller body size and delayed renal excretion in the elderly (EWY et al., 1969).

VII. Atrial Fibrillation

Of particular interest is the apparent special relation between atrial fibrillation and digitalis toxicity, since fatal arrhythmias can follow the application of electrical countershock in the presence of excessive or even normal amounts of digitalis (LOWN, 1967). In attempting to explain the occurrence of digitalis-induced ventricular irritability after successful cardioversion in patients receiving therapeutic doses of digitalis, it has been suggested that the amount of electrical energy required to produce ventricular tachycardia is reduced in the digitalized heart (LOWN et al., 1965) and that electrical discharge affects myocardial cellular membranes, resulting in a leakage of intracellular potassium which thereby enhances the background for digitalis toxicity (LOWN and WITTENBERG, 1968). An alternative possibility is that cardiac toxicity due to digitalis is reduced in atrial fibrillation, thereby allowing a greater tolerable maintenance dose, and that after cardioversion the level of digitalis is no longer tolerable and leads to ventricular irritability. This latter view is supported by the finding that in dogs the toxic dose of the glycoside producing ventricular tachycardia is greater during atrial fibrillation than during normal sinus rhythm (ELKINS et al., 1967).

Concerning the use of direct current countershock to convert supraventricular tachyarrhythmias to normal sinus rhythm electively in the presence of maintenance digitalis without pre-cardioversion glycoside toxicity, the degree of discharge energy required to produce life-threatening ventricular tachycardia is reduced in the digitalized heart (LOWN et al., 1967). Thus, some clinicians have reduced or withdrawn maintenance digitalis before the use of electroconversion. In contrast, our approach is to continue the drug therapy, because of the risk of exacerbating heart failure and developing a rapid ventricular rate before cardioversion, and to use small amounts of electrical energy at the time of electroshock (HUGHES et al., 1970). The frequency of ventricular tachyarrhythmias after electroconversion of supraventricular arrhythmias in the presence of digitalis can be reduced, if the shock energy is at the lowest level necessary to achieve conversion (Fig. 1). This is done by using initially small, incrementally increasing energy levels of countershock for restoring sinus rhythm and using lidocaine to suppress any premature ventricular contractions occurring with direct current shock.

E. Potassium–Digitalis Interactions

The serum potassium concentration is known to influence the actions of digitalis significantly (MASON et al., 1971). Both the toxic and contractile actions of digitalis are increased in the presence of hypokalemia (COHN et al., 1967; GOLDSMITH et al., 1969; PRINDLE et al., 1971). Consistent with this observation is the finding that both the toxic and contractile effects of digitalis are reduced when the glycoside is administered during hyperkalemia (GOLDMAN et al., 1973; MATSUI and SCHWARTZ, 1968; MORGAN and BINNION, 1970). The relationship between potassium and the contractile action of digitalis was recently examined in isolated, supported cat papillary muscles in our laboratories (LEE et al., 1977). The linear dose–contractile response curve for acetylstrophanthidin added to a muscle bath with an extracellular

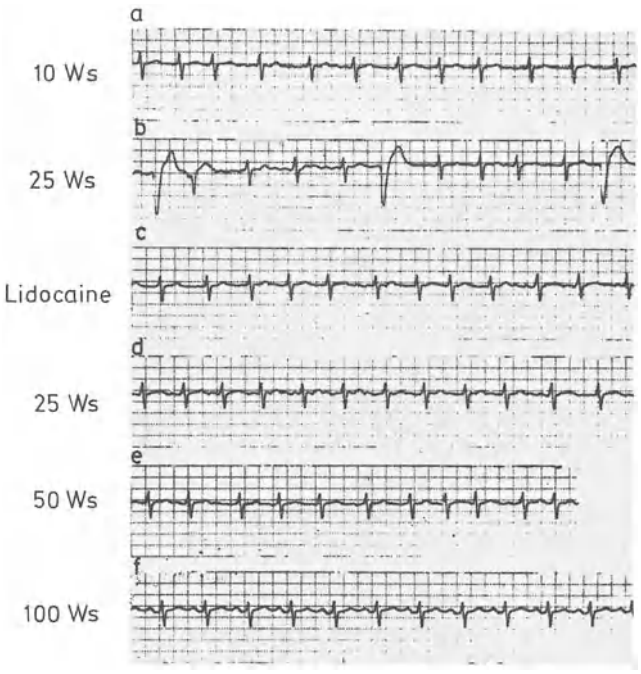

Fig. 1 a–f. Sequence of initially small, incrementally increasing levels of direct current (DC) in cardioversion of atrial fibrillation in presence of maintenance digitalis in patient with congestive heart failure. Atrial fibrillation persists after 10 W s countershock (**a**). After 25 W s (**b**), atrial fibrillation remains, now with premature ventricular contractions which are abolished by intravenous lidocaine (**c**). Atrial fibrillation still not terminated after repeat 25 W s discharge (**d**) and 50 W s countershock (**e**). Sinus rhythm restored following 100 W s direct current discharge (**f**)

potassium concentration of 3.5 m*M* was markedly depressed when the drug was added to an extracellular bath of 7.0 m*M* potassium concentration, demonstrating attenuation of inotropic stimulation by digitalis after pretreatment with potassium (Fig. 2). In contrast, increasing the extracellular potassium concentration in the muscle bath from 3.5 to 7.0 m*M* after pretreatment with digitalis did not alter the force of cardiac muscle contraction.

From these (Lee et al., 1977) and related (Allen and Schwartz, 1971) observations, potassium and digitalis appear to compete for myocardial binding sites. However, potassium is relatively loosely bound to the myocardium, and it delays subsequent digitalis binding. In contrast, digitalis is firmly bound to myocardial receptors, and thus potassium has little effect on glycoside already attached to the heart when potassium is administered. Translated into the clinical setting, alterations in serum potassium levels effected before treatment with digitalis have marked influences on the toxic and contractile actions of the glycoside. However, potassium has relatively little influence on the toxic and contractile effects of digitalis when it is given after the glycoside has been taken up by the heart.

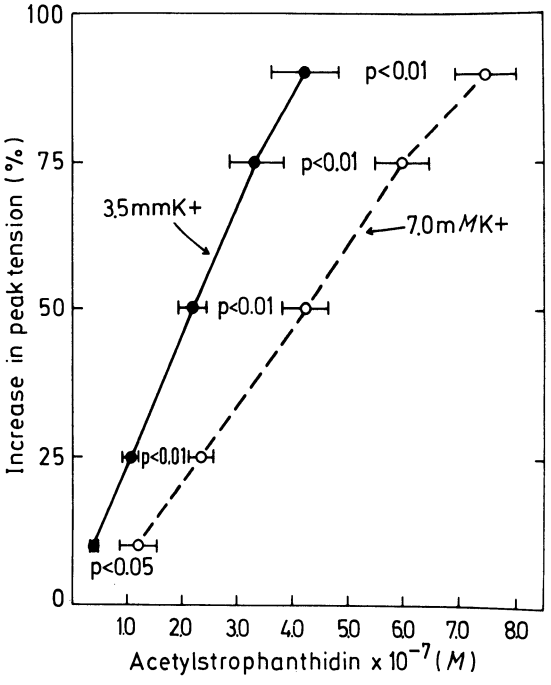

Fig. 2. Effects of cumulative doses of acetylstrophanthidin on percentage increases of peak tension above control peak tension in the 3.5 m*M* K^+ (*closed circles* and *solid line*) compared with the 7.0 m*M* K^+ (*open circles* and *broken line*) media. (LEE et al., 1977)

F. Quinidine–Digoxin Interactions

The recent observation that the institution of quinidine in patients receiving standard maintenance digoxin therapy frequently results in considerable (often two-fold) elevation of serum glycoside levels (DOERING and KÖNIG, 1978) in consort with the onset of extracardiac and myocardial electrophysiologic manifestations of digitalis toxicity (LEAHEY et al., 1978) has suggested important kinetic disturbances of digoxin by physiologically meaningful doses of oral quinidine. In additional clinical studies, initial quinidine dosage appeared to displace the glycoside rapidly from extracardiac tissue binding sites into the circulation, as well as to delay renal excretion of digoxin in the absence of kidney disease (LEAHEY et al., 1979). Further investigation of this phenomenon in patients has also shown that this quinidine–digoxin response is quite common, and is in agreement with the mechanisms being glycoside displacement from body tissue and quinidine inhibition of digoxin renal excretion (HAGER et al., 1979); average steady-state quinidine plasma levels were only minimally increased in the presence of digoxin.

Other workers using experimental animals have confirmed that redistribution of digoxin from its major depots is the basis of the digoxin–quinidine interaction (DOHERTY et al., 1979); these canine studies showed that quinidine administration resulted in considerable reduction of tritiated digoxin from skeletal and cardiac muscle with consequent increase in brain glycoside levels, evidence suggesting that

quinidine-induced digitalis toxicity is neurally mediated and may be associated with lessened contractile effect of digoxin. In view of these important new findings concerning altered digoxin pharmacokinetics, digoxin toxicity appears to be enhanced in the presence of quinidine and, therefore, substantial reduction of conventional digoxin maintenance dosage is required with concomitant quinidine treatment, as well as frequent assessment of plasma digoxin concentrations and careful attention to the appearance of clinical symptoms of digitalis intoxication.

G. Digitalis-Induced Arrhythmias

Although digitalis is probably capable of producing every type of cardiac arrhythmia, certain abnormalities occur more frequently than other (CHOU, 1970; CHURCH et al., 1962; DREIFUS et al., 1963; FISCH and KNOEBEL, 1970; MASSUMI et al., 1972). Present evidence now indicates that the mechanism of digitalis-induced cardiac ectopy is due in part to heightened adrenergic activity resulting from glycoside action on the brain stem and on the sympathetic ganglia (GEORGE et al., 1974; HELKE et al., 1979; STORSTEIN et al., 1979; WEAVER et al., 1976). Two of three rhythm disorders are premature ventricular systoles; particularly characteristic are bigeminy (Fig. 3) and multifocal ectopic beats. Manifestations of AV block appear in about one-third of patients with digitalis-induced arrhythmias. Less common but more indicative of digitalis toxicity are paroxysmal atrial tachycardia with block, junctional tachycardia (Fig. 4), AV dissociation in which the ventricular pacemaker exhibits a faster frequency than the atrial rate without block, sinoatrial arrest, sinoatrial block (Fig. 5), ventricular tachycardia, and ventricular fibrillation. Bidirectional ventricular tachycardia is particularly characteristic of severe digitalis over-

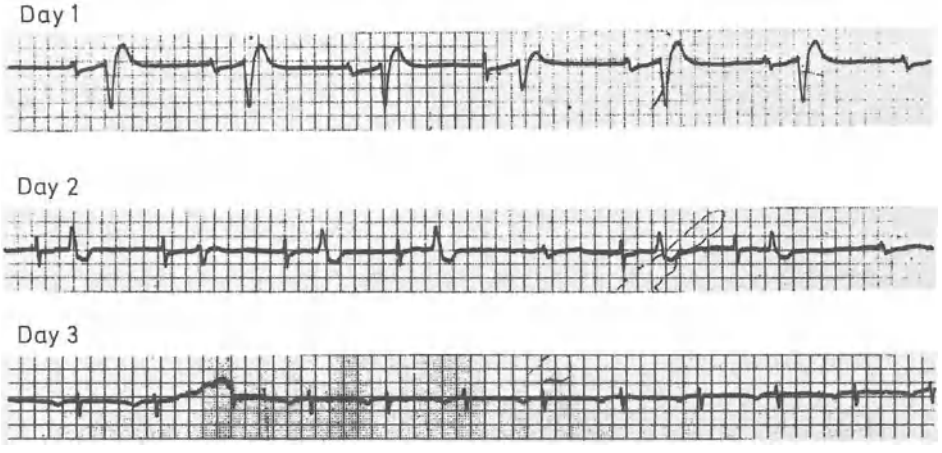

Fig. 3. Sequential daily electrocardiograms (precordial monitor lead) from a 67-year-old patient after self-administered intentional overdose of oral digitoxin. The excessive dose of digitoxin produced, in addition to a toxic serum level of digitoxin (90 ng/ml), a toxic serum level of digoxin (3.5 ng/ml) due to metabolism of digitoxin. Cardiac rhythm progressed from sinus bradycardia (day 1), to idioventricular rhythm with bigeminy (day 2), and finally to normal sinus rhythm (day 3). Successful treatment consisted of prophylactic temporary pacemaker ventricular overdrive

