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Evaluation of Analytical, Pharmacokinetic and Pharmacodynamic Methods for the Study of Digoxin

Santosh John Vetticaden

Department of Pharmacy and Pharmaceutics
School of Pharmacy
Medical College of Virginia
Virginia Commonwealth University

This is to certify that the dissertation prepared by Santosh J. Vetticaden entitled "Evaluation of Analytical. Pharmacokinetic and Pharmacodynamic Methods for the Study of Digoxin" has been approved by his committee as satisfactory completion of the dissertation requirement for the degree of Doctor of Philosophy.

[redacted]

Director of Dissertation

[redacted]

Committee Member

[redacted]

Committee Member

[redacted]

Committee Member

[redacted]

Committee Member

[redacted]

Dean of the School of Pharmacy

[redacted]

Chairman of the Medical College of Virginia
Graduate Committee and Dean. School of Basic Sciences

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by

Santosh John Vetticaden

B.Pharm. (Hons.), Banaras Hindu University, 1980
Banaras. India

Director: William H. Barr. Pharm.D., Ph.D.,
Chairman. Department of Pharmacy and Pharmaceutics

Virginia Commonwealth University

Richmond, Virginia, USA.

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Abstract

EVALUATION OF ANALYTICAL, PHARMACOKINETIC
AND PHARMACODYNAMIC METHODS
FOR THE STUDY OF DIGOXIN

Santosh J. Vetticaden

Medical College of Virginia–Virginia Commonwealth University, 1985.

Major Director: William H. Barr Pharm.D., Ph.D.

The primary objective of the research was to investigate the pharmacodynamics of digoxin in dogs. Initially an assay specific for digoxin in the presence of its major metabolites, viz., digoxigenin, digoxigenin mono-digitoxoside, digoxigenin bis-digitoxoside and dihydrodigoxin was developed using HPLC-RIA. Methodology for non-invasive measurement of left ventricular ejection time (LVET) and other systolic time intervals (STI) in beagle dogs were developed. This involved surgery for exteriorization of the carotid artery in the dogs and subsequent measurement of LVET and STI after recovery. STI, heart rate (HR) and digoxin levels were monitored in normal beagle dogs administered 0.05 mg/kg or 0.025 mg/kg i.v., infused uniformly over a 5 min. period. The STI did not lend itself to pharmacodynamic modelling. The LVET, QS_2 and P-R interval were found to be inversely, but linearly, related to the heart rate. Therefore, the bradycardic response to digoxin was

extensively investigated in beagle dogs. Pharmacodynamic models evaluated for modelling the bradycardic response to digoxin were: the pharmacokinetic model with a direct linear link, the linear model, the physiologic-pharmacokinetic model with direct linear link and the effect compartment model. The physiologic pharmacokinetic model was simulated using SPICE2 which uses network thermodynamics to simulate biological systems. Criteria for the selection of appropriate models were established. Using the established criteria, the effect compartment model was demonstrated to be the best model. The implications and applications of pharmacodynamic models in general and specifically of the pharmacodynamic model for the bradycardic response to digoxin are discussed.

AIMS AND OBJECTIVES OF PROGRAM

The primary aim of the research project was to investigate, develop and evaluate the methods and means for pharmacokinetic and pharmacodynamic studies with digoxin. Specifically, the objectives of the project were:

1. To develop an assay specific for digoxin in the presence of its major metabolites digoxigenin, digoxigenin mono- digitoxoside, digoxigenin bis- digitoxoside and dihydrodigoxin.
2. To investigate and evaluate methods for the measurement of systolic time invervals (STI) and other pharmacologic responses to digoxin in dogs.
3. To characterize digoxin pharmacokinetics in dogs.
4. To investigate digoxin pharmacodynamics in dogs.
5. To develop and evaluate mathematical models. computer programs and simulation programs for describing digoxin pharmacokinetics and pharmacodynamics in dogs.
6. To establish criteria for the selection of appropriate model(s) when more than one pharmacokinetic-pharmacodynamic model may be representative of the obtained data.
7. To evaluate applications and implications of investigated models.

CHAPTER 1

LITERATURE SURVEY

REVIEW OF DIGOXIN PHARMACOKINETICS

History:

Digoxin is one of the digitalis glycosides. The introduction of digitalis glycosides has been attributed to Withering who published the first monograph on the action of digitalis in 1785. Since then digitalis therapy has become well established and digoxin, the most widely used of digitalis glycosides, is an indispensable drug in the treatment of cardiac insufficiency. Despite this long history of digoxin usage, implementation and maintenance of digoxin therapy in patients is still complicated. This is due to various pharmacokinetic factors that have to be considered in designing the optimal dosage for each individual.

1) Review of Digoxin Pharmacokinetics

In the absence of sensitive analytical methodology, estimation of the quantity of glycoside in the body was possible only by observing the pharmacologic effect. More precise estimations became possible with the introduction of radiolabeled digoxin. The subsequent development of sensitive radioimmunoassays and HPLC assays have resulted in a greater understanding of pharmacokinetics in animals and humans. However, radioimmunoassay procedures are also non-specific and measure some of the metabolites of digoxin, making them unsuitable for accurately characterizing the pharmacokinetics of digoxin. Hence, non-specificity of the assay procedure used

is one of the factors that has to be considered when reviewing the existing body of literature on digoxin pharmacokinetics.

Following an intravenous dose, factors that contribute to the observed digoxin pharmacokinetics in animals or humans may be broadly classified as follows:

a) Absorption

b) Excretion

c) Distribution

d) Protein Binding

e) Metabolism

a) Absorption:

Digoxin is absorbed from the gastrointestinal tract by a passive, nonsaturable transport process (Greenberger and Caldwell, 1972). The authors determined that 40-60% of the dose of digoxin is absorbed in the duodenum and upper jejunum and only 10% is absorbed in the stomach. The first order rate constant for absorption is several times greater than the elimination rate constant (Jelliffe, 1967) and, therefore. variation in the rate of absorption has little clinical relevance (Keys, 1980). Studies indicate negligible metabolism of digoxin during "first pass"through the liver (Beerman *et al.* 1972; Greenblatt *et al.* 1976). Reviews on the bioavailability of digoxin have indicated that the average bioavailability of digoxin tablets is 68% to 70% and that for the elixir is about 77% (Collaizzi, 1977; Greenblatt *et al.* 1976).

b) Excretion:

Digoxin is eliminated primarily by renal excretion. Biliary elimination makes a smaller contribution to the total elimination of digoxin.

1) Renal excretion:

60%-70% of the dose of digoxin administered as ^{3}H-digoxin is recovered in the urine over a period of 5-7 days (Huffman and Azarnoff, 1972; Greenblatt *et al.* 1973, 1974; Sanchez *et al.* 1973; Marcus *et al.* 1976; Lindenbaum 1973; Bochner 1977; Danon *et al.* 1977; Koup *et al.* 1975; Falch 1973; Falch *et al.* 1973; Gilfrich *et al.* 1973; Greenwood *et al.* 1975; Iisalo and Dahl, 1974; Iisalo and Ruikka, 1974; Johnson *et al.* 1976; Ochs *et al.* 1975 and Wittrell *et al.* 1974). However changes in the amount of digoxin excreted in the urine may also represent differences in the bioavailability of the different dosage forms. In a group of six healthy subjects, digoxin renal elimination varied from 50%-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).

2) Renal excretion of metabolites:

Most of the dose of digoxin appears in the urine as unchanged drug. Major metabolites of digoxin that have been identified in the urine include digoxigenin, digoxigenin bis-digitixoside, digoxigenin mono-digitoxoside and reduced forms of digoxin and the corresponding metabolites (Clark and Kalman 1974; Peters *et al.* 1978; Watson *et al.* 1973; Reitbrock and Abshagen, 1973; Marcus *et al.* 1966 and Zilly *et al.* 1975).

3) Factors influencing renal elimination:

Since a major part of digoxin is excreted by the kidney, a good correlation exists between digoxin clearance and creatinine clearance. It has been generally well accepted that 60%-70% of the plasma digoxin passes into the ultrafiltrate, and creatinine clearance is used as a measure of digoxin clearance (Jelliffe 1967,Iisalo 1977). However, a few studies have shown that digoxin may also be eliminated by

active tubular secretion (Steiness 1974, Waldorf *et al.* 1978). These observtions do not detract from the usefulness of creatinine clearance as an index of digoxin clearance, since creatinine itself may be subject to active transport (Weiner 1973).

Gibson *et al.* 1984, however, found no evidence of concentration-dependent renal excretion of digoxin. This is in agreement with similar findings by Ochs *et al.* 1978 and Wagner *et al.* 1981. However, Cl_{dig}/Cl_{inulin} (digoxin clearance/inulin clearance) exceeded 2.00 in all subjects indicating that net tubular secretion is responsible for a large amount of the digoxin excreted in the urine. A strong correlation between Cl_{dig} and renal plasma flow as measured by Cl_{pah} (para- amino hippurate clearance) was also observed and may be further evidence for the involvement of a secretory mechanism in overall Cl_{dig}. Net tubular secretion of digoxin was also found in other studies (Cogan *et al.* 1981, Jelliffee *et al.* 1967) and can account for up to 60% of the total digoxin secreted in the urine.

Malcolm *et al.* (1977), however, observed no significant effect for paired comparisons on the digoxin renal excretion in 8 healthy subjects receiving a single 0.75 mg oral dose of digoxin before and during the administration of oral furosemide 40 mg daily. Their results seem to agree with those by Bissett *et al.* (1973) who showed that digoxin excretion is not dependent on urine flow rate. The above two studies are supported by those 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. All these studies involved an increase in urine flow rate as compared to patients with congestive heart failure (CHF). CHF patients usually have a low urine flow rate and hence might have a higher than normal digoxin tubular reabsorption. Moreover Tsutsumi *et al.* produced evidence that furosemide inhibits the tubular secretion of digoxin, thus further confusing the issue. Halkin *et al.* (1975) in studies in 35 patients found that the variation in digoxin clearance

in these patients was more related to urine flow and urea clearance than to creatinine clearance. Urea clearance may therefore be a better way of estimating digoxin clearance in patients.

Another drug affecting digoxin renal elimination is quinidine, which produces a reduction in renal clearance and a corresponding increase in serum digoxin concentration of about 2.5 times by an action which may involve inhibition of digoxin secretion (Doering 1979, Hayer *et al.* 1979).

4) Extrarenal excretion:

Koup *et al.* (1975) found a mean value for total body clearance of digoxin (Cl_{tbc}) of 188 $\pm$ 44 ml/min/1.73 m^2. which was significantly higher than that for digoxin renal clearance 144 $\pm$ 41 ml/min/1.73 m^2 indicating that digoxin may also be eliminated through non-renal routes. These results together with similar results from Sumner and Russel (1976) indicate that around 18%-28% of digoxin is eliminated by non-renal routes in patients with normal renal function.

Based on the ratio of non-renal to total body clearance (Cl_{nr}/Cl_{tbc}), it is estimated that 25% of the elimination of digoxin occurs via non-renal routes (21.6% based on the ratio of total amount of unchanged digoxin excreted in the urine and the dose administered i.e., $Xu_{inf.}$/dose). These values are somewhat lower than the value commonly used by Jelliffe (1967). The data is in agreement with that by Sheiner *et al.* (1972), which suggest that only 26% of the Cl_{tbc} of digoxin is accounted for by non-renal routes.

According to Caldwell and Cline (1976), about 30% of an intravenous dose of ^3H-digoxin reaches the digestive tract in 24 hr. About 45%-50% of the radioactivity recovered in the stools after a dose of labeled digoxin consists of unchanged drug, 10%-20% as digoxigenin bis-digitoxoside and 25% as digoxigenin mono-digitoxoside (Doherty and Kane, 1975).

In patients with renal failure. the reduced renal elimination rate results in a greater fraction of the dose being excreted through the non-renal mechanisms. 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 GI absorption. Also. Doherty *et al.* 1967, observed an increase in fecal elimination of ^{3}H-digoxin in anephric subjects.

c) Tissue Distribution:

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 the blood and that in the myocardium ?

Studies on the distribution of digoxin have revealed large variations in the concentration of digoxin in cardiac tissue. This variation in the estimation of digoxin concentration in cardiac tissue can be explained by ratio of muscle tissue to fat and connective tissue in the sample taken, patient groups studied, dosage and compliance, time of sampling, different methods of sample preparation and analysis, variations in electrolyte levels in the blood, or changes in myocardial concentrations due to various pathologic states. However after steady state conditions have been established there exists a constant ratio between serum and myocardial levels of digoxin which permit plasma digoxin levels to be reliably used in the control of digoxin therapy.

Doherty *et al.* (1967). in a study involving 11 patients found that highest tissue/serum ratio of digoxin in heart, kidney and liver and lowest for brain. Dry weights of tissues were also obtained in an attempt to determine if fluid-retention in tissues would affect the concentrations of ^{3}H-digoxin found in the tissue. There was no correlation between patients with varying degrees of CHF and the dry/wet

tissue ratio showing that this factor is unimportant in relation to the distribution of digoxin. Anephric patients and those with renal failure showed highest tissue/serum ratio in heart muscle followed by liver and kidney and lowest in brain. The low concentration in brain tissue suggests the presence of a blood brain barrier for digoxin.

Considerable differences in the distribution of digoxin have been found in the CNS. Andersson *et al.* (1975) found a mean digoxin concentration of 32 ng/ml wet weight in the telencephalon region of the brain in humans during steady-state. This was comparable to that in the skeletal muscle, but was 3 times higher than the concentration in fatty tissue. A concentration seven times that in the telencephelon was recorded for the choroid plexus where the concentration exceeded even that in the myocardium by a factor of 1.7 (Kuhlmann *et al.* 1979). Digoxin distribution studies on various brain tumors by Williams *et al.* (1976) indicate the importance of blood-brain barrier and Na^+, K^+ -ATPase in glycoside uptake. Corresponding to the significantly higher ATPase in meningiomas in comparison with malignant blastomas (Laws and O'Connor, 1970; Agren *et al.* 1971) the concentrations of digoxin were 21.8 ± 7.3 ng/gm. and 5.7 ± 5.2 ng/gm wet weight respectively.

In dogs there is a distinct concentration difference between the cerebrum, cerebellum and different sections of the brain stem (Kuhlmann *et al.* 1979). On a daily maintenance dose, digoxin accumulation in the dog brain is higher than in other tissues (Reitbrock and Alken, 1980), a large part of it being bound non-specifically.

The reported values for cerebrospinal fluid : serum ratio in patients on maintenance doses of digoxin vary between 0.03 and 0.3 (Allonen *et al.* 1977, Gayes *et al.* 1978, Schott *et al.* 1976). Children have the same ratio as adults (Allonen *et al.* 1977).

d) Protein binding:

Using equilibrium dialysis. Storstein *et al.* (1976), determined protein binding of digoxin to be 22.4 ± 2.1% and that for digoxigenin mono-digitoxoside to be 18.8 ± 1.9%. Protein binding of digoxin may be altered in serum of individuals with Kwashiorkor (Buchanan *et al.* 1976). and in uremic patients on hemodialysis (Hawlina and Rahn 1974).

e) Metabolism:

Studies have shown that digoxin metabolism involves reactions ranging from cleavage of sugar molecules to conjugation reactions. The major pathways are :

1) cleavage of the digitoxose residues

2) conjugation reactions

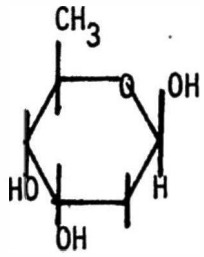
3) Hydrogenation

Structures of digoxin and some of its major metabolites is given in fig. 1.1

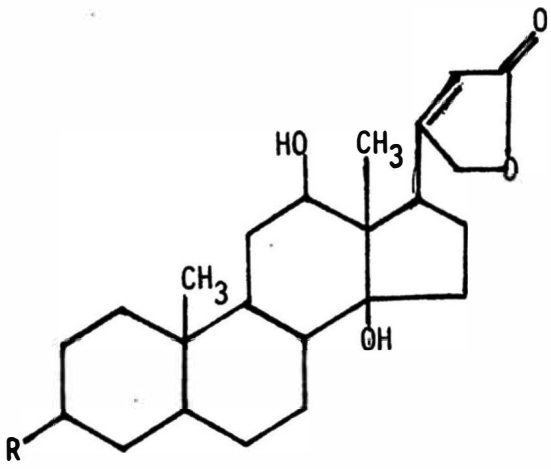
1) Cleavage of digitoxose residues

One of the earliest reports of the cleavage of cardiac glycosides into the genin and sugar moiety by enzymatic hydrolysis has been by Kelsey (1957). The first conclusive analytical investigations however, came from the research activities of Okita *et al.* (1975), and Repke (1959, 1966, 1970).

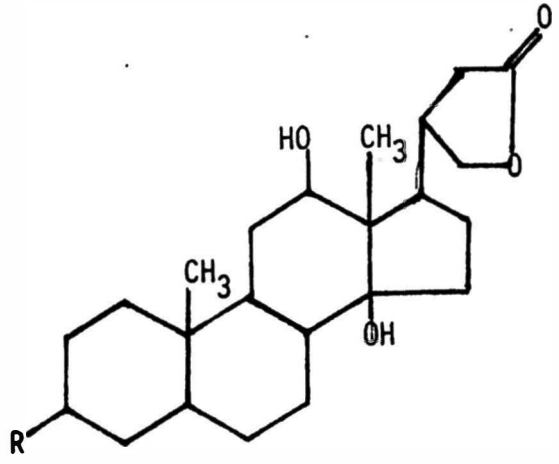
The liver is the principal site for the cleavage of the β-glycoside bond. The precise nature of the enzyme however is not yet clearly explained. A cleavage by β-glucuronidase is unlikely since this enzyme has an absolute specificity for carbon atom 3 which in digoxin has the –OH group linking the sugars in the epimer form (Repke 1970). The cleavage of a sugar chain is therefore probably not a simple hydrolysis step. Investigations on rat liver microsomal preparations have shown that the digitoxose group of digitoxin is only released in the presence of NADPH



Digitoxose



Digoxin



Dihydrodigoxin

Digoxin	R = 3 digitoxose residues
Dihydrodigoxin	R = 3 digitoxose residues
Digoxin bis-digitoxoside	R = 2 digitoxose residues
Digoxin mono-digitoxoside	R = 1 digitoxose residue
Digoxigenin	R = OH

Fig. 1.1 Structure of digoxin and its metabolites.

and that the reaction can be inhibited by the microsomal inhibitor SKF-525A and by carbon monoxide (Schmoldt *et al.* 1975). The neonate liver is unable to carry out the cleavage reaction, thus supporting the participation of these postnatally acquired enzymes (Hermann and Repke, 1963a). Schmoldt *et al.* (1980), by incubating digoxigenin (dg-0), digoxigenin bis-digitoxoside (dg-2), and digoxigenin mono-digitoxoside (dg-1) and digoxin (dg-3), with rat microsomes found that microsomal monooxygenases can oxidize the axial hydroxyl group of the terminal digitoxosyl of digoxin, or bis- or mono-digitoxosides to an oxo group. Only after this oxidation can the terminal sugar be split off, presumably by β-elimination. Therefore for the degradation of dg-3, three successive cytochrome P-450 catalyzed oxidations are neccessary before dg-0 can be obtained.

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 (Hermann and Repke 1963 a,b; Kuhlmann *et al.* 1974).

The possibility of direct conjugations with tri-digitoxosides and their sugar deficient metabolites has also been considered. Almost all the relevant studies left room for contradictory interpretations. 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 lack of definitive methodological alternatives, thin layer 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 (Hermann and Repke, 1963a,b; Reitbrock and Vohringer, 1974). Both reaction steps lead to a considerable increase in elimination rate (Repke 1970). It is safe to assume that due to the higher polarity of the conjugates their elimination rates

are high and their accumulation comparatively low. This is in agreement with the half-lives of 1.2 hr., 11.5 hr., 8.5 hr., and 2 hr. for dihydrodigoxin, digoxin bis-digitoxoside. digoxin mono-digitoxoside and digoxigenin respectively, reported by Gault *et al.* (1979). That the metabolites of digoxin have a shorter half-life than that for digoxin was also brought out by Gierke *et al.* (1980), who studied the disposition of digoxin bis-digitoxoside in dogs before and during chronic azotemia. The bis-digitoxoside was eliminated primarily by non-renal mechanisms. The $t_{1/2}$ for the bis-digitoxoside was 18.5 hours and was not significantly increased in azotemic dogs.

3) Hydrogenation

There have been several reports on the occurrence 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 bond between C_{20} and C_{22} is effected by intestinal microorganisms (Hermann and Repke 1968).

The microorganisms responsible are probably anaerobic since saturation of the lactone ring becomes completely suppressed when lanatoside C is incubated with cecum contents in the presence of oxygen (Hermann and Repke 1968). Since the hydrogenation occurs in the lower intestine, the appearance of the cardenolide in urine is observed 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 th of that for digoxin (Hermann and Repke 1968).

Watson *et al.* (1973), succeeded in the determination of dihydrodigoxin in plasma and urine using gas chromatography - mass spectroscopy (GC-MS). A high dihydrodigoxin concentration in plasma was detected in 3 of 150 patients. In one

case it amounted to 30% of the total glycoside and was suprising in view of the report from Luchi and Gruber (1968), that none of the patients required an unusually high 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. daily. From 2% - 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. However, studies by Magnusson *et al.* (1982) in 8 healthy men administered oral tritiated digoxin revealed that dihydrodigoxin constituted only 0.7 ± 0.4% of the total radioactivity excreted in the urine. Gault *et al.* (1979) determined the elimination of digoxin and its metabolites in 6 subjects with normal renal function and 6 patients with minimal renal function. Dihydrodigoxin constituted 0.3% and 0.03% of ingested radioactivity in these two groups.

The clinical importance of such dihydrodigoxin compounds must be evaluated from two aspects. The first is with regard to the radioimmunoassay of glycoside concentrations in plasma and urine since it is known that digoxin antibodies cross-react with digoxin metabolites (Kramer *et al.* 1976; Stoll *et al.* 1972).

The second aspect remains to be investigated and is whether individual differences in transformation of digitalis glycosides into their dihydrodigoxin 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 (Rietbrock *et al.* 1981). Furthermore the presence of a plasma-tissue concentration gradient of approximately 1.0 indicates the absence of an organ-specific distribution.

Peters *et al.* (1978), also indicated that the occurence of significant quantities of dihydrodigoxin in the urine could be dependent on diet or gut flora. That

the gastrointestinal flora play an active role in the hydrogenation of digoxin and metabolites to give digoxin reduction products (DRP's) was proven by Lindenbaum *et al.* (1981). He found that in approximately 10% of the patients given digoxin, substantial conversion of the drug to cardioinactive DRP's occurs. Stool cultures from subjects known to make DRP's in vivo (excretors), converted digoxin to DRP's; cultures from non-excretors did not. Three excretors were given digoxin tablets for 22 - 29 days. In these excretors urinary excretion of DRPs was greatest after administration of the poorly absorbed tablet and least after i.v. administration. A 5-day course of erythromycin or tetracycline after a baseline period of 10 - 17 days, markedy reduced or eliminated DRP excretion in urine and stool. Serum digoxin concentration rose as much as two fold after antibiotics were given. It is thus evident that the enteric flora is responsible for the hydrogenation of digoxin to give DRP's and that changes in the enteric flora may alter digoxin levels.

Effect of acidity:

Magnusson *et al.* (1982), studied the 3-day urinary excretion of digoxin. its conjugated and unconjugated hydrolytic metabolites and dihydrodigoxin, in 8 healthy men after oral administration of tritiated digoxin. Analysis was performed by HPLC. 42.4 ± 2.7% of the dose of ^{3}H-digoxin was recovered before and 44.0 ± 2.2% after deconjugation of the urine samples. Digoxin and dihydrodigoxin constituted 40.3 ± 2.9% of the total radioactivity recovered in the urine of which 0.7 ± 0.4% was dihydrodigoxin. The sum of the hydrolytic metabolites was 2.1 ± 0.3% before and 3.4 ± 0.15% after deconjugation. No correlation was found between fasting gastric pH measured just prior to the administration of the dose of digoxin and the production of hydrolytic metabolites.

Magnusson *et al.* (1984), studied the metabolic profile of digoxin in patients with a low gastric hyperacidity (GH), and a group of patients on digoxin treatment in coronary care. The overall metabolic profile of the two groups was not significantly different. For the patients with low gastic pH the percentage of total radioactivity excreted in the urine in 24 hrs. constituted 21.3 ± 0.6% of the dose. 75.2 ± 8.5% was excreted as digoxin and dihydrodigoxin. Dihydrodigoxin represented only 0.7 ± 0.1% of the digoxin-dihydrodigoxin fraction. Digoxigenin and its conjugated version constituted 7 ± 2.5% of total radioactivity, digoxigenin mono-digitoxoside and its conjugate constituted 1.5 ± 0.5% and digoxigenin bis-digitoxoside and its conjugate constituted 4.3 ± 0.8% of the dose. The metabolic profile for the coronary care group was similar. The pH in the gastric hyperacidity (GH) group ranged from 1.15 to 2.04. It thus seems that the gastric acid degradation of digoxin is small and the clinical importance of the hydrolysis of the glycoside seems limited.

Effect of renal function:

Gault *et al.* (1984), studied the effect of renal function on digoxin biotransformation. Group I patients consisted of 10 dialysis-dependent chronic renal failure patients with creatinine clearance ranging from 0 to 5 ml/min. and Group II consisted of 5 patients with creatinine clearance ranging from 38 to 100 ml/min. Patients were on maintenance doses of digoxin. They were then administered 150 μCi of ^{3}H-digoxin-12-α. Blood and urine collected over 24 hours was assayed by HPLC. The variation in the degree of biotransformation among patients was great in both groups and no significant differences between the means for digoxin for the two groups or for the metabolites was found. The mean percentages for digoxigenin, 3-keto-digoxigenin, 3-α-(epi)-digoxigenin and for the mono- and bis-digitoxosides

were less than 3% of the total radioactivity in both groups. Polar metabolites constituted a mean percentage of 28% of the total radioactivity in Group I and 22% of the total radioactivity in Group II.

Studies on CAPD patients have shown that means for percent digoxin and metabolites for CAPD patients did not differ significantly from those on hemodialysis and that CAPD removes only relatively small amounts of digoxin from the body (Vitali *et al.* 1981).

In conclusion, it appears that the biotransformation of digoxin involves all the classic metabolic reactions. In one route there is initially hydrolysis with release of the sugars, either in the stomach (Gault *et al.* 1980), or in the liver, to produce 3-β-digoxigenin. This is then followed by oxidation to produce 3-keto-digoxigenin, epimerization to give the 3-α-(epi)-digoxigenin, and finally conjugation, as with glucuronic acid, to give polar end-metabolites. It is possible that conjugation of the mono-digitoxoside takes place (Kuhlmann *et al.* 1974), and that steriod ring hydroxylation may also take place. Reduction may occur in the lactone ring to give significant amounts of dihydrodigoxin in some patients. Clearly biotransformation of digoxin to polar metabolites occurs to a major extent in some patients and may result in an appreciable component of the serum digoxin concentration measured by RIA being metabolites some of which may be cardioinactive.

Compartmental Analysis

The digoxin concentration-time curve after intravenous injection or infusion can 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 initial decline in plasma concentration is the rapid distribution in to the peripheral compartment from the central or plasma compartment. This distributive phase has a half-life which varies from 20-60 mins. Extensive distribution into the peripheral compartment is indicated by an average volume of distribution at steady state which greatly exceeds the volume of the central compartment and the volume of body water (Reuning *et al.* 1973; Nyberg *et al.* 1974; Koup *et al.* 1975). The peripheral compartment is mainly the skeletal muscle. The deep compartment includes erythrocytes and brain where the equilibrium concentration is reached later than in most other tissues (Gorodischer *et al.* 1976: Kuhlmann *et al.* 1979). The volume of distribution, calculated at steady state (Vd_{ss}). varies in healthy subjects between 5.1 and 8.1 l/kg.: in patients with cardiac failure it is approximately 5.0 l/kg.: and in renal failure, 3.3 to 4.4 l/kg. (Reuning *et al.* 1973; Nyberg *et al.* 1974; Koup *et al.* 1975. 1976). Infants and neonates seem to have a higher volume of distribution at steady state than found in adults.