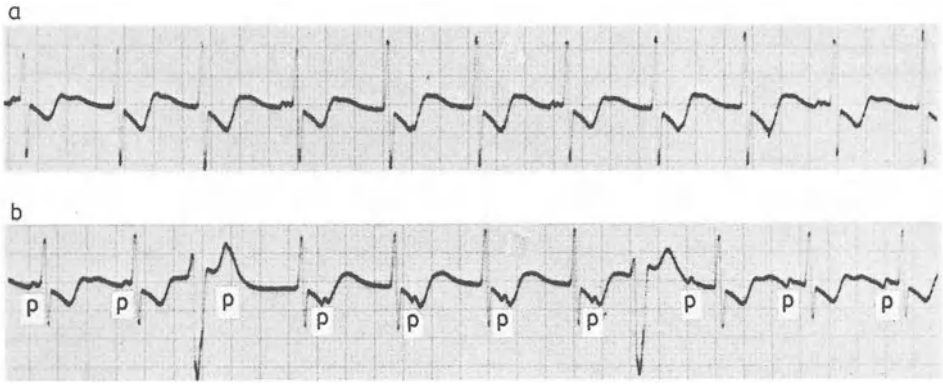

Fig. 4 a, b. This man with chronic lung disease, ischemic heart disease, and renal insufficiency had blood urea nitrogen of 90 mg/100 ml and serum creatine of 4.2 mg/100 ml. The maintenance dose of 0.25 mg/day digoxin led to emergence of junctional rhythm at rate of 96 beats/min in presence of sinus rate of 90 per minute. **a** and **b** are continuous monitor leads. Increased automaticity of the AV junction related to direct effects of high levels of digitalis on the AV junction. This nonparoxysmal junctional tachycardia is highly characteristic of digitalis excess and generally represents mild to severe intoxication. In this patient the drug was discontinued for 5 days, by which time sinus rhythm returned. Cardiac output was reduced during the junctional rhythm and increased when sinus rate returned. (MASSUMI et al., 1972)

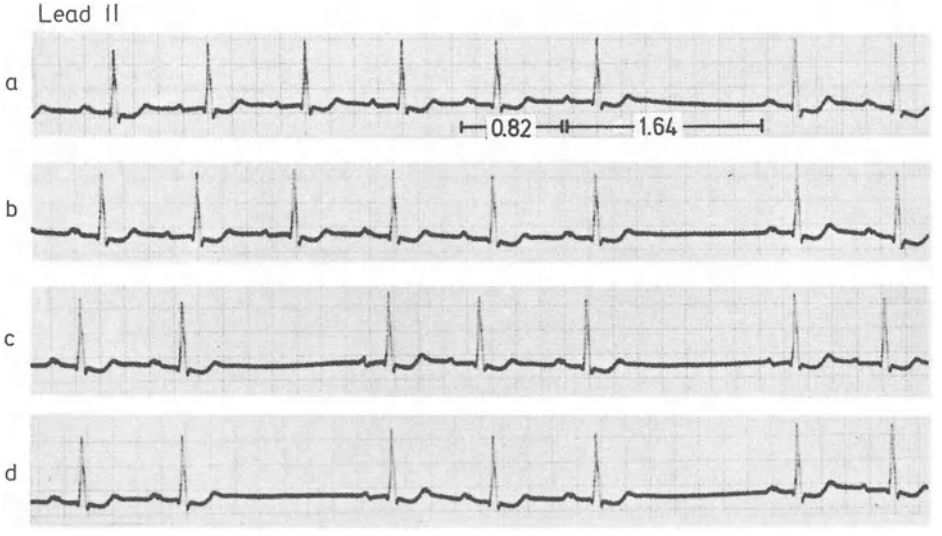

Fig. 5 a–d. Four strips of lead II taken from a 53-year-old man with idiopathic hypertrophic subaortic stenosis, taking digitalis leaf 0.1 g daily and who had recently exhibited anorexia and weakness. Electrocardiogram shows first degree AV block with PR interval 0.24 s and periodic appearance of a long cycle with no visible P waves. The length of these PP cycles is almost exactly twice the normal PP interval, indicating that one discharge from the sinoatrial node is blocked, which is characteristic of digitalis excess. Heavier intoxication usually adds greater degree of AV block and causes markedly slowed heart rate with low cardiac output. (MASSUMI et al., 1972)

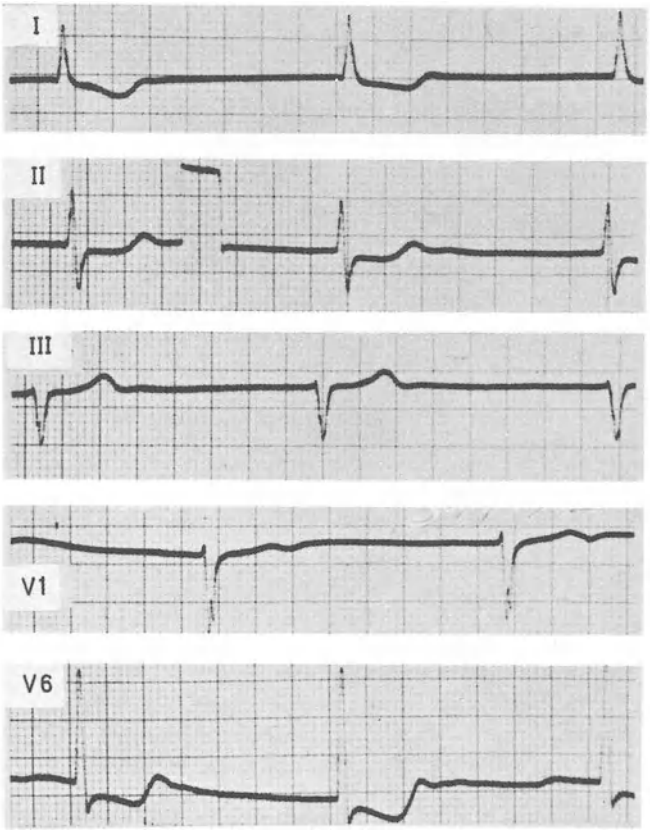

Fig. 6. Selected leads from a 78-year-old woman taking 0.25 mg/day digoxin for chronic congestive heart failure who developed increasing weakness and lassitude. She was found to have atrial fibrillation with a very slow, fixed ventricular rate of 40 beats/min suggesting complete heart block secondary to digitalis toxicity. Digoxin serum level was 3.1 ng/ml. (MASSUMI et al., 1972)

dose and results from alterations in intraventricular conduction, junctional tachycardia with aberrant intraventricular conduction or, on rare occasions, alternating ventricular pacemakers. The incidence of atrial fibrillation due to digitalis toxicity is higher than is commonly appreciated. Thus, slow regular ventricular rates induced by digitalis in the treatment of atrial fibrillation might be due to complete heart block (Fig. 6) or drug-prolonged concealed conduction of fibrillatory waves into the AV junction.

A spectrum of electrophysiologic manifestations resulting from digitalis administration is observed on the standard electrocardiogram, beginning with ST-T wave changes that often accompany the salutary contractile effects of the drug and ending with ectopic impulses and conduction disturbances considered to be evidence of digitalis excess (MASON et al., 1971). Isolated prolongation of the PR interval is not considered a toxic manifestation. Reducing conduction velocity and pro-

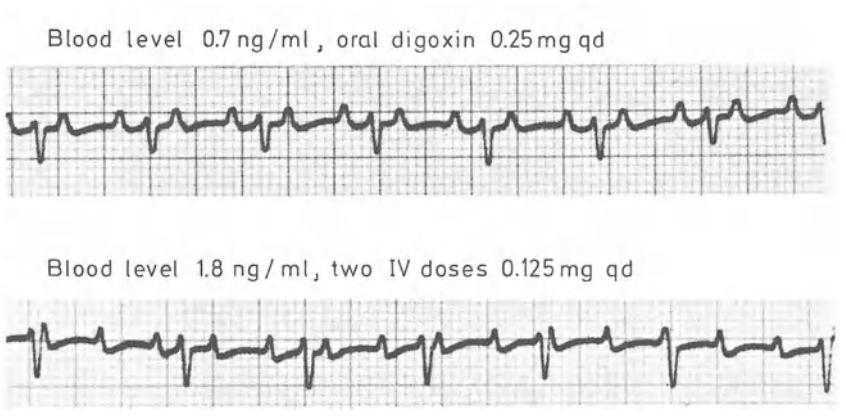

Fig. 7 a, b. Two strips of V_1 in a 59-year-old man with previous myocardial infarctions, mitral regurgitation, and left ventricular aneurysm, complicated by severe congestive heart failure and anasarca. Atrial flutter is present in both strips. The ventricular rate of 124 beats/min on admission persisted for several days while receiving oral digoxin 0.25 mg/day (**a**). However, when digoxin was given intravenously 0.125 mg/day for 2 days, the ventricular rate decreased to 90–110 beats/min and cardiac function improved remarkably (**b**). Digoxin serum level, which was only 0.7 ng/ml on oral medication, rose to 1.8 ng/ml on intravenous therapy. This and similar experiences in other edematous patients have led us to the impression that digitalis may not be absorbed adequately from the edematous gut in some individuals with right-heart failure, in whom an intravenous route is prudent. (MASSUMI et al., 1972)

longing the functional refractory period in the AV node is beneficial in decreasing the rapid ventricular rate observed with supraventricular tachycardias and atrial fibrillation (Fig. 7). In congestive heart failure, the slowing of sinus tachycardia by digitalis is attributable to improvement of impaired hemodynamics and accompanying sympathetic withdrawal, not to a direct or vagal action of the drug upon the sinus pacemaker (MASON and AWAN, 1979; MASON and BRAUNWALD, 1968; MASON et al., 1969). However, when extrasystoles are isolated or occur in runs, or when it is concluded that advanced AV block is being produced by the glycosides, digitalis toxicity should be recognized and appropriate management of these electrical disorders instituted.

H. Treatment of Toxicity

Although glycoside toxicity can now be reversed with rapidly excreted Fab fragments of sheep digitalis antibodies in desperate potentially lethal situations refractory to conventional management (SMITH et al., 1976), no practical specific antidote is currently available for widespread application in the too common problem of digitalis electrical intoxication in patients. However, special advantages pertain to the use of potassium (MASON et al.,1971), phenytoin (DAMATO, 1969), lidocaine (BIGGER and HEISSENBUTTEL, 1969), propranolol (GIBSON and SOWTON, 1969), bretylium (AMSTERDAM et al., 1972), and cardiac pacing (ZELIS et al., 1970). Although the administration of potassium or anti-arrhythmic drugs after the onset of electrical toxicity in experimental animals can suppress digitalis-induced

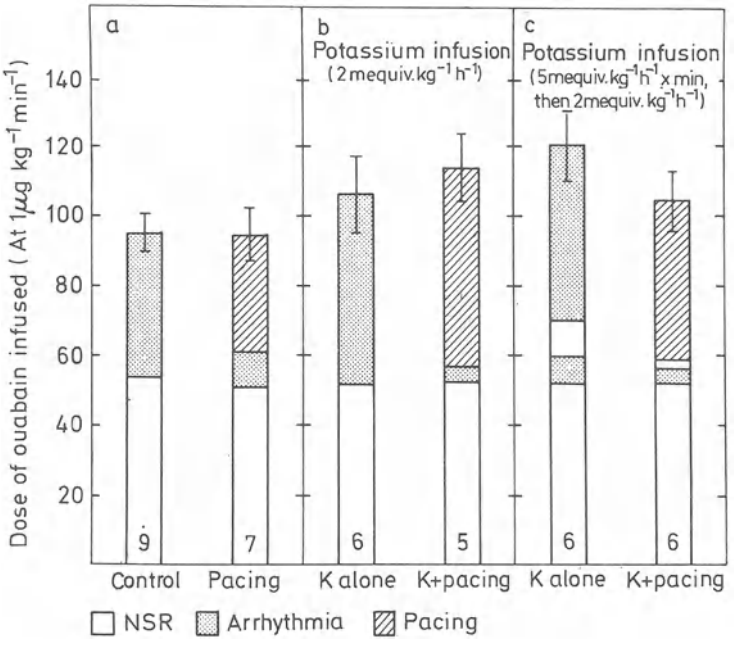

Fig. 8a–c. Doses of ouabain required to produce arrhythmias and death in control animals *(first vertical bar)*, with single electrical pacing of the ventricle carried out at the onset of arrhythmia *(second bar)* (**a**); and in 4 additional groups of dogs (**b, c**) during potassium administration with or without pacing *(third through sixth bars)*. NSR = normal sinus rhythm. (MASON et al., 1971)

tachyarrhythmias, studies in our laboratories (ZELIS et al., 1970) have shown that the accumulated dose of digitalis at which these arrhythmias become refractory and fatal is not increased by this treatment (Fig. 8). Similarly, ventricular overdrive pacing begun at the onset of ouabain-induced tachyarrhythmias transiently overcame these rhythm disorders, but the maximal tolerated dose of ouabain prior to death was not altered compared with that in control animals receiving digitalis in the same manner (Fig. 8).

In clinical practice, however, digitalis is discontinued at the onset of toxicity, and anti-arrhythmic measures are employed to suppress ectopic activity until the glycoside is excreted or metabolized. Any hypokalemia should, of course, be corrected. Even in the absence of hypokalemia, potassium should be administered whenever ventricular irritability is present, since ventricular arrhythmias are related to intracellular potassium loss, potassium delays further binding of the glycosides to the myocardium, and the cation has the anti-arrhythmic effect itself of reducing diastolic depolarization of ectopic pacemakers (FISCH et al., 1964).

I. Quinidine and Procainamide

Although both quinidine and procainamide are useful in reducing increased automaticity produced by digitalis, these anti-arrhythmic drugs may induce or worsen

AV block. In using procainamide for ventricular tachyarrhythmias, it may become necessary to discontinue the drug because of the development of excessive widening of the QRS complex. Continued suppression of the arrhythmia can be obtained by adding lidocaine or phenytoin without exacerbating the conduction delay, while increasing depression of ectopic automaticity (BIGGER and HEISSENBUTTEL., 1969; DAMATO, 1969).

II. Lidocaine and Phenytoin

Lidocaine is more effective than procainamide or quinidine in the treatment of digitalis-induced ventricular tachycardias (HILMI and REGAN, 1968) and is reported not to affect the conduction velocity in the AV node and the ventricular myocardium (BIGGER and HEISSENBUTTEL, 1969). Phenytoin is particularly useful for premature ventricular contractions associated with digitalis toxicity, since this anti-arrhythmic drug depresses enhanced ventricular automaticity without affecting intraventricular conduction. Phenytoin also tends to reverse the glycoside-induced prolongation of AV conduction. Phenytoin has been shown to dissociate the inotropic and arrhythmic actions of digitalis (HELFANT et al., 1967), thus depressing digitalis-induced tachyarrhythmias without diminishing the contractile effects of the glycoside. In addition, phenytoin can terminate supraventricular tachycardias induced by digitalis (CONN, 1965), whereas lidocaine has not been as useful in these conditions.

Quinidine, procainamide, lidocaine, and phenytoin are useful in terminating ectopic impulses resulting from disorders of either impulse formation or conduction, since each of the drugs diminishes diastolic depolarization, thereby reducing automaticity, and alters conduction velocity and the refractory period. However, since quinidine and procainamide depress conduction velocity and lengthen the refractory period, whereas lidocaine and phenytoin shorten the refractory period, these latter two drugs may be effective when procainamide and quinidine are not; the reverse may also be true.

III. Propranolol

Propranolol is particularly effective in the treatment of certain digitalis-induced tachyarrhythmias (GIBSON and SOWTON, 1979), and current evidence suggests that the β-adrenergic blocking action, rather than the direct membrane effect of the agent, is most important in this regard (COLTART et al., 1971; SEIDES et al., 1974). Although propranolol is useful in the treatment of both supraventricular and ventricular arrhythmias due to digitalis toxicity, it has been most successful in terminating premature ventricular extrasystoles (GIBSON and SOWTON, 1969). In patients who have digitalis-induced atrial tachycardia with AV block, propranolol usually has restored sinus rhythm, although depressed nodal conduction has been exacerbated. Thus, phenytoin is preferable to propranolol in the initial treatment of glycoside-induced atrial tachycardia with block. Since propranolol, the quinidine-like drugs, and potassium diminish conduction velocity, their use appears to be limited to supraventricular and ventricular extrasystoles and tachycardias not associated with serious degrees of AV block. In patients with digitalis-in-

duced paroxysmal supraventricular arrhythmias with or without AV block, propranolol can be used to slow rapid ventricular rate by decreasing the atrial rate or by reducing the conduction velocity and increasing the functional refractory period of the AV node.

IV. Bretylium and Cholestyramine

Bretylium also has been reported to be useful in digitalis-induced ventricular tachyarrhythmias (AMSTERDAM et al., 1972; BACANER, 1968). A new approach in the treatment of digitalis toxicity is the use of cholestyramine, which diminishes the absorption and enterohepatic circulation of digitoxin (CALDWELL and GREENBERGER, 1971; BROWN et al., 1978).

V. Ventricular Pacemaker Overdrive

Electrical pacemaker ventricular overdrive by electrode catheter has been successful in suppressing digitalis-induced ventricular tachyarrhythmias (ZELIS et al., 1970). However, neither ventricular pacing nor potassium alters the maximal tolerated dose of digitalis before the development of fatal arrhythmias (ZELIS et al., 1970). More important is that rapid ventricular pacing with a single electrical stimulus effectively overcomes serious digitalis-induced arrhythmias (Fig. 9). Al-

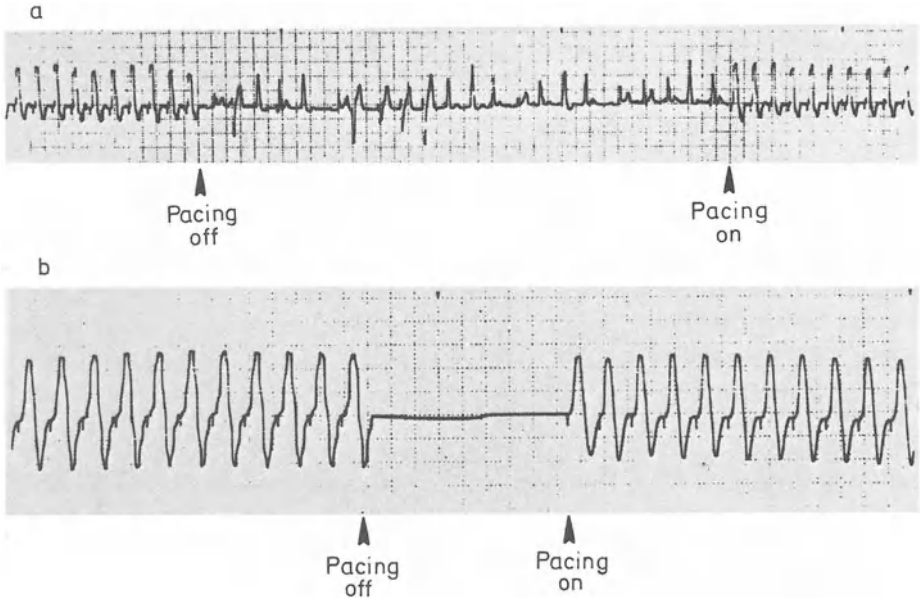

Fig. 9. a Electrocardiogram of an animal taken after the onset of digitalis-induced tachyarrhythmia. At the beginning and end of the tracing, the multifocal premature ventricular impulses were completely suppressed by single electrical ventricular stimulation. **b** Electrocardiogram of an animal with atrial and ventricular arrest induced by digitalis, observed during the temporary interval in which the ventricular pacemaker was turned off. The pacemaker had been started previously because of the development of ventricular tachycardia induced by digitalis. (MASON et al., 1971)

though the total fatal dose of digitalis is not changed by electrical pacing, digitalis is discontinued at the onset of toxicity, and anti-arrhythmic drugs in addition to ventricular overdrive are employed to suppress ectopic activity until the glycoside is metabolized or excreted. Ventricular pacing should not be utilized as the initial approach in the treatment of digitalis-induced ventricular tachycardias; rather, it should be employed only when standard measures fail, since digitalis lowers the threshold for spontaneous repetitive ventricular extrasystoles in response to pacemaker stimuli (LOWN et al., 1967). The administration of phenytoin to suppress automaticity without encouraging re-entry mechanisms mediating ectopic tachycardias might eliminate the electrical hazard of provoking spontaneous arrhythmias (HELFANT et al., 1968).

VI. Rapid Right Atrial Pacing

Rapid right atrial pacing has been reported to be a useful means of terminating supraventricular tachycardias due to digitalis toxicity without enhancing the tendency for glycoside-induced ventricular tachyarrhythmias (LISTER et al., 1968). Although paired electrical ventricular pacing has been shown to be an effective means of overcoming digitalis-induced arrhythmias in experimental animals (FROMMER et al., 1965), this technique has not been used clinically because of the danger of inducing ventricular fibrillation. Precordial electrical countershock is contraindicated in the presence of digitalis toxicity since there is an increased propensity for glycoside-related ventricular fibrillation after electroshock (LOWN, 1967; LOWN et al., 1967).

VII. Atrioventricular Block

In the management of digitalis-induced conduction abnormalities with advanced degrees of heart block and slow ventricular rate, it is important to discontinue the drug and consider protective therapy. Since potassium itself prolongs impulse conduction and lengthens the refractory period in the AV node (FISCH et al., 1966), the cation is usually not given in this manifestation of digitalis toxicity. Atropine may be effective in terminating AV block induced by excessive vagal action of digitalis (FISCH and KNOEBEL, 1970). Isoproterenol has the disadvantage of increasing ventricular ectopic activity in the presence of toxic levels of digitalis (FISCH and KNOEBEL, 1970). Cardiac stimulation with an endocardial electrode catheter has been used successfully in the management of digitalis-induced ventricular asystole with complete heart block (Fig. 9).

J. Conclusions

Digitalis toxicity is among the most common adverse drug reactions, and may cause arrhythmias and conduction disturbances in as many as one in five patients. Particularly responsible are the electrophysiologic properties of digitalis in increasing the automaticity of subsidiary pacemakers, reducing the refractory period and prolonging conduction velocity in the atria and ventricles, and delaying conduction in the atrioventricular node. Underlying these electrophysiologic efffects are digi-

talis-influenced alterations of cardiac cell transmembrane movements of sodium and potassium; the induction of ectopic impulses due to increased automaticity appears to be caused by inhibition of the activity of the Na^+, K^+-pump. Digitalis can provoke every type of cardiac arrhythmia, and no specific disorder of rhythm can be considered absolutely pathognomonic of digitalis toxicity. The factors that predispose to digitalis toxicity, as well as the onset and duration of action of the agent, must be taken into account. Potassium has relatively little influence on the toxic and the contractile actions of digitalis when the cation is administered after the glycoside has been taken up by the myocardium. In contrast, alterations in serum potassium effected before treatment with digitalis may have drastic effects on the electrophysiologic and contractile actions of the glycoside; hyperkalemia reduces the binding of digitalis to the myocardium. Phenytoin, lidocaine, and propranolol are effective in terminating digitalis-induced tachyarrhythmias, usually without inducing or worsening atrioventricular block. Atrial or ventricular pacing to achieve electrical overdrive of the ectopic focus may be used if standard measures fail. In complete heart block, potassium should not be administered; atropine and electrical pacing should be used.

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CHAPTER 14

Interactions Between Cardiac Glycosides and Other Substances in the Body*

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A. Introduction

It is well documented that cardiac glycosides require careful dosage adjustment in the individual patient to achieve satisfactory clinical responses without toxic symptoms. Many factors contribute to the variation in dosage between patients, for instance, variable absorption, differences in disposition pharmacokinetics, and severity of the heart disease. In addition, interactions with other substances have been found to explain observations of resistance or increased sensitivity to treatment with cardiac glycosides.

The literature contains an overwhelming body of reported interactions that come from speculation, in vitro experiments on isolated organs, animal experiments, or irrelevant concentrations/doses of drug. Translation of such information into the therapeutic situation must be undertaken with caution. We have therefore focused our attention predominantly on clinically interesting experiences. Nonhuman experiments will be discussed to explain mechanisms or when established pharmacologic or physicochemical principles give a rationale for a possible interaction.

Concurrent treatment with other agents may alter the glycoside level at the receptors or the reactivity at a given glycoside concentration. Interactions that influence the amount of drug reaching the site of action (pharmacokinetic interactions) and those affecting the response by acting at the receptor level (pharmacodynamic interactions) are often discussed separately. In the following, we will keep to this distinction, but as the text will show, some interacting substances cannot readily be put into only one of these two groups. Generally, interactions with the pharmacokinetics can often be discussed in mechanistic terms, which is not that common when dealing with pharmacodynamic interactions.

B. Interactions with Cardiac Glycosides Influencing the Amount of Active Drug Available at the Site(s) of Action (Pharmacokinetic Interactions)

Before the active drug substance reaches its site(s) of action, it must go through a number of events. The amount of drug available at the receptor then depends on the balance between supply and elimination, i.e., between absorption and dis-

* This review covers the literature through 1978. Some discussions contain more recent information

tribution on the one hand and redistribution, metabolism, and excretion on the other. These processes are summarized in the term pharmacokinetics; consequently, pharmacokinetic interactions occur with agents that influence the active drug substance on its way to or from the receptor.

With few exceptions, reports on pharmacokinetic interactions in the literature deal with effects of other agents on cardiac glycosides, and not vice versa. This is probably because cardiac glycosides are administered in very small doses and are highly distributed to different tissues, thereby having little potential to affect the pharmacokinetics of other drugs.

I. Interactions in the Gastrointestinal Tract

Interactions with cardiac glycosides before or during absorption, particularly in the gastrointestinal tract, are among the most widely investigated pharmacokinetic drug interactions. Research interest was greatly stimulated by the discovery of variable bioavailability of digoxin tablets (MANNINEN et al., 1971; LINDENBAUM et al., 1971) and its connection with dissolution rate (BERTLER et al., 1972; WAGNER et al., 1973; JOHNSON et al., 1973; SHAW et al., 1973). Cardiac glycosides can interact with other substances in the stomach or intestine via several mechanisms. Most of them decrease the amount of glycoside that reaches the blood.