Investigators have used 2 or 3 compartment models (Sumner *et al.* 1976, Shenfield *et al.* 1977. Koup *et al.* 1975). The two compartment model consists of a central and peripheral (or tissue) compartment while the 3 compartment model has 2 peripheral compartments one being a deep compartment (Sumner *et al.* 1976). The central compartment is usually regarded as being the serum and highly perfused organs such as liver, kidneys and lungs. Skeletal and cardiac tissue are regarded as belonging to the peripheral compartment (Iisalo 1977).

Kramer *et al.* (1974). fit plasma concentration vs. time profiles for digoxin in 5 healthy volunteers. to appropriate equations describing two and three compartment open models using weighted non-linear least squares regression analysis. The comparison of two and three compartment model indicates that the three compartment open model is the simplest model consistent with the observed data. The improved fit using the three compartment model, as evidenced by F-tests, weighted

residual plots, and semilogarithmic plots of predicted and experimental serum digoxin concentrations yields more meaningful parameter estimates and an improved estimate of the half-life of the terminal exponential phase of the serum digoxin concentration-time data.

Balant *et al.* (1980), in an excellent article, observed that the results of previous workers indicated that a three compartment (mammillary) model more accurately describes digoxin disposition after an i.v. dose than do simpler models and that the more accurate model. consistent with data is needed when blood and/or tissue concentrations are to be correlated with the acute effects of digoxin on left ventricular ejection time, heart rate or on the duration of electromechanical systole.

However in the postdistributive phase after single or multiple dosing, digoxin disposition may be adeqately described by a mono-exponential or bi-exponential equation. Therefore, although a three compartment model is kinetically most accurate in describing digoxin kinetics in man, the simpler two and one compartment model are clinically useful as long as its limitations are appreciated.

Effect of Disease on Digoxin Pharmacokinetics

a) Renal insufficiency

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). Ochs *et al.* 1978a, noted that the age related increase in serum levels probably reflects the decline in renal function in the elderly.

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

population (Shapiro *et al.* 1969) and as high as 40% in patients with severe renal impairment (Ogilvie and Ruedy 1972).

It is well accepted that renal clearance of the intact drug accounts for the total digoxin clearance (Bodem and Ochs 1979; Greenblatt *et al.* 1974). 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; Waldorg *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 tubular secretion of digoxin is reduced (Steiness 1978). This also occurs with combined treatment of digoxin and furosemide. Tubular secretion is enhanced by potassium supplementation.

Jusko and Weintraub (1974), using radioimmunoassay studied the relationship of myocardial digoxin distribution at autopsy and the ante-mortem renal function. The myocardial serum concentration ratio became progressively smaller in patients with decreasing creatinine clearance. These altered tissue-serum concentration ratios probably explain the changes in volumes of distribution (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 in heart, kidney, liver and the lowest for brain. Anephric patients and those with renal failure showed the 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 stools over a 7-day period. 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). Differences in volume of distribution (Vd), may also account for changes in half-life since changes in half-life depends on both clearance and Vd. Koup *et al.* (1975), found Vd values ranged 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 in 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.* 1978b). Ohnhaus *et al.* (1979a) reported that absolute bioavailability of oral digoxin was not altered in patients with renal failure despite a 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 9 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.

Koup *et al.* (1975b) found a value of 47.7 ml/min/1.73 m^2 for the metabolic or non-renal clearance. This value agrees very well with the average reported by Koup *et al.* (1975a) in patients with severe renal failure. This suggests that the metabolic clearance of digoxin may be constant in renal failure.

b) 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 (Wenkebach 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 (CHF) (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 in Na^+ and K^+ -dependent ATPase (Lindsay and Marker 1976). However, alterations in glycoside kinetics is another possible explanation and have been evaluated by several investigators using either radioactive glycosides (Doherty and Perkins 1966; Eichenbusch *et al.* 1970). or radioimmunoassay (Croxon and Ibertson 1975; Gilfrich and Meinertz 1978; Shenfield *et al.* 1977). Interpretation of results obtained with radioactive glycosides is difficult because the methods used in the isolation and quantitation of the various metabolite fractions differ in specificity (Eichelbaum 1976).

Doherty and Perkins (1966) investigated digoxin pharmacokinetics in relation to 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 hypothyroid, intermediate in euthyroid and highest in hyperthyroid patients, suggesting that these pharmacokinetic changes might explain altered sensitivity. The serum half-lives in the 3 groups was not significantly different. nor were the half-lives for urinary excretion. In 2 hyperthyroid patients who became hypothyroid following treatment with radioiodine, and one hypothyroid patient who became euthyroid following treatment with thyroid hormone;

no significant changes in serum half-lives for urinary excretion were observed. To explain the lower or higher plasma digoxin levels in thyrotoxicosis and myxedema respectively, Doherty and Perkins proposed an altered volume of distribution in these conditions.

Ismail-Beigi and Edelman (1971) showed higher tissue levels of Na^+, K^+-ATPase in thyrotoxicity. Therefore, the suggestion of changes in tissue concentration 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 (RIA) studied steady-state serum digoxin concentrations in 17 hyperthyroid and 16 hypothyroid patients 24 h after the final dose. These patients had been treated with 0.5 mg. digoxin daily for 7 days. Serum concentrations and plasma half-lives of digoxin were significantly different between the two groups.

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 observations were confirmed by Lawrence *et al.* (1977) who administered tritiated digoxin to 9 hyperthyroid and hypothyroid patients. Mean plasma radioactivity did not differ between the 2 groups, but digoxin clearance and glomerular filtration rate (GFR) 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/day i.v. and

0.75 mg/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 RIA were also observed by Shenfield *et al.* (1977) in 4 hyperthyroid patients after oral administration of a 0.5 mg. dose as compared to normal patients. Gilfrich and Meinertz (1978) administered 1 mg. digoxin i.v. to 8 patients with thyrotoxicosis. The same patients were studied after they became euthyroid with treatment. Urinary excretion half life was approximately 24 h in the thyrotoxic state as compared to 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 dose) than after normalization of thyroid function (78%). Since the drug was given i.v. a decrease in the extent absorption during hyperthyroidism cannot account for these differences.

Another possible explanation for the differences in levels are due to altered volumes of distribution (Doherty and Perkins 1966). Increased volume of distribution in hyperthyroid patients and decreased volume of distribution in hypothyroid patients were demonstrated by Shenfield *et al.* (1977), and Gilfrich and Meinertz (1978), showed a larger Vd in hyperthyroid patients than in euthyroid patients. The findings of decreased serum and liver levels of tritium in the hyperthyroid group, but increased cardiac levels, supports the concept of changes in the distribution of digoxin in hyperthyroidism. There is considerable evidence to suggest that there are changes in glycoside receptors with altered thyroid status. The nature of the cardiac receptors remains unresolved but there is still doubt that the Na^+, K^+-ATPase is intimately involved (Coltart 1978) and it has also been shown that there is an increase in Na^+,K^+-ATPase activity in hyperthyroidism (Curfman, Crawley and Smith 1977).

Lindsay and Parker (1976), showed that pre-treatment with thyroid hormones or digoxin in rats resulted in increase Na^+, K^+-ATPase activity but that pretreatment with both was not additive suggesting that T_4 and digoxin both competed for the same pathway. Therefore with increased ATPase activity more digoxin may be needed to achieve the degree of inhibition necessary for a therapeutic response. In addition, increased Na^+, K^+-ATPase activity resulting in increased digoxin binding to tissues such as skeletal muscle could account for the observed differences in serum levels and Vd of ^3H-digoxin.

There is evidence of liver enzyme induction in hyperthyroidism for a number of drugs (Kato 1977, Crooks *et al.* 1973, Eichelbaum 1976) and an increase in biliary excretion of both unchanged digoxin and metabolites in hyperthyroid rats has been demonstrated (Huffman *et al.* 1978). It is possible that in hyperthyroidism, digoxin metabolites are more avidly taken up into cardiac tissue than is the parent drug and this possibility has not been excluded by any of the present studies since ^3H-digoxin was used or digoxin was assayed by RIA both of which measure a fair amounts of metabolites. Liver enzyme induction is also suggested by Varadi and Foldes (1976), who observed increased D-glucaric acid secretion in most hyperthyroid patients as compared to normal subjects. Since an increased D-glucaric acid excretion is a result of the induction of the glucuronic acid pathway (Hunter *et al.* 1971), it was concluded that in thyrotoxicosis the enzymes of the glucuronic acid sequence are stimulated and the hepatic transformation of digoxin can be enhanced by thyroid hormones as endogenous enzyme inducers.

The hyperdynamic state in hyperthyroidism may also be involved in the altered cardiac tissue level, heart rate for example has been implicated in glycoside binding (Lloyd and Taylor 1978, Roth-Schechter *et al.* 1970) and there is an increased heart rate in hyperthyroidism.

Thus there are several possible explanations for the increased cardiac binding of digoxin in hyperthyroidism. The two most likely hypotheses are :- 1) Changes in sensitivity to digoxin with altered thyroid status are due to changes in serum level. 2) Changes in sensitivity to digoxin with altered thyroid status are due to a direct effect of thyroid hormone of the heart inducing increased Na^+, K^+-ATPase. As a result more glycoside is needed to achieve the same response.

c) Potassium Imbalance

Potassium ions influence electrophysiologic events in the heart. Excess myocardial potassium reduces conduction in the atria and subnodal system and depresses automaticity. Despite this, in clinical situations, conduction disturbances during hyperkalemia are rarely seen, except in digitalized patients, indicating that interaction between potasium 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). Potassium ions also contribute to the transmembrane resting and action potential of the myocardial cell. These facts may explain the relationship between hypokalemia and digitalis toxicity. Also, clinical benefit has often been achieved by the administration of potassium in patients with digoxin-induced arrythmias. The fact that hyperkalemia can also result from digoxin 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 the 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 confirmed clinically.

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). This may partly explain why hypokalemia pre-disposes to digoxin toxicity. 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.

Cohn *et al.* (1967), studied the effect of K^+ depletion on myocardial concentration of tritiated digoxin. He found that there was no significant differences between the myocardial ^3H-digoxin of the central and potassium-depleted groups at 0.5, 1, 2, 6 or 16 hours, but at 24 hours, a significantly higher concentration of digoxin was present in the potassium deficient mouse hearts (23.6 ± 5.2 μg/gm.) than in the control group (3.1 ± 1.5 μg/gm.). Potassium depleted mice with total renal failure, showed increased myocardial ^3H-digoxin levels at 20 hours as compared to mice with renal failure but without potassium deficiency. It is thus evident that diminished renal function produced by potassium-depletion is not the sole mechanism of this retention of cardiac ^3H-digoxin.

The mechanism by which digitalis inhibits the influx of potassium is still under investigation. However, it has been established that the active transport of Na^+ and K^+ across the cell membrane is associated with the splitting of ATP by membrane ATPase. It has been postulated that Na^+ and K^+ activate ATPase by combining at sites which are more or less selective for these ions. The transport ATPase is readily inhibited by concentrations of the cardiac glycosides in the range from less than 10^{-8}M to more than 10^{-4}M (Glynn 1964). At lower concentrations of digitalis, the degree of inhibition of K^+ influx appears to diminish as extracellular K^+ increase (Page 1964, Glynn 1956). This observation may be explained by competition of cardiac glycoside and K^+ for a binding site, possibly on the cell membrane (Page

1964, Repke 1965, Glynn 1957). In addition, Ebert *et al.* (1967), reported that K^+ can displace digoxin from the heart. This is in agreement with the studies of Marcus *et al.* (1969). The authors determined digoxin disposition in 2 groups of dogs. In group I hyperkalemia was produced prior to the injection of digoxin and was continued to 1 hour or 4 hours before sacrifice. In group II infusion continued for 150 mins. Serum K^+ was in the range of 6-9 mEq./L. There was less digoxin in the myocardium of hyperkalemic dogs in group I. There was a decrease in the amount in the kidney, but an increase in digoxin concentration in the skeletal muscle. Group II dogs displayed no difference in concentrations of digoxin in the myocardium, kidney and skeletal muscle. The authors postulated that the change in body distribution of digoxin in the dogs made hyperkalemic prior to the administration may be explained in part by competetive inhibition of K^+ and digitalis for similar receptor sites. Once digitalis is bound to the cell, hyperkalemia does not alter its retention. Therefore the mechanism of K^+ in reversing digitalis induced arrythmias may be other than physical displacement of digoxin from the heart. It is also possible that the portion of digoxin that is pharmacologically active is a minute portion of that present in the myocardium and that K^+ displaces this fraction, diminishing pharmacologic activity but releasing amounts of digoxin which are not measureable by methods available then.

d) Obesity

Ewy *et al.* (1971), studied the effects of obesity on digoxin distribution by administration of a single i.v. dose to five obese patients before and after a mean loss of weight of 102 lbs. There was no significant difference in the blood concentration of digoxin before and after weight reduction. The finding is consistent with the hypothesis that fat-free body weight is a more important determinant of the blood concentration of digoxin than total body weight.

INTRODUCTION TO PHARMACODYNAMICS

Pharmacokinetics involves the study of movement of drugs into, through and out of the body. It describes the processes and rates of drug movement from the site of absorption into the blood, distribution into the tissues and elimination by metabolism or excretion. Pharmacodynamics refers to the description of drug effects once the drug has reached its site of action. An aphorism that is appropriate is, "Pharmacokinetics is what the body does to the drug : Pharmacodynamics is what the drug does to the body." Therefore, to investigate the pharmacodynamic behavior of a drug the primary requirement is that the drug have a biological response that can be monitored.

Biological responses

Pharmacodynamic parameters that can be monitored with time following acute administration of cardioactive agents include blood pressure, heart rate and left ventricular ejection time (LVET) (Shapiro *et al.* 1970; Weisler *et al.* 1966; Hinderling and Garrett 1976; Thiobonnier *et al.* 1984). The extent of antagonism of isoproterenol action may permit the quantification of beta-blocker action. Ocular pressure is a measure of the action of carbonic anhydrase inhibition (Lehmann *et al.* 1970), and pupil dilation is a proper measure of mydriatic action (Smolen *et al.* 1971). Depth or duration of anesthesia can be measured for the action of anesthetics (Levy *et al.* 1966; Brodie *et al.* 1967).

However, in many cases, an acute pharmacodynamic response cannot be quantified. Many drugs have cumulative effects that can only be appraised clinically with long term or chronic therapy. For example it is not feasible to quantify anticancer

drug action (e.g. methotrexate. fluorouracil) within the body on acute administration by following the regression of a solid tumor or loss of viable cancer cells (although it may be feasible to monitor the toxicity with successive white blood cell counts). The diminution of a lesion by an anti-inflammatory drug is a slow process compared to the acute pharmacokinetics of the anti-inflammatory agent. Also, appropriate pharmacodynamic responses that can instantaneously reflect the action of an active agent at a receptor site are sometimes limited. An example is the long term effect in groups of pregnant animals acutely challenged with various doses of a teratogenic agent. The frequency of anomalies in the litter serves as an index of the toxicological effect (Jusko, 1972). In these instances correlation of plasma levels with biological action on chronic dosing may be the only effective procedure.

Provided the drug under investigation does evoke a monitorable biologic response, it should be possible to model the effect. Descriptions of pharmacokinetics and pharmacodynamics rely heavily upon the use of models. Models are usually mathematical equations, the parameters of which represent factors which we believe to be important in determining drug concentration effects. Models are preferred that not only describe the observations but also offer some insight into the underlying biological processes responsible. However, we are frequently forced to use empirical models with no clear biological counterpart.

Having decided upon a model, the values of its parameters quantify the pharamacokinetic or pharmacodynamic differences and similarities among drugs and the influence of other factors, such as other drugs and disease states. As noted by Hennis and Stanski (1985), the factors influencing pharmacokinetic-pharmacodynamic models could therefore be simply classified as:

1) Pharmacokinetic factors

2) Pharmacodynamic factors

1) Pharmacokinetic factors:

Immediately after an intravenous administration there is almost instantaneous mixing of the drug into blood. This phase occurs within one or two circulations of the drug in the body and is rapid. This very rapid mixing phase defines the peak drug concentration observed in blood one-half to two minutes after adminstration of the drug. After the peak blood concentrations are achieved. one or more phases are visible comprised of distributive and elimination phases where the blood concentrations decline at a slower rate.

The distributive phases represent the equilibration of the blood concentration (after the immediate mixing phase that defines the peak concentration) with tissues that are less well perfused. During this phase, distribution equilibrium has not been established between the blood concentration and drug concentrations in most of the tissues of the body. Drug is being distributed and redistributed between various tissues, with several factors governing the rate at which the tissue concentrations equilibrate with the blood concentration. These include the perfusion of the tissues and the partitioning or solubility of the drug in the tissues. If the drug has limited ability to penetrate membranes (low lipid solubility, high degree of ionization, large molecular weight), diffusion factors can also become rate limiting in the equilibration of the blood concentration with the tissues (Stanski *et al.* 1985).

After the drug has been distributed-redistributed throughout the body, the elimination phase becomes obvious. During this phase the decline of the blood concentration is slower relative to the distribution phase. The decline of the drug concentration is due to the irreversible removal of drug from the blood. This can be due to metabolism in the liver (blood or other tissues can also metabolize drug) to generally less active compounds and/or excretion of the unchanged drug via the kidney or bile. Because the change of the blood concentration is relatively slow in

the elimination phase compared with the distribution phase, a pseudoequilibrium is said to exist between drug in the blood and drug in the various tissues.

Drug response is generally related to the steady state concentrations to emphasize the clinical implications of the defined relationship. Steady state is defined as a situation in which the blood concentration is constant because the rate of drug elimination is equal to the rate of drug administration. Since the steady-state blood concentration is in equilibrium with all of the tissue drug concentrations, the biophase, or site of action, concentrations will also be constant (an exception would be any organ that eliminates the drug). Since the drug effect occurs in the tissues, at steady state the drug concentration at the site of action (biophase) will be constant.

2) Pharmacodynamic factors:

Some of the factors that can cause time delay between change in the blood concentration and the corresponding change in drug effect are:

a) Perfusion or delivery of drugs to the biophase.

If the perfusion to the biophase is high, drug delivery will be enhanced, and therefore the rate of equilibration between the blood concentration and the biophase concentration will be enhanced.

b) Diffusion of drug from the capillary lumen to the biophase.

Once in the capillary lumen of the region of the biophase, the drug must diffuse into the specific tissue or site where it can act. If physiochemical factors (ionization, molecular size) significantly limit the rate of membrane penetration (diffusion) into the biophase, this can translate into a time delay between change in blood concentration and corresponding change in the drug effect.

c) Partitioning of the drug between the blood and the biophase.

Once the drug has reached the biophase, several processes can occur. The drug can reach the receptor sites within the biophase and induce the drug effect. The

drug can also be nonspecifically bound to various (nonreceptor) proteins within the biophase analogous to the drug-protein binding that occurs in blood. If the drug is bound to proteins that are not relevant in creating drug effect. the drug is effectively "unavailable" to induce a pharmacologic effect.

d) Time to achieve receptor events and create the drug effect.

After the drug reaches the biophase, the actual time of receptor association, dissociation and translation of the receptor events into a pharmacologic effect become important. If the time to achieve receptor events is slow, then a significant delay between the change in blood concentration and the corresponding change in drug effect will be obvious.

Techniques of Pharmacodynamic Modelling

The estimation of the parameters of pharmacodynamic models using observations of drug effect require, first of all, a description of drug concentrations at the time of the effect. Usually this description is obtained from a pharmacokinetic model. If the model linking drug concentration to effect is known to be appropriate, then in theory the observations of drug concentration and drug effect should be used simultaneously to estimate the parameters of both the pharmacokinetic and pharmacodynamic model. However, most frequently the pharmacokinetic model is better defined than the pharmacodynamic model, and more reliable estimates of its parameters can be obtained from drug concentrations alone, because these are usually more precise and easier to obtain than measures of drug effect. It is therefore often easier to test different pharmacodynamic models by estimating parameters using fixed values for the pharmacokinetic parameters, which have been estimated from drug concentrations alone.

The use of computer-based model-fitting is strongly recommended for estimation of the pharmacokinetic and pharmacodynamic parameters (Johnson, 1974; McIntosh and McIntosh. 1980).

Selection of a suitable model is often helped by a plot of concentration against effect. Information about linearity. equilibration delays or tolerance may be acquired by this simple method.

Pharmacodynamic Models

The term 'pharmacodynamic models' has been used to describe models that utilize concentrations at the effector site (generally obtained from ***in vitro*** or isolated organ studies), whereas, the term 'pharmacokinetic-pharmacodynamic' models is used to describe those models in which the drug concentrations at the effector site are not known or cannot be estimated without knowledge of the drug effect (Holford *et al.* 1982). Pharmacodynamic models usually require the assumption that drug concentration, (e.g. in a tissue bath) is in equilibrium, or at steady state condition ***in vivo***. If no assumption about equilibrium can be made, a combined pharmacokinetic-pharmacodynamic model is then required.

Fixed Effect Model

The simplest pharmacodynamic model relates drug concentration to a fixed effect. No assumptions have to be made about the form of the relationship between concentration and effect, because only one degree of effect is considered, and the effect 'model' has only one parameter: the concentration at which the effect occurs. The model may be improved to include the statistical probability of the occurence of effect.

The Linear Model

The simplest relationship between concentration and effect which can describe a range of effect is the linear function

$$E = S \cdot C \qquad ...(1.1)$$

where E is intensity of effect, C is concentration and S is the slope parameter. Implicit in this model is zero effect at zero concentration. If the measured effect has some value when drug is absent, e.g. blood pressure. then the model may be expressed as

$$E = S \,.\, C + E_0 \qquad ...(1.2)$$

where, E_0 is the effect without drug. The parameters of this model are easily obtained by linear regression. However if E_0 is known with much greater reliability than the measurements of E obtained in the presence of the drug, then E_0 should not be estimated as a parameter, but used in the form

$$(E - E_0) = S \cdot C \qquad ...(1.3)$$

to estimate S alone. This avoids the problem of estimating a value for E_0 which is different from a reliably known effect in the absence of drug. Kramer *et al.* (1979) noted this and concluded that a linear model was not appropriate to describe the effect of digoxin on systolic time intervals, even though the relationship appeared to be linear in the presence of digoxin. The linear model predicted an effect of digoxin at zero concentration which was nearly 40 % of the maximum change in electromechanical systole (QS_2) observed in the presence of digoxin.

A linear relationship between drug concentration and prolongation of the Q–T interval has been reported for disopyramide (Whiting *et al.* 1980) and quinidine

(Holford *et al.* 1981). Eichelbaum *et al.* (1980), have found a similar relationship for the effect of verapamil on prolongation of the P–R interval.

Transformation of drug effect measurements may also allow the use of a linear model: Whitfield and Levy (1980) used the logarithm of the activated partial thromboplastin time (APTT) and found a linear relationship with heparin concentration. The basis for the transformation is empirical. and is dictated by use of a response which tends towards infinity as the drug concentration increases. The use of an alternative transformation, e.g. the reciprocal of the APTT may also be considered in defining the effect of heparin because heparin action is directly related to inhibition of coagulation, and the prolongation of the APTT may be expected to be inversely related to this inhibition.

A linear relationship between the area under the warfarin concentration *vs.* time curve and the area under the logarithm of prothrombin time *vs.* time curve has been used to compare the potencies of the R(+) and S(-) enantiomorphs of warfarin (O'Reilly, 1974). The logarithmic transformation of response presumably was used to correct for the obviously nonlinear relationship between prothrombin time and the coagulation factors.

Because the relationship between warfarin concentration and its effect is complicated by the time course of coagulation factor synthesis and degradation, O'Reilly used the areas under the log prothrombin–time *vs.* time and warfarin concentration *vs.* time after a single dose of each enantiomorph. This procedure effectively removes the time dependence for the effect of warfarin on coagulation.

Falliers (1979) used a linear relationship between forced expiratory volume, in one second, FEV_1 (expressed as a percentage of predicted 'normal') and theophylline concentrations using the two-parameter model. The observations were made in a group of 18 patients over a concentration range of 2–18 mg/l. The intercept value

(E_0) was estimated as 58.2%, which is in agreement with the pre-drug mean FEV_1 of 55.2%. The slope of the line was 1.23% per mg/l.

The Log–Linear Model

The concentration–response relationship of isolated tissues has formed the basis of current theories of pharmacodynamics (e.g. Ariens and Simonis, 1964a,b). Graphical representation of responses, obtained over a wide range of concentrations, is conveniently obtained by using the logarithm of concentration, which effectivly compresses the scale of the abscissa. The log transformation has a second property, that of linearity of the log concentration–effect curve between 20–80% of the maximum effect. However, there are problems associated with this approach: The equation describing this relationship is given below:

$$E = S \cdot logC + I \qquad ...(1.4)$$

where E, S and C are the same as in Eqn. (1.1), and I is a constant. The first obvious problem with this log–linear model is its inability to predict E when C is zero, and the second problem is its inability to predict a maximum effect. The estimation of the maximum effect and the influence of interventions such as antagonists on the maximum is an inherent part of pharmacodynamic investigation. In addition, if the measurement of effect is to be restricted to the range of 20–80% of the maximum and the model used only to summarize the observations within these bounds, then the only problem is how to the estimate the maximum effect to assure only the 20–80% responses are used. If it is not possible to estimate the magnitude of the maximum effect because of restrictions on the highest concentration possible to use, then there is no guarantee that the model is being applied within the 20–80% range. Shephard *et al.* (1979) compared the effects of warfarin in young and old people using the log–linear model. The effect of warfarin was expressed as the log

of the synthesis rate. and the slopes of the straight lines fitted to the log of the total warfarin concentration were the same in both groups. The rate of prothrombin complex elimination was 50% lower in the older group, but the concentration of warfarin required to reach the same inhibition of synthesis (50%) was the same in both groups.

Ishizaki *et al.* (1980). who studied the beta blockade effects and absolute reduction of HR following exercise using sotalol in elderly hypertensive subjects found that the log-linear model adequately modeled the reduction in HR.

The E_{max} Model

This model incorporates the existence of a maximum effect. The model is described by the following hyperbolic function:

$$E = \frac{E_{max} \cdot C}{EC_{50} + C} \qquad ...(1.5)$$

where E_{max} is the maximum effect attributable to the drug and EC_{50} is the concentration producing 50% of the maximum effect. This model also predicts the absence of an effect when the concentration, C, is zero. It may be modified, like the linear models described above, with the addition of a constant term, E_0, describing the baseline effect existing in the absence of drug

$$E = E_0 + \frac{E_{max} \cdot C}{EC_{50} + C} \qquad ...(1.6)$$

In this case, E_{max} refers to the maximum effect attributable to the drug whereas the maximum absolute effect in the presence of drug is $E_{max} + E_0$. The E_{max} model may be justified not only on empirical grounds described above, but also as the expression of simple receptor theory (e.g. Ariens and Simonis, 1964a). However, the E_{max} model cannot be used to prove the existence of a specific receptor and

estimates of the receptor dissociation constant obtained *in vivo* (EC_{50}) are unlikely to reflect the the events at the receptor sites (Holford and Sheiner, 1982).

If the drug effect is inhibition of a physiological phenomenon, e.g. lowering of exercise heart rate by a β-blocker, then Eqn. (1.6) can be modified

$$E = E_0 - \frac{E_{max} \cdot C}{IC_{50} + C} \qquad ...(1.7)$$

where IC_{50} is now the concentration producing 50% inhibition of E_{max}. The study of Mitenko and Ogilvie (1973) on the effects of theophylline on airways obstruction in asthmatic patients illustrates the value of the E_{max} model. They determined the change in forced expiratory volume (FEV_1) over a range of theophylline serum concentrations in a group of six patients. The effect was expressed as a percentage of the expected FEV_1 in a normal patients. The maximum effect predicted by the model was 63%. This suggests the presence of a non-reversible component of airway obstruction, which would be a reasonable expectation in this group of patients. The concentration required to achieve 50% of this maximum was 10 mg/l; doubling this concentration to the upper limit of the therapeutic range (20 mg/l.), predicts two thirds of the maximum achievable response. The biggest increases in expiratory flow rate were observed at concentrations up to 10 mg/l.

The E_{max} model may also be applied to the data of Singh *et al.* (1980), who studied the effects of timolol on exercise and resting heart rate. The workers used the log-linear model to correlate the effect to timolol serum concentration and concluded that there was only a weak correlation. However, when the results are plotted in a non-logarithmic fashion, the existence of a maximum response is evident. The lowest heart rate achievable by beta-blockade (E_0), estimated during maximal exercise, was 68 beats/min, and at rest was 56 beats/min. E_0 reflects the influence of factors affecting the heart rate other than β-receptor activation.

The Sigmoid E_{max} Model

This is a modification of the E_{max} model and is useful in that it allows for differences in the concentration-effect relationship as defined by the E_{max} model. The equation is described by

$$E = \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n} \qquad ...(1.8)$$

where, n is a new parameter affecting the shape of the curve. If n is greater than 1 then the curve will be S-shaped, and if it is less than 1 the initial portion has a slope greater than the hyperbola and beyond the EC_{50} the slope is less.

This function was proposed on an empirical basis by Hill (1910) to describe the association of oxygen with hemoglobin. The sigmoid E_{max} model can be derived from receptor theory where n is the number of drug molecules combining with each receptor molecule. However, non-integer values of n are sometimes found in practice. This does not detract from the model's value as an empirical description of the interactions of the drug with its effect site (Wagner, 1968).

The work of Stanski *et al.* (1979) has shown that the *in-vivo* effects of *d*-tubocurarine on muscle strength in patients undergoing surgery may be described by a sigmoid E_{max} model with a value of 2.3 for n. Although the receptor basis for the mechanism of action of *d*-tubocurarine is well established, no physical interpretation of this number in terms of the drug–receptor interaction seems feasible. Meffin *et al.* (1977) used the Sigmoid E_{max} model to describe the antiarrythmic effects of tocainide in man. They found a very steep concentration-response curve requiring values of n from 2.3 to 20.1. Interpretation of these values is not possible in terms of a tocainide receptor, but they do serve to quantify the steepness of the relationship.

Pharmacokinetic-Pharmacodynamic Models

Pharmacokinetic-pharmacodynamic models provide insight into the properties and nature of that body compartment that contains the biophase. The biologically active receptor sites reside within the biophase, a volume where the effect of drug on a site is only limited by its free diffusion. Optimum dosage regimens can be designed to give biophasic compartmental levels of the active drug necessary for the proper magnitude and duration of the desired pharmacological response with minimum toxicities.

If such compartmental levels that provide effective pharmacodynamic activity are the same for all species, optimum dosage regimens for humans can be effectively predicted from animals by the use of appropriate scaling factors.

Concentration is the link between the pharmacokinetic model (PK), relating dose to the concentration, and the pharmacodynamic model. PD, relating concentration to effect. Some of the models used to link the PK and the PD models are discussed below:

a) The Direct Link

The simplest link is achieved by a direct relationship between the measured concentration and the observed effect. If the effect is measured at the same time as drug concentration is measured, e.g. in plasma, then no formal pharmacokinetic model is required. The compartment where the receptor sites reside and where the drug exercises its pharmacodynamic action could be either the central, shallow or deep compartment (Garrett, 1977).

The central compartment is defined as that volume in the body where an amount of drug is instantaneously homogenously distributed on administration. An example is when an intravenously administered bolus of a drug is monitored in the plasma with time and the highest plasma level is observed as close to time zero

as is practically possible and the apparent volume of distribution is the administered dose divided by the initial concentration at time zero. The central compartment would then consist of the plasma water and all instantaneously equilibrating body tissues which may include plasma protein, erythrocytes and in many cases, the extracellular water accessible to the well-vascularized tissues.

The shallow compartment is that apparently homogenous volume which readily, but not instantaneously, equilibrates with the central compartment. It frequently contains the intracellular water. It is characterized by a relatively rapid decrease of drug concentration in the equilibrated central compartment associated with plasma. The decline is largely due to the rate determining elimination of drug from the body where the re-equilibration between the central and shallow compartment is a relatively fast process.

The deep compartment consists of that volume within the body which slowly equilibrates with the central and/or shallow compartment at rates comparable to rates of elimination. It's presence is indicated by the fact that the monitored cumulative urine or excreta demonstrates a prolonged and slow increase. It may contain non-vascularized fatty tissues, tightly binding enzymes or tissues, or bone.

It is also possible that no pharmacokinetically significant amount of drug is in a deep compartment that contains the biophase, since only small amounts of drug may be necessary to give biological activity and this small amount may be insignificant relative to the total mass balance.

Biophase in the central compartment

A relatively instantaneous onset of pharmacodynamic action which decreases rapidly and parallels the loss of drug from the central compartment, indicates that this compartment contains the biophase. An example is thiopental (Stanski, 1984),

a short acting drug, whose half-life of pharmacodynamic action is much shorter than the plasma half-life generally obtained from the dispostion curve.

It may be considered that there is no blood-brain barrier to thiopental, that the drug in the central compartment and the brain are in relatively instantaneous equilibration. The primary cause of dimunition of brain concentrations of drug are its loss, through the central compartment, to the relatively rapid equilibrating tissues of the shallow compartment. Slow infusions or oral administrations of thiopental are thus contraindicated since large doses would be necessary to maintain effective drug concentrations in the central compartment. If toxicities were engendered in the peripheral comparment, adverse reaction would result from such routes of administration.

The drug concentration in the central compartment of a pharmacokinetic model may be linked to a pharmacodynamic model by simple substitution for C in one of pharmacodynamic models described before. Using a linear pharmacodynamic model for simplicity and the sum of a series of exponentials to describe the central compartment concentration of drug we obtain, for example

$$E(t) = S \cdot \sum_{i=1}^{n} C_i exp(-\lambda_i \cdot t) \qquad ...(1.9)$$

where $E(t)$ is the effect at time t and C_i and λ_i are the constants of a sum of n exponentials.

b) The Indirect Link

Graphical display of the relationship between plasma or central compartment concentrations and drug effect may reveal a counter–clockwise hysteresis loop when the points are joined in time sequence. This form of hysteresis is characteristic of a delay in equilibration between plasma drug concentration and the concentration of

active substance at the effect site. The active moiety maybe the drug, its metabolite or some other indirect effect, e.g. protein synthesis. leading to the observed effect.

Biophase in the shallow compartment

Garrett (1979), noted that if the time course of pharmacodynamic action parallels the time course of drug in the shallow compartment, a tissue component of this compartment is the biophase. The classical half-life of this phase is equal to the biological half-life of the active agent and denotes that plasma level is a proper determinant of drug efficacy on both acute and chronic administration. When the pathogenic organisms are in the readily available tissues, plasma levels of antibiotics on acute as well as chronic administration are proper criteria of antibacterial efficacy.

Hinderling and Garrett (1977), showed that the pharmacokinetics of β-methyl digoxin can be characterized by a three compartment body model. The time course of the average decrease in heart rate paralleled the time course of drug in the shallow compartment on both intravenous and oral administration and the biophase was definitely not in the central or deep compartments.

If a multicompartmental model is used to describe plasma drug concentration, it is possible to calculate the time course of a quantity proportional to drug concentration in the peripheral or tissue compartments of the model (Benet 1972). With the assumption of unit partition coefficient between compartments and no loss of drug from the tissue compartment, then the amount of drug in the tissue compartment can be used directly used to estimate the model parameters. The time course of the tissue compartment 'concentration' can then be linked to a suitable pharmacodynamic model by the use of an expression such as Eqn. 1.9 to describe the tissue compartment.

The major drawback of the tissue compartment link is the requirement that the equilibration of drug with the effect site must have the same time course as drug concentrations in a compartment derivable from measurements made in plasma and/or some other compartment, e.g. urine. The time course of equilibration is governed by the perfusion, diffusion and partition properties of the effect site. If the organ in which this resides is large or shares properties with a pharmacokinetically significant organ or tissue, then the tissue–compartment link may be valid. But there is no *a priori* reason to expect this to occur. Neverthless, the tissue compartment approach has been used with some success to describe the time course of lysergic acid (LSD) on mental performance in human volunteers (Wagner *et al.* 1968). The peripheral compartment of a two-compartment pharmacokinetic model for LSD, linked to a linear pharmacodynamic model, adequately described the time course of drug effect. A similar approach was used by Reuning *et al.* (1973), to describe the time course of the effect of digoxin on electromechanical systole of the heart (QS_2) after a single i.v. bolus dose. Although the time course of effect was clearly related more closely to the concentrations predicted in the tissue compartment, systematic time–related deviations (from a linear pharmacodynamic model) were seen. This illustrates the limitations of the tissue compartment approach, which cannot account for equilibration with the effect site, when the latter is either faster or slower than equilibration with one of the identifiable pharmacokinetic compartments. The use of a three–compartment model to describe digoxin pharmacokinetics by Kramer *et al.* (1979) improved the ability of the tissue compartment to describe the effects of digoxin on QS_2. The concentrations in the more superficial of the two tissue compartments had a time course similar to that of drug effect, but the interpretation of the data is made difficult by the use of pooled observations from a group of subjects.

Biophase in the deep compartment

A delay in the appearance and maximum of drug action in contrast to the plasma level data and the amount of drug in the shallow compartment implies that the biophase is in a deep compartment. The quantification of pharmacodynamic activity with time would follow the time course of the drug in the deep compartment. Such a deep compartment may not be pharmacokinetically discernible since insignificant amounts of drug may be responsible for the pharmacodynamic action and the actual blood level on acute administration would have no direct relationship with the activity of the drug. However, the magnitude of the activity at any time would still be dose related and mediated by the time course of the drug in the observable compartments. In general, there would be no strict proportionality between pharmacodynamic activity and plasma level on acute drug administration until the rate determining step of drug elimination from the body is the release of drug from this deep compartment. Only if the deep compartment can be equilibrated with the plasma on chronic administration would plasma level be indicative of drug action at all times.

A pharmacokinetically observed deep compartment is not necessarily the same as, or equivalent to, the biophase which may be in a deep compartment. However, evidence of parallelism in the time course of biological activity and drug amounts in such a compartment is indicative. Levy *et al.* (1969), observed that LSD pharmacodynamic action paralleled the time course of drug in a slowly accessible pharmacokinetic compartment.

Another example of a deep compartment biophase occurs with β-methyldigoxin when the pharmacodynamic parameter monitored in healthy humans was the per cent decrease in left ventricular ejection time index (LVETI). The time course of LVETI action correlated with the time course of β-methyldigoxin and its active

metabolite, digoxin. in their deepest pharmacokinetic compartment and not their plasma levels on acute dosing (Hinderling and Garrett, 1977).

Kelman and Whiting (1980) have proposed a method using a multicompartment model to describe drug concentrations in each compartment to partition different fractions of the effect among the separate compartments. However, this approach requires the acceptance of the theory that single drug effect is mediated by drug concentrations at different effect sites.

Shortcomings of the above methods:

The above classical methods suffer from the following drawbacks in that it cannot adequately model pharmacodynamics in the following cases:

1) When the amount of drug necessary to evoke a response is pharmacokinetically negligible, the observed effect over time may not correlate with observed disappearance from any of the pharmacokinetically discernible compartments.
2) When the biological receptor sites are saturated the response will not correlate with the concentration in any of the kinetic compartments although the biophase may kinetically correspond to one of the same compartments.
3) When there is a delay in equilibrium with the receptor sites the time delay to onset of action or time to maximal action is not readily explained by classical methods.

The Effect Compartment Model

Forrester *et al.* (1974) realized that the time course of the effect could be used to define the rate of drug movement to the effect site. This concept was used by Forrester *et al.* to explain the observations of Shapiro *et al.* (1970) of the time course of effect of various cardiac glycosides. Forrester and co-workers used the

onset of drug effect to estimate the halftime for achievement of peak effect, which varied from a few minutes (oubain) to nearly an hour (digitoxin).

Holford and Sheiner (1982), noted that if drug concentration in the plasma is suddenly changed from zero to some value C, the effect E. will increase in proportion to the concentration at the effect site until the plasma and the effect site come into eventual equilibrium at the effect E_c. If drug loss from the effect site is controlled by a rate constant k_{e0}, then the half time for achievement of E_c will be ln $2/k_{e0}$. More often, however, we are concerned with changing concentrations in the plasma and hence at the effect site. In this case the pharmacokinetic model can be used as an input function to the effect site, to describe the time course of drug effect. An ingenious way of doing this has been proposed by Sheiner *et al.* (1979) which used an effect compartment as an extension of the pharmacokinetic compartment model. The rate of change of drug amount in the hypothetical effect compartment (A_e) can be expressed as

$$\frac{dA_e}{dt} = k_{1e}A_1 - k_{e0}A_e \qquad ...(1.10)$$

where A_1 is the amount in the central compartment of a pharmacokinetic model, lined to the effect model, with rate constants k_{1e} and k_{e0} (Fig. 1.2). The exact form of the pharmacokinetic model is irrelevant as long as it adequately describes the central compartment concentration. A generalized derivation of the relevant equations for the effect compartment model follows.

For a drug whose concentration *vs.* time profile in the central compartment follows exponential kinetics, the amount of drug in the central compartment following the administration of an i.v. bolus may be represented as

$$A_1 = D\sum_{i=1}^{n} X_i e^{-\alpha_i t} \qquad ...(1.11)$$

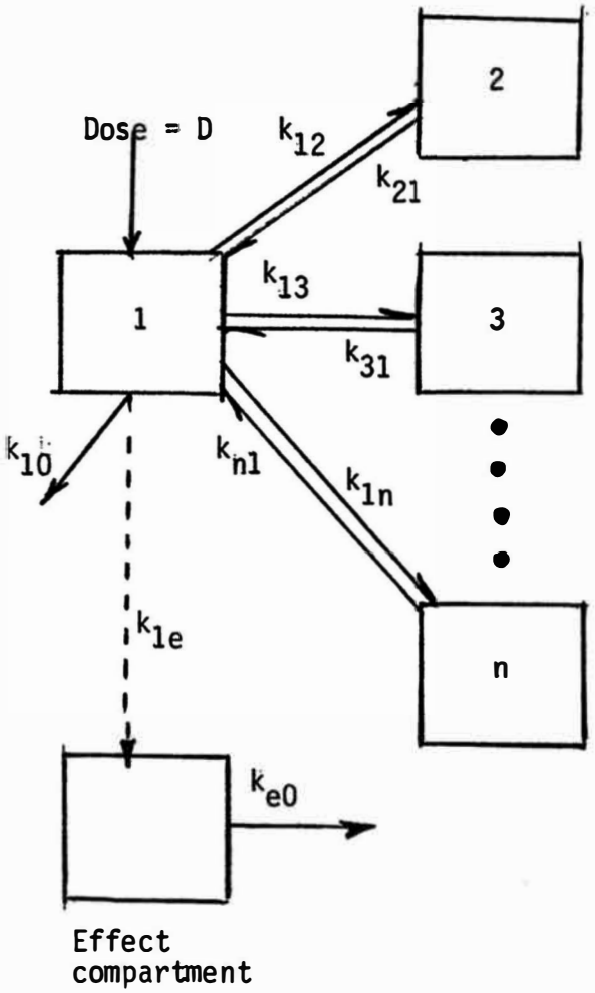


Fig.1.2 THE EFFECT COMPARTMENT MODEL

where X_i and α_i are constants, D is the dose and A_1 is the amount of drug in the central compartment any time t. The amount of drug in the effect compartment at any time is then given by,

$$A_e = k_{1e} \cdot D \sum_{i=1}^{n} \frac{X_i}{(k_{e0} - \alpha_i)} \left[e^{-\alpha_i t} - e^{-k_{e0} t} \right] \qquad ...(1.12)$$

The concentration of drug, C_e, in the effect compartment is obtained by dividing A_e by the effect compartment volume, V_e

$$C_e = k_{1e} \cdot D \sum_{i=1}^{n} \frac{X_i}{V_e(k_{e0} - \alpha_i)} \left[e^{-\alpha_i t} - e^{-k_{e0} t} \right] \qquad ...(1.13)$$

At equilibrium, the rates of drug transfer between the central and effect compartments are equal, i.e.

$$k_{1e} \cdot A_1 = k_{e0} \cdot A_e \qquad ...(1.14)$$

or

$$k_{1e} \cdot V_1 \cdot C_1 = k_{e0} \cdot V_e \cdot C_e \qquad ...(1.15)$$

If the partition coefficient, K_p, equals C_e/C_1 at equilibrium, then we rearrange Eqn.(1.15) to obtain,

$$V_e = \frac{k_{1e} \cdot V_1}{K_p k_{e0}} \qquad ...(1.16)$$

Substituting for V_e in Eqn.(1.13)

$$C_e = k_{e0} \cdot D \cdot K_p \sum_{i=1}^{n} \frac{X_i}{V_1(k_{e0} - \alpha_i)} \left[e^{-\alpha_i t} - e^{-k_{e0} t} \right] \qquad ...(1.17)$$

Since, $K_p = C_e/C_1$, substituting for K_p in eqn. (1.17) gives,

$$C_1 = k_{e0} \cdot D \sum_{i=1}^{n} \frac{X_i}{V_1(k_{e0} - \alpha_i)} \left[e^{-\alpha_i t} - e^{-k_{e0} t} \right] \qquad ...(1.18)$$

where, C_1 is now the equivalent steady state concentration of drug in the central compartment for a corresponding steady state concentration C_e in the effect compartment.

Also,

$$\frac{E}{E_{max}} = \frac{{C_1}^{\delta}}{{C_1}^{\delta} + {Cp_{ss(50)}}^{\delta}} \qquad ...(1.19)$$

where, E_{max} is the maximum response, $Cp_{ss(50)}$ is the concentration that produces 50% of the maximal response and E is the response. The value of EC_{50} then, as estimated from the data, will be the central compartment concentration producing 50% of the maximum effect at equilibrium and at steady state conditions. Clinically, this is more important than the concentration at the effect site producing 50% of the response. This model has been used extensively in recent years to model the pharmacokinetics-pharmacodynamics of *d*-tubocurarine (Hennis *et al.* 1985; Sheiner *et al.* 1979), thiopental (Stanski *et al.* 1984) and Δ-9-tetrahydrocannabinol (Chiang and Barnett, 1984).

Scott *et al.* (1985) studied the effects of fentanyl and alfentanil on EEG changes in humans. They observed a temporal lag (or hysteresis) between changes in serum narcotic concentration and changes in spectral edge. They therefore coupled the pharmacologic model with a pharmacokinetic model with a separate effect compartment.

They noted that hysteresis could be due to:

1) perfusion (drug delivery to the brain)
2) diffusion (crossing the blood brain barrier and cell membranes to reach the opoid receptors)
3) receptor events (affinity, dissociation constants)

c) The Model Independent Link

The methods described above have used pharmacokinetic models to describe the concentrations at the effect site, and linked this to the pharmacodynamic model. Model-independent methods have been described by Smolen *et al.* and Smolen (1976a,b). This approach is based on the assumptions that the pharmacokinetic processes involved are linear, and secondly, that the same concentration at the effect site produces the same response. The approach uses convolution and deconvolution techniques. The dose-concentration-effect relationship is obtained by convolution of the drug input with the pharmacodynamic processes. The techniques of numerical deconvolution are powerful tools although they are complex and not readily understood by many pharmacokineticists.

Tolerance and Sensitization

Tolerance and sensitization are the phenomena of increased and decreased responses by the animal when rechallenged with the same drug under identical conditions respectively. These are usually due to adaptive changes by the receptor in its number and affinity. The term "down-regulation" is now used to describe a decrease in receptor number. The opposite process of increased sensitivity to drug action with continued exposure to the drug is not well defined. A likely mechanism for this event is the accumulation of an active metabolite of the parent drug or a physicochemical change in the receptor. Mathematical models to describe tolerance and sensitization have not been developed. A clockwise hysteresis loop on plotting the response *vs.* concentration is indicative of tolerance or is due to the cumulation of an antagonistic metabolite.

Applications of Pharmacodynamics

Duration of Effect

Pharmacokinetic–pharmacodynamic models predict the time course of drug effect. These models can hence be used to predict the duration of effect for any dosage regimen. If the concentration in the effect site parallels the concentration in the central compartment, then the pharmacokinetic model suffices to predict the duration of effect. If the concentration in the effect compartment does not parallel the concentration in the central compartment, then the duration of effect will not be related simply to the dose, especially when repeated doses are administered.

For example, if the pharmacodynamics of a drug can be described by an E_{max} model, then we can identify three phases of the time course of drug action during the terminal elimination phase of the drug from the body. When concentrations are much larger than the EC_{50}, the effect will be close to the maximum effect and concentrations in the plasma may change several–fold with little change in effect: e.g. a ten–fold change in concentration from 90 to 9 times the EC_{50} will reduce the effect by less than 9%. When concentrations lie between 20% – 80% of of the EC_{50} then effect will be almost directly proportional to the concentration. Below these concentrations, effect will be almost directly proportional to concentration, and will decline in an exponential fashion.

Influence of Dosing Regimen

With pharmacokinetic–pharmacodynamic models it is now possible to adequately describe the influence of dosing regimen on overall drug effect. Wagner (1968) pointed out that a dosing regimen producing wide fluctuations in concentrations would have a reduced overall drug effect as compared to a dosing regimen

producing minimal fluctuations in concentration. This can be explained by observing that for the E_{max} model, the cumulative drug effect ($\overline{E}$) is an integral of the effect term (Eqn. 1.5) as given below:

$$\overline{E} = E_{max} \int \frac{C(t)}{EC_{50} + C(t)} \cdot dt. \qquad ...(1.20)$$

If C is constant, e.g. at steady state following a continous infusion of drug, then the integrated effect will be the same as the instantaneous effect predicted by the average steady state concentration of the drug. If dosing is now changed from a constant infusion to intermittent dosing, the same daily dose will produce the same average steady state concentrations, but concentrations will fluctuate about this average. It can be shown mathematically that the increased effect at concentrations above the average will not fully compensate for the decreased effect at concentrations below average. The total effect for the day will therefore be less with intermittent dosing than for continous infusion, for the same average steady state concentration. Holford *et al.* (1982), have noted that the differences are not large if the concentrations are always above the region of EC_{80}, but becomes marked when the concentrations are in the region of EC_{50}. Clinical experience with dosing regimens of various drugs have confirmed these predictions. e.g. in the treatment of cranial arteritis, efficacy is much worse when prednisolone is administered on alternate days, compared with the same averge daily dose given every day (Hunter *et al.* 1975). On the other hand, efficacy in the treatment of the nephrotic syndrome appears to be the same when alternate–day and daily dose regimens are compared, but toxicity is reduced compared with the alternate–day regimen (Hunter *et al.* 1975). Holford *et al.* (1982), hypothesize that this could be explained if the concentrations of prednisolone are around the EC_{80} for the treatment of nephrotic syndrome and

in the region of the EC_{50} for the toxicity. They further hypothesized that it is possibly for similar reasons that the reduction in creatinine clearance in dogs produced by gentamicin or tobramycin is greater when a constant infusion is given, compared with aminoglycoside concentration resulting from the same daily dose given every 24 hr. It was implied that the average aminoglycoside concentration produced by the infusion must be in the EC_{20} to EC_{50} range for renal toxicity. In general, the influence of dosing regimen can be expected to be of greatest significance when the average concentration is in the neighborhood of the EC_{50} and the dosing interval is several times longer than the drug elimination half–life.

Metabolite Activity

The metabolite of a drug may contribute to the observed effect. This may be challenged by comparing the time course of the activity with that of the time course of the metabolite obtained on drug administration. The correlation of the pharmacokinetics of the administered metabolite and the time course of pharmacodynamic activity is demanded. This could be confounded by the fact that the contribution may be agonistic or antagonistic. These factors need to be understood and incorporated into the pharmacodynamic model. Examples of drugs where the metabolite may be active are quinidine (Holford *et al.* 1981), disopyramide (Whiting *et al.* 1980), lorcainide (Meinertz *et al.* 1979), verapamil (Eichelbaum *et al.* 1980) and 3-methyldigoxin (Hinderling and Garrett, 1976).

Drug Combinations

Pharmacodynamic models can be derived that incorporate the effect of drug combinations and the receptor mechanisms involved.

Separate Receptor-Separate Mechanism

When two drugs act by different mechanisms and contribute to the same overall response, their combined effect can be described as the sum of their individual effects

$$E_{AB} = \frac{E_{max,A} \cdot A}{EC_{50,A} + A} + \frac{E_{max,B} \cdot B}{EC_{50,B} - B} \tag{1.21}$$

where the subscripts A and B stand for the E_{max} model parameters for the drug A and drug B respectively. Equations for cases involving seperate receptor–common mechanism and common receptor–common mechanism have been described by Holford *et al.* (1982).

REVIEW OF DIGOXIN RESPONSES

Systolic Time Intervals

The responses most commonly measured clinically as indices of cardiac funtion are the sytolic time intervals. It was therefore intended to monitor systolic time intervals in the pharmacodynamic studies to be conducted with digoxin.

The term 'systolic time intervals' (STI) is generally used to describe time intervals or measurements obtained from an electrocardiogram, a phonocardiogram, a carotid pulse tracing, or from simultaneous recordings of one or more of the same. Some of the STI are: a) left ventricular ejection time (LVET) b) duration of electromechanical systole (QS_2) c) duration of heart sounds (S_1S_2) d) pre-ejection period (PEP) e) iso-volumic contraction time (ICT) f) P-R interval g) R-R interval.

The P-R and R-R intervals are measured from the electrocardiogram. The P-R interval is measured as the time interval from the start of a P-wave to the immediately following R-wave, and the R-R interval is measured as the time interval from one R-wave to the next. Measurement of other STI mentioned above is discussed later. STI corrected for heart rate are viz., left ventricular ejection time index (LVETI), pre-ejection time index (PEPI) and QS_2 index (QS_2I).

Although it is difficult to pinpoint the first use or application of systolic time intervals (STI), Garrod (1874–75) can be credited with the first graphic recording of the pulse to determine the relationship between HR and the duration of left ventricular ejection. From then on the development of STI progressed. Katz *et al.* (1921), using the then modern electronic techniques, simultaneously recorded the EKG, phonocardiogram and the subclavian pulse. This set the stage for the techniques we apply today.

Systolic time invervals (STI) measurements are sensitive indices of myocardial contractility. They reflect a number of factors such as stroke volume, aortic and left ventricular, end-diastolic pressures (Diament and Kippip, 1970), or the level of adrenergic activity which may affect the measured values in opposing directions (Lewis *et al.* 1972).

Attempts to use STI in complex situations – for instance in the course of acute myocardial infarction – has proved not very satisfactory. The most useful application appears to be in the documentation of the effect of positive and negative inotropic agents in patients who can act as their own controls and who are hemodynamically stable.

As an archetype of positive inotropic drugs, digitalis has been extensively studied with the use of the STI. The first cardiac glycoside was studied almost four decades ago by measuring the changes in STI during treatment with strophanthin. In the 1960's Weissler and co-workers led the revival of this technique for use in clinical-pharmacologic testing of digitalis (Weissler *et al.* 1964,1966,1968).

Other cardiac glycosides more recently studied were strophanthin K (0.125 0.25 mg, i.v.) and lanatoside G (0.4, 0.8 and 1.6 mg). STI and impedance plethysmographic (IP) values were recorded in ten patients with compensated coronary heart disease (Matos *et al.* 1975). The heart rate decreased significantly during the 2-hr. study in the supine position for both the glycoside and placebo groups; blood pressure remained unchanged.

The QS_2I, the LVETI and the PEPI showed no change after placebo. The indices were obtained from the measured values by using the regression equations for heart rate by Weissler *et al.* (1969). The glycosides caused no change in LVETI. QS_2I was significantly shortened only after 1.6 mg of lanatoside C. PEPI and isovolumic contraction time (ICT) were significantly shortened by both doses of strophanthin K and by all doses of lanatoside C; the effects were dose-related.

The amplitude of the IP curve and the relative pulse volume showed positive and negative changes without any trend following administration of placebo or glycoside. It was suggested that the usual therapeutic doses of cardiac glycosides do not cause significant changes in the peripheral circulation in patients with compensated coronary heart disease. Yet their action on STI is quite marked, showing a positive inotropic effect.

Our present knowledge about the effect of drugs acting on the autonomic nervous system in man is based to a considerable extent on data based on STI measurements. Matos *et al.* (1976), studied the chronotropic and inotropic efffects of i.v. administration of 5 μg. of isoproterenol and of 0.5 mg of atropine in 12 patients (4 men and 8 women; mean age 55 years) with symptomatic sinus bradycardia (SSB), and 12 match-paired subjects. Heart rate, blood pressure, and STI were measured before and after drug administration.

There was no difference in the baseline STI values between the SSB patients and the controls. The two groups differed significantly only in heart rate.

Isoproterenol had a significant positive chrono-and inotropic action on patients with SSB as well as the controls: PEPI, PEP/LVET, and ICT/QS_1 values were shortened. The effects were of similar magnitude in both groups. There was an increase in heart rate following injection of atropine in both groups. There was no significant difference in the response of the two groups. QS_1 lengthened significantly after atropine in SSB patients but remained unchanged in the controls.