1. Chemical Interactions

a) Hydronium Ion

The hydronium ion (H_3O^+) is the smallest chemical entity that interferes with the pharmacokinetics of cardiac glycosides. In vitro studies have shown that digitalis and scilla glycosides are degraded in acid milieu at a rate proportional to the hydrogen ion activity. Thus, at pH 1 and 37 °C, cleavage of digoxin and digitoxin and inactivation of proscillaridin A proceed with a half-life of about 15 min, and at pH 2, ten times slower (STERNSON and SHAFFER, 1978; HOSSIE et al., 1977; BERGDAHL and ANDERSSON, 1977). β-Methyldigoxin is hydrolyzed and methylproscillaridin inactivated at about the same rate (KUHLMANN et al., 1973; BERGDAHL and ANDERSSON, 1977), whereas acid-catalyzed hydrolysis of β-acetyldigoxin appears to be somewhat slower (KUHLMANN et al., 1973).

Because of differences in pharmacokinetics and intrinsic activity between parent glycosides and their hydrolysis products, the clinical consequences of the in vitro findings have recently attracted considerable interest. Under provocative experimental conditions, such as left-side recumbent position of the (fasting) subjects to retard gastric emptying (LOO et al., 1977) or that position combined with pentagastrin injection to stimulate secretion of gastric juice (GAULT et al., 1977), considerable hydrolysis of orally given digoxin could be demonstrated. Results from trials under more normal conditions did not indicate much hydrolysis in the gastrointestinal lumen of digoxin (BEERMANN et al., 1972; GAULT et al., 1976), digitoxin (VÖHRINGER and RIETBROCK, 1974), or β-acetyldigoxin (FLASCH et al., 1977).

Experiences with other cardiac glycosides regarding acid-catalyzed hydrolysis in man are less conclusive. Gastric aspirates obtained after oral administration of a solution of β-methyldigoxin to fasting healthy subjects contained, on average,

about 20% hydrolysis products (BEERMANN, 1972b). As the samples were taken 10–52 min after administration, when a considerable amount of drug must have left the stomach, hydrolysis calculated as a fraction of the administered dose should have been smaller. This would agree with an estimate of, on average, 13% hydrolysis of β-methyldigoxin ascribed to processes in the gastrointestinal tract (HINDERLING et al., 1977).

Achlorhydric subjects had, on average, higher plasma concentrations of proscillaridin A than normal subjects up to 12 h after a single oral dose (ANDERSSON et al., 1977a). The subject groups were not matched, e.g., the achlorhydrics were considerably older. In a mainly crossover study, enteric-coated tablets gave higher steady-state plasma concentrations of proscillaridin A then plain tablets (ANDERSSON et al., 1975). The authors suggested that the differences might have arisen as a consequence of less acid-catalyzed degradation in the achlorhydric subjects or when the drug was protected by enteric coating.

After oral administration of lanatoside C, two peaks in the plasma concentration profile (measured as digoxin) are usually seen. The first peak has been ascribed to acid hydrolysis of the otherwise poorly absorbed drug. This is reasonable, as hourly antacid treatment up to 4.5 h after administration resulted in a substantial decrease of that first peak in five of seven subjects (ALDOUS and THOMAS, 1977). The interaction is not of clinical importance, the totally absorbed amount not being affected, probably because of subsequent hydrolysis in the gut by microbial activity (cf. below).

The general impression from the investigations performed so far is that, under normal clinical conditions, hydrolysis of cardiac glycosides by acid in the gut is not therapeutically important. This is probably because, even in the fasting state, only few subjects have critically low pH values in the stomach (KUNA, 1964) and also because gastric emptying is often rapid enough to transfer most of the drug intact into the less acidic intestine.

b) Enzyme Activity

Human digestive enzymes are not known to be very active against cardiac glycosides. However, enzymes from bacteria – particularly enterococci existing in the lower human small intestine – have degrading ability (HERRMANN and REPKE, 1969; HAWKSWORTH et al., 1971). By their activity, lanatoside C can be transformed into α-acetyldigoxin, which in turn can be deacetylated to digoxin. This degradation seems to explain the second peak of the plasma concentration profile after oral administration of lanatoside C and part of the different metabolic patterns observed in urine after oral and intravenous administration of the drug (ALDOUS et al., 1972; BEERMANN, 1972a; DENGLER et al., 1973).

2. Physical Interactions

a) Activated Charcoal

The in vitro binding of cardiac glycosides to activated charcoal is high and increases with decreasing polarity of the substances (BELZ, 1974). In man, intake of 2 g of activated charcoal soon after digoxin substantially reduced absorption of the glycoside, whereas intake of 50 g virtually prevented it (HÄRTEL et al., 1973;

NEUVONEN et al., 1978). Even given 1 h after digoxin, 50 g of charcoal considerably reduced absorption (NEUVONEN et al., 1978). Being less polar than digoxin, digitoxin should be adsorbed at least as well by orally given charcoal, but experimental evidence seems to be lacking. Charcoal has been found to influence the pharmacokinetics of methylproscillaridin, presumably by adsorbing the drug or its metabolites after excretion in the bile (BELZ and BADER, 1974).

b) Anion-Exchange Resins

The strong anion-exchange resin cholestyramine is used therapeutically because of its ability to bind bile acids. Beside binding by coulomb forces, the resin can bind substances hydrophobically to its matrix (LINDENBAUM and HIGUCHI, 1975). This seems to explain the adsorption of digoxin and digitoxin to cholestyramine found in vitro (SARAL and SPRATT, 1967; CALDWELL and GREENBERGER, 1970; BAZZANO and BAZZANO, 1972a; BINNION, 1973), despite the fact that the digitalis glycosides, unlike the bile acids, do not form anions. The binding of digoxin and digitoxin to cholestyramine is very weak compared with that to charcoal. Nonetheless, interaction could occur, as the resin is usually given in large amounts.

Simultaneous administration of cholestyramine and digoxin reduced bioavailability of the glycoside, on average, 20–30% depending on the resin dose (4 and 8 g). Temporal separation of the agents by giving cholestyramine 8 h after digoxin eliminated the interaction (BROWN et al., 1978). An even shorter period (1.5 h) was said to be enough to avoid significant interaction between cholestyramine or colestipol, which is a weak anion-exchange resin, and digoxin or digitoxin during long-term treatment (BAZZANO and BAZZANO, 1972b).

Despite close administration of digoxin and cholestyramine, HALL et al. (1977), in a crossover balance study, were unable to reveal an effect of the resin on net digoxin absorption. Combined intake of β-methyldigoxin and cholestyramine gave no significant interaction (HAHN and REINDELL, 1974). Factors other than time of administration therefore seem to be important. It is noteworthy that HALL et al. (1977) gave cholestyramine together with orange juice. As the binding of digoxin to the resin is weak and unspecific, constituents of the juice might have been adsorbed to cholestyramine, thereby blocking digoxin binding. This idea is substantiated by the observation that chloride and glycocholic acid can compete with presumably hydrophobic binding to cholestyramine (ANDERSEN and SCHJØNSBY, 1978). In addition, the adsorptive capacity of activated charcoal has been found to be strongly reduced upon mixing the adsorbent with ice cream or lemon sherbet (LEVY et al., 1975).

Because of the potential for interaction between cardiac glycosides and anion-exchange resins seen with digoxin, it would seem prudent to avoid coadministration of the agents. Administration of adjuvants together with the resin might, besides making it more palatable, reduce the risk of interaction; however, the possibility cannot be excluded that this might also counteract its therapeutic effect.

c) Fibers and Bulk-Forming Agents

Among psyllium preparations (FINGL, 1975), the dried, mucilaginous husks of the seeds from the Indian plant *Plantago ovata* are in common use, particularly in el-

derly patients, to give volume and softness to the feces. It has been suspected that drugs could adhere to the husks or be occluded during their water uptake, but steady-state plasma concentrations of digoxin were not affected by simultaneous administration of this type of bulk-forming agent (WALAN et al., 1977). With regard to bran, used for the same therapeutic purpose, in vitro studies have shown a considerable uptake of digoxin to this material (FLOYD, 1978). In a group of healthy volunteers, the bioavailability of a single oral dose of digoxin decreased about 20% by giving the drug together with 5 g of crude bran fibers (BROWN et al., 1978, 1979). No interaction was seen when digoxin was administered 15–30 min before the intake of bran (WOODS and INGELFINGER, 1979).

d) Antacids and Antidiarrheals

In vitro binding studies suggested that antacids and an antidiarrheal preparation containing kaolin and pectin might impair bioavailability of digitalis glycosides (BINNION, 1973; KHALIL, 1974). Simultaneous administration of high doses of such drugs and digoxin to healthy subjects reduced absorption of the glycoside (BROWN and JUHL, 1976; ALBERT et al., 1978). In addition, the kaolin-pectin suspension made absorption more variable between subjects (ALBERT et al., 1978). The dose of antacid or antidiarrheal influenced the magnitude of the interaction. Thus, mean bioavailability decreased about 40% with 60 ml of a kaolin-pectin suspension of usual strength (BROWN and JUHL, 1976) and about 60% with 90 ml of a more concentrated suspension (ALBERT et al., 1978). Normal doses of antacids did not interact with digoxin (VÖHRINGER et al., 1976; COOKE and SMITH, 1978) or with β-acetyldigoxin (BONELLI et al., 1977). As with cholestyramine and bran, interaction could be avoided by administering the kaolin-pectin suspension later than digoxin. A 2-h temporal spacing proved sufficient (ALBERT et al., 1978).

3. Physiologic Interactions

a) Gastric Emptying Time and Intestinal Motility

α) *Food.* Intake of food prolongs absorption of digoxin without significantly influencing the totally absorbed amount of glycoside (WHITE et al., 1971; SANCHEZ et al., 1973; GREENBLATT et al., 1974; BROWN et al., 1978; JOHNSON et al., 1978 b). Meals containing a large amount of fiber are exceptional, being capable of impairing the extent of absorption of digoxin given concomitantly (BROWN et al., 1978, 1979). This is probably due to physical binding as discussed above. As with digoxin, absorption from a solution of lanatoside C tended to be delayed by food intake, but the total amount absorbed did not significantly differ in the fasting and postprandial state (ALDOUS and THOMAS, 1977).

β) *Drugs Changing Bowel Motility.* Digoxin administered in a readily soluble form (solution or rapidly dissolving tablets) to patients having undergone partial stomach resection or small intestinal reconstructions was satisfactorily absorbed despite the loss of absorptive mucosa (BEERMANN et al., 1973; OCHS et al., 1975). Therefore, it seems consistent that in subjects without gastrointestinal defects, the bioavailability of easily soluble digoxin was not affected by motility-changing agents, such as propantheline (MANNINEN et al., 1973 a, b; JOHNSON et al., 1978 a)

or metoclopramide (JOHNSON et al., 1978a). Similarly, the total absorbed amount from a solution of lanatoside C was not influenced by the anticholinergic oxyphencyclimine (ALDOUS and THOMAS, 1977).

With decreasing dissolution rate of digoxin, the potential for motility-induced variation in bioavailability increases, as the drug will require more transit time for dissolution. Thus, bioavailability of slowly dissolving digoxin tablets was augmented by propantheline, presumably because of delayed transport through the gut. Conversely, it was impaired by metoclopramide (MANNINEN et al., 1973a; MEDIN and NYBERG, 1973; JOHNSON et al., 1978a). Even digoxin tablets with a moderate dissolution rate can lose bioavailability because of hypermotility of the gut (HEIZER et al., 1971; KOLIBASH et al., 1977).

b) Damaged Mucosa

HEIZER et al. (1971) found that patients with different malabsorption syndromes had reduced absorption of digoxin. Impaired digoxin absorption has also been associated with damage of the intestinal mucosa after radiotherapy (SOKOL et al., 1978). As neomycin is known to damage the intestinal wall, a similar mechanism might therefore explain the reported interaction between this drug and digoxin (LINDENBAUM et al., 1976). When the two substances were given together, digoxin bioavailability decreased, on average, by 42% and steady-state plasma concentrations of digoxin just before dosing were reduced by about 30%. This seems to parallel the finding that in patients treated with large oral doses of neomycin, the 7-day urinary excretion from a single oral dose of lanatoside C was only about one fifth that in healthy subjects (BEERMANN, 1973). However, in that study, another mechanism could not be excluded, namely, that the polar and poorly absorbable lanatoside C may not have been split sufficiently in a sterilized gut to yield less polar and better absorbed substances (particularly digoxin) (HERRMANN and REPKE, 1969; DENGLER et al., 1973).

Concomitant digoxin and para-aminosalicylic acid (PAS) administration decreased bioavailability of the glycoside by about 20%, probably by interference with the function of the intestinal wall (BROWN et al., 1978). This was suggested because it was found that the absorption of D-xylose was also substantially decreased upon treatment with PAS.