In this well-defined and selected SSB subgroup of sick sinus syndrome, the response to isoproterenol and atropine was uniform and reproducible. According to the STI values, myocardial contractility and the response to isoproterenol and atropine were no different in patients with SSB as compared with control subjects of the same age and sex.

Despite the early introduction of the method, clinical application of the STI recieved little attention until recent years. Their acceptance as useful quantitative measures of left ventricular performance in individual patients has indeed evolved slowly. In part, this reflects the different nature of the STI as a physiologic measure, one which denotes the timing of the events of the cardiac cycles rather than the more conventionally applied dimensions of pressure, flow, and volume. In addition, the fact that STI changes occur with sudden alterations in ventricular loading, such as changes in end-diastolic wall stress and impedance to systolic emptying (as do virtually all other physiologic measures of chamber performance), has discouraged some from accepting these measures as stable physiologic expressions. Probably the major deterrent to the more rapid clinical applications of STI is the fact that until the recent decade, the significance of the STI in a quantitative sense had not been critically tested.

Clinical applications of STI:

Weissler *et al.* (1980), determined the relative frequency of residual symptoms and signs and of abnormal STI among 37 patients who had convalesced from a previously documented injury to the left ventricular myocardium. The patients had recovered from acute transmural myocardial infarction occuring 3–6 months previously.

Compared with the values for a group of 25 normal subjects studied concurrently, the pre–ejection period index (PEPI) was prolonged, the left ventricular ejection time index (LVETI) was shortened, and the PEP/LVET ratio was increased. For each of these indices, the differences between the controls and patients were statistically significant ($P<0.001$). Of the three, PEP/LVET allowed best discrimination between normal and abnormal groups. Of interest was the observation that in asymptomatic patients – that is, those who did not have dyspnea, fatigability,

or angina pectoris, but all of whom had convalesced from a previously documented myocardial infarction, the majority exhibited abnormal left ventricular performance as defined by the PEP/LVET measurement.

Having defined this high incidence of abnormality in left ventricular performance among asymptomatic patients, Weissler *et al.* attempted to detect this abnormality by other common clinical indicators viz., the presence of an S_3 or S_4 gallop or of cardiomegaly on chest X-ray. The presence of S_3 or S_4 could not reliably detect the patients with abnormal left ventricular performance. Thus among the patients who had convalesced from a previous insult to the myocardium, the STI revealed the presence of residual abnormality when clinical and radiological evidence for such cardiac abnormality is absent.

STI and other methods of assessing left ventricular performance: A comparison

Weissler *et al.* studied 29 patients with angina pectoris who had not experienced myocardial infarction (MI). These patients were evaluated by conventional and by STI methods in 54 patients with angina pectoris who had survived an acute attack of MI. Among the patients with a normal coronary arteriogram and a normal left ventricle, the EF was 0.68 ± 0.08 (mean $\pm$ SD). Among the same patients the PEP/LVET was 0.34 ± 0.04 (mean $\pm$ SD). These values correspond to norms previously established for these measures. Highly significant deviations ($P<0.001$) in EF, PEPI, LVETI, and PEP/LVET from the normal occured among the patients with coronary artery disease who had a previous myocardial infarction. In contrast, among patients with documented coronary artery disease but without a record of a previous myocardial infarction, there was no significant difference from normal

in these measures. The STI and the EF were therefore accurately predicted in patients (except for patients with coronary artery disease and a previous history of myocaridal infarction).

The final test of the accuracy of the STI is provided in a determination of the agreement between the EF and the PEP/LVET in defining the presence of an abnormal ventricle among various subsets of patients with coronary artery disease. The data indicated that the prevalence of abnormal left ventricular performance as detected by the PEP/LVET in the four group of patients was virtually identical to that determined by EF. Agreement between PEP/LVET and EF in estimating the prevalence of abnormal function exceeded 90% in each subset.

It was evident that PEP/LVET as a noninvasive measure of left ventricular performance is superior to current clinical approaches in detecting the presence of normal or abnormal ventricular performance in patients with coronary artery disease.

Limitations of Systolic time intervals for evaluation of cardiac function

STI are influenced by several anthropometric, hemodynamic, and pharmacologic factors which can limit the usefulness of STI for the evaluation of cardiac function in individual patients. The hemodynamic factors are largely unknown in individual patients. To appropriately use STI the following facts have to be kept in mind.

Regression Equations

No universally acceptable regression equation is as yet available for the relationship between the PEP, LVET, and QS_2 (interval between the onset of depolarisation and onset of aortic component of the second heart sound, on one hand, and the heart rate (HR), on the other. No large randomized population group has been studied

taking into account age, sex, blood pressure, anthropometric factors, the supine or upright positions etc.

Different equations have been published regarding the relationship between LVET and HR. Significant differences in the slope of the regression equation exist, resulting even at HR's between 60 and 90 beats/minute (bpm) in important differences in the calculated LVET. In heart failure LVET, expressed as a percent of normal, also differs depending on the regression equation used for the comparison. At an HR of 60 bpm, LVET would still be within normal limits, i.e., ± 10% of normal.

Different slopes have been calculated for the relationship between LVET and HR between men and women (Willems and Kesteloot, 1967), after digitalis administration (Kesteloot *et al.* 1969), and during exercise (Vanderhoeven *et al.* 1973, Gleichmann *et al.* 1976).

Age and systolic blood pressure have been shown by multiple regression analysis to have independent influence on LVET when HR is included, (Willems *et al.*, 1967). Different slopes have been calculated for the relationship of QS_2 and HR (Weissler *et al.* 1968, Kesteloot 1968).

Nearly all authors have found that PEP is virtually independent of HR. A logarithmic relationship between LVET and R-R interval has been described during atrial fibrillation in patients with mitral disease (Keteloot and Denef 1970, Baragan *et al.* 1976). Different slopes have been calculated for the relationship of LVET and QS_2 to HR during consecutive days in myocardial infarction (Spodick and Kumar, 1968). As a result of the above observations, no single equation can be used to correct LVET or QS_2 for HR changes in different clinical situations.

There is evidence which indicates that even between HR's of 60–100 bpm, a linear regression equation is not the best fit. The relationship is better characterized by a polynomial or hyperbolic regression equation as shown by Willems and

Kesteloot (1967). This could explain the differences in slope between males and females; the lower slope can be predicted in normal groups with a higher mean HR. However, between HR's of 50–110 bpm the deviation becomes important, when the regression equations are used for the extrapolation or correction of the data.

The correlation coefficients of the relationship between LVET and HR vary between 0.75 and 0.85. This means that 30–45% of the total variation of LVET and QS_2 remains unexplained. As a consequence, the confidence with which conclusions can be made in an individual case is reduced. Multiple regression equations with the introduction of other relevant parameters besides HR can increase the predictive power of the regression equation.

Interactions of influencing factors

Increases in the HR due to physiologic variations, emotions, exercise, atrial pacing, atropine, isoproterenol, and like have different influences on preload, afterload, sympathetic tone and cardiac output. Interactions between different factors influencing STI can make the interpretation difficult.

The only reported study of STI in dogs is by Zamella *et al.* (1980). 27 anesthetized mongrel dogs were studied and correlations were obtained between various measures of cardiac function and STI. The regression equations were extremely susceptible to pre-load changes and were applicable to 2 to 3 dogs, but none to all the dogs.

Wolf (1980), in an excellent review of correction methods for STI indicated that the relationship between HR and STI is curvilinear and not linear. The R-R interval (RRI) and HR are connected by a simple inversion, the graph of the relation being a hyperbola. Thus if there is a linear dependency from one of these parameters, e.g. RRI, the dependency on the other one, e.g. HR, will be hyperbolic. Because of

Weissler's great influence (Weissler *et al.* 1974), many assume a linear relationship between HR and STI.

Cardus *et al.* (1974), demonstrated the linearity of the dependency of RRI and STI and the hyperbolic curve for HR and STI. Kesteloot and Willems (1967), also discussed the possible models for the relationship. The data support the hypothesis of the nonlinear relationship for HR and STI, the linear function of RRI and STI and the hyperbolic curve for HR and STI. Statistical analysis by these authors did not show a significantly better fit for any one of several different functions. Linearity between HR and STI was therefore retained. Data of Wolf *et al.* (1978) point in the same direction. It is sometimes possible that the assumption of the linear relation between HR and STI could lead to incorrect results. For example, in a study by Belz *et al.* (1978), 12 groups of 10 persons were given different doses of cardiac glycosides. The first analysis was done using the correction formula of Weissler and statistical analysis of variance. Overall differences between the groups was not significant. The second analysis used analysis of covariance with RRI as covariate. The differences then were significant. Thus an analysis of covariance using RRI as covariate instead of linear regresion using HR is the better method.

SUMMARY

In summarizing the value of the STI as a predictive, diagnostic and research tool it is appropriate to include the concluding comments of Weissler at the International conference on systolic time intervals (Weissler *et al.* 1980). Weissler noted that the current methods for assessing the duration of the sequential phases of the cardiac cycle in man are quite accurate. Thus, whether the duration of the STI are measured by the conventional triple recording of the electrocardiogram, phonocardiogram and carotid arterial pulsation, or by the echocardiographic approach, the measurements derived yield data that are indistinguishable from that determined by

direct interventions in the cardiac chambers. There remain some differences of view as to the most appropriate mathematical expressions for denoting the relationship between HR and the duration of STI. However, the linear regression relationships between HR and QS_2, LVET are quite adequate for clinical applications.

The relationship of the pre-ejection period (PEP) to HR is somewhat more controversial. Although it is likely that the PEP diminshes with increasing HR, the magnitude of change with increasing HR is certainly less than that for the QS_2 and LVET intervals. The minimal slope of the regression relationship between PEP and HR limits our capacity to demonstrate a close correlation between those two variables. However, it is generally agreed that the ratio PEP/LVET requires no correction for HR for clinical application. It is possible that this ratio, of two intervals, each of which is influenced by HR, must also be influenced by HR. But, for practical purposes, in the HR range of 50 to 110 bpm, the correction is not employed because of the minimal influence of the HR.

It is evident from a number of papers mentioned before that both the PEP and STI are influenced by ventricular loading. In this respect, the STI behave no differently than other noninvasive and invasive methods. Because of the influence of loading conditions, changes in STI cannot be readily interpreted as reflecting changes in the intrinsic contractility of the myocardium. However in chronic disease states the LVET and changes in STI accurately reflect the level of left ventricular performance.

DIGOXIN AS THE INVESTIGATIONAL DRUG

Digoxin was selected as the investigational drug due to the following reasons:

1) Digoxin has measureable pharmacodynamic responses:

The systolic time intervals (STI), have been used extensively to monitor the pharmacodynamic responses of digoxin for clinical and investigational purposes since its introduction in 1921 by Katz *et al.*. A revival of the technique by Weissler *et al.* (1964, 1966, 1968), has led to the now prevalent use of STI for clinical, investigational testing of cardiac glycosides. A more extensive review of STI and its applications has been presented earlier.

2) Lack of correlation between STI and digoxin serum levels.

The first study in which it was attemped to study digoxin pharmacokinetics and pharmacodynamics simultaneously was in 6 normal subjects by Shapiro *et al.* (1970). The authors found a negative correlation between LVET and plasma digoxin values ($r = 0.77$, $P<0.01$). Significant decreases in LVETI and QS_2 were seen 30 min. after injection. The LVETI shortening progressed to its maximum at 3 and 4 hours. While the 24 and 48 hr values were significantly less than control, they were returning toward the initial value. A similar pattern of change was noted in the QS_2 interval. The mean PEP decreased within the first 2 hours, but was not materially different from control afterward. Heart rate, ejection fraction, S_1S_2, isovolumic contraction time, and Q_1 were not significantly altered. The $t_{1/2}$ of the ΔLVETI derived from the slope of these values was approximately 29 hrs.

The authors observed that the maximal inotropic effects seen at 3 and 4 hours, as determined from the maximum ΔLVETI, occurred as plasma digoxin declined

into a slow excretion phase. During the rapid early fall of the plasma level, inotropic effects began. During the subsequent slow decline, the inotropic effects, as reflected in shortened LVETI and QS_2 , paralleled the plasma digoxin values. This was in contrast to earlier views that plasma concentration of digoxin may be of little importance concerning myocardial effects, particularly since the plasma to myocardial ratio of digoxin is large i.e., at least 1:30 (Doherty and Perkins, 1966; Binion *et al.* 1969).

However Reuning *et al.* (1973), showed that simultaneous experimental determinations of the change in the left ventricular ejection time index (ΔLVETI) as a function of time, do not correlate with the plasma level-time curve when digoxin is distributing between plasma and tissues. In contrast, levels of digoxin predicted for the tissue compartment correlate closely with the ΔLVETI during the distribution. They observed a good correlation between values of these two parameters (correlation coefficient=0.91). A similar analysis of the change in the QS_2 interval, ΔQS_2, as a function of time after a single intravenous dose of digoxin indicated that this measure of pharmacologic effect does not correlate with changes in digoxin plasma levels during the distributive phase.

That the pharmacodynamic responses of digoxin are possibly related to tissue levels and not to serum levels was further borne out in a study by Forester *et al.* (1974), who studied the onset and magnitude of the contractile response of cedilanid-D, oubain, digoxin and digitoxin in normal volunteers after i.v. administration of the glycosides. The onset of QS_2I shortening on a mole basis was described by an exponential equation for the glycosides. The maximum shortening of QS_2I/mole assuming no excretion and metabolism in the first hour was the same for all the glycosides. However the time constant for the maximal effect (the time at which 63% of the total response has been achieved) was 5.8, 7.2, 23 and 56 mins. for oubain, cedilanid-D, digoxin and digitoxin respectively. The observed time lag to

peak effect may reflect the fact that the receptors for the observed effect are in a shallow or deep compartment and could therefore be an indication of the time required for the concentration of glycoside in the biophase to reach the minimum concentration required to elicit a response.

In another study Jogestrand *et al.* (1981), related the plasma and skeletal muscle levels of digoxin to the response. The authors had previously shown (Jogestrand, 1980), that highly significant correlation exists between the digoxin concentration in skeletal muscle and the right atrial myocardium using biopsies taken during open heart surgery. The approach was meritorious considering the following 3 reasons:

1) The major depot for digoxin is the skeletal muscle. About 50% of the total body content of digoxin is bound to this tissue.
2) The effect of cardiac glycosides on skeletal muscle is similar to that on the heart, with an increased contractile force being produced by a high concentration of glycoside in the tissue (Smulyan and Eich 1976).
3) The percutaneous needle biopsy technique proposed by Bergstrom (1962) allows sampling of skeletal muscle for digoxin assay without surgical intervention.

The results of the study indicated that a significant correlation between ΔQS_2I and skeletal muscle digoxin concentration exists. There was also a comparatively high r-value (-0.58) for the correlation between ΔLVETI and skeletal muscle digoxin concentration, although this correlation was not statistically significant. In all subjects in the present study, in whom the serum and skeletal muscle digoxin concentrations were measured at two different dose levels, the increase in serum and skeletal muscle digoxin concentration was associated with shortening of QS_2I and the shortening QS_2I was significantly greater at the higher than at the lower dose.

Hinderling and Garrett (1977), modeled the pharmacokinetics - pharmacodynamics of digoxin and β-methyl digoxin in seven normal subjects. They observed

significant decreases in LVET and HR after an oral and i.v. administration of β-methyl digoxin. The time course of this action correlated with the time course of β-methyl digoxin and its active metabolite, digoxin, in their deepest compartments and not with their plasma levels. The relative peak effect and area under the ejection time-time curves (for doses of 0.3 and 0.6 mg.) indicated a linear dose-response relationship on intravenous administration. The time course of HR action correlated (8.3 and 12.5% decreases with 0.3 and 0.6 mg. i.v., respectively; 6.5 and 9.5% decreases with 0.3 and 0.6 mg. p.o., respectively) with the time course of β-methyldigoxin and its metabolite digoxin in shallower pharmacokinetic compartments (peaks at approx. 80 min. i.v. and 135 min. p.o.). Significant effects had disappeared by 10 hr. after drug administration. This finding indicated that the biophases differ for ejection time and heart rate action.

The authors observed that the time course of the apparent negative chronotropic effect observed from heart rate measurements was remarkably different from LVETI measurements after β-methyldigoxin administration. Therefore the authors suggest that these two receptor-sites containing biophases are kinetically different.

The most extensive study on modelling digoxin pharmacokinetics - pharmacodynamics was by Kramer *et al.* (1979), who conducted pharmacokinetic - pharmacodynamic studies with digoxin in 12 volunteers. The authors fitted the digoxin concentrations vs. time to a 3-compartment model. The levels of digoxin in the deep compartment that were predicted from this fit were found to relate closely to the intensity of the response as measured by QS_2I. The authors found that a nonlinear relationship (langmuir type), between ΔQS_2I and the level of digoxin in the more slowly equilibrating tissue compartment yielded a better simultaneous fit than the linear relationship attempted. The results of their study established a close relationship between digoxin levels in the slowly distributing (deep) peripheral compartment and the inotropic response (as estimated by ΔQS_2I).

Although the data in their study established a relationship between digoxin levels in the deep peripheral compartment and ΔQS_2I, several aspects related to the work require further substantiation or improvement. The variability inherent in the response measurement, both intrasubject and intersubject limits the quantitative treatment of ΔQS_2I data. Although the relationship is established for averaged data from 12 subjects, the same relationship has not been established for individual subjects. Also, the limited range of the changes in ΔQS_2I coupled with the intersubject variability makes it difficult, even for averaged data, to distinguish among different possible mathematical relationships between drug level and response. Thus the results of this study suggest that further efforts designed to develop more reproducible response measurements for digoxin would be desirable. A second aspect of the response measurements that requires further substantiation is the relationship between the ΔQS_2I and the degree of inotropy obtained after administering digoxin. Although a linear relationship has been clearly demonstrated in a previous study between ΔQS_2I and the direct measurement of the rate of pressure change in the left ventricle obtained after digoxin administration, further studies designed to test this apparent link between ΔQS_2I and inotropy are needed.

Repke, *et al.* have pointed out that any proposed mechanism for digitalis action must explain both the lag phase of myocardial response and the absence of a direct correlation between glycoside level in blood and glycoside activity. There appear to be at least two possible mechanistic explanations for lack of direct correlation of digoxin serum levels with response, the lag phase of myocardial response, and the observation in their study of an apparent correlation of inotropy with digoxin levels in the deep compartment of a three-compartment pharmacokinetic model. One possibility is that the receptor for digoxin is sufficiently remote from the digoxin in serum so that the time needed for distribution to the receptor is similar to that needed for distribution to the deep compartment. A second possibility is that the

mechanism of the inotropic response to digoxin involves a sufficient delay such that the time course of inotropy is similar (perhaps fortuitously) to the time course of drug levels in the deep compartment. Repke *et al.* have presented evidence that the mechanism consisting of an inhibition of transport ATPase by cardiac glycosides followed by an increase in intracellular calcium is at least consistent with the idea of a response mechanism involving considerable delay in the development of inotropy.

3) Altered pharmacokinetics-pharmacodynamics of digoxin in altered physiological states.

A comprehensive review of altered digoxin pharmacokinetics in disease states is given earlier in the review on digoxin pharmacokinetics. The implications of these altered physiological states to pharmacodynamics is as follows.

a) Renal Function:

Results of studies in patients and in normal subjects compiled by Reuning *et al.* (1973), indicate that the apparent volume of distribution obtained by extrapolation (Vd), is about 52% as large in subjects with severe renal insufficiencyis as compared to the Vd in normal subjects; with the exception of the studies of Ueda *et al.* (1967) in Japanese subjects. In these subjects the Vd of subjects with renal insufficiency was only 16% of that observed in subjects with normal renal function. Reuning *et al.* further suggested that although the extensive data of Doherty *et al.* (1964), did not permit a numerical calculation of Vd, their results suggest that the Vd in subjects with renal failure is about one third that of normal subjects. Since the apparent volume of distribution relates the plasma concentration of drug to the amount of drug in the body, it is evident from the results that the relationship between plasma level and body level of digoxin is altered considerably in subjects with severe renal impairment. Since the decreased numerical value of Vd in renal

failure could be a direct kinetic result of the decreased rate of digoxin elimination, a second measure of the apparent volume of distribution Vd_{ss}, was determined. The value of this parameter, which is independent of the degree of renal impairment, was also depressed in subjects with severe renal insufficiency to about 65% of normal. The apparent volume of the central compartment, Vc, was depressed to a similar extent. The apparent volume of distribution in all the cases was very large (up to 2100 liters), indicating extensive binding of digoxin in tissues.

The implications of this observed differences in Vd of patients with renal failure compared to those with normal renal function is significant. These differences in Vd may be differences at the biophase, or in a peripheral storage site. Pharmacokinetic-pharmacodynamic models need to be developed that can discern these differences in Vd and indicate the adjustment in dosage required.

b) Potassium imbalance

Potassium ions influence electrophysiologic events in the heart. Excess myocardial potassium reduces conduction in the atria and subnodal system and depresses automaticity. Despite this, in clinical situations, conduction disturbances during hyperkalemia are rarely seen, except in digitalized patients, indicating interaction between potasium 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).

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). This may partly explain why hypokalemia pre-disposes to digoxin toxicity. All patients were recieving both digoxin and furosemide maintanence treatment and inulin clearance

was used as the baseline for changes in clearance. When patients recieved potassium supplements, the tubular secretion was restored to normal.

Cohn *et al.* (1967), studied the effect of K^+ depletion on myocardial concentration of tritiated digoxin. He found that there was no significant differences between the myocardial ^{3}H-digoxin of the control and potassium-depleted groups at 0.5, 1, 2, 6 or 16 hours, but at 24 hours, a significantly higher concentration of digoxin was present in the hearts of potassium deficient mice (23.6 ± 5.2 μg/gm.) than in the control group (3.1 ± 1.5 μg/gm.). Potassium depleted mice with total renal failure, showed increased myocardial ^{3}H-digoxin levels at 20 hours as compared with mice with renal failure but without potassium deficiency. It is thus evident that diminished renal function produced by potassium-depletion is not the sole mechanism of this retention of cardiac ^{3}H-digoxin.

The mechanism by which digitalis inhibits the influx of potassium is still under investigation. However, it has been established that the active transport of Na^+ and K^+ across the cell membrane is associated with the splitting of ATP by membrane ATPase. It has been postulated that Na^+ and K^+ activate ATPase by combining at sites which are more or less selective for these ions. The transport ATPase is readily inhibited by concentrations of the cardiac glycosides in the range from less than $10^{-6}M$ to more than $10^{-4}M$ (Glynn 1964). At lower concentrations of digitalis, the degree of inhibition of K^+ influx appears to diminish as extracellular K^+ increase (Page 1964, Glynn 1956). This observation may be explained by competition of cardiac glycoside and K^+ for a binding site, possibly on the cell membrane (Page 1964, Repke 1965, Glynn 1957). In addition, Ebert *et al.* (1967), reported that K^+ can displace digoxin from the heart.

Marcus *et al.* (1969) postulated that the change in body distribution of digoxin in the dogs made hyperkalemic prior to the administration of digoxin as compared to normal dogs may be explained in part by competetive inhibition of K^+ and

digitalis for similar receptor sites. Once digitalis is bound to the cell, hyperkalemia does not alter its retention. Therefore the mechanism of K^+ in reversing digitalis induced arrythmias may be other than physical displacement of digoxin from the heart. It is also possible that the portion of digoxin that is pharmacologically active is a minute portion of that present in the myocardium and that K^+ displaces this fraction, diminishing pharmacologic activity but releasing amounts of digoxin which are not measureable by methods available then.

Serum potassium poorly reflects intracellular potassium (Lown *et al.* 1951, Lown and Levine 1954a, Moore *et al.* 1954). Potassium ions also contribute to the transmembrane resting and action potential of the myocardial cell. These facts may explain the relationship between hypokalemia and digitalis toxicity. Also, clinical benefit has often been achieved by the administration of potassium in patients with digoxin-induced arrythmias. The fact that hyperkalemia can also result from digoxin 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 the changes in serum potassium. The influence of potassium on ionotropy is not well understood. In laboratory experiments, potassium was found to lessen the ionotropic response (Prindle *et al.* 1971, Lee *et al.* 1977), but this has not been confirmed clinically.

The results indicate the need for the development of pharmacokinetic - pharmacodynamic models to better characterize the effects of K^+ in altering the pharmacokinetics and pharmacodynamics of digoxin.

c) Thyroid dysfunction

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 (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 (CHF) (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 Na^+, K^+-dependent ATPase (Lindsay and Marker 1976). However, alterations in glycoside kinetics is another possible explanation and has been evaluated by several investigators using either radioactive glycosides (Doherty and Perkins 1966: Eichenbusch *et al.* 1970) or radioimmunoassay (Croxon and Ibbertson 1975; Gilfrich and Meinertz 1978; Shenfield *et al.* 1977). Some of these studies have been reviewed earlier.

The hyperdynamic state in hyperthyroidism may also be involved in the altered cardiac tissue level. Heart rate for example has been implicated in glycoside binding (Lloyd and Taylor 1978, Roth-Schechter *et al.* 1970) and there is an increased heart rate in hyperthyroidism.

Thus there are several possible explanations for the increased cardiac binding of digoxin in hyperthyroidism. The two most likely hypotheses are :- 1) Changes in sensitivity to digoxin with altered thyroid status are due to changes in serum level. 2) Changes in sensitivity to digoxin with altered thyroid status are due to a direct effect of thyroid hormone on the heart inducing increased Na^+, K^+-ATPase. As a result more glycoside is needed to achieve the same response.

Another possible explanation for the differences in levels are due to altered volumes of distribution (Doherty and Perkins 1966). Increased volume of distribution in hyperthyroid patients and decreased volume of distribution in hypothyroid patients were demonstrated by Shenfield *et al.* (1977), and Gilfrich and Meinertz (1978), showed a larger $Vd.$ in hyperthyroid patients than in euthyroid patients.

The findings of decreased serum and liver levels of ^{3}H-digoxin in the hyperthyroid group, but increased cardiac levels supports the concept of changes in the distribution of digoxin in hyperthyroidism. There is considerable evidence to suggest that there are changes in glycoside receptors with altered thyroid status. The nature of the cardiac receptors remains unresolved but there is still doubt that the Na^{+}, K^{+}-ATPase is intimately involved (Coltart 1978) and it has also been shown that there is an increase in Na^{+}, K^{+}-ATPase activity in hyperthyroidism (Curfman, Crawley and Smith 1977).

The existing body of literature on the effect of thyroid dysfunction on digoxin kinetics and disposition are far from conclusive and indicate the need for development of pharmacokinetic-pharmacodynamic models to determine whether the changes in digoxin effects due to thyroid dysfunction are due to altered pharmacokinetics, altered levels at the biophase, or both.

4) Lack of a specific assay for digoxin

Finally, there is the possibility that metabolites of digoxin may interfere with the radioimmunoassay of digoxin serum levels if these metabolites are present in serum at a sufficient concentration. It has been demonstrated that the active metabolites digoxigenin bis-digitoxoside and digoxigenin mono-digitoxoside and the inactive metabolite dihydrodigoxin are all capable of interfering with the RIA to varying degrees (Stoll *et al.* 1972; Kramer *et al.* 1976). It has not been established whether there are significant concentrations of these metabolites in serum because of the extremely low concentrations of assayable compounds achieved after digoxin administration in man. Also, most of the pharmacokinetic and pharmacodynamic studies have been conducted using these non-specific assays for digoxin so that the contribution of the parent drug to the response has not been reliably determined.

5) Use of the dog as an experimental model

Although the dog has been used extensively as an experimental model for digoxin pharmacokinetics and pharmacodynamics using invasive techniques (Zamella *et al.* 1980; Horwitz *et al.* 1977; Rubenson *et al.* 1984), there has been no study yet that has used non-invasive techniques of measuring STI in the dog. Further there has been no study in the dog where the pharmacokinetics and pharmacodynamics have been simultaneously studied for pharmacodynamic model development. Most studies that have measured digoxin responses in the dog, have been pharmacologic studies, where the objective usually has been the demonstration of an observable pharmacologic effect, rather than pharmacodynamic modelling.

Altered physiologic states may be easily induced in the dog, and could provide insight into altered pharmacokinetics-pharmacodynamics due to these altered physiological states. The dog thus seemed to be an adequate experimental model for our current and future studies.

6) Lack of pharmacokinetic-pharmacodynamic studies in existing literature

Toxicity occurs in over 20% of the patients receiving digoxin. Also, patients with altered renal function have varying needs of digoxin. Resistance to digoxin is an accepted phenomenon in hyperthyroid and hyperkalemic patients, while sensitivity to digoxin is common in hypokalemic and hypothyroid patients. In spite of these existing problems in digoxin dosing adequate pharmacokinetic-pharmacodynamic models have not yet been developed to assess digoxin needs in such patients. Studies that have attempted to model digoxin pharmacokinetics-pharmacodynamics suffer from the following drawbacks:

1) Use of a non-specific assay for digoxin since lack of a specific assay confounds the contribution of the cardioactive metabolites of digoxin to the response.

2) Previous studies have not monitored the plasma levels and response of digoxin simultaneously over time thus preventing mathematical definition of the relationship between the levels and responses.

It is for all of the above mentioned reasons that digoxin was chosen as the investigational drug in the dog.

REVIEW OF EXISTING ANALYTICAL METHODS

Analytical methods for digoxin can be divided into 2 groups. In the μg. range, the classical methods of photometry and chromatography still have an established place in the pharmacoepoeias and in control laboratories for the quantative determination of content and purity. Pharmacokinetic investigations utilize methods that will detect digoxin in the ng. range. Some of the methods used are isotope assays, gas chromatography (GC), gas chromatography - mass spectrometry (GC-MS), high pressure liquid chromatography (HPLC), radioimmunoassay (RIA) and rubidium (Rb)-uptake by erythrocytes.

I. Chemical and Chromatographic methods

Initial methods involved converting digoxin to colored products by the Baljet or Keller-Kiliani reactions which were measured by photometry. Or, digoxin was treated with strong acid and then measured by fluorimetry. The fluorimetric method was the most sensitive. These methods are no longer used because of their lack of specificity and lack of sensitivity. Since 1950's chromatographic methods have been used for qualitative and quantative analysis of digoxin. Initial methods used paper chromatography and subsequently thin layer chromatography (TLC). Here again the major drawback was lack of sensitivity. Some of the TLC procedures were those by Fuch *et al.* 1958, Stahl 1961, and Carvalhas and Figueira 1973.

II. Gas Chromatography

The first GC analyses of digoxin (Jelliffe and Blankenhorn 1963, Wilson *et al.* 1967), were effected by converting digoxin to its trimethylsilyl ether derivative. Watson and Kalman (1971), developed a GC assay for digoxin. Although this assay

enables analysis of ng. levels of digoxin in plasma, it requires 10 ml of plasma and elaborate processing and derivatization with heptafluorobutyrate prior to injection. Neverthless GC in conjunction with MS is of great value. The technique enables metabolites to be identified and quantified. (Watson *et al.* 1973).

III. Liquid Chromatography

Gravity column chromatography with silica-gel and aluminium oxide has been employed for many years. Other column filling materials have been used including Sephadex LH-20 (Gault *et al.* 1976) and DEAE-Sephadex LH-20 (Sugden *et al.* 1976). Due to its accuracy, reproducibility and speed of operation HPLC has been the preferred choice over liquid chromatography (LC), at normal pressure. A number of HPLC procedures for digoxin and its metabolites have been published using various columns including silica-gel, ion-exchangers and reversed phase columns.

IV. Double Isotope Dilution Derivative Assay

This method originally used for the analysis of amino acids and steroids has also been applied to the analysis of digoxin (Lukas 1973).

The sample is spiked with ^{3}H-digoxin for recovery calculations. The sample is extracted and converted to its acetate derivative with 1-^{14}C-acetic anhydride. Finally. the digoxin content is calculated from the carbon-14 and tritium content of the acetate derivative. the specific activity of the I-^{14}C-acetic anhydride and fraction of tritium recovered. Several extractions and chromatographic clean-up procedures are necessary before and after derivatization. The precision of this assay is poor at levels of 2 ng/ml. of digoxin or lower.

V. Enzyme Immunoassay

The enzyme-immunoassay technique (EIA), differs from RIA in that an enzyme-labeled drug (i.e. a drug covalently attached to an enzyme) rather than an isotope-labeled drug is used as the antigen competing with the unlabeled drug to be analyzed (Rubenstein *et al.* 1972). The antibody bound to the labeled drug then sterically inhibits the enzyme activity; the higher the concentration of unlabeled drug, the more competition there is for binding sites on the antibody and thus more release of enzymatic activity, which serves as the indicator for immunologic competition.

This technique was introduced for the determination of digoxin by Chang *et al.* (1975), and made commercially available. The kit has been evaluated (Rosenthal *et al.* 1976), and compared to RIA (Rosentahl *et al.* 1976; Sun and Spiehler 1976). Comparison of digoxin concentrations determined with both EIA and RIA revealed no systematic differences. About 20 specimens can be analysed within 2.5 hr. However, the antibody used in EIA also crossreacts with digoxin metabolites viz., digoxigenin, digoxigenin mono-digitoxoside and digoxigenin bis-digitoxoside (Linday and Drayer, 1983).

VI. Radioactively Labeled Digoxin

Most of our present knowledge on the pharmacokinetics and biopharmaceutics of digoxin is due to the use of radioactive labeled digoxin (Marcus *et al.* 1976; Doherty *et al.* 1961, 1966, 1967; Harrison *et al.* 1966; Hernandez et al. 1963). The technique is easy, inexpensive, highly sensitive and extremely precise. However its 2 major drawbacks have curtailed its use in recent years, viz., 1) non-specificity, i.e., radioactivity of a plasma or urine sample collected after administration of a labeled drug may not only represent the drug but the sum total of different species formed by chemical, or biologic decomposition or by metabolic transformation. 2) Restrictions on the use of radioactive materials.

VII. Radioimmunologic methods

Basic principles:

Based on the work of Yalow and Berson (1959) on the detection of insulin, radioimmunoassay (RIA) has now developed to be the most widely used method for the detection of trace amounts of biologically active substances (antigens).

In the assay a constant amount of antibody (which is selected in order to bind only part of the amount of added antigen) is incubated with a constant amount of tracer and antigen to be determined. In the course of the competetive reaction more labeled antigen will be bound by the antibody with decreasing amounts of unlabeled antigen present in the system and vice versa; according to the law of mass action. After a set time, ideally at equilibrium - the antibody-bound antigen is separated from the free antigen and the radioactivity in one of the fractions is measured, in order to determine the unknown concentration of the antigen from the measured count rates. This requires that both the antigen present in the standard and the unknown displace antigen from an antibody-labeled-antigen immune complex, but identical behaviour of tracer and unknown or standard antigens is not necessary.

Antibodies:

Digoxin is a small molecule (mol. wt. 780.92), and is too small to be immunogenic by itself. To obtain antibodies it is therefore necessary to couple it to protein carriers or haptens. Butler and Chen (1967) were the first to succeed in obtaining antibodies against digoxin. Antibodies have also been developed by Smith *et al.* (1970). The process involves oxidation of the OH groups on the digitoxose molecules by metaperiodate, yielding a dialdehyde derivative. The dialdehyde is then coupled to albumin to yield a conjugated, immunogenic molecule. Antibodies against dihydrodigoxin have also been developed (Butler *et al.* 1982), although they have not been commercially marketed.

Tracers

The original digoxin RIA method (Smith et al. 1969) employed 12-α-^{3}H-digoxin. ^{3}H- as a beta-emitter requires quench correction; so that the unit cost per assay is higher compared with labels that allowed gamma-counting. For these reasons ^{125}I-labeled digoxin is used in virtually all digoxin RIA kits today. The shorter half-life of ^{125}I (62 days) does not present a serious restriction.

VIII. Radioimmunoassay

The first published RIA for digoxin (Smith et al. 1969) employed high-affinity anti-digoxin antibody, ^{3}H-digoxin as tracer and dextran-coated charcoal (DCC) as the separation system. A serum volume of 1 ml. was required with a 15 min. incubation. Centrifugation of the free digoxin bound to DCC, denaturation of antibodies by heating at 60 °C, and quench corrections had also be be carried out. In contrast modern assays use less serum (0.1 ml or less) and by the use of iodinated tracers the counting process has become much more convenient. The general capabilities and limitations of digoxin RIA have been summarized (Shapiro *et al.* 1975, Ravel 1976). Considerable difficulty has been encountered due to the lack of specificity of ^{125}I-digoxin kits. The antibody cross reacts with some of the digoxin metabolites (Stoll et al. 1972) including dihydrodigoxin (Kramer *et al.* 1976).

IX. ATPase for the Determination of Cardiac Glycosides

The existence of an Na^{+}, K^{+} - activated ATPase (Mg^{++} - dependent, Na^{+}, K^{+} - activated ATP-phosphohydrolase) was discovered by Skou in 1957. Soon afterwards he discovered cardiac glycosides are able to inhibit ATPase activity by binding to the enzyme (Skou 1960). This enzyme is specifically associated with active cation transport, and becomes phosphorylated during the transport reaction. The ATPase molecule also possesses a binding site for oubain.

Both reactions - The dephosphorylation of ATP and the binding of oubain - may be employed as tools to measure the concentration of cardiac glycosides. The first method measures the inhibition of ATP hydrolysis; the second is based on the displacement of radiolabeled oubain from its binding site by other cardiac glycosides.

Comparison of the results obtained by ATPase activity and RIA

Since some known metabolites of digoxin are able to inhibit ATPase and to bind the antibodies respectively, both methods lack specificity. However with respect to the minor extent to which the metabolites occur in plasma (Marcus *et al.* 1966; Vohringer and Reitbrock 1974) the results obtained by both assays should be comparable. Results obtained from urine samples by both methods might show differences since polar and nonpolar metabolites occur in remarkably high concentrations in the urine (Marcus *et al.* 1966; Vohringer and Reitbrock 1974).

After administration of a dose of 0.5 mg. tritiated digoxin to each of 5 human volunteers the total radioactivity was determined in the polar and in the chloroform-extractable fractions of the urine. Measurements using ATPase activity as well as RIA's were performed for the same samples (Gundert-Remy et al. 1978). No systematic deviation could be detected between the results obtained from the three different assays. This indicates that both radioimmunologic and enzymatic assays are non-specific to the same extent since the results are not statistically different from the data obtained by measurement of total radioactivity.

Comparison of ATPase and RIA methods

The ATPase enzyme is not stable for more than 3 months and hence it is neccessary to prepare batches of enzyme from time to time to characterize them for use in the assay procedure. Using ATPase according to Booka and Jelliffe (1972), it takes

60 mins. and according to Mardh (1973) 90 mins. to run a sample by displacement and activity assay respectively. Large series of plasma samples can be determined more conveniently using RIA due its short incubation times and because an extraction procedure is not neccessary. However with the ATPase enzyme, more than one cardiac drug or its metabolites can be measured using the same enzyme system.

X. Rubidium Uptake in Erythrocytes

Active uptake by human erythrocytes of potassium measured by flame photometry has been demonstrated to be inhibited by cardiac glycosides (Schatzmann 1953). This effect was shown to be due to an inhibition of membrane Na^+ - K^+ - ATPase activity (Post *et al.* 1960; Dunham and Glynn 1962; Glynn 1957).

Therefore Lowenstein (1965) introduced the principle of inhibition of active cation uptake by erythrocytes to measure plasma concentrations of cardiac glycosides. Potassium, however had to be replaced by rubidium, since available potassium isotopes are impractical for standard laboratory techniques. Since ^{86}Rb has a half-life of 19.7 days and emits beta and gamma-rays, it is very appropriate for routine laboratory use. ^{86}Rb is taken up by human erythrocytes in the same way as potassium (Love and Burch, 1953).

Some of the disadvantages of the assay, are:

1. Variability in batches of erythrocytes - since uptake of ^{86}Rb is dependent on the source of the erythrocytes e.g. blood group, age, race, and disease states of the donor.
2. The assay requires 8 hrs., which is much longer than the time required to do RIA's.
3. Has a dichloromethane extraction step, which can contribute to errors.
4. Large volumes of plasma are required to assay low digoxin concentrations.

XI. Recent HPLC Assays

Initial studies measured digoxin concentrations by administration of ^{3}H-digoxin to patients (Beall et. al. 1963; Doherty *et al.* 1961; Doherty et al. 1969; Hernandez *et al.* 1963). All subsequent studies have used RIA for measuring digoxin concentrations (Biddle *et al.* 1978; Coltart *et al.* 1974; Gorodischer *et al.* 1976; Gullner *et al.* 1974; Jusko *et al.* 1974; Karjalainem *et al.* 1974; Krasula *et al.* 1974; Park *et al.* 1982).

It has been demonstrated that the digoxin metabolites: digoxigenin, digoxigenin mono-digitoxoside, digoxigenin bis-digitoxoside; cross-react extensively using RIA (Stoll *et al.* 1972). Kramer *et al.* (1976), showed that dihydrodigoxin also cross-reacts with the commerically available RIA's.

Recent methods have separated digoxin from its metabolites by HPLC . By collecting the digoxin fraction at the end of the HPLC column, and by either applying RIA or measuring radioactivity (when radiolabeled digoxin is administered), specific assay methods for digoxin have been made available (Eriksson *et al.* 1981; Loo *et al.* 1977, 1981; Morais *et al.* 1981; Nelson *et al.* 1979).

Gibson and Nelson (1979, 1980) reported that plasma digoxin concentrations measured by specific HPLC assay and direct ^{125}I-RIA are essentially identical when glomerular filtration rate is 40 ml/min. or more: in contrast, the ratio of HPLC-RIA/RIA values was 0.83 ± 0.12 in renal failure patients. Wagner et al. (1981), reported no significant differences between results with a specific assay and RIA with groups of 17 randomly selected plasma samples and 34 randomly selected urine samples from a study in normal adult male subjects. Loo et al. (1981), reported a mean ratio of HPLC-RIA/RIA digoxin in serum of 0.84 ± 0.12 in 14 digitalized patients of unknown renal status. Wagner *et al.* (1983), reported a mean ratio of HPLC-RIA/RIA digoxin in a mean and range 0.74 (0.23 to 2.63) in serum and 0.81 (0.068 to 1.38) for atrial tissue.

CHAPTER 2

EXPERIMENTAL

An Improved Method For Assaying Digoxin In Serum Using HPLC-RIA

The following section describes the developed HPLC-RIA assay for digoxin in the presence of its major metabolites using information detailed in the preliminary studies. Details of these preliminary studies which include, the development of HPLC procedures, and validation of the selectivity of the RIA kit (Diagnostic Products Corporation) to digoxin in the presence of dihydrodigoxin may be found in appendix B. References to the various materials used may be found in appendix A.

Introduction:

Most of the existing 'specific' assays for digoxin have inherent drawbacks. Procedures involving gas chromatography - mass spectrometry although sensitive are expensive and tedious. The HPLC methods by Loo *et al.* (1977) and Nelson *et al.* (1979), Gault *et al.* and Sugden *et al.* require ^{3}H-digoxin as an internal standard, or require ^{3}H-digoxin be administered to patients.

The developed assay has some advantages over currently available procedures. The extraction of digoxin from serum is over 90%, from serum as compared to previous assays where the extraction varied from 54% - 78% (Nelson *et al.* 1979). Loo *et al.* (1974) and Gault *et al.* (1976), also reported mean extraction percentages of around 70% - 80% . The assay does not require ^{3}H-digoxin to be administered

to the subjects or patients, or to be used in the assay, except for ^{125}I-digoxin used in the RIA procedure.

Kuhlman *et al.* 1973, and Sternson *et al.* 1978, have shown that digoxin hydrolyses to give rise to digitoxosides, viz., digoxigenin bis-digitoxoside, digoxigenin mono-digitoxoside and digoxigenin . There is a possibility that digoxin hydrolysis may occur in assays using reverse phase HPLC (Morais *et al.* 1981, Eriksson *et al.* 1981, Nelson *et al.* 1979, Loo *et al.* 1981, Wagner *et al.* 1983), since the mobile phase in such systems is comprised largely of water. Use of a normal phase HPLC system (where the mobile phase is comprised of organic solvents) eliminates the possibility of hydrolysis during the assay. Furthermore, after collection of eluate fractions, the solvent can be easily evaporated to dryness prior to reconstitution for RIA. Evaporation of aqueous solvents (in reverse phase HPLC) is extremely cumbersome and further enhances the possibility of degradation.

Morais, *et al.* (1977), Loo *et al.* (1981) and Eriksson *et al.* (1981), have reported partial or complete separation of dihydrodigoxin from digoxin using HPLC. Little success was had in reproducing this separation. Due to its very low absorbance, nanogram quantities of dihydrodigoxin cannot be monitored using ultraviolet (UV) spectrophotometry. Hence, during the developmental stages of the assay the retention times for dihydrodigoxin were monitored using spectrofluorometry (Wells and Myers, 1961).

Experimental:

Materials:

All solvents used for the extraction or chromatography were HPLC grade from Fisher Scientific Co. (Pittsburgh, PA, USA). Digoxin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Digoxigenin, digoxigenin mono-digitoxoside

and digoxigenin bis- digitoxoside were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind., USA). Dihydrodigoxin was a gift from H.Hull (Burroughs Wellcome, Research Triangle, N.C, USA). RIA was performed using the Digoxin RIA kit (Diagnostic Products Corporation LA, CA, USA).

Methods:

Extraction:

1 ml. of serum was extracted with a combination of 0.5 ml. methylene chloride and 4 ml. chloroform in a screw-capped 10 ml. glass tube. The tube was agitated for 15 mins. on a rotary mixer and then centrifuged at 1000 g for 5 mins. The organic layer was pipetted into a 12 ml. conical centrifuge tube. The aqueous layers were re-extracted and centrifuged again using the procedure above. The aqueous layer was aspirated and the organic layer was pipetted out. The combined organic extract was evaporated to dryness on a vortex evaporator. The residue was reconstituted in 170 μl. of mobile phase and was injected on to the HPLC column using a fixed volume 100 μl. loop injector.

Chromatography

The HPLC system was a Waters M - 6000 A solvent delivery system. The separation was acquired with a Lichosorb SI-100 silica column (Hewlett Packard, 5 μm. particles, 20 cm. length, 3 mm. internal diameter). The mobile phase was comprised of 75% hexane, 18% ethanol and 7% methylene chloride. The flow rate was monitored at 3 ml./min. and pressures ranged from 1,500 to 3,000 psi. The retention times for digoxigenin, digoxigenin mono-digitoxoside, digoxigenin bis- digitoxoside and digoxin varied slightly with each batch of mobile phase that was prepared but typically were 5.2, 7.2, 9.8 and 13.2 mins. respectively. Dihydrodigoxin is not separated from digoxin using the above procedure. Retention times during development of the assay were monitored by injecting a mixture containing 2.5 μg. of digoxin

and its metabolites on the column and detected using a Gilson variable wavelength UV spectrophotometer at 230 nm. Retention times (prior to assaying each batch of samples) were determined by injecting 20 ng. of each of the metabolites in the mobile phase on the column and collecting 1/2 min. fractions of the eluant over a period of 20 mins. Each fraction was evaporated to dryness, reconstituted in 0.5 ml. of blank plasma and assayed by RIA to confirm that digoxin was indeed separated from its metabolites

Digoxin cannot be detected spectrophotometrically in the ng./ml. range, but can be assayed by RIA. Hence, post injection, the eluant fraction corresponding to the retention time of digoxin was collected off the HPLC column. This fraction was evaporated to dryness. The residue was reconstituted in 0.5 ml. of blank plasma and this was assayed for digoxin using the radioimmunoassay kit mentioned before.

Radioimmunoasay

RIA was conducted on the eluant fractions. The fraction corresponding to digoxin (typically 12-15 mins.) was collected in 12 ml. centrifuge tubes and was evaporated to dryness on a vortex evaporator. The residue was reconstituted in 0.5 ml. plasma and assayed by RIA. The RIA kit used was found to be highly selective for digoxin in the presence of dihydrodigoxin.

Analyses:

The standard curve and samples were done in duplicate. The standard curve was constructed using serum standards containing 0.5, 1.0, 2.0 and 4.0 ng/ml. digoxin. The standards (containing known concentrations of digoxin in serum) were extracted and assayed by HPLC-RIA to obtain measured concentrations. The standard curve was then obtained by regression of the measured and the known concentrations. A set of controls (frozen serum samples spiked with 0.50 ng/ml. and 3.50 ng/ml. digoxin) was run before and after each batch of 12-13 samples to

be assayed. The concentrations of the samples and controls were obtained from the standard curve for that day.

Results:

Chromatography:

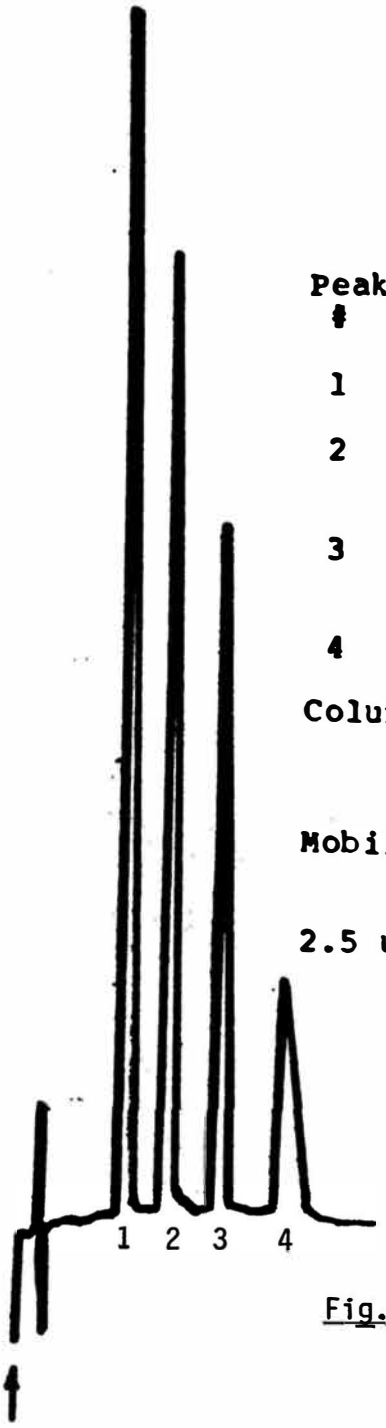
Digoxin was separated from its metabolites (except dihydrodigoxin) on the column. A typical chromatogram showing separation of digoxin from its major metabolites is shown in Fig. 2.1.

Extraction:

Various solvents were tried for optimizing extraction of digoxin from serum. Most of the systems exhibited nonlinearity, low recoveries or non-reproducibility. A double extraction using 4 ml. chloroform and 0.5 ml. of methylene chloride gave almost complete extraction which was reproducible. A typical extraction curve is given in Fig. 2.2. The regression line has a slope of 1.04 ± 0.11 and a mean value for the the intercept not significantly different from zero (The hypothesis H_o : $Mean = 0$ was not rejected. $p = 0.6$). The correlation coefficient for the regression line was 0.99 The slope of the line indicates an extraction efficiency of around 100% While the extraction procedure is tedious, it is accurate and reproducible, and requires no internal standard. Furthermore the organic solvents used are easily evaporated.

Linearity:

A typical standard curve is shown in fig. 2.3. The slopes and intercepts are given in Table 2.1. It is evident that the standard curve remained essentially constant over a eight month period. The low standard deviations for the slope and the intercept indicate that the standard curve remained essentially constant over a eight month period.



Peak #		Retention Time (mins)
1	digoxigenin	5.2
2	digoxigenin mono-digitoxoside	7.2
3	digoxigenin bis-digitoxoside	10.0
4	digoxin	13.6

Column : Lichosorb SI-100
Int. dia. 3mm
length 20 cm.

Mobile phase : Hexane : Methylene chloride : Ethanol (75:17:8)

2.5 ug. of each on column.

(a.u.f.s. = 0.1)

Fig. 2.1 TYPICAL CHROMATOGRAM
Arrow indicates injection.

EXTRACTION CURVE

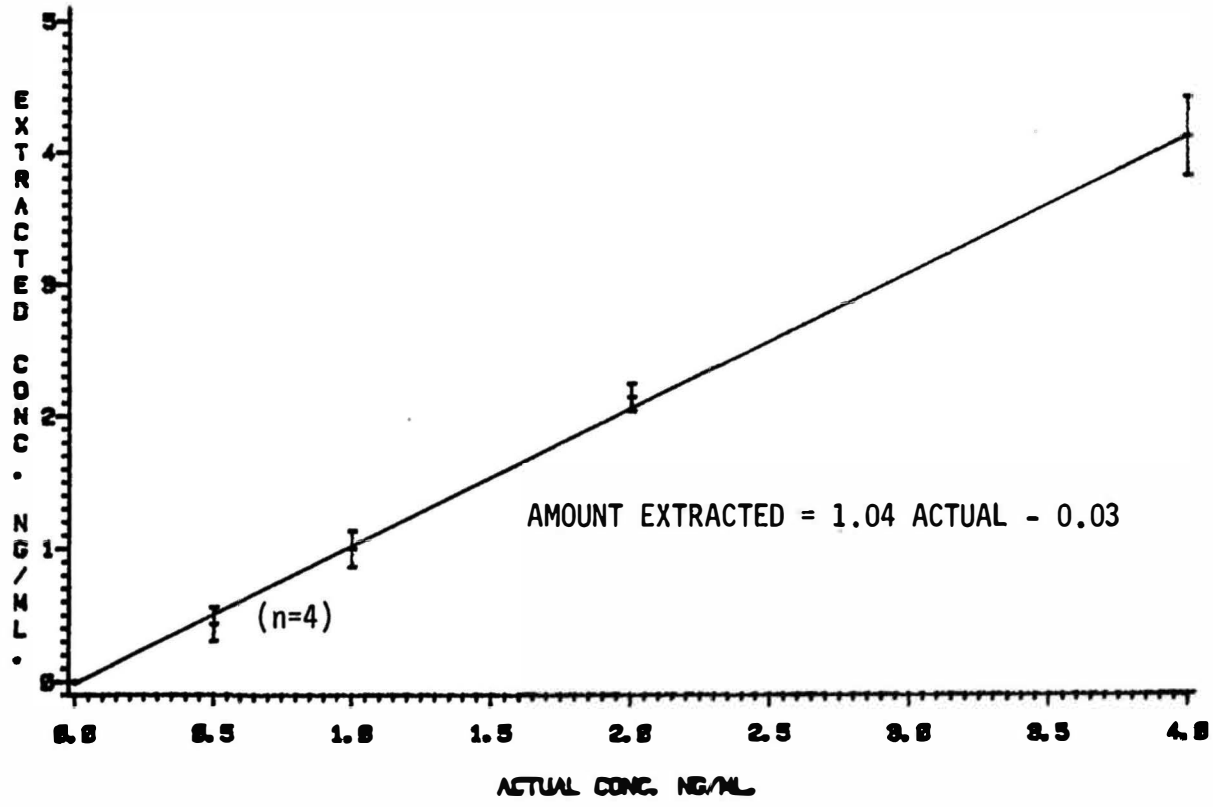


Fig. 2.2 Figure indicates extraction efficiency and reproducibility of extraction procedure. (bars indicate mean ± s.d. at that level). Intercept is not significantly different from zero (p=0.6).