Low serum concentration of digoxin in a patient simultaneously treated with sulfasalazine prompted a study of a possible interaction (JUHL et al., 1976). Following a 6-day pretreatment period, administration of sulfasalazine depressed bioavailability of a single oral dose of digoxin elixir, on average, by about 20% in ten normal subjects. The mechanism of the interaction was not established, but the drug's use for treatment of inflammatory mucosal diseases suggests that the absorptive function of the mucosa could have been affected.

II. Interactions with Systemic Drug Disposition

Distribution and elimination of drugs after entrance into the systemic blood are often described as disposition. All cardiac glycosides are widely distributed in the body, but their affinity to different tissues varies. Some are mainly excreted un-

changed in the urine; others are largely metabolized in the liver or gut mucosa before excretion. Polarity is an important determinant of the systemic disposition of the different cardiac glycosides.

1. Plasma Protein Binding

Digitoxin in human plasma is about 97% bound to proteins (LUKAS and DE MARTINO, 1969; STORSTEIN, 1976; PETERS et al., 1977). The binding, almost exclusively to albumin, is endothermic, probably because it induces conformational changes in the protein (LUKAS and DE MARTINO, 1969). The binding site is highly specific, which explains the very few interactions with other drugs at similar concentrations (SJÖHOLM et al., 1979). Also, at clinically encountered concentrations, there is little or no competition for binding with otherwise strong displacers (SOLOMON et al., 1971 b). Heparin injection causes a transient increase in the free fraction, which persists during hemodialysis because of repeated administration of the anticoagulant (STORSTEIN and JANSSEN, 1976). The reason for the interaction is obscure.

Other cardiac glycosides are bound to plasma proteins to a lesser extent than digitoxin, which reduces the potential for clinically important interactions. However, even with digitoxin, changing the protein binding in plasma would cause only minute changes in the total amount of free glycoside in the body. This is because of the large distribution volume of the unbound drug (PERRIER et al., 1977). Nonetheless, caution is advisable when serum concentrations are interpreted for therapeutic guidance, as they comprise the sum of free and protein-bound drug.

2. Tissue Binding

Potassium and probably also spironolactone and quinidine interact with the binding of cardiac glycosides to tissues. However, they also interfere with glycoside elimination and are therefore discussed in a separate section below. The same section also includes thyrostatic agents and thyroid hormones, because patients made euthyroid distribute and eliminate cardiac glycosides in a changed manner.

3. Metabolism

Some of the cardiac glycosides depend to a considerable extent on liver function for their elimination. For instance, hydroxylation and conjugation are important elimination pathways for digitoxin. Scilla glycosides are largely conjugated (RIETBROCK and STAUD, 1975; STAUD et al., 1975; ANDERSSON et al., 1977b), mostly it seems in the gut wall after oral administration (ANDERSSON et al., 1977c).

a) Hydroxylation

An early report described how phenobarbital stimulates 12-hydroxylation of digitoxin to digoxin (JELLIFFE and BLANKENHORN, 1966). Others subsequently confirmed this (SOLOMON et al., 1971 a). Administration of phenylbutazone in one patient and phenytoin in another, both on continuous digitoxin therapy, depressed the plasma concentrations of digitoxin. When the other drug was withheld, the concen-

trations of the glycoside again rose (SOLOMON et al., 1971 b). These interactions were suggested to occur via induction of the hepatic mixed function oxidase system.

b) Conjugation

Besides hydroxylation, digitoxin (with its hydrolytic and hydroxylated metabolites) is conjugated to a large extent (STORSTEIN and AMLIE, 1977). Tuberculostatic treatment with rifampin, on average, halved the steady-state plasma level of digitoxin (PETERS et al., 1974). The observation was first ascribed to stimulation of 12-hydroxylation, but later the investigators proposed increased conjugation as the mechanism, because the polar (not extractable with dichloromethane) fraction of the drug in urine increased between 50% and 100% (PETERS et al., 1978).

4. Excretion

The more polar glycosides, i.e., ouabain and digoxin, are mainly excreted in urine. With decreasing polarity, metabolism becomes more important, and particularly scilla glycosides and digitoxin are to a considerable extent excreted as conjugates in the bile.

a) Renal Excretion

Since cardiac glycosides are not protolytes, changes in urinary pH should not affect their excretion in urine. Digoxin, the most widely investigated glycoside, is mainly excreted by glomerular filtration but also by tubular secretion (FALCH and TEIEN, 1973; STEINESS, 1973, 1974). In addition, it appears that reabsorption of digoxin occurs in the tubules (HALKIN et al., 1975). All these mechanisms have been seen in animal experiments (DOHERTY et al., 1969; ROMAN and KAUKER, 1976). Potassium, spironolactone, quinidine, and agents affecting the thyroid state interfere with the renal excretion of cardiac glycosides. As they interact also with other disposition parameters, they are discussed separately below.

α) *Furosemide.* Changes in urinary digoxin excretion rate have been observed after furosemide administration. At extremely brisk diuresis, excretion of the glycoside is accelerated (MCALLISTER et al., 1976), presumably because of reduced reabsorption in the renal tubules. With moderately increased urine flow, urinary digoxin clearance is diminished (TSUTSUMI et al., 1979). This might be due to the observed decreased glomerular filtration rate of digoxin during furosemide treatment (TILSTONE et al., 1977). The effects of furosemide on digoxin clearance are transient; in a normal clinical setting, it appears that furosemide does not alter net renal excretion of digoxin to any important extent (SEMPLE et al., 1975; BROWN et al., 1976; MALCOLM et al., 1977), nor is the excretion of β-methyldigoxin affected (BACZYŃSKI and KOKOT, 1978).

β) *L-Dopa.* L-Dopa given three times daily in a dose of 0.5 g depressed steady-state plasma digoxin (MANNINEN et al., 1973 c). The mechanism was not elucidated, but the authors proposed that increased tubular secretion was responsible.

b) Biliary Excretion and Enterohepatic Circulation

Cholestyramine increased the survival rates in groups of rats and guinea pigs injected with high doses of digitoxin (CALDWELL and GREENBERGER, 1971). In man,

cholestyramine shortened the half-life of digitoxin and also promoted a more rapid return to baseline measurements of cardiac function (CALDWELL et al., 1971). Digitoxin is subject to considerable enterohepatic circulation (OKITA et al., 1955) and is presumably adsorbed to the resin after excretion in the bile as conjugates or after their splitting in the gut to less polar compounds (KATZUNG and MEYERS, 1965, 1966). The same mechanism probably underlies a report describing successful treatment of digitoxin intoxication with the weak anion-exchanging resin colestipol (BAZZANO and BAZZANO, 1972a).

According to KOLIBASH et al. (1976) and KLOTZ and ANTONIN (1977), cholestyramine did not increase the elimination of intravenously given digoxin. This could reflect minor biliary excretion of this glycoside (DOHERTY, 1968; KLOTZ and ANTONIN, 1977). However, CALDWELL and CLINE (1976) estimated that about 30% of an intravenous dose of digoxin was excreted in the bile in 1 day. If their figure is correct, other explanations must be sought for the failure of cholestyramine to affect digoxin elimination. As discussed earlier in this chapter, the resin can adsorb the glycoside, but its adsorptive capacity might be reduced by concomitant administration of some adjuvant other than water. It is not reported whether adjuvants were used in the studies by KOLIBASH et al. (1976) and KLOTZ and ANTONIN (1977). However, it is interesting that contrary to the study described above (BAZZANO and BAZZANO, 1972a), colestipol given in juice did not increase digitoxin elimination rate (VAN BEVER et al., 1976).

In a crossover study, administration of activated charcoal three times daily reduced mean plasma methylproscillaridin by about 40% from 10 h after intravenous administration of the drug (BELTZ and BADER, 1974). Ther interaction probably implied that the drug or its metabolites were adsorbed to the charcoal during enterohepatic circulation (RIETBROCK and STAUD, 1975; STAUD et al., 1975; ANDERSSON et al., 1977b).

5. Effects on Both Distribution and Elimination

Substances that interfere with the binding of cardiac glycosides to tissues have been found also to affect their elimination. These interactions may or may not influence the elimination half-life, it being directly proportional to the volume of distribution and inversely proportional to clearance.

a) Potassium

α) *Distribution.* In vitro experiments have shown that potassium influences the binding of cardiac glycosides to Na^+, K^+-ATPase, which may be due to an allosteric effect (SCHWARTZ et al., 1968). Thus, the binding of ouabain to enzyme preparations was shifted by potassium toward a lower equilibrium state (AKERA et al., 1978), and extracellular potassium reduced digitalis binding in intact cells (AKERA and BRODY, 1978).

In vivo, the effect of potassium on the tissue binding of cardiac glycosides, mostly investigated in dogs, is obscured because of differences in experimental conditions used by various authors (MARCUS et al., 1969, 1971; FRANCIS et al., 1974; STEINESS, 1978a). Whether changes in body potassium are acute or chronic, induced before or after digitalization, seems to influence the results. The timing of the sampling procedures and the representativeness of the samples for the partic-

ular tissue are other critical factors. Also, the effects of potassium are different in the heart and other tissues, e.g., skeletal muscle. It seems fair to conclude, however, that once that cardiac glycosides are bound to the tissues, changes in potassium status have little effect on their binding (OKITA, 1973).

Conversely, digitalis affects potassium distribution in the body. By interfering with the cellular transmembrane ion transport, cardiac glycosides cause intracellular depletion of potassium. This occurs even at therapeutic doses (MICHEL, 1966) and may be conveniently studied in erythrocytes from digitalized subjects (ASTRUP, 1974; WESSELS et al., 1974; LOES et al., 1978).

β) *Renal Tubular Secretion.* In a small group of patients, lowered tubular secretion of digoxin was observed during hypokalemia (STEINESS, 1978 b). The tubular secretion increased when the patients were made normokalemic. It was speculated that the findings were due to competition for a steroid excretion pathway in the tubular cell. This interaction might promote toxicity, particularly since hypokalemia is arrhythmogenic in itself.

b) Spironolactone

α) *Digoxin.* Spironolactone (100 mg daily) was found to block renal tubular secretion of digoxin so that, after correction for plasma protein binding, renal digoxin clearance equalled inulin clearance (STEINESS, 1974). In a group of eight subjects, the same intravenous dose of digoxin gave higher plasma concentrations of the glycoside when repeated after 5 days of treatment with spironolactone (WALDORFF et al., 1978). On the average, both renal and plasma clearance fell by about one fourth. Pharmacokinetic analysis according to a three-compartment open model revealed that the total volume of distribution at steady state, V_D^{ss}, was smaller during spironolactone treatment. As this volume is independent of changes in elimination rate, the finding suggests that spironolactone reduces tissue binding of digoxin. The changes in clearance and distribution of digoxin may require dosage adjustment, unless spironolactone also decreases the affinity of digoxin to its receptors.

β) *β-Methyldigoxin.* High doses of spironolactone (400–500 mg daily) did not influence the elimination half-life of β-methyldigoxin, nor did the treatment significantly affect the cumulated amount of drug excreted in urine or feces during 7 days or the metabolic pattern in urine and bile (ABSHAGEN et al., 1976). The authors did not discuss whether spironolactone affected clearance of the glycoside.

γ) *Digitoxin.* High doses of spironolactone (400 mg daily) shortened digitoxin half-life by about 20%, possibly by metabolic induction (TAYLOR et al., 1972) as indicated by a significant increase in hydrophilic metabolites in the urine (WIRTH et al., 1976). No significant changes in clearance of digitoxin (renal or plasma) were observed.

c) Quinidine

During combined therapy with digoxin and quinidine, elevated plasma or serum concentrations of the glycoside were measured (EJVINSSON, 1977, 1978 a; HOOYMANS and MERKUS, 1978; KAUFMANN, 1978; LEAHEY et al., 1978). Similar changes were observed when β-methyldigoxin and quinidine were given together (DOERING and KÖNIG, 1978). Conversely, digoxin administration raises plasma concen-

trations of quinidine but, as it seems, to a proportionally much lesser extent (HAGER et al., 1979). The rise of the concentration of the cardiac glycosides depends on the quinidine dose (DOERING, 1979), but with a conventional therapeutic dosage (1.2 g of quinidine sulfate given daily), the increase of the steady-state concentrations is, on average, about 90% (EJVINSSON, 1977; HOOYMANS and MERKUS, 1978; SCHENCK-GUSTAFSSON and DAHLQVIST, 1981). Several patients with digoxin serum concentrations elevated by quinidine above the usual therapeutic range experienced extracardiac intoxication symptoms, which were resolved by reducing or stopping digoxin or by discontinuing quinidine (LEAHEY et al., 1978). Similarly, enhanced cardiac action was seen with the increased digoxin concetrations (LEAHEY et al., 1978, 1979). In the average patient, a 50% reduction of the digoxin maintenance dose therefore seems to be rational. Successful application of this concept was recently described (DOERING, 1979).