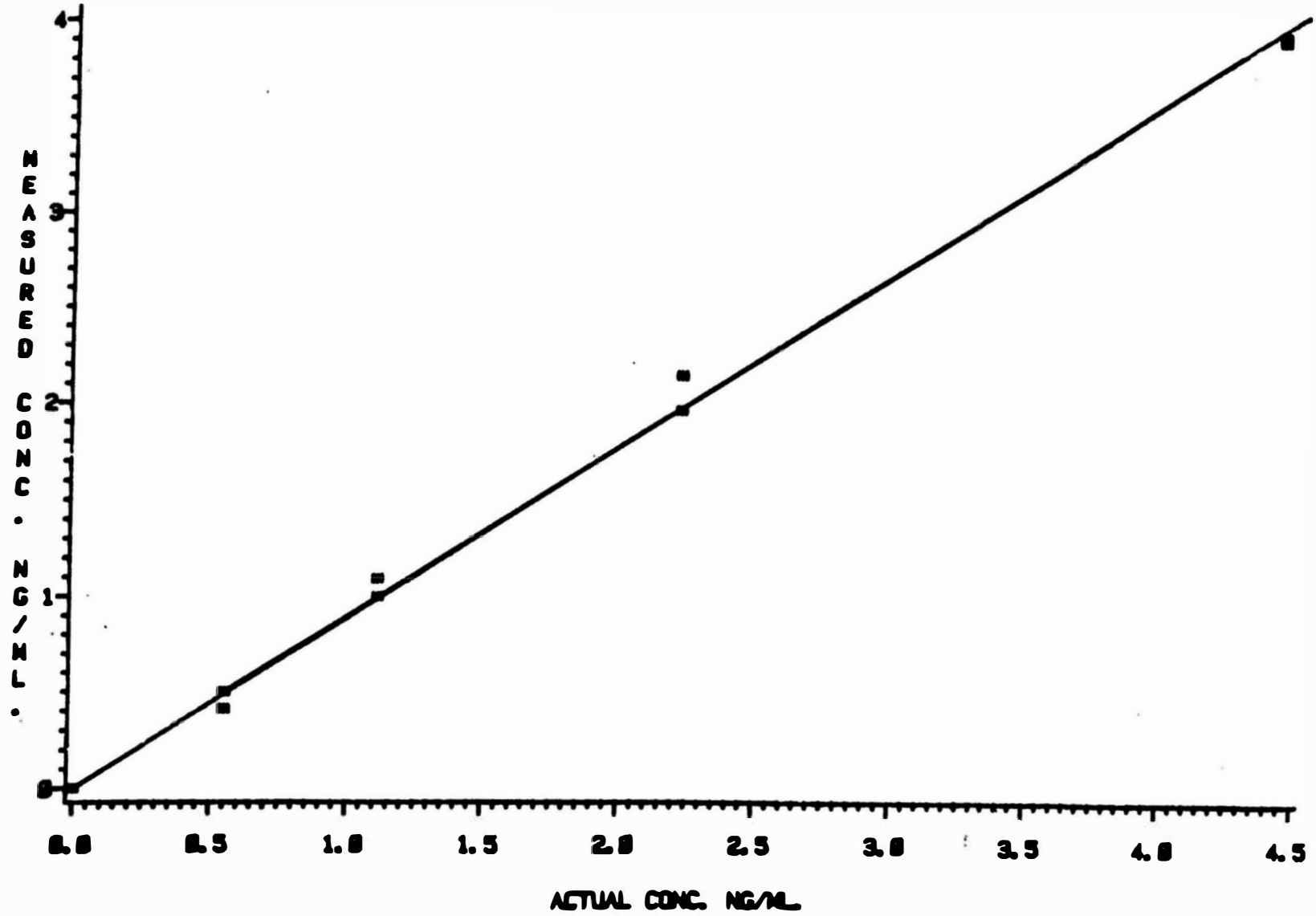


Fig. 2.3 TYPICAL STANDARD CURVE. (Standards were run in duplicate).

TABLE 2.1

Reproducibility of the standard curve.

DAY	NO. OF STDS.	SLOPE	INTERCEPT	CORR. COEFF.
1	5	1.096	-0.044	0.99
2	5	0.974	-0.106	0.99
3	4	0.865	-0.06	0.99
4	5	0.88	0.023	0.99
5	5	0.997	0.053	0.99
	MEAN	0.96	-0.03	
	±			
	s.d.	0.09	0.06	

Recovery:

The precision of the method is estimated from the coefficients of variation for the repeated measurements made on two sets of controls. This was achieved by running a control from each set at the start and completion of each batch of samples. The recovery for these repeated measurements is given in Table 2.2. The recovery at a spiked concentration of 3.5 ng/ml. was 99.91% ± 8.97% ng/ml. The overall day to precision is indicated by the low standard deviations associated with the recovery of the controls. The mean recoveries of 100.17% and 99.91% at 0.50 and 3.50 ng/ml. is indicative of the accuracy of the assay. The in-day precision is obtained from the recovery (calculated for day 2) and is 107.5 ± 3.0 and 98.71 ± 9.69 at concentrations of 0.50 and 3.50 ng/ml. respectively. The values for the controls compared well with the values for the same controls obtained by direct RIA.

Application:

Serum samples obtained from beagle dogs administered digoxin for pharmacokinetic and pharmacodynamic studies (described later in this chapter) were assayed using both HPLC- RIA and by direct RIA. Over 40 samples were assayed by both methods. A linear relationship was observed between the values obtained by both methods, with a slope of 1.02 and a mean intercept not significantly different from zero (The hypothesis H_o : $Mean = 0$ was not rejected $p = 0.78$). Values obtained by using direct RIA were plotted on the abscissa. (Fig. 2.4).

The results are similar to those of Gibson and Nelson (1979, 1980) in patients. They reported the values obtained by direct RIA and those obtained by HPLC-RIA were essentially identical in patients with glomerular filtration rates of greater than 40 ml/min. They observed that the differences in the values may be significant

TABLE 2.2

Recovery of controls.

	CONTROLS BY HPLC-RIA			
	(Spiked conc. = 0.5 ng/ml.)		(Spiked conc. = 3.5 ng/ml.)	
DAY	Measured conc.	Recovery %	Measured conc.	Recovery %
1	0.44	88.00	3.22	92.00
	0.39	78.00	3.25	92.86
	0.43	86.00	3.31	94.57
	**		3.72	106.28
2	0.55	110.00	3.05	87.14
	0.55	110.00	3.30	94.28
	0.52	104.00	3.74	106.86
	0.53	106.00	3.73	106.57
3	0.54	108.00	4.05	115.71
	0.54	108.00	3.92	112.00
4	0.48	96.00	3.25	92.86
	0.55	110.00	3.54	101.14
	0.49	98.00	3.38	96.57
Mean ± s.d.	0.50 ± 0.05	100.17 ± 10.97	3.50 ± 0.31	99.91 ± 8.79

**indicates lost sample due to spillage.

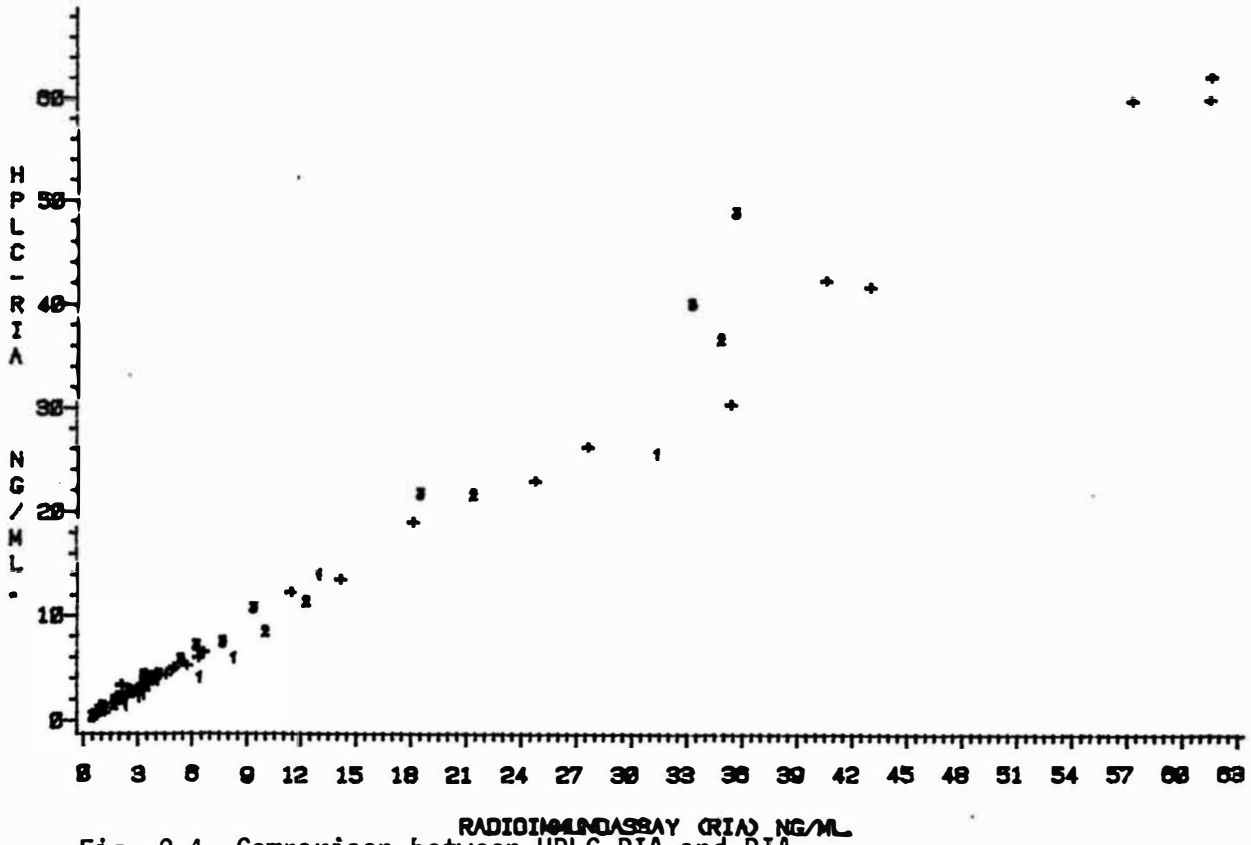


Fig. 2.4 Comparison between HPLC-RIA and RIA.
The regression line is given by: HPLC-RIA = 1.02 RIA - 0.09
The intercept was found not to be significantly different from zero (p=0.78).

in patients with renal failure presumably due to accumulation of cross-reacting metabolites.

Conclusion:

Described here is an accurate and precise method for assaying digoxin in serum. The assay has major advantages over existing assays, viz.:

1) The extraction procedure has high recovery and a high degree of reproducibility.
2) The possibility of cross-reactivity to metabolites has been minimized by: (a) an HPLC procedure that separates digoxin from its metabolites and (b) by using a RIA kit that is highly selective for digoxin in the presence of dihydrodigoxin.
3) Hydrolysis of digoxin on column is minimized since the mobile phase for the HPLC procedure is comprised of organic solvents.

These represent substantial improvements over existing assays. The improved selectivity of the assay could be of clinical value in patients with renal failure and accumulation of metabolites of digoxin. This assay was developed for application to pharmacodynamic studies in dogs, to assure that cardioactive metabolites were not present in sufficient quantities, to contribute significantly to the pharmacodynamic response being monitored.

METHODS

Initial Pharmacokinetic Studies.

Initial studies were done in 3 normal beagle dogs (Dog no.1, 13.2 kg., Dog no.2, 10.0 kg. and Dog no.3, 9.75 kg.). These studies were essentially pilot pharmacokinetic studies conducted to determine digoxin dispostion in these dogs. Information pertaining to materials used in these studies may be found in appendix A. These studies were conducted with the dogs in a conscious, unanesthetized state. The forelegs of the dogs were shaved. A tourniquet was applied above the elbow and a heparin lock was introduced into the saphenous vein. After removal of the tourniquet the lock was kept patent at all times by flushing it periodically with a solution of heparin (100 U/ml.) in normal saline. The dogs were administered 0.05 mg/kg. digoxin (Lanoxin$^{(R)}$, 0.25 mg/ml.) i.v as an infusion (manually) over 5 mins. The lock was then flushed with 3 ml. of normal saline to rinse the syringe and the tubing (associated with the heparin lock) of residual digoxin. Blood samples were drawn at specific times as follows: Using a sterile 3 ml. syringe, 2 ml. of blood was drawn via the heparin lock. This syringe was discarded and 5 ml. of blood was again drawn via the heparin lock using a 10 ml. sterile syringe. The lock was flushed with 2 ml. of heparin. The contents of the 10 ml. syringe was transferred to a sterile vacutainer$^{(R)}$ (10 ml., red top). Typically the sampling times were 0.5, 1.0, 1.5, 2.0, 3.5, 5.5, 7.5, 9.5, 16.0, 24.0, 32.0, 48.0 hrs. post-infusion. After each blood sample was withdrawn, 10 mins. was allowed for clotting. The samples were then centrifuged for 10 mins. and the serum was pipetted out into polystyrene tubes (Falcon$^{(R)}$) and frozen for future analysis. Prior to being assayed the serum

samples were thawed to room temperature and assayed for digoxin content using RIA (Diagnostic Products, LA, CA.). Compartmental analysis was conducted on the concentration vs. time data using the nonlinear regression program, NONLIN (Metzler *et al.* 1974).

Pharmacodynamic studies

Responses measured

Dogs used in these studies were normal dogs as determined by blood tests and tests conducted for detection of heart worm. The responses recorded were electrocardiograms (EKG), heart sounds and carotid pulse tracings. The electrocardiogram used was a VS4 Portable Electrocardiograph. The electrodes for the electrocardiogram were placed as follows: The insides of the dogs fore and hind legs were shaved. Cambridge electrode jelly was then applied to the inside of the legs where the electrodes were to be placed. A small amount of electrode jelly was also applied to the electrodes. The electrodes were kept in position by rubber straps that attached to pairs of hooks on the electrodes. The straps were tightened just enough to hold the electrodes in position and to facilitate conduction of the body's electrical currents. Standard Lead I and Lead II electrocardiograms were usually recorded.

Heart sounds were measured using a microphone pick-up unit. This high fidelity microphone was usually placed externally in between the 3rd or the 4th intercostal space in a position optimal to measuring the high frequency vibrations of the heart sounds. The optimal position was also determined by listening to the heart sounds with a stethophone.

Left ventricular ejection times (LVET's) were obtained either invasively (initial studies) or non-invasively (later studies). The LVET's may be non-invasively obtained in humans by measurements made on the carotid pulse recording obtained by

the placement of a pressure transducer externally over the carotid artery. A similar procedure could not be used in dogs by virtue of the fact that the carotid artery is not as superficial in dogs as compared to humans. In fact, the carotid artery is very deep-seated in the dog's neck, being in a deep paratracheal position. Therefore the pulsations in the carotid artery could not be obtained by external pick-up. LVET's were therefore obtained by invasive catheterization of the left auricle. Invasive measurement of the LVET involved introduction of a pigtail catheter (5 french) into the left auricle after introduction via the femoral artery. The catheter was connected to a strain-gauge transducer through a dome. The pressure changes in the left auricle were recorded on an Electronics for Medicine EKG recording system.

Evolution of methodology led to methods for the non-invasive measurement of the LVET. This involved exteriorization of the carotid artery in dogs by surgery. The carotid pulse tracings were then obtained by placement of a strain-gauge type pressure transducer with a funnel-shaped pickup unit placed externally over the exteriorized carotid artery. The LVET's could then be obtained by measurements made on the carotid pulse tracing.

The procedure used for exteriorization of the carotid artery was basically that of Dueck *et al.*, (1982). The retaining suture used was 2-Prolene (cutting needle blue monofilament polypropylene suture, 50 cm). The suture used for closure of the incision was 00 black silk. The procedure involves making a longitudinal incision in the neck of an anesthetized dog. The carotid artery is then transferred from a deep paratracheal position to a ventrolateral subcutaneous position and is protected from the underlying mattress sutures by the sternomastoid muscle.

Responses measured

The responses monitored by measurement and/or calculations on the recordings obtained are: LVET (left ventricular ejection time), S_1S_2 (duration of heart sounds), QS_2 interval (duration of total electromechanical systole), P-R interval, R-R interval, Q-1 and the PEP (pre-ejection period). The measurements were made as described by Weissler *et al.*, (1968). However the the heart sound tracing, the carotid pressure tracing and the standard EKG tracing were not recorded simultaneously. The VS4 electrocardiograph possesses a single writing channel. However, any two tracings may be simultaneously recorded by superimposing them on each other. Therefore the heart sound tracing and the carotid pulse tracing, or the standard EKG and the carotid tracing, or the standard EKG tracing and the heart sound tracing may be recorded together simultaneously. Therefore in the studies conducted the carotid pressure tracing and the standard EKG and the heart sounds were recorded singly and the heart sound tracing and the standard EKG simultaneously recorded was also obtained. The following systolic time intervals were measured from the recorded tracings:

1) The R-R interval was obtained from the standard EKG tracing. It was measured from the uppermost tip of the QRS wave (i.e. the R wave) to the R wave immediately following it (Fig. 2.5).
2) S_1S_2 which is the interval between the heart sounds, was obtained from the heart sounds tracing. It was measured from the onset of the first heart sound to the begining of the high amplitude of the second heart sound (Fig. 2.6).
3) LVET was obtained from the carotid pressure tracing. It was measured from the beginning of the upstroke to the dicrotic notch or the incisura (Fig. 2.7).
4) The QS_2 interval was obtained from the simultaneously recorded tracing of the heart sounds and the standard EKG. It was measured from the onset of

the Q-wave to the beginning of the high amplitude of the second heart sound (Fig. 2.8).

5) The isovolumic contraction time (ICT), is derived by subtracting LVET from S_1S_2.

6) The pre-ejection period is obtained by subtracting LVET from QS_2

7) The time between the beginning of depolarization to the first heart sound (Q-1), is obtained by subtracting S_1S_2 from QS_2.

All measurements were an average of ten simultaneous recordings. Only well defined waves were used to obtain the measurements. All recordings were carried out at a chart speed of 100 mm/sec.

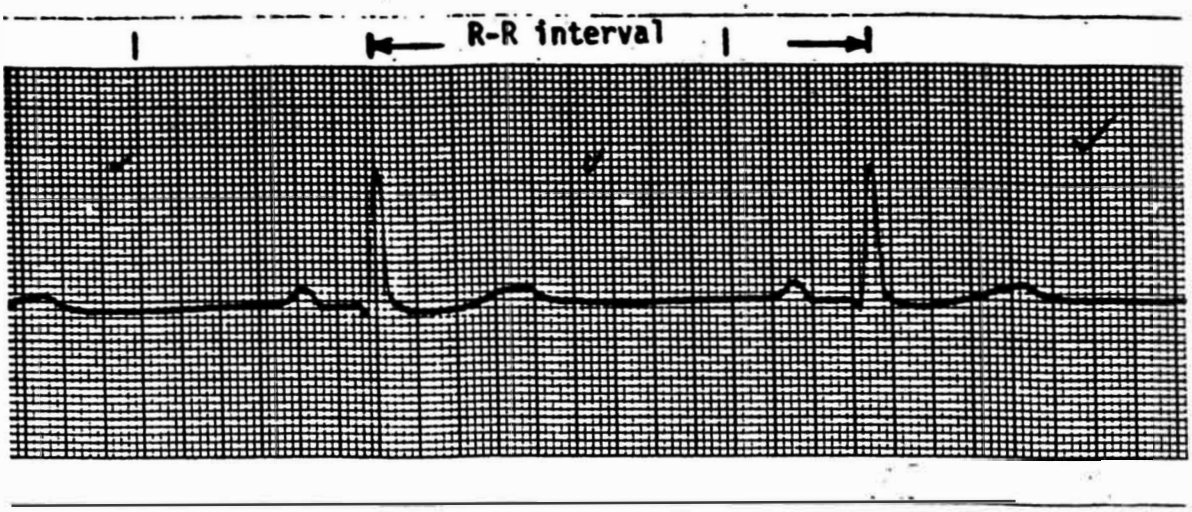
Study No. 1A

Dog no.: 4

Weight: 20.45 kg.

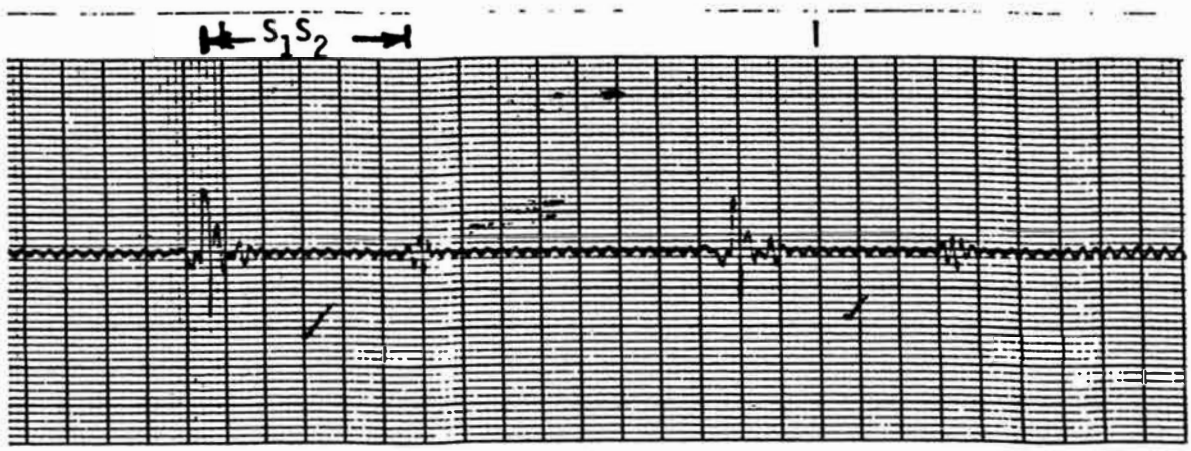
Sex: Male

This was a pilot pharmacokinetic-pharmacodynamic study conducted in a mongrel dog. The dog was anesthetized using 30 mg/kg. pentobarbital. The femoral artery was catheterized and a pig-tail catheter (5 fr.) was introduced and threaded into the left auricle. The catheter was hooked to a strain gauge pressure transducer so that the LVET could be measured invasively as described before. Digoxin (0.05 mg/kg.) was administered i.v. as a (manually administered) uniform infusion over 5 mins. Blood samples were drawn at 0, 15, 30, 45, 60, 75, 90, 120, 180, 210, 240 and 270 mins. post infusion from the catheter. The blood samples drawn were treated as described as before. The pressure tracings, heart sound tracings and Lead II EKG tracings were recorded each time a blood sample was drawn. After the completion



A standard EKG tracing.

Fig. 2.5



A typical phonocardiogram.

Fig. 2.6

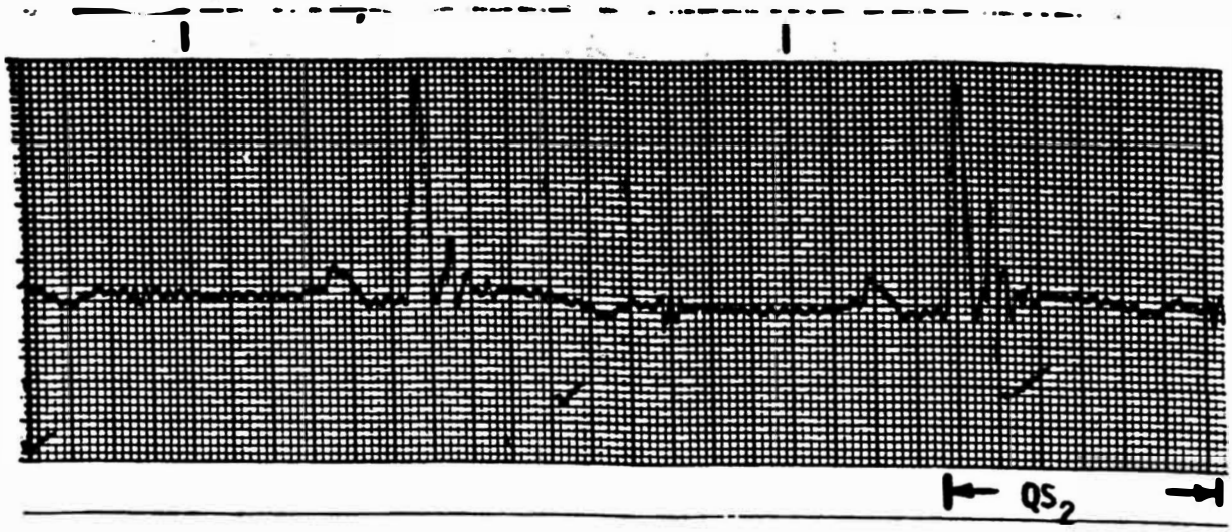


Fig. 2.8 Superimposed standard EKG and heart sounds tracing (simultaneously recorded).

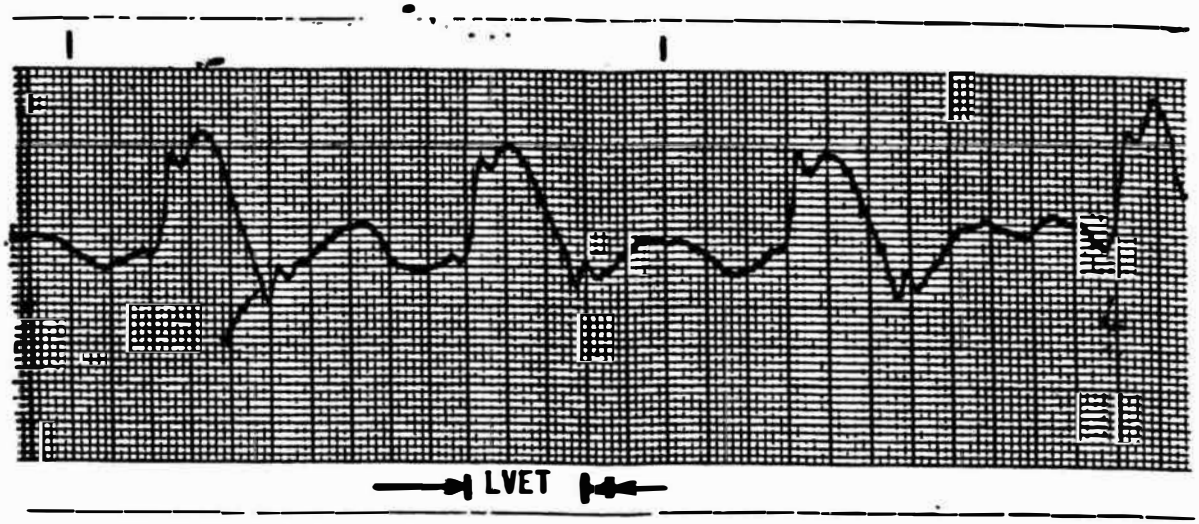


Fig. 2.7 A carotid pressure tracing.

of the study the dog was sacrificed by i.v administration of a saturated solution of KCl in normal saline.

Study No. 1B

Dog no.: 5

Weight: 19.3 kg.

Sex: Female

This was again a pilot pharmacokinetic-parmacodynamic study and hence was conducted using a mongrel dog. The dog was conscious and in a sling. The LVET was measured using the non-invasive techniques (after adequate recovery from surgery) described previously. In contrast to the previous study blood samples were drawn and the pharmacodynamic responses measured simultaneously over a period of 78 hrs.

Study Nos. 2, and 3

Study no. 2

Dog no.: 2

Weight: 10 kg.

Sex: Female

Study no. 3

Dog no.: 1

Weight: 13.0 kg.

Sex: Male

These studies were conducted in beagle dogs. The studies were conducted after the dogs had adequately recovered from surgery, for exteriorization of the carotid artery. The dogs had recovered with no infection and seemed quite normal in their

behavior and seemed to have the same friendly disposition they had before undergoing surgery. The study was conducted after the dog was anesthetized with pentobarbital (30 mg/kg.). After the dog was anesthetized an endotracheal tube was introduced in the trachea of the dog. The balloon in the endotracheal tube was inflated to keep the tube in position. Whenever necessary, the dog was maintained on artificial respiration by attachment of the external end of the tube to a respirator pump which delivered 200-300 cc/stroke at the rate of 15 strokes per minute. inflated Digoxin (0.05 mg/kg) was then administered as an i.v. infusion over a period of 5 mins. through a heparin lock placed in the saphenous vein of the forearm of the dog. The EKG electrodes and the heart sound microphone were placed in position. The pressure transducer was connected to the electrocardiogram but the funnel pick-up was not placed in position until just before recording the carotid pulse tracing. This is because a perfect seal is required between the funnel pick-up of the pressure transducer and the surface where the pulsations of the carotid artery are measured. This could only be accomplished manually, since the use of a belt resulted in excessive pressure around the neck region. Also, use of a belt resulted in minute changes in the position of the funnel pick-up, resulting in deterioration of the quality of the carotid pulse tracing obtained. Blood samples were drawn and the Lead I or II electrocardiograms, phonocardiogram and the carotid pulse tracings were simultaneously obtained at specific time intervals over a period of 72 hours. The dog was given maintenance doses of pentobarbital on the first day until collection of the 12 hour sample. Pentobarbital was then discontinued and the dog recovered from the anesthesia. The dog was again anesthetized with pentobarbital each time before blood samples were drawn and the pharmacodynamic responses simultaneously measured at 24, 32, 48, 56 and 72 hrs. Five ml.'s of blood were drawn at each of the pre-determined intervals. The resulting serum was used to assay for digoxin content by RIA. After assaying by RIA, aliquots of the serum

were diluted with blank serum so that their digoxin concentrations were between 0.5 and 4 ng/ml. These samples were then assayed using HPLC.

In a similar study dog no. 3 died of respiratory depression prior to digoxin administration.

Study No. 4

Dog no.: 1

Weight: 13 kg.

Sex: Male

This study was conducted similar to studies 2 and 3. The most important difference between this study and the previous studies is in the sampling times. Blood samples were drawn frequently and the pharmacodynamic responses measured simultaneously over a period of 10 hrs. As in previous studies the dog was under anesthesia through the 10 hrs. of the study. After administration of anesthesia the heart rate (HR) was allowed to stablize. HR was monitored frequently and after it had reached a stable value (usually after 30-60 mins.), digoxin was administered. The dose of digoxin administered was 0.05 mg/kg. i.v. given over a period of 5 mins.

Study No. 5

Dog no.: 2

Weight: 11.5 kg.

Sex: Female

Dog 2 died of respiratory depression after administration of pentobarbital and prior to the administration of digoxin.

Study No. 6

Dog no.: 1

Weight: 13.0 kg.

Sex: Male

This study is identical to study no. 4. Dog no. 1 was again used in the study. The study was aborted 2.5 hrs. after adminstration of digoxin because sites could not be found in the fore and hind legs for placement of a heparin lock. The dose of digoxin administered was 0.025 mg/kg. i.v. over a period of 5 mins.

Study No. 7

Dog no.: 1

Weight: 13.0 kg.

Sex: Male

Since study no. 6 was prematurely aborted, the study was repeated (a month later) using dog no. 1. The procedures used were identical to those used in study no. 6 The dose of digoxin administered was 0.025 mg/kg. i.v. as an infusion over 5 mins. Blood samples were drawn and pharmacodynamic responses measured frequently over a period of 10 hrs.

Study No. 8

Dog no.: 6

Weight: 17.0 kg.

Sex: Male

This study is identical to that of study nos. 2 and 3. The digoxin dose administered was 0.05 mg/kg. i.v. as an infusion over 5 mins. followed by 3 ml. of saline to flush the tubing.

Study No. 9

Dog no.: 7

Weight: 9.75 kg.

Sex: Female

The study is identical to that of study nos. 2 and 3. The dose of digoxin administered was 0.05 mg/kg. i.v. as an infusion over 5 mins. followed by 3 ml. of normal saline.

Study no. 10

Dog no.: 6

Weight: 16.4 kg.

Sex: Male

This study was a repetition of study no. 8. The study was repeated since analysis of the data from study no. 8 revealed that the HR of the dog was not stable at the time of administration of digoxin. Therefore, in this study the HR was frequently monitored after induction of anesthesia to determine that the HR had indeed stabilized and did not show any large fluctuations over time. After this was verified digoxin was administered (0.05 mg/kg. i.v. as an infusion over 5 mins. followed by 3 ml. of saline to flush the tubing).

CHAPTER 3

RESULTS

Pharmacokinetic modelling

Computer programs are generally used to conduct regression procedures on the concentration-time data obtained from pharmacokinetic studies. These procedures permit characterization of the polyexponential equations used to describe the concentration-time profile. They also permit accurate determination of parameters used in the polyexponential equations.

Nonlinear regression procedures (NONLIN - 1976) were conducted on the plasma concentrations (C_t) as a function of time obtained from the initial pharmacokinetic studies. The digoxin plasma concentration *vs.* time data obtained in the initial pharmacokinetic studies are tabulated in tables 3.1, 3.2 and 3.3 Bi-exponential and tri-exponential equations were fit to the data. The tri-exponential equation was found to best fit the data. The following bi-exponential and tri-exponential equations were fitted to the data:

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} \qquad ...(3.1)$$

$$C_t = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \qquad ...(3.2)$$

A typical NONLIN "deck" (excluding the data) submitted to the computer is given in table 3.4 The values of the parameters obtained by nonlinear fitting are listed in table 3.5

The model was selected based on on the F-test (Boxenbaum *et al.*,1974) AIC (Akaike information criterion, Akaike H. 1973,1976: Yamaoka K. *et al.*,1978) and

Dog no.: 1

TABLE 3.1
Initial Pharmacokinetic Studies

TIME (hrs.)	CONC. (ng./ml)
0.50	31.30
1.00	12.94
1.50	8.20
2.00	6.38
3.50	4.04
5.50	3.56
7.67	3.28
9.58	3.02
16.92	2.31
24.20	1.36
30.75	1.25
47.83	0.86

Plasma concentration vs. time data following administration of 0.05 mg/kg of digoxin i.v. (uniformly over a period of 5 mins.) to a 13.2 kg normal beagle dog.
TIME = time post-infusion

Dog no.: 2

TABLE 3.2
Initial Pharmacokinetic Studies

TIME (hrs.)	CONC. (ng./ml)
0.25	34.80
0.50	21.32
1.00	12.20
1.50	9.95
3.50	6.28
7.50	3.86
11.00	3.24
17.07	1.94
23.92	2.08
31.00	1.64
38.00	1.16
54.00	0.96
77.00	0.55

Plasma concentration vs. time data following administration of 0.05 mg/kg. digoxin i.v. (uniformly over a period of 5 mins.) to a 10.0 kg normal beagle dog.
TIME = time post-infusion

Dog no.: 3

TABLE 3.3
Initial Pharmacokinetic Studies

TIME (hrs.)	CONC. (ng./ml)
0.25	35.62
0.50	18.55
1.00	9.38
1.50	6.26
3.50	4.13
7.50	3.32
11.00	2.49
17.00	1.94
24.13	1.04
31.03	1.08
38.00	0.87
54.50	0.81
78.00	0.41

Plasma concentration vs. time data following administration of 0.05 mg/kg. digoxin i.v (uniformly over a period of 5 mins.) to a 9.75 kg. normal beagle dog.
TIME = time post-infusion

```
JOB CARD
/ STEP EXEC FORTXCLG
//FORT.SYSIN DD *
         SUBROUTINE PLOTS(X)
        ENTRY PLOT(X)
         ENTRY AXIS(X)
         ENTRY SYMBOL(X)
         ENTRY WHERE(X)
        ENTRY DATE(X)
        RETURN
        END
C       ..........................................................
C        NONLIN    NONLIN    NONLIN    NONLIN    NONLIN    NONLIN    NONLIN
C
C          THIS IS FOR   3    COMPARTMENT MODEL    STUDY #9
C
C        NONLIN    NONLIN    NONLIN    NONLIN    NONLIN    NONLIN    NONLIN
C          ..........................................................
        SUBROUTINE DFUNC(F,P,CON,VAL,X,I,J,ISPEC,XVEC,Y,W,NOBS)
        IMPLICIT REAL*8 (A-H,O-Z)
        DIMENSION ISPEC(1),NOBS(1)
        DOUBLE PRECISION P(1),VAL(1),F,CON(1),Y(1),W(1),X,XVEC(1)
       A1=P(1)
       ALPHA=P(2)
       B1=P(3)
       BETA=P(4)
       C1=P(5)
       GAMMA=P(6)
       E2=BETA*(X)
       E1=ALPHA*(X)
       E3=GAMMA*(X)
       IF(E1.GT.170.)E1=170.
       IF(E2.GT.170.)E2=170.
       IF (E3.GT.170)E3=170
       F=A1/DEXP(E1) + B1/DEXP(E2)+ C1/DEXP(E3)
        RETURN
        END
//LKED.SUB DD DSN=VCU.STATPGMS.DISP=SHR
//LKED.SYSIN DD *
     ENTRY MAIN
    INCLUDE SUB(NONLN1,NONLN2,MISC)
//GO.FT03F001 DD UNIT=SYSSQ,SPACE=(1200,(100,100))
//GO.PLOTTAPE DD DUMMY
//GO.SYSIN DD *
        .
        .
        .
       DATA
        .
        .
        .
```

TABLE 3.4 A typical NONLIN "deck" submitted for fitting a tri-exponential equation to the data.

TABLE 3.5
NONLIN ANALYSES
Initial pharmacokinetic studies

DOG NO.	3		2		1	
	2-COMP.	3-COMP.	2-COMP.	3-COMP.	2-COMP.	3-COMP.
A (ng/ml.)	7.49(2.16)*	52.06(11.32)	11.68(2.68)	34.23(8.42)	8.14(3.35)	66.56(12.33)
B (ng/ml.)	1.42(0.28)	4.62(0.92)	2.01(0.34)	4.42(1.24)	1.98(0.54)	4.32(0.39)
C (ng/ml.)		1.54(0.28)		1.44(0.42)		0.85(0.19)
α (hr^{-1})	0.17(0.053)	2.65(0.43)	0.19(0.037)	1.44(0.32)	0.24(0.13)	1.98(0.21)
β (hr^{-1})	0.014(0.0053)	0.13(0.018)	0.014(0.0038)	0.74(0.014)	0.015(0.016)	0.074(0.014)
γ (hr^{-1})	—	0.015(0.0039)		0.012(0.0038)	—	0.0032(0.0016)
SSW	1.36	0.15	1.71	0.40	3.41	0.18
d.f.	9	7	9	7	8	6
N	13	13	13	13	12	12
R^2	0.99	1.00	0.99	1.00	0.99	1.00
CORR	0.78	0.997	0.86	0.99	0.78	0.998
F		28.23		11.46	—	53.43
AIC	12.0	-12.66	14.97	0.09	22.72	-8.6
WT.	$1/Y^2$	$1/Y^2$	$1/Y^2$	$1/Y^2$	$1/Y^2$	$1/Y^2$

AIC = Aikaike information criterion
SSW = weighted sum of square deviations
* numbers in brackets are standard deviations

the SSW (weighted sum of squared deviations). The criteria for the selection of models is discussed in greater detail later in this chapter. The value for AIC was calculated as follows:

$$AIC = N \cdot ln(SSW) + 2p$$

where,

N is the total number of observations used for regression,

p is the number of parameters in the equation, and,

SSW is the weighted sum of square deviations

The best fit was obtained using a tri-exponential equation. It is evident therefore that digoxin follows tri-exponential kinetics in the beagle dog. This is in agreement with existing pharmacokinetic studies of digoxin in humans (Kramer *et al.*,1974; Sumner and Russell, 1976; Balant *et al.*,1980) and dogs (Kuhlman *et al.*,1979; Bruznock 1973; Morgan and Binnion 1970).

Similar nonlinear regression fitting was conducted on the data obtained in studies 4, 7, 8, 9 and 10. The results are summarized in table 3.6.

Again using the F-test, AIC and SSW the tri-exponential equation was found to give the best fit. Regression analysis was not conducted on the serum concentration as a function of time data, obtained from Study no. 6. due to a lack of sufficient number of data points required for such analyses. The values in the last column in table 3.6 are the mean and standard deviations of the pharmacokinetic parameters listed for studies 4, 8, 9, 10. The small standard deviations indicate that the pharmacokinetic profile of digoxin in the 3 beagle dogs is quite similar and constant.

Pharmacodynamic Modelling

The plasma concentration vs. time data and the response vs. time data for studies 1A, 1B, 2-4 and 6-10 are given in tables 3.7-3.16 respectively.

TABLE 3.6
NONLIN ANALYSES
(Studies 4, 7-10)

DOG NO.	1		6	7	6	
STUDY NO.	4	7	8	9	10	(4,8,9,10)
DOSE (mg/kg.)	0.05	0.025	0.05	0.05	0.05	0.05
A (ng/ml.)	60.98(6.45)	33.51(2.69)	27.38(9.7)	65.24(3.09)	35.53(6.32)	40.88(5.49)
B (ng/ml.)	24.97(2.42)	11.20(0.55)	31.57(5.78)	11.97(1.09)	41.79(2.35)	31.04(2.29)
C (ng/ml.)	5.02(0.41)	2.29(0.03)	6.06(0.40)	7.53(0.35)	3.73(0.11)	5.37(0.23)
α ($min.^{-1}$)	0.064(0.0072)	0.11(0.005)	0.053(0.0094)	0.044(0.0016)	0.10(0.014)	0.061(0.0049)
β ($min.^{-1}$)	0.012(0.00049)	0.017(0.006)	0.018(0.0016)	0.012(0.0005)	0.018(0.00057)	0.016(0.00068)
γ ($min.^{-1}$)	0.00099(0.00019)	0.00057(0.00004)	0.0012(0.00016)	0.0018(0.000099)	0.00043(0.000059)	0.0011(0.000087)
RSQUARE	1.00	0.99	0.99	1.00	1.00	0.99
CORR.	0.99	0.99	0.99	0.99	0.99	0.99
SSW	1.62	0.11	1.8	0.77	1.80	29.96
WT.	$1/Y^2$	$1/Y^2$	$1/Y^2$	$1/Y^2$	$1/Y^2$	$1/Y^2$
AUC	8104.35	4981.00	7320.49	6663.56	11351.38	7684.16(2350.85)
V_1 (ml/kg.)	549.56	531.90	769.11	574.89	620.65	609.22(95.41)

* numbers in brackets are standard deviations
AUC = area under the plasma conc. time curve (ng. min./ml.)
SSW = sum of wieghted square deviations

TABLE 3.7
Study 1A

TIME (mins.)	CONC. (ng./ml)	LVET (± s.d.) (msec.)	QS2 (± s.d.) (msec.)	RR (± s.d.) (msec.)	PR (± s.d.) (msec.)	S1S2 (± s.d.) (msec.)
-5.0	0.0	121.7 *2.89*	190 *0*	335 *21.21*	78.33 *7.64*	170 *0*
15	53.17	— —	— —	— —	— —	— —
30	12.72	119 *3.94*	193 *4.83*	349 *3.16*	85 *0*	178.5 *3.37*
45	7.85	147.5 *3.59*	215 *7.07*	350 —	85 —	190 —
60	5.88	143 *4.83*	194.5 *2.84*	353 *4.83*	86 *2.11*	176 *5.16*
75	4.6	147.5 *4.25*	190 *2.36*	346.5 *4.12*	80 *0*	170 *0*
90	3.78	145 *4.71*	188.5 *2.42*	330.5 *1.58*	82 *2.58*	166.5 *4.12*
120	3.06	131.5 *3.37*	183 *5.37*	321 *3.16*	75 *5.27*	160.5 *1.28*
210	1.93	100.5 *5.99*	167 *2.58*	280.5 *1.58*	80 *0*	148.5 *3.37*
240	1.64	125 *6.67*	169.5 *1.58*	306.5 *4.74*	80.5 *1.58*	153 *4.22*
270	1.5	118 *2.58*	170 *0*	298.5 *3.37*	80 *0*	145 *0*

Plasma concentration vs. time and response vs. time data following administration of 0.05 mg/kg digoxin i.v. (uniformly over 5 min) to a anesthetized normal mongrel dog. TIME = time post-infusion, LVET = left ventricular ejection time, QS2 = duration of total electromechanicl systole, RR = R-R interval, PR = P-R interval and S1S2 = duration between first and second heart sounds.
— indicates intervals were not measured.

TABLE 3.8
Study 1B

TIME (hrs.)	CONC. (ng/ml.)	LVET (± s.d.) (msec)	QS2 (± s.d.) (msec)	RR (± s.d.) (msec)	PR (± s.d.) (msec)	S1S2 (± s.d.) (msec)
-0.08	0	240.00 *0.0*	888.00 *126.67*	1074.67 *129.06*	246.15 *15.02*	440.00 *0.0*
0.33	33.28	320 *0.0*	1003.33 *317.47*	1280 *190.69*	266 *16.46*	445.45 *18.09*
1.33	7.65	268.57 *19.51*	990 *82.11*	1231.11 *76.88*	257.78 *21.08*	400 *0.0*
1.83	5.36	212.5 *18.32*	1246.67 *190.01*	1480 *197.58*	280 *0.0*	443.64 *21.57*
3.08	3.76	212.5 *10.35*	1280 *174.61*	1460 *110.45*	268 *16.86*	415 *29.76*
4.92	3.30	213.33 *17.32*	1256 *173.44*	1464 *184.61*	266 *18.97*	448.89 *17.64*
7.00	3.10	231.42 *27.94*	1065 *157.8*	1285 *173.11*	265 *10.0*	400 *0.0*
23.33	1.70	200 *0.0*	1155.56 *213.02*	1371.11 *207.63*	245.45 *15.72*	404 *8.34*
30.83	2.00	224 *8.94*	1112.73 *227.56*	1327.27 *245.16*	240 *0.0*	440 *0.0*
48.0	1.20	238.18 *10.99*	1245 *178.16*	1405.71 *114.14*	250 *15.12*	440 *0.0*
54.75	0.91	246.67 *24.49*	1047.69 *110.62*	1263.68 *118.56*	243.08 *7.51*	444.62 *27.27*
72.42	0.71	245 *9.04*	1034.28 *135.74*	1241.23 *122.65*	248.5 *18.75*	440 *0.0*
78.5	0.49	237.14 *17.99*	1138.67 *194.71*	1354.67 *193.09*	253.33 *17.99*	424.44 *19.44*

Plasma concentration vs. time and response vs. time data following administration of 0.05 mg/kg digoxin i.v. (uniformly over 5 min) to a normal female mongrel dog. TIME= time post-infusion. LVET = left ventricular ejection time, QS2 = duration of total electromechanicl systole, RR = R-R interval. PR = P-R interval and S1S2 = duration between first and second heart sounds.

TABLE 3.9
Study 2

TIME (hrs.)	CONC. (ng./ml)	LVET (± s.d.) (msec.)	RR (± s.d.) (msec.)	PR (± s.d.) (msec.)	QS2 (± s.d.) (msec.)	S1S2 (± s.d.) (msec.)
-0.08	0	185.60 *1.77*	808.88 *16.16*	132.50 *2.64*	380.00 *0*	320.91 *3.02*
0.33	35.53	230.9 *9.17*	882.33 *18.12*	129.58 *3.5*	401.82 *6.15*	330 *0*
0.63	19.25	195.71 *15.12*	750.77 *18.12*	123.00 *3.5*	386 *6.15*	300 *0*
0.92	55.22	220.5 *3.69*	873.75 *80.52*	121 *3.94*	398.12 *10.33*	317.22 *6.18*
1.50	7.58	183.57 *12.49*	1010.56 *58.76*	145 *4.08*	444.5 *5.99*	376.5 *3.37*
3.83	4.54	254 *2.11*	1470.6 *352.85*	137.22 *3.63*	455.71 *9.32*	371.36 *5.04*
7.50	3.47	292 *9.49*	1341.87 *305.55*	135.5 *3.69*	476.5 *11.32*	381.67 *6.12*
24.83	1.68	150 *0*	465 *0*	123.5 *4.74*	325 *61.37*	290 *6.32*
31.83	1.65	139.28 *10.17*	523.75 *29.61*	113.33 *5.36*	314.54 *6.50*	213.63 *0*
48.00	1.11	160 *0*	634.28 *79.73*	132.08 *8.38*	339.17 *9.73*	210 *0*
55.58	0.81	151 *2.11*	500.5 *15.54*	123.5 *4.12*	293 *16.36*	203.5 *7.47*
72.83	0.69	141.42 *14.64*	537.73 *12.52*	131.5 *3.37*	319.5 *3.69*	220.5 *1.58*

Plasma concentration vs. time and response vs. time data following administration of 0.05 mg/kg digoxin i.v. (uniformly over 5 min) to a normal beagle dog. TIME= time post-infusion. LVET = left ventricular ejection time, QS2 = duration of total electromechanicl systole, RR = R-R interval, PR = P-R interval and S1S2 = duration between first and second heart sounds.

TABLE 3.10
Study 3

TIME (hrs.)	CONC. (ng./ml)	LVET (± s.d.) (msec.)	RR (± s.d.) (msec.)	PR (± s.d.) (msec.)	QS2 (± s.d.) (msec.)	S1S2 (± s.d.) (msec.)
-0.08	0.0	135.00 *7.07*	440.40 *9.4*	140.42 *1.44*	347.92 *6.2*	191.36 *12.67*
00.25	31.50	189.50 *1.58*	551.00 *27.36*	151.50 *5.8*	223.75 *6.94*	220.50 *3.69*
00.45	16.83	200.00 *0.0*	488.33 *13.69*	143.88 *4.17*	— —	211.43 *0.78*
00.92	***	144.17 *4.91*	502.00 *13.16*	150.00 *4.71*	220.00 *0*	208.57 *3.78*
1.25	5.58	140.00 *0*	468.00 *9.48*	140.00 *0*	338.00 *11.35*	192.00 *8.88*
1.75	3.24	132.27 *2.61*	470.00 *3.87*	144.09 *5.84*	341.11 *8.58*	198.18 *4.04*
3.58	1.62	140.00 *5.0*	461.11 *5.46*	140.00 *0*	214.44 *5.27*	230.00 *4.63*
7.75	2.48	161.67 *2.5*	550.00 *0*	135.00 *2.36*	249.00 *9.07*	222.78 *3.63*
12.00	2.01	207.27 *6.07*	625.50 *3.69*	149.50 *1.58*	400.50 *12.12*	243.00 *4.83*
16.00	1.99	205.45 *6.87*	642.00 *4.22*	150.00 *0*	297.00 *4.83*	255.55 *5.27*
24.33	1.55	112.72 *5.18*	371.36 *3.23*	157.73 *3.44*	180.45 *1.51*	163.57 *3.78*
36.00	1.12	130.00 *0*	472.00 *15.31*	149.50 *5.85*	204.00 *5.31*	178.50 *8.51*
48.00	0.61	133.00 *2.58*	461.67 *8.66*	140.55 *1.67*	214.00 *2.24*	196.67 *12.25*
60.25	0.49	133.18 *3.37*	461.82 *11.47*	143.64 *6.36*	204.09 *6.64*	183.63 *3.93*
72.00	0.37	132.78 *3.63*	500.50 *34.52*	151.50 *2.42*	212.00 *4.22*	181.50 *4.74*

Plasma concentration vs. time and response vs. time data following administration of 0.05 mg/kg digoxin i.v. (uniformly over 5 min) to a normal beagle dog. TIME = time post-infusion, LVET = left ventricular ejection time, QS2 = duration of total electromechanicl systole. RR = R-R interval. PR = P-R interval and S1S2 = duration between first and second heart sounds. (***) indicates not monitored and (—) indicates not measureable due to ill-defined landmarks on recording.

TABLE 3.11
Study 4

TIME (mins.)	CONC. (ng./ml)	LVET (± s.d.) (msec.)	RR (± s.d.) (msec.)	PR (± s.d.) (msec.)	QS2 (± s.d.) (msec.)	S1S2 (± s.d.) (msec.)
-45	0.0	187.50 *4.25*	507.78 *6.2*	143.50 *4.7*	245.00 *7.1*	201.40 *10.3*
-40	0.0	188 *2.6*	593.9 *18.3*	133 *5.4*	257.5 *9.9*	225 *5.5*
-10	0.0	209 *5.2*	638.6 *31.9*	145.9 *4.9*	290 *8.2*	281 *6.5*
10	58.01	233 *4.8*	810 *40.3*	156.5 *10.3*	312.8 *3.9*	293 *6.7*
20	40.56	255 *5.3*	989.4 *230.2*	152 *4.2*	331.2 *6.3*	293.3 *5.8*
30	31.76	257.5 *12.7*	1067.7 *189.7*	159.2 *5.6*	320 *5.3*	291.4 *4.5*
40	26.64	249.3 *6.1*	895 *118.1*	158.1 *5.3*	319.4 *6.8*	291.4 *16.0*
50	21.36	251.5 *8.2*	1009.4 *178.7*	155 *3.5*	336 *8.9*	301.7 *7.5*
60	19.76	266 *5.2*	1060.4 *255.6*	157.5 *6.3*	342.1 *7.6*	301.4 *9.0*
70	16.08	244.4 *12.6*	892.8 *83.0*	152.8 *4.4*	338.1 *10.0*	296.2 *12.7*
80	15.04	250.7 *5.3*	775.6 *42.7*	150 *0.0*	303.3 *8.7*	278.6 *8.7*
90	12.24	245.7 *5.3*	791.5 *42.7*	150.6 *1.8*	308.8 *4.8*	279 *10.2*
100	12.16	277.2 *8.3*	1110 *31.2*	160.5 *6.0*	346 *13.4*	321.4 *37.6*
110	11.12	281.1 *11.4*	1153.8 *354.3*	157.2 *5.0*	358.7 *15.5*	363.3 *15.0*

TABLE 3.11 (contd.)

TIME (mins.)	CONC. (ng./ml)	LVET (± s.d.) (msec.)	RR (± s.d.) (msec.)	PR (± s.d.) (msec.)	QS2 (± s.d.) (msec.)	S1S2 (± s.d.) (msec.)
120	11.36	236.7 *4.3*	728.1 *15.1*	147.8 *7.9*	— —	287.5 *11.6*
130	10.16	272.1 *4.9*	832 *41.0*	145.9 *44.2*	342.2 *6.7*	308.6 *13.4*
150	7.95	231.5 *6.7*	680.5 *25.7*	145 *5.0*	315.6 *10.4*	293.5 *8.8*
170	8.01	250.5 *4.4*	803.1 *97.8*	150 *6.7*	332.7 *2.6*	305 *7.1*
190	7.22	237.5 *4.2*	853.6 *162.4*	143.5 *9.7*	333.5 *10.0*	298.5 *15.3*
210	6.44	242.7 *6.5*	743.6 *62.4*	143 *6.3*	324.4 *9.2*	301.1 *6.0*
230	5.82	234.5 *6.8*	685 *18.4*	153 *12.3*	330 *4.1*	286 *5.7*
250	5.51	233 *4.2*	633 *6.3*	143 *8.9*	309 *7.4*	280 *4.7*
270	5.17	238 *7.9*	675.5 *4.4*	160.5 *12.8*	320 *3.3*	280 *3.2*
290	4.69	240 *0*	688 *6.3*	150.5 *2.8*	328.5 *2.4*	291 *9.7*
310	4.48	238.5 *2.4*	627.5 *2.6*	150 *0*	307.5 *3.5*	279 *5.2*
330	4.18	232 *4.2*	633.5 *5.8*	148.5 *6.2*	310.5 *1.6*	280 *9.4*
350	3.62	231 *3.2*	640 *0*	148 *2.7*	310 *14.6*	313.9 *9.1*
390	3.43	232.5 *8.9*	704.5 *6.0*	150 *4.1*	310 *5.8*	282.5 *4.2*

TABLE 3.11 (contd.)

TIME (mins.)	CONC. (ng./ml)	LVET (± s.d.) (msec.)	RR (± s.d.) (msec.)	PR (± s.d.) (msec.)	QS2 (± s.d.) (msec.)	S1S2 (± s.d.) (msec.)
410	3.33	240 *0*	730.5 *1.6*	152 *6.3*	324.5 *7.6*	281.5 *2.4*
450	3.22	244 *5.7*	784.5 *13.8*	158.5 *5.8*	330 *2.3*	314 *5.7*
470	3.28	250 *0*	767.5 *2.6*	154.5 *2.8*	342.5 *5.9*	308.5 *5.8*
510	3.14	247.5 *4.2*	729 *5.2*	153.5 *4.7*	336.1 *4.8*	301 *3.2*
530	3.17	249 *2.1*	697.1 *4.9*	150.6 *5.6*	325 *5.0*	295 *5.8*
550	3.10	238 *4.8*	656.9 *10.0*	154.4 *6.8*	313.5 *6.2*	288 *6.3*
570	2.92	240.5 *5.0*	685 *26.2*	150 *7.1*	320 *2.4*	291.5 *4.7*
590	2.89	240 *4.1*	713.6 *7.5*	147.1 *3.9*	328 *3.5*	287 *4.3*
610	2.77	248 *6.7*	689.4 *3.9*	149.5 *2.8*	328.5 *2.4*	292 *4.2*

Plasma concentration vs. time and response vs. time data following administration of 0.05 mg/kg digoxin i.v. (uniformly over 5 min) to a normal beagle dog. TIME = time post-infusion. LVET = left ventricular ejection time, QS2 = duration of total electromechanicl systole, RR = R-R interval, PR = P-R interval and S1S2 = duration between first and second heart sounds.

(—) indicates that interval could not be measured due to ill-defined landmarks on recording.