The exact mechanisms behind these observations are not known, Cinchona alkaloids show affinity to glandular tissues (HIATT and QUINN, 1945), and it seems probable that secretory processes are involved in the interaction with cardiac glycosides since it was established that quinidine reduces renal clearance of digoxin (HOOYMANS and MERKUS, 1978, 1979; HAGER et al., 1979; SCHENCK-GUSTAFSSON and DAHLQVIST, 1981) and β-methyldigoxin (DOERING, 1979) without a change in creatinine clearance. Total body clearance of digoxin also falls (HAGER et al., 1979; SCHENCK-GUSTAFSSON and DAHLQVIST, 1981) – to an extent that suggests an even larger influence of quinidine on nonrenal digoxin elimination. Nonrenally, digoxin is eliminated in the bile (DOHERTY et al., 1970; CALDWELL and CLINE, 1976), but the importance of active secretion of the substance into the gut lumen (LAUTERBACH, 1975) may have been underestimated. It is therefore possible that in the reduction of nonrenal digoxin clearance, too, inhibition of secretory pathways by quinidine plays a role.

There has been much speculation about whether quinidine displaces digoxin from its binding sites in the body. The apparent distribution volume of digoxin calculated from the area under the plasma concentration-time curve and its terminal slope (V_D^β) is reduced during quinidine treatment, but this provides no evidence for displacement (JUSKO and GIBALDI, 1972). It is noteworthy, however, that quinidine reduced the volume of the central compartment of digoxin in multi-compartmental pharmacokinetic models of the experimental findings (HAGER et al., 1979; SCHENCK-GUSTAFSSON and DAHLQVIST, 1981). This indicates competition for binding sites but is apparently in conflict with in vitro studies showing no effect of quinidine on distribution of digoxin to human erythrocytes (HOOYMANS and MERKUS, 1979) or of ouabain to sarcolemma fractions of lamb myocardium (DOERING, 1979). Moreover, the seeming parallelism described between effect and increased serum concentration of digoxin suggests that displacement is not important at the receptor level. More experimental information is needed to see if quinidine displaces cardiac glycosides in specific tissues, which should offer an explanation for the volume decrease in the pharmacokinetic models.

d) Thyrostatic Agents and Thyroid Hormones

Thyroid hormones increase the basal metabolic rate and probably also kidney perfusion and glomerular filtration rate (BRADLEY et al., 1974). This could explain the

many observations of enhanced body and/or renal clearance of cardiac glycosides in thyrotoxic patients (DOHERTY and PERKINS, 1966; EICKENBUSCH et al., 1970; CROXSON and IBBERTSON, 1975; SHENFIELD et al., 1977; BONELLI et al., 1978; GILFRICH and MEINERTZ, 1978). In hypothyroidism, compared with the euthyroid state, glycoside clearance is slightly reduced (DOHERTY and PERKINS, 1966; EICKENBUSCH et al., 1970) or unchanged (SHENFIELD et al., 1977). The clearance changes are associated with changes in the volume of distribution of the cardiac glycosides in the body (DOHERTY and PERKINS, 1966; SHENFIELD et al., 1977; GILFRICH and MEINERTZ, 1978), possibly with the reported parallelism between the number of Na^+, K^+-ATPase molecules in the cell and the functional state of the thyroid (EDELMAN, 1976; HEGYVARY, 1977). Conflict exists as to whether the elimination rate of the glycosides, too, is thyroid-dependent. The influence of thyroid hormones on the clearance of digitalis drugs calls for attention to their dosage in thyroid patients. Adjustment of the dose must be considered when they are made euthyroid, particularly by thyrostatic treatment.

C. Interactions with Cardiac Glycosides at the Receptor Level (Pharmacodynamic Interactions)

Pharmacodynamic interactions occur when the effects of one agent are altered by the pharmacologic action of another. A unidirectional interaction implies that the pharmacologic response is increased, whereas a bidirectional interaction reduces the effect. Interactions can occur at a single site or at different (multiple) sites and can be mediated by agonists or by antagonists (RAWLINS, 1977).

All cardiac glycosides have in common the ability to increase the force of myocardial contraction, i.e., a positive inotropic action. Heart rate will also be reduced, in therapeutic situations, as a consequence of improved circulation and enhanced vagal tone. Glycoside effects on electric phenomena in the heart are variable, dependent on location, dosage, and electrolyte status, and cannot be categorized in general terms. In addition, extracardiac effects occur, affecting the vascular and the central nervous system. This complexity of cardiac glycoside action can make it difficult to interpret reported interactions.

Animal experiments indicate that, generally, the toxic effects of different cardiac glycosides are additive (NEUMANN, 1949). This is also the basis for the acetylstrophanthidin tolerance test (LOWN and LEVINE, 1954b). Given intravenously in man, cardiac glycosides differ in the rapidity of onset of action but would apparently produce the same maximum inotropic effect per mole if elimination could be neglected (FORESTER et al., 1974). For therapeutic purposes, summation of the effects of different cardiac glycosides should therefore be adequate, with due recognition of differences in bioavailability and disposition pharmacokinetics (BIGGER and STRAUSS, 1972).

I. Substances Associated with Electrolyte and Acid-Base Balance

The heart's reactivity to cardiac glycosides can be modified by changes in electrolyte and acid-base status. Acid-base balance is closely associated with electrolytes. Clinically important derangements in electrolyte and acid-base status can

occur during the natural course of certain diseases, as side-effects to therapy, or as a result of inappropriate diet. Changes in only one parameter are rare but are almost inevitably coupled with other changes in homeostasis (KASSIRER et al., 1965; FLEAR, 1966; MICHEL, 1966).

1. Ions Influencing Cardiac Function

Cardiac glycosides inhibit Na^+, K^+-ATPase leading to decreased cellular potassium, increased cellular sodium, and a consequent enhancement of the rapidly exchangeable calcium pool in the myocardial cell. It is beyond the scope of this presentation to discuss whether this is the basic biochemical mechanism leading to increased inotropic effect (REPKE et al., 1973; WALLICK et al., 1977; AKERA and BRODY, 1978) or not (OKITA, 1977). The general agreement that these processes are involved in glycoside-induced changes of the action potential of myocardial cells is sufficient to indicate the crucial role of electrolytes in the action of cardiac glycosides.

a) Potassium

Apart from their ability to interfere with the binding of cardiac glycosides to the tissues (see pharmacokinetic interactions), potassium ions influence electrophysiologic events in the heart. Excess myocardial potassium reduces conduction in the atria and the subnodal system and depresses automaticity. Despite this, in clinical situations, conduction disturbances during hyperkalemia are rarely seen – except in digitalized patients – indicating interaction between potassium and the cardiac glycosides (FISCH and KNOEBEL, 1966). Intracellular depletion of potassium appears to interact directly with the action of cardiac glycosides promoting toxicity (LOWN et al., 1951).

Serum potassium poorly reflects intracellular potassium (LOWN et al., 1951; LOWN and LEVINE, 1954a; MOORE et al., 1954). Besides potassium, other ions contribute to the transmembrane resting and action potential of the myocardial cell. These facts, together with variable interindividual sensitivity to cardiac glycosides and to potassium, would seem to explain the many observations of inconsistent relations between hypokalemia and digitalis toxicity. Nevertheless, the tendency of hypokalemia to increase the frequency of digitalis intoxications with a shift to more serious toxic symptoms is well documented in the literature. Also, clinical benefit has often been achieved by the administration of potassium in patients with digitalis-induced arrhythmias. Such administration must be done with some caution because of the risk of producing hyperkalemia in these patients. The fact that hyperkalemia can also result from digitalis intoxication (GAULTIER et al., 1968; CITRIN et al., 1972; RUMRACK et al., 1974) complicates the situation and stresses the importance of understanding the etiology behind changes in serum potassium. The influence of potassium on inotropy is not well understood. In laboratory experiments, potassium was found to lessen the inotropic response (PRINDLE et al., 1971; LEE et al., 1977), but this has not been documented clinically.

b) Magnesium

Magnesium is needed for normal potassium homeostasis (SEELING, 1972; DYCKNER and WESTER, 1979). Excess magnesium can produce rhythm and conduction distur-

bances similar to those seen during hyperkalemia (MORDES and WACKER, 1978). Administration of magnesium has often been found to abolish arrhythmias caused by cardiac glycosides (SZEKELY and WYNNE, 1951; NEFF et al., 1972; MORDES and WACKER, 1978). In dogs, acute hypomagnesemia reduced the arrhythmogenic dose of acetylstrophanthidin; restitution of sinus rhythm was rapid after intravenous administration of magnesium sulfate (SELLER et al., 1970). There has been considerable speculation about the clinical role of magnesium, as epidemiologic evidence does not strongly indicate increased sensitivity of the heart to cardiac glycosides during hypomagnesemia (BELLER et al., 1974; STORSTEIN et al., 1977; OCHS et al., 1978).

c) Sodium

Several electrophysiologic events in the heart depend on sodium (HOFFMAN and BIGGER, 1971; ROBERTS and KELLIHER, 1972; REPKE et al., 1973; WALLICK et al., 1977; AKERA and BRODY, 1978). In laboratory experiments, hyponatremia altered the binding of cardiac glycosides to myocardium (DUTTA and MARKS, 1969; HARRISON and WAKIM, 1969). In experiments with dogs, cardioactive glycosides increased natriuresis by direct renal action (HYMAN et al., 1956; STRICKLER et al., 1961). Despite these findings, no clinically important interactions between sodium and cardiac glycosides have been reported.

d) Calcium

The general opinion is that the positive inotropic effect of cardiac glycosides depends on an increase in rapidly exchangeable intracellular calcium (LEE and KLAUS, 1971). Increased ventricular automaticity has been observed in hypercalcemia (NALBANDIAN et al., 1957). Studies in isolated Purkinje fibers suggest that, under the influence of toxic concentrations of cardiac glycosides, calcium can incite a depolarizing afterpotential that can reach threshold (KASS et al., 1978). In clinical practice, potentiation of the effects of cardiac glycosides by hypercalcemia is rarely seen, probably because the calcium excess is seldom large enough to precipitate arrhythmias (NOLA et al., 1970). Nonetheless, infusion of calcium salts in patients receiving cardiac glycosides is best avoided.

In hypocalcemia, cardiac glycosides are less effective (CHOPRA et al., 1977; BRENTON et al., 1978). This is consistent with the observation that hypocalcemic agents, such as disodium edetate, antagonize both the therapeutic and toxic actions of cardiac glycosides (JICK and KARSH, 1959; SURAWICZ et al., 1959; SZEKELY and WYNNE, 1963; COHEN et al., 1965).

e) Lithium

During lithium therapy, ECG findings mimicking those caused by cardiac glycosides have been found (SCHOU, 1962; WELLENS et al., 1975; WILSON et al., 1976). In laboratory experiments, lithium was found to interact with the function of the membrane sodium pump (KU et al., 1978). It therefore appears that the possibility for interaction between lithium and the cardiac glycosides should be considered, even if there is no well-documented evidence given in the literature.

2. Acid-Base Balance

Animal experiments indicate that alkalosis can increase the toxicity of cardiac glycosides (WARREN et al., 1968; GALMARINI et al., 1973). The most common alkalotic state in patients with heart failure is the hypochloremic metabolic alkalosis caused by potassium-depleting diuretics (cf. below). In normokalemic patients with serum concentrations of digoxin usually not considered toxic, alkalosis, even if moderate, considerably increased the incidence of arrhythmias attributable to digoxin (BRATER and MORRELLI, 1977). This was ascribed to intracellular depletion of potassium and again emphasizes that potassium homeostasis can be disturbed without being reflected by the serum concentration.

Respiratory acidosis enhanced the arrhythmogenicity of cardiac glycosides in cats, probably because of increased catecholamine activity (KÖHLER and GREEFF, 1972). However, in clinical practice, the effect of acidosis on cardiac glycoside toxicity is not sufficiently investigated. Moderate acidosis apparently plays an insignificant role (GREEN and SMITH, 1977).

3. Diuretics

Diuretics are often used in the treatment of congestive heart failure, alone or in combination with cardiac glycosides. The combination gives multiple-site interactions: unidirectional and beneficial on cardiac output, bidirectional and potentially toxic on electrolyte status. In advanced heart disease, electrolyte disturbances already exist prior to treatment (FLEAR, 1966), which increases the risk of adverse reactions during combination therapy with cardiac glycosides and diuretics. Potassium depletion is particularly crucial. This has led to a classification into potassium-depleting agents, acting on the proximal tubule (metolazone), the loop of Henle (furosemide, ethacrynic acid, bumetanide), or the cortical diluting segment (thiazides, chlorthalidone, metolazone) and potassium-sparing agents (spironolactone, amiloride, triamterene) whose site of action is the distal tubule.

a) Potassium-Depleting Diuretics

Substances in this group have in common the ability to deplete body potassium. Several epidemiologic studies have documented a considerable increase in the incidence of cardiac arrhythmias during concomitant treatment with these agents and cardiac glycosides (TAWAKKOL et al., 1967; HURWITZ and WADE, 1969; SHAPIRO et al., 1969; JØRGENSEN and SØRENSEN, 1970; LEHMANN et al., 1978). Potassium depletion, however, does not alone appear to be responsible for the increased number of adverse reactions. When patients need both diuretics and cardiac glycosides, the underlying heart failure is often advanced, rendering these patients more liable to adverse reactions. Moreover, it is known that loop diuretics, e.g., furosemide, and thiazides can induce hypochloremic metabolic alkalosis (KASSIRER et al., 1965) and hypomagnesemia (DUARTE, 1968; JACKSON and MEIER, 1968; SELLER et al., 1970; LIM and JACOB, 1972; SULLIVAN et al., 1978); thiazides can also cause hypercalcemia (GURSEL, 1970; DUARTE et al., 1971; SULLIVAN et al., 1978). Such changes can also contribute to adverse interactions.

It has been found experimentally that loop diuretics can inhibit Na^+, K^+-ATPase and transmembrane ion fluxes (DUNN, 1973; MARTINEZ-MALDONADO et al., 1974; CUNARRO and WEINER, 1978). It is not known whether this can influence the clinical effects of the cardiac glycosides.

b) Potassium-Sparing Diuretics

Pharmacokinetic interactions with spironolactone are described above. In addition, laboratory experiments and occasional clinical observations have indicated pharmacodynamic antagonism between cardiac glycosides and potassium-sparing diuretics, even independent of potassium. In therapeutic doses, however, it appears that pharmacodynamic interactions between the cardiac glycosides and potassium-sparing diuretics in man are rare. This includes the potential risk of hyperkalemia and hyperchloremic acidosis – at least in patients with normal renal function. Conversely, the use of potassium-sparing diuretics can remove or lessen adverse interactions initiated by potassium wasting.

4. Miscellaneous Agents

a) Insulin and Glucose

Although the physiologic control of external and internal potassium balance is incompletely understood, it seems that insulin plays at least a permissive role in intracellular potassium homeostasis (COX et al., 1978). Insulin may also be required for normalization of other ion gradients, including calcium and sodium, across the cell membranes (ZIERLER, 1966). Thus, insulin has been said to be sometimes effective in treatment of congestive heart failure, possibly because of extrusion of sodium, chloride, and water from the myocardial cells (FLEAR, 1966).

Usually, the effect of administration of insulin and/or glucose on body potassium is short-acting and does not greatly influence cardiac glycoside therapy. However, in digitalized patients with severe potassium depletion, slow intravenous infusion of potassium in 5% glucose led to further lowering of serum potassium and worsening of arrhythmias (PAGE, 1955; KUNIN et al., 1962); the use of saline instead of glucose is therefore recommended (BIGGER and STRAUSS, 1972). Based on results from dogs, the combined use of glucose-potassium-insulin in treating digitalis toxicity was advocated (DE MICHELI et al., 1971). The clinical usefulness of this treatment is not adequately documented but might be supported by its present use during the acute phase of myocardial infarction.

b) Cathartics and Liquorice

Excessive use of liquorice or abuse of cathartics can lead to potassium losses larger than the daily intake (FLEISCHER et al., 1969; SALVADOR et al., 1970), which can enhance digitalis toxicity owing to depletion of body potassium stores.

II. Drugs Known to Affect the Autonomic Nervous System

Apart from direct effects on cardiac tissue, much of the action of cardiac glycosides is mediated by the autonomic nervous system (GOLD et al., 1939; ROBERTS et al.,

1976; GILLIS et al., 1978). The potential for interaction is therefore great, particularly as cardiac glycosides can act on all levels of the autonomic reflex arc. This includes effects on chemoreceptors and baroreceptors, the central nervous system, ganglia, nerve endings, and postsynaptic receptors (GILLIS et al., 1978). Many of these effects are evident during the therapeutic use of cardiac glycosides and are exaggerated after overdose. More experimentation is needed to elucidate the extent to which the autonomic nervous system participates in the production of the inotropic and the hemodynamic effects of the cardiac glycosides (GILLIS and QUEST, 1978).

Usually, interactions between cardiac glycosides and drugs affecting the autonomic nervous system are predictable because of known pharmacologic effects. Adverse interactions during modern drug therapy are infrequent: instead, several beneficial uni- and bidirectional interactions are seen.

1. Sympathomimetic Amines

The heart contains both β_1- and β_2-adrenoceptors. Much of the inotropic action brought about by sympathomimetics is mediated by the β_1-receptors, being predominant both in the sinoatrial node and in the ventricular muscle. Cardiac β_2-receptors occur mainly in the node; therefore β_2-stimulation gives higher chronotropic than inotropic response at a given dose (CARLSSON et al., 1977; HEDBERG et al., 1980).

It appears that cardiac glycosides and sympathomimetic amines increase cardiac contractility by independent mechanisms (KOCH-WESER et al., 1964; BEISER et al., 1970; HOUGEN and SMITH, 1978). Studies in dogs with the β_1-selective sympathomimetic amine prenalterol and ouabain indicate additive inotropic action (EK et al., 1979). In arrhythmogenesis, cardiac glycosides and catecholamines seem to potentiate each other's effect (BECKER et al., 1962; TANABE, 1968; RAPER and WALE, 1969), an interaction that may be reduced by use of selective β_1-agonism (ARINIEGO et al., 1980). In patients with atrial fibrillation, however, β_1-stimulation may increase the risk of ventricular tachycardia because of facilitated atrioventricular conduction (SONNENBLICK et al., 1979). Better understanding of hemodynamics and improved therapy, such as the introduction of pacemakers, have rationalized the use of potent sympathomimetics in cardiology, thereby decreasing the frequency of adverse interactions with the cardiac glycosides.

Sympathomimetic amines are commonly used for relief of bronchospasm. In patients treated with cardiac glycosides, some caution is recommended with regard to inhalation of nonselective bronchodilating amines. This is becaues of the transiently high concentrations of amine reaching the heart immediately after administration (TATTERSFIELD and MCNICOL, 1969; FREEDMAN and HILL, 1971; SHIM and WILLIAMS, 1975). Although systemic effects of, for instance, inhaled isoprenaline are usually minimal at conventional dosage, excessive use during acute bronchospasm can compound the risk. The increasing role of selective β_2-stimulants for bronchodilatation should reduce cardiovascular effects of inhalant therapy (TATTERSFIELD and MCNICOL, 1969; HUHTI, 1972; AMORY et al., 1975; TASHKIN et al., 1975).

2. β-Adrenoceptor Blocking Drugs

The β-adrenoceptor blocking drugs exert several effects that can assist or antagonize therapy with cardiac glycosides. Their ability to decrease heart rate and depress several arrhythmias is well established. They also exert a negative inotropic action, which can precipitate heart failure during β-blockade of cardiac receptors. Different opinions are held about whether β-adrenoceptor blocking drugs influence the uptake of cardiac glycosides in the heart (CAGIN et al., 1973; BELLER et al., 1975; BINNION and DAS GUPTA, 1975; LLOYD and TAYLOR, 1975; MARZO et al., 1976). Augmentation of digoxin uptake by the myocardium has been observed during increased cardiac load (LLOYD and TAYLOR, 1976), presumably because an increased number of membrane depolarizations promote transport of cardiac glycosides (AKERA and BRODY, 1978). Thus, when decreased glycoside binding is seen during β-blockade, it could be a consequence of lowered myocardial work.

The ability of both β-adrenoceptor blocking drugs and cardiac glycosides to decrease heart rate is seldom troublesome in clinical practice, even though bradycardia and atrioventricular block may occasionally develop (BIGGER and STRAUSS, 1972). Instead, the unidirectional pharmacologic response can give therapeutic advantage. In some patients with atrial fibrillation, inadequately controlled with cardiac glycosides alone, adding a β-adrenoceptor blocking drug has effectively reduced ventricular rate by further increasing the nodal refractory period (GIBSON and SOWTON, 1969; DREIFUS and WATANABE, 1972; YAHALOM et al., 1977). The combination may give additional benefit to patients with mitral stenosis by giving a longer diastole for ventricular filling.

Also the antagonistic effects of the two types of drug have proved beneficial in combination. Successful treatment with β-adrenoceptor blocking drugs of certain arrhythmias induced by cardiac glycosides is clinically well documented (SINGH and JEWITT, 1974). The effect appears to be mediated not only by blocking the cardiac receptors but also by reducing the glycoside-induced release of catecholamines from the adrenal medulla (ROBERTS et al., 1976). Cardiac glycosides can augment heart pump function in patients liable to cardiac failure during β-blockade (DUNÉR and PERNOW, 1973; EKELUND et al., 1973; NECHWATAL et al., 1977). In myocardial infarction, β-adrenoceptor blocking drugs may help to dampen sympathetic overactivity (MAROKO and BRAUNWALD, 1973; GOLD et al., 1976; HEIKKILÄ and NIEMINEN, 1978) but this, in conjunction with the muscle damage, can precipitate heart failure. Even during the acute phase of the infarction, cardiac glycosides are not contraindicated (LOWN et al., 1972; BACHOUR and HOCHREIN, 1975; RAHIMTOOLA and GUNNAR, 1975; REIČANSKY et al., 1976), if augmentation of cardiac contractility is important. However, because cardiac glycosides may increase the infarct size, as judged from accumulated creatine phosphokinase activity (VARONKOV et al., 1977), it is a sine qua non that pump failure be unequivocally proved before they are used in the first 24 h of the infarction (LESCH, 1976). From a metabolic and circulatory point of view, β-adrenoceptor blocking drugs and cardiac glycosides appear to combine beneficially during coronary ischemia (RAINA et al., 1978; VATNER and BAIG, 1978; KÖTTER et al., 1978).

It is well known that cardiac glycosides induce typical electrocardiographic changes (NORDSTRÖM-ÖHRBERG, 1964). During concomitant therapy with β-adre-

noceptor blocking agents, these signs can be masked (FRICK et al., 1972; LE WINTER et al., 1977). The interactions with drugs blocking the β-adrenergic receptors illustrate the importance of the adrenergic nervous system for the action of cardiac glycosides. The interactions are both unidirectional (slowing of the heart rate) and bidirectional (arrhythmogenesis and inotropy). As already described, much clinical benefit can be achieved by appropriate concurrent use of these two types of drug. However, the potential of the β-adrenoceptor blocking drugs to produce bradycardia, atrioventricular conduction disturbances, and congestive heart failure must be considered.

3. α-Adrenoceptor Blocking Drugs

Drugs that have α-adrenoceptor blocking ability, such as phentolamine and prazosin, beneficially interact with cardiac glycosides in the treatment of congestive heart failure. As discussed later, by acting as vasodilators, these drugs can augment cardiac performance and diuresis leading to clinical improvement of the failure.

4. Adrenergic-Neuron Blocking Drugs

In animal experiments, depleting catecholamines by reserpine pretreatment resulted in increased tolerance to cardiac glycosides (ROBERTS et al., 1963, 1976). On the other hand, administration of reserpine to patients on cardiac glycoside therapy has induced arrhythmias, presumably because of initial catecholamine release (LOWN et al., 1961; DICK et al., 1962; SOFFER, 1965). Occasionally, depletion of catecholamines in digitalized patients, by repeated treatment with rauwolfia alkaloids, guanethidine, or bethanidine, led to bradycardic or hypotensive episodes (BIGGER and STRAUSS, 1972). Despite these reports, in normal clinical situations, interactions between adrenergic-neuron blocking drugs and cardiac glycosides do not seem to be of much importance. Moreover, because more appropriate hypotensive alternatives are now available, the combination has become less common.

The antiarrhythmic agent bretylium accumulates in postganglionic adrenergic neurons and initially stimulates the release of noradrenaline. This stimulation can aggravate toxicity to cardiac glycosides, as can hypersensitivity to catecholamines that may later develop (Medical Letter, 1978).

5. Cholinergic and Anticholinergic Drugs

Early studies by GOLD et al. (1939) documented the fact that vagal tone is important in the slowing of ventricular rate seen with moderate doses of cardiac glycosides. With larger doses, extravagal effects increase. This explains the antagonistic effect of atropine on heart rate (MILLER, 1969) but implies that the effect may be attenuated in digitalis intoxications. It also explains why other anticholinergics usually have only small effects on bradyarrhythmias during therapy with cardiac glycosides. As discussed later, interactions between succinylcholine and cardiac glycosides might involve enhancement of vagal tone.

III. Antiarrhythmic Drugs

Antiarrhythmic drugs are commonly used together with cardiac glycosides in the treatment of rhythm disturbances in diseased hearts or to treat arrhythmias caused by cardiac glycosides. Based predominantly on differences in effect on membrane responsiveness and conduction velocity observed in laboratory experiments, the antiarrhythmic drugs have been classified into two main groups (HOFFMANN and BIGGER, 1971; DREIFUS et al., 1974). Quinidine is the model substance for group 1; other drugs in this group are disopyramide, procainamide, and ajmaline. Group 2 contains lidocaine, phenytoin, tocainide, mexiletine, and aprindine. The classification is undoubtedly an oversimplification (KOCH-WESER, 1978). It must be recognized that the electrophysiologic effects are nonuniform and difficult to predict in the diseased, innervated human heart. In addition, other drugs, such as β-adrenoceptor blocking agents, bretylium, and verapamil have antiarrhythmic properties. The concentrations of drug in affected areas of the heart are also important; for instance, the β-adrenoceptor blocking agent propranolol shows a quinidine-like action when given in large doses.

1. Group 1 Antiarrhythmic Drugs

By decreasing automaticity and prolonging the effective refractory period, group 1 antiarrhythmic drugs, i.e., quinidine and quinidine-like drugs, counteract ectopic and premature contractions. All drugs of this type have been administered together with cardiac glycosides, and usually combination is safe. However, it should be recognized that their negative inotropic action may precipitate cardiac failure and hypotension (HOFFMAN and BIGGER, 1971). Also, ajmaline has been said to impart a risk of cardiac standstill upon intravenous administration of the drug to digitalized patients (BARILLON and GRAND, 1976). In digitalis toxicity, the drugs are less suitable, as they can enhance conduction disturbances (BIGGER and STRAUSS, 1972). Because of the more pronounced anticholinergic properties of disopyramide (HARRISON et al., 1977), this drug might be safer during digitalis intoxication, but clinical documentation is insufficient.

As discussed under pharmacokinetic interactions, quinidine has been found to alter the disposition of digoxin and β-methyldigoxin. This necessitates dose reduction of the cardiac glycosides to avoid toxicity. Occasional syncopal episodes have been reported during quinidine therapy (DAVIS and SPRAGUE, 1929; SELZER and WRAY, 1964; EJVINSSON, 1978b) but can probably occur with all group 1 drugs. The syncope is caused by ventricular tachycardia or fibrillation (KOSTER and WELLENS, 1976; EJVINSSON, 1978b). The majority of the patients were simultaneously treated with cardiac glycosides, creating the suspicion of pharmacodynamic interaction (DAVIS and SPRAGUE, 1929; GOLD et al., 1932). However, in a study of adverse reactions to quinidine, combined use of digitalis and quinidine did not appear to be a predisposing factor to arrhythmias (COHEN et al., 1977). Syncope also occurs with quinidine alone (KOSTER and WELLENS, 1976). Moreover, the arrhythmias leading to quinidine syncope are not uniform in their electrocardiographic appearance. Some of them fit well to the description of *torsade de pointes* (DESSERTENNE, 1966; KRIKLER and CURRY, 1976; KOSSMANN, 1978). This syndrome appears to be precipitated by factors that prolong the QT interval but not by the cardiac glycosides. Therefore, the etiology behind quinidine syncope is probably complex.

For instance, most of the patients described were also treated with diuretics. A possible influence from disturbances in potassium distribution (GERTLER et al., 1956; LEE, 1960) or multiple-drug interactions must therefore also be considered.

2. Group 2 Antiarrhythmic Drugs

The group 2 antiarrhythmic drugs lidocaine and phenytoin are in common use in treating ventricular extrasystoles and tachycardia, including those caused by the cardiac glycosides. They have less negative inotropic action than the group 1 drugs (HOFFMAN and BIGGER, 1971). In acute cases, its easy administration and short duration of action may render lidocaine more advantageous. Both phenytoin and lidocaine have high efficacy and, given as recommended, a very low incidence of toxicity. Neither of these drugs in conventional doses has depressive effects on the node. Rather, during digitalis intoxication, phenytoin has been found to improve atrioventricular conduction (BIGGER and STRAUSS, 1972). In addition, phenytoin might antagonize both the glycoside-induced cardiac potassium loss (LOH et al., 1976) and attenuate the glycoside effects on the central nervous system (EVANS and GILLIS, 1975). Other drugs in group 2 were more recently introduced, and their potential for interaction with the cardiac glycosides is obscure.

IV. Other Drugs Used in Cardiovascular Therapy

1. Vasodilatating Drugs

Vasoactive drugs of the nitrite type have been used as antianginal agents for the past century. Recently, the concept of total cardiocirculatory performance as one integrated system has made it more common to consider vasodilatating drugs in the treatment of cardiac failure together with cardiac glycosides and diuretics (CHATTERJEE and PARMLEY, 1977; COHN and FRANCIOSA, 1977 a, b). Clinically used vasodilators affect both preload (venous bed) and afterload (arterial bed) with or without preference for either side. Thus, nitroglycerin and isosorbide act mainly on the venous bed, hydralazine and phentolamine on arterial resistance, whereas nitroprusside and prazosin affect preload and afterload to about the same extent.

The combination of vasodilatating drugs with diuretics and cardiac glycosides illustrates a unidirectional, multiple-site interaction with salutary results. Despite some earlier suggestions, such combination does not appear to induce unpredictable, hazardous side-effects (MENTZ and FÖRSTER, 1972), though documentation with the newer compounds is scarce.

2. Calcium Antagonists

Verapamil and nifedipine are two drugs, often classified as calcium antagonists, that have gained considerable clinical use. Observations indicate that they reduce the amount of active calcium in the heart cells by inhibiting the flux of the ion across the cell membrane (FLECKENSTEIN, 1977). Verapamil is said also to influence sodium transport (ANDERSSON, 1978; SINGH et al., 1978). Both drugs are used as antianginal agents. In addition, verapamil has antiarrhythmic properties.

Laboratory work has suggested that the positive inotropic effects of cardiac glycosides are attenuated but not nullified by verapamil (SINGH and VAUGHAN

WILLIAMS, 1972; SCHÜMANN et al., 1977), indicating that cardiac glycosides may, at least in part, reverse the depressant effects of verapamil in heart failure. Verapamil was found to antagonize arrhythmias caused by cardiac glycosides (SCHÜMANN et al., 1977) and the drug has, by its ability to reduce atrioventricular conduction (SINGH et al., 1978), found some use in treating rapid atrial fibrillation resistant to cardiac glycosides. Clinically, adverse reactions between verapamil and cardiac glycosides are rare (SCHAMROTH et al., 1972; HENG et al., 1975), though some caution seems prudent because of the nodal depression.

Because nifedipine has vasodilatating properties not restricted to the coronary vessels (LYDTIN et al., 1975; MOSTBECK et al., 1975), the net effect of the drug on cardiac output is positive (LICHTLEN, 1975). Clinical experience indicates that nifedipine has little or no influence on atrioventricular conduction (LYDTIN et al., 1975), which is in agreement with animal experiments (TAIRA et al., 1975). The findings are consistent with the observation that patients tolerate concomitant treatment with cardiac glycosides and nifedipine well (LYDTIN et al., 1975).

V. Miscellaneous Drugs

1. Doxorubicin

Doxorubicin (adriamycin) is an antibiotic used as a cytotoxic agent in neoplastic diseases. Its use is limited by the high incidence of fatal cardiomyopathy that develops with cumulative doses (LEFRAK et al., 1973; FRIEDMAN et al., 1978). Experiments with isolated organs and intact animals have shown that cardiac glycosides can interact with doxorubicin by reducing its uptake to the heart (ARENA et al., 1972) and by counteracting its inhibition of the rapidly exchangeable calcium fraction in the myocardial cell (VILLANI et al., 1978). Pretreatment with strophanthin depressed the mortality rate with doxorubicin in mice and increased the threshold dose of the cytotoxic agent in dogs. The antitumor effect of doxorubicin was apparently unchanged in the presence of strophanthin (ARENA et al., 1972).

Based on measurements of systolic time intervals, acute cardiotoxicity of doxorubicin was prevented in patients by digitalization with β-methyldigoxin (VILLANI et al., 1976). The clinical usefulness of adding digoxin to treatment with doxorubicin was recently demonstrated (GUTHRIE and GIBSON, 1977). In cancer patients, daily digoxin treatment prevented the outbreak of cardiomyopathy even at cumulative doses of doxorubicin above those usually considered critical. In no patient on digoxin were electrocardiographic changes attributable to doxorubicin observed, unlike control patients and patients treated with ouabain instead of digoxin. Digoxin was also found to correct cardiac failure precipitated by the cytotoxic agent. In addition, the incidence of voluntary muscle weakness was largely reduced. Ouabain was not effective with the dosage used, presumably because of its shorter duration of action.

2. Thyrostatic Agents and Thyroid Hormones

Clinical impression, supported by adequate documentation (FRYE and BRAUNWALD, 1961), shows that patients with hyperthyroidism often need larger than nor-

mal doses of cardiac glycosides against rhythm disturbances or cardiac failure. The reason for the altered need has not been fully elucidated. As discussed earlier in this chapter, changes in the pharmacokinetic disposition of cardiac glycosides appear to play a roll. At the cellular level, several mechanisms of thyroid hormone action are possible (STERLING, 1979). It seems that the sensitivity to catecholamines is not changed (MORROW et al., 1962; MCDEVITT et al., 1978). Instead, the observation that the number of Na^+, K^+-ATPase molecules per cell depends on thyroid state (EDELMAN, 1976; CURFMAN et al., 1977; HEGYVARY, 1977) could offer an attractive explanation for the changed sensitivity to cardiac glycosides in thyroid disease.

If patients with hypothyroidism need treatment with cardiac glycosides, there is a trend in the pharmacokinetic observations suggesting that smaller than conventional doses might be required. With respect to the pharmacodynamics, experiments in hypothyroid dogs suggest that small doses could efficiently improve contractility but that treatment of rhythm disturbances would require the same doses as in the euthyroid state (MORROW et al., 1963).

As changes in thyroid function affect a dosage regimen with cardiac glycosides, correction of the disturbance by thyrostatic agents or thyroid hormones must be accompanied by proper adjustment of the glycoside dosage. Sometimes the need for digitalis therapy may even be eliminated by making the patients euthyroid.

3. Xanthines

Xanthines are often used in the treatment of bronchospasm to relax airway smooth muscle. Theophylline is the most common representative of this group of drugs. It enhances ventricular performance by increasing the ejection fraction and rate, an effect also seen in subjects without cardiopulmonary disease (MATTHAY et al., 1978). Laboratory findings suggest that the positive inotropic actions of theophylline and the cardiac glycosides occur by different mechanisms and can be summed (SIMAAN and FAWAZ, 1973). Theophylline has been observed to worsen arrhythmias, but it is obscure whether judicious administration of conventional doses can enhance ventricular automaticity to a clinically important extent (GREEN and SMITH, 1977). The laboratory finding of ouabain potentiating theophylline-induced ventricular arrhythmias (SIMAAN and FAWAZ, 1973) needs further exploration in the clinical situation.

4. Tricyclic Antidepressive Drugs

It was recently demonstrated that the tricyclic antidepressive drug imipramine in therapeutic doses has quinidine-like antiarrhythmic properties (BIGGER et al., 1977). This implies a possibility for arrhythmogenic interaction between tricyclic antidepressants and cardiac glycosides. That risk should be recognized, particularly because tricyclic antidepressants are not infrequently used in overdose with suicidal intent. On the other hand, cardiac glycosides might help to increase cardiac contractility, if it has been reduced by treatment with a tricyclic antidepressive drug (GREEFF and WAGNER, 1971).

5. Drugs Used During Anesthesia

Preoperative digitalization is often advocated, but the positive inotropic action of cardiac glycosides may be overshadowed by their potentially harmful arrhythmogenic effects (VATNER et al., 1971). Even without cardiac glycosides, rhythm disturbances during anesthesia are common. It can therefore be difficult to discriminate possible arrhythmias due to interactions between cardiac glycosides and drugs used in anesthesia. Imbalances in electrolyte and acid-base status should also be considered as complicating factors in analyzing the real role of drug interaction during anesthesia. In spite of much speculation in the literature, mostly based on laboratory or animal experiments, there is no convincing evidence for important adverse reactions between inhalant anesthetics and cardiac glycosides in routine clinical situations. Administration of a depolarizing agent, succinylcholine, to patients treated with cardiac glycosides has met with troublesome bradyarrhythmias. The reason might be increased vagal tone or disturbances in potassium homeostasis (ROTH and WÜTHRICH, 1969; WEINTRAUB et al., 1969).

D. Concluding Remarks

The frequent use of cardiac glycosides in medicine apparently creates a large potential for interactions with other substances, which might be serious, particularly in view of the narrow therapeutic range of digitalis. We have tried to review the topic as completely as possible, even if some reports of importance may have escaped notice. Moreover, future research can be expected to generate more information. Nonetheless, the impression is that clinically important adverse interctions with the cardiac glycosides are relatively few, increasing in frequency with age and severity of the heart disease. This suggests that other factors, such as interpatient variability, bioequivalence problems between drugs, and poor compliance with the drug regimens, would generally influence therapy to a larger extent.

As the review has shown, interactions are not infrequently therapeutically beneficial and useful for combination therapy. There are several examples when judicious administration of cardiac glycosides can reduce undesired effects of other drugs, particularly when cardiac contractility is at risk. On the other hand, several agents can counteract the arrhythmogenic effects of digitalis.

The purpose of this chapter has been to make a contribution to a safer and better use of the cardiac glycosides. Awareness of their possible interactions should help the clinician not only to find the appropriate dosage in different situations and hence to avoid toxicity, but also to use beneficial combinations with therapeutic intention.

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