TABLE 3.12
Study6

TIME (mins.)	CONC. (ng./ml)	LVET (± s.d.) (msec.)	RR (± s.d.) (msec.)	PR (± s.d.) (msec.)	QS2 (± s.d.) (msec.)	S1S2 (± s.d.) (msec.)
-55.00	0.0	187.00 *2.73*	558.30 *7.07*	140.56 *3.91*	266.25 *5.18*	250 *8.66*
-45.00	0.0	176.11 *6.51*	569.00 *14.3*	141.50 *4.12*	275.50 *4.97*	243 *3.5*
-35.00	0.0	190.00 *7.07*	606.5 *28.48*	138.5 *5.3*	247.5 *2.63*	250 *0*
-25	0.0	175 *4.08*	570.83 *30.59*	138.0 *4.83*	248 *4.22*	234 *3.94*
-15	0.0	178 *2.58*	542.27 *14.21*	134.17 *4.17*	250 *0*	234.58 *2.57*
0	***	174.44 *1.67*	574.38 *21.78*	136.11 *4.86*	257.14 *9.06*	242.22 *4.41*
10	27.6	— —	934.64 *323.95*	151.5 *5.8*	287.86 *5.67*	247 *18.14*
20	***	213.75 *8.54*	1068.18 *427.27*	145.56 *4.64*	298.12 *5.30*	274 *15.16*
30	11.6	195 *7.07*	1050.5 *305.48*	153 *9.19*	298.57 *6.27*	274 *5.98*
40	7.6	212.5 *6.12*	1036.07 *320.75*	151.07 *8.81*	297.14 *8.59*	274.44 *11.30*
50	5.76	195 *17.61*	844 *186.16*	148 *3.5*	295.56 *4.64*	275 *5.98*
70	***	223.75 *4.79*	600.45 *19.03*	138.18 *3.37*	276.25 *3.54*	229.44 *6.82*
85	5.44	— —	737.78 *63.69*	144 *8.43*	281.43 *3.78*	228.33 *7.07*
100	3.8	200 0.0	691.67 *18.37*	145.56 *3.91*	281.25 *6.94*	241.67 *9.01*

TABLE 3.12 (contd.)

TIME (mins.)	CONC. (ng./ml)	LVET (± s.d.) (msec.)	RR (± s.d.) (msec.)	PR (± s.d.) (msec.)	QS2 (± s.d.) (msec.)	S1S2 (± s.d.) (msec.)
115	***	188.89 *7.41*	588.12 *2.59*	141.88 *2.59*	262.14 *5.67*	228.89 *7.82*
130	***	187.5 *41.24*	661.5 *36.52*	139.44 *7.68*	276.88 *7.04*	237.78 *8.33*
145	***	209 *2.11*	— —	— —	— —	247.86 *11.13*

Plasma concentration vs. time and response vs. time data following administration of 0.025 mg/kg digoxin i.v. (uniformly over 5 min) to a normal beagle dog. TIME = time post-infusion, LVET = left ventricular ejection time, QS2 = duration of total electromechanicl systole, RR = R-R interval, PR = P-R interval and S1S2 = duration between first and second heart sounds.

(***) indicates serum concentrations were not monitored.

(—) indicates intervals could not be measured due to ill-defined peaks on recording.

TABLE 3.13
Study 7

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
-54	0.0	384.28 *1.82*
-45	0.0	403.57 *6.33*
-35	0.0	393.33 *4.44*
-25	0.0	381.15 *3.0*
-20	0.0	396.54 *3.15*
-5	0.0	392.86 *4.69*
8	24.72	476.92 *7.23*
15	18.08	480.77 *10.58*
25	11.84	528.33 *14.2*
35	8.48	503.21 *7.5*
45	7.86	545 *13.84*
55	6.73	586.43 *26.34*
65	5.42	617.14 *32.45*
75	5.63	603.85 *16.6*

TABLE 3.13 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
85	4.88	660 *51.96*
95	4.52	662.31 *37.28*
105	4.37	683.75 *38.44*
115	3.66	718.46 *38.32*
125	3.42	684.62 *40.8*
140	3.26	673.46 *15.99*
155	2.98	607.31 *29.97*
170	2.58	593.85 *36.29*
185	2.53	525 *39.58*
200	2.26	505 *11.48*
215	2.46	508.08 *10.32*
230	2.08	467.86 *8.92*
250	2.27	432.5 *12.52*
270	2.11	444.5 *7.76*

TABLE 3.13 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
290	1.98	421.15 *2.19*
310	***	437.08 *4.5*
320	1.88	423.75 *10.9*
330	***	418.08 *5.6*
340	***	416.92 *3.25*
350	1.83	413.46 *10.68*
360	***	424.23 *9.97*
370	***	412.69 *5.99*
380	1.78	410 *11.18*
395	***	415.77 *14.98*
410	1.91	386.53 *10.28*
440	1.97	390 *8.55*
460	***	396.92 *5.22*
470	1.84	381.15 *15.3*

TABLE 3.13 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
485	***	378.85 *15.3*
500	1.75	377.86 *12.36*
515	***	379.23 *14.84*
530	1.63	396.67 *4.88*
545	***	393.93 *4.46*
560	1.66	386.92 *2.53*
575	***	383.21 *4.64*
590	1.59	372.5 *5.46*
605	***	366.78 *3.17*
620	***	356.67 *3.26*
637	***	350 *4.08*
650	***	340.77 *2.77*
690	***	320 *0*

Plasma concentration vs. time and response vs. time data following administration of 0.025 mg/kg digoxin i.v. (uniformly over 5 min) to a normal beagle dog. TIME = time post-infusion, RR = R-R interval.
(***) indicates serum concentrations were not monitored.

TABLE 3.14
Study 8

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
-105	0.0	463.82 *6.5*
-90	0.0	527.06 *31.82*
-80	0.0	578 *47.08*
-70	0.0	572.69 *65.53*
-60	0.0	615.29 *54.47*
-45	0.0	654.21 *61.22*
-35	0.0	679.12 *80.45*
-20	0.0	728.57 *49.08*
-10	0.0	766.78 *137.04*
10	53.5	827.31 *76.04*
20	34.8	907.67 *119.15*
30	27.8	910 *119.13*
40	25.3	944.06 *84.25*

TABLE 3.14 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
50	19.4	610.48 *29.91*
60	20.49	742 *64.28*
70	18.1	919.23 *171.22*
80	12.9	703.75 *17.27*
90	11.3	912.33 *59.43*
100	10.6	909.06 *185.47*
110	9.8	610.94 *20.83*
125	9.15	524.83 *94.11*
140	7.3	620.29 *12.31*
155	5.8	668.66 *10.93*
170	7.33	669.06 *28.47*
185	6.85	639.05 *51.95*
205	5.71	704.06 *41.07*
230	5.37	668.33 *51.01*

TABLE 3.14 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
250	4.94	715.28 *64.89*
260	***	623.53 *8.43*
270	4.5	568.95 *35.18*
280	***	606.47 *27.08*
290	4.55	623.12 *42.38*
300	***	610.28 *46.51*
310	4.32	650.36 *15.12*
330	4.05	602.89 *25.29*
340	***	537.14 *24.0*
350	3.8	559.44 *36.76*
360	***	577.63 *5.10*
370	3.78	520.87 *11.93*
405	4.28	569.38 *7.93*
420	***	547.67 *23.82*

TABLE 3.14 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
435	3.29	589.33 *4.27*
450	***	601.92 *9.02*
465	3.27	585.62 *34.78*
485	***	592.14 *14.64*
495	3.33	634.33 *19.07*
510	***	532.25 *18.24*
525	3.21	542.5 *28.6*
540	***	407.04 *6.69*
555	3.10	493.04 *26.45*
570	***	562.81 *40.21*
585	3.11	601.67 *50.15*
600	***	410.26 *8.24*
615	2.8	532.81 *32.66*
630	***	346.54 *5.96*

Plasma concentration vs. time and response vs. time data following administration of 0.05 mg/kg digoxin i.v. (uniformly over 5 min) to a normal beagle dog. TIME = time post-infusion, RR = R-R interval.
(***) indicates serum concentrations were not monitored.

TABLE 3.15
Study 9

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
-142	0.0	575 *4.08*
-130	0.0	540.5 *16.41*
-120	0.0	629.5 *19.64*
-110	0.0	696 *35.5*
-100	0.0	678 *21.88*
-90	0.0	681.5 *24.6*
-80	0.0	615.5 *43.81*
-70	0.0	664 *36.12*
-60	0.0	620.5 *28.72*
-50	0.0	708 *52.4*
-40	0.0	637 *28.5*
-30	0.0	676 *40.4*
-20	0.0	694 *37.18*
-10	0.0	738.5 *76.05*

TABLE 3.15 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
10	61.6	704.5 *9.56*
20	42.99	668 *11.83*
30	31.98	727 *20.16*
40	25.63	730 *16.33*
50	21.00	756 *26.12*
60	16.31	839 *89.96*
70	15.25	796 *46.36*
80	12.89	826 *29.14*
90	11.62	894 *135.33*
100	10.10	811.5 *33.34*
110	9.56	903.5 *90.89*
120	9.03	902.5 *70.37*
135	8.70	824.5 *28.52*

TABLE 3.15 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
150	7.83	808.89 *17.46*
165	7.22	996.11 *48.84*
180	6.6	1193 *181.17*
195	6.36	913.5 *66.25*
210	5.69	1010.5 *91.66*
225	5.62	956.5 *47.9*
240	5.46	931 *37.4*
260	5.48	1035.5 *165.84*
270	***	944.44 *216.12*
280	4.89	918 *32.93*
290	***	861.5 *23.58*
300	4.54	827.5 *32.77*
310	***	1015.5 *114.47*
320	4.2	818 *32.51*

TABLE 3.15 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
330	***	926.5 *56.37*
340	4.47	921 *136.8*
350	***	972 *248.19*
360	4.05	881.5 *16.84*
375	***	822.5 *12.96*
390	3.8	664.5 *13.83*
405	***	803.5 *17.96*
420	3.58	784.5 *22.78*
435	***	750 *75.46*
450	3.54	947 *47.44*
465	***	788.5 *12.92*
480	3.15	870.5 *63.79*
515	***	652 *5.37*
510	2.84	646 *12.87*

TABLE 3.15 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
525	***	830 *109.14*
540	2.77	625.5 *14.62*
555	***	721.67 *30.21*
570	2.64	595.5 *22.78*
585	***	563 *7.15*
600	2.62	495 *4.71*

Plasma concentration vs. time and response vs. time data following administration of 0.05 mg/kg digoxin i.v. (uniformly over 5 min) to a normal beagle dog. TIME = time post-infusion, RR = R-R interval.
(***) indicates serum concentrations were not monitored.

TABLE 3.16
Study 10

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
-150	0.0	343.5 *8.83*
-140	0.0	346 *5.16*
-130	0.0	365.5 *4.97*
-120	0.0	359.5 *3.69*
-110	0.0	364.5 *5.5*
-100	0.0	344 *7.74*
-90	0.0	335 *5.77*
-80	0.0	352 *6.32*
-70	0.0	375 *7.07*
-60	0.0	386 *6.99*
-50	0.0	375.5 *4.38*
-40	0.0	375.5 *7.24*
-30	0.0	351.5 *5.3*
-20	0.0	361.5 *6.69*

TABLE 3.16 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
10	57.3	392 *6.75*
20	35.34	427 *4.22*
30	28.62	373.5 *5.78*
40	23.60	365.5 *7.98*
50	22.44	380 *3.33*
60	20.26	359 *5.16*
70	15.8	391.5 *4.12*
80	13.4	445.5 *12.8*
90	11.53	425.5 *4.97*
100	10.97	443.5 *6.69*
110	10.04	496.5 *16.17*
120	9.21	441.5 *3.37*
130	8.02	499 *6.58*

TABLE 3.16 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
150	6.7	494.5 *7.98*
165	5.7	497.5 *20.44*
180	5.00	474.5 *14.99*
195	4.5	473.5 *17.33*
210	4.25	493.5 *10.29*
225	3.88	466.5 *9.73*
240	3.86	535.5 *13.43*
260	4.03	504.5 *4.38*
270	***	479 *6.58*
280	3.8	436 *5.68*
290	***	439.5 *3.69*
300	3.8	434 *10.49*
310	***	468 *4.83*
320	3.64	436.5 *9.14*

TABLE 3.16 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
330	***	416.5 *7.09*
340	3.59	437.5 *7.17*
350	***	443 *5.87*
360	3.3	387 *20.44*
375	***	433 *4.83*
390	3.25	433 *16.36*
410	***	382.5 *5.89*
420	3.22	430.5 *3.69*
435	***	415.45 *13.12*
450	2.92	451.5 *10.55*
465	***	396.5 *6.25*
480	2.95	408.5 *7.83*
495	***	365 *6.67*
510	3.04	378.18 *9.56*

TABLE 3.16 (contd.)

TIME (mins.)	CONC. (ng./ml)	RR (± s.d.) (msec.)
525	***	419.5 *8.96*
540	3.04	374 *9.37*
555	***	358.5 *10.55*
570	2.80	372.5 *11.12*
585	***	340.5 *10.39*
600	2.84	368 *10.85*

Plasma concentration vs. time and response vs. time data following administration of 0.05 mg/kg digoxin i.v. (uniformly over 5 min) to a normal beagle dog. TIME = time post-infusion, RR = R-R interval.
(***) indicates serum concentrations were not monitored.

LVET, QS_2, P-R interval and the R-R interval *vs.* time and serum concentration of digoxin were plotted. The data appeared to be extremely noisy and no readily discernible relationship between the serum levels of digoxin and the response was apparent. The inherent noise in the data made simultaneous pharmacokinetic-pharmacodynamic analysis impractical. However a linear direct relationship between LVET. QS_2 and P-R interval and the HR was observed (*fig. 3.1, 3.2 and 3.3*).

Results of the regression analysis are as follows:

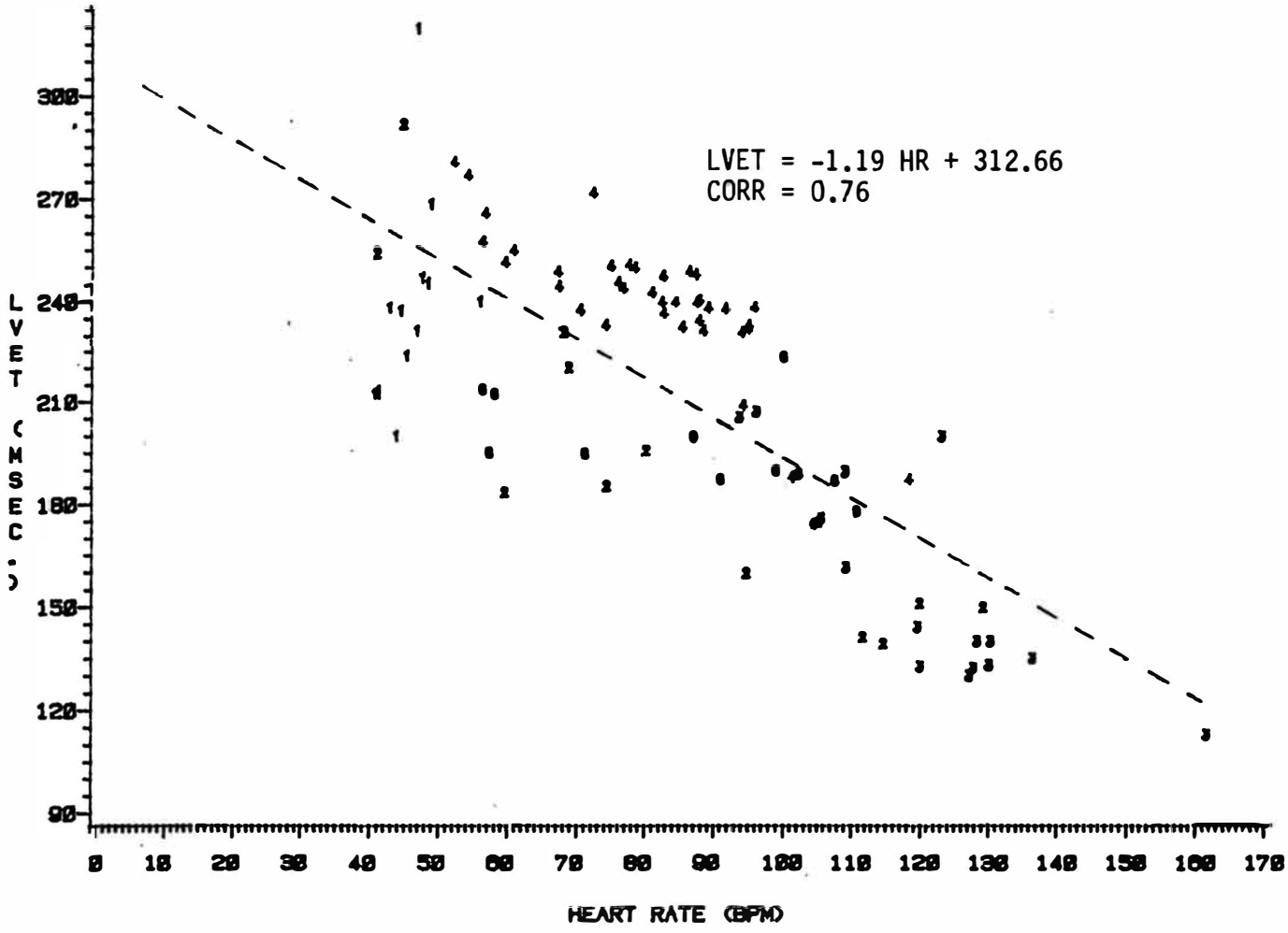
$$LVET = -1.19HR + 312.66 \qquad corr. = 0.76$$

$$QS_2 = -1.49HR + 442.46 \qquad corr. = 0.66$$

$$PR = -0.13HR + 155.21 \qquad corr. = 0.26$$

Although similar relationships have been observed between QS_2, LVET, PR interval and the HR in humans by Weissler *et al.*,(1968), similar relationships have not previously been reported in the literature for dogs. This observed relationship could be useful to investigators who intend to use the dog as a model for digoxin. It is observed (fig. 3.1) that Dog no.5 (Study no. 1B) does not follow the same linear relationship as expressed by the data from other studies. Hence the data obtained from study no. 1B was not incorporated in the regression analyses of LVET, QS_2, and PR interval with HR. Possible reasons for dog no. 5 not expressing the same linear relationship may be one or more of the following:

1) Dog no. 5 was not anesthetized, whereas all other studies were conducted with the dogs under pentobarbital anesthesia.
2) Dog no. 5 had a low basal heart rate, and hence did not show a large change in heart rate as compared to the other dogs.



LEGEND: STUDY NO. 1 1 1 1 2 2 2 2 3 3 3 3 4 4 4 4 6 6 6 6

FIG. 3.1 Relationship between left ventricular ejection time (LVET) and heart rate (beats per minute) in normal dogs administered 0.05 mg/kg. digoxin i.v. (as an infusion over 5 mins).

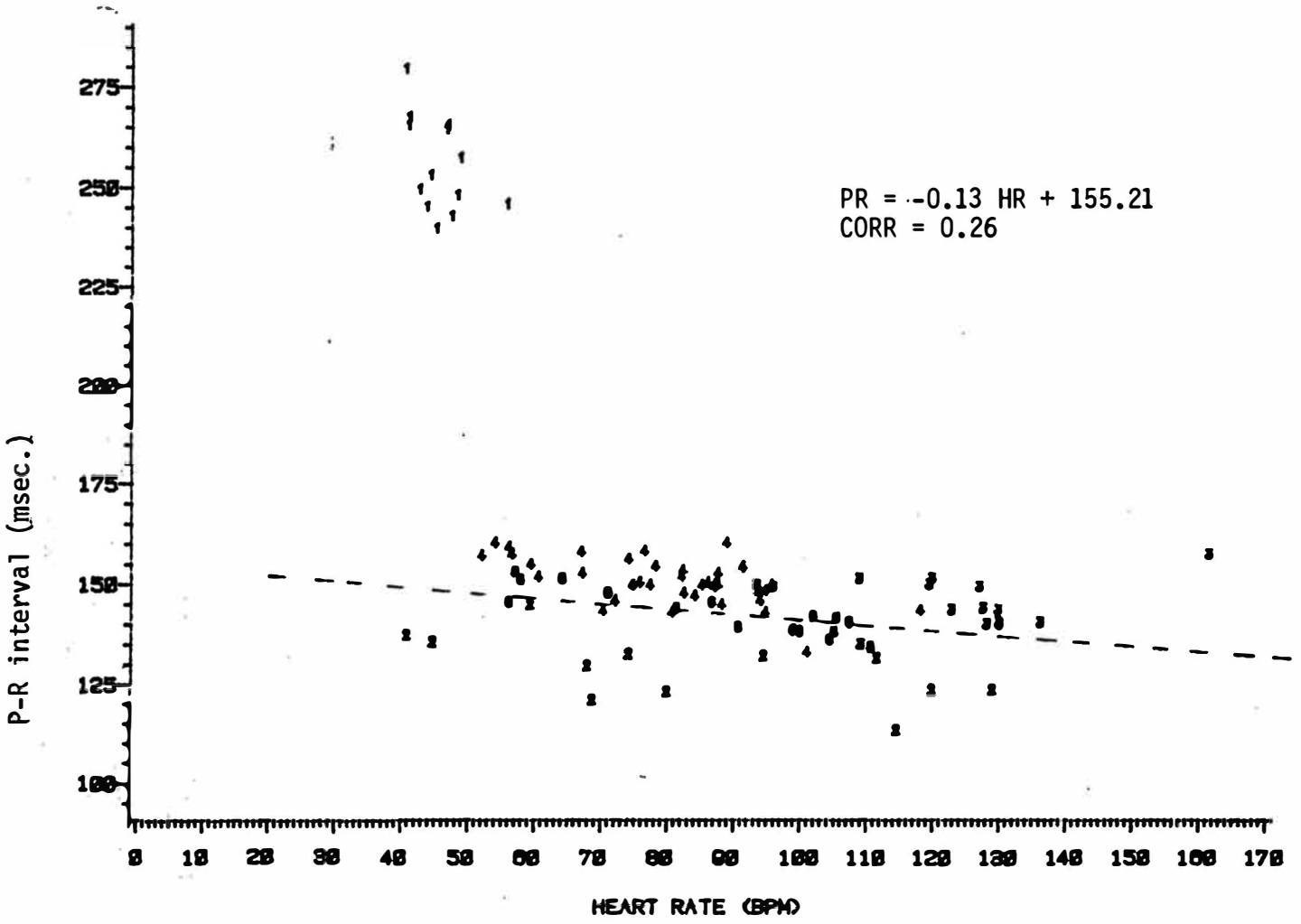


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FIG. 3.2 Relationship between P-R interval and heart rate (beats per minute) in normal dogs administered 0.05 mg/kg digoxin i.v. (infusion over 5 mins). Points from study 1B are not included in the regression line.

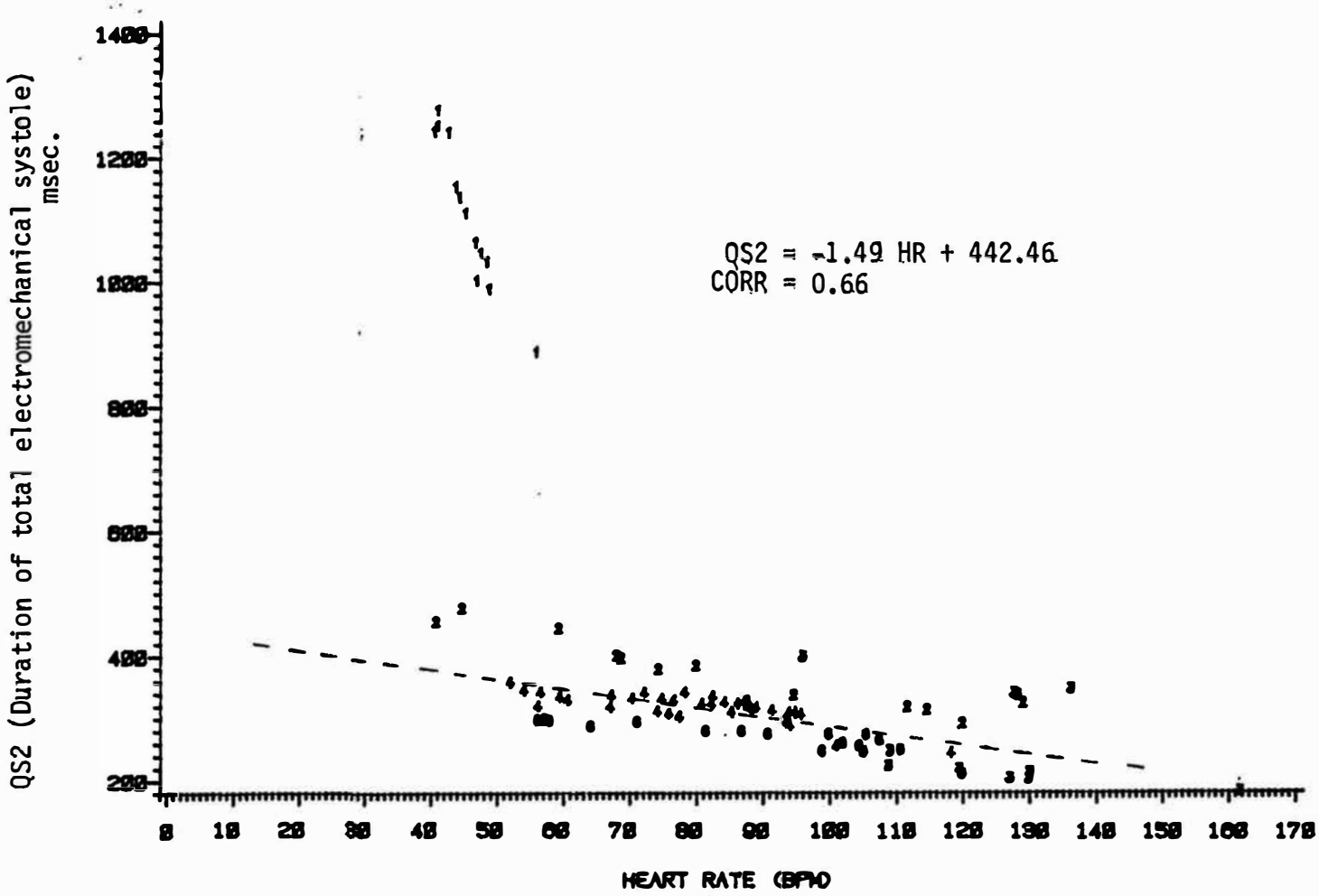

FIG. 3.3 Relationship between QS2 and heart rate (HR) in normal dogs administered 0.05 mg/kg. digoxin i.v. (infusion over 5 mins.). Points from study 1B are not included in the regression line.

3) Dog no. 5 was a mongrel, whereas the dogs used in all other pharmacodynamic studies were beagles.

Since there was a linear relationship between the measured pharmacodynamic responses viz., LVET QS_2 and PR-interval and heart rate, it was decided to identify pharmacodynamic models for heart rate so that these models may be incorporated in models for LVET, QS_2 and other relevant pharmacodynamic responses in future studies. This was one of the primary reasons for conducting studies 4-10, where the serum concentrations of digoxin and HR were primarily measured.

Pharmacodynamic modelling of heart rate

The models investigated were:

1) The linear linking model.
2) The physiologic-pharmacokinetic model.
3) The effect compartment model.

The linear linking model:

Relationships were sought between the serum concentrations (concentrations in the central compartment) and response. No readily discernible relationships were evident. Peak bradycardic effects of digoxin were not observed until 60-120 mins. after the administration of the dose of digoxin and an anticlockwise hysteresis loop was observed on plotting the response and the serum concentrations. This implied that the biophase may be in one of the more slowly equilibrating pharmacokinetic compartments. It was therefore decided to simulate digoxin concentrations in the pharmacokinetic shallow and deep compartments and to correlate these levels to the observed response. Since, it was established that digoxin obeys tri-exponential kinetics in the dog, the following three compartment versions of the following pharmacokinetic models were investigated:

a) The mammillary model.

b) The first pass model.

c) The catenary model.

Schematic representations of the above models are given in *fig. 3.4*

The equations for the mammillary model were obtained from the literature (Gibaldi and Perrier, 1982) and are as follows:

$$X_1 = \frac{D(k_{21} - \alpha)(k_{31} - \alpha)}{(\beta - \alpha)(\gamma - \alpha)} e^{-\alpha t} + \frac{D(k_{21} - \beta)(k_{31} - \beta)}{(\alpha - \beta)(\gamma - \beta)} e^{-\beta t} + \frac{D(k_{21} - \gamma)(k_{31} - \gamma)}{(\alpha - \gamma)(\beta - \gamma)} e^{-\gamma t} \quad ...(3.3)$$

$$X_2 = \frac{-Dk_{12}(\alpha - E_3)}{(\alpha - \beta)(\alpha - \gamma)} e^{-\alpha t} + \frac{Dk_{12}(\beta - E_3)}{(\alpha - \beta)(\beta - \gamma)} e^{-\beta t} + \frac{-Dk_{12}(\gamma - E_3)}{(\beta - \gamma)(\alpha - \gamma)} e^{-\gamma t} \quad ...(3.4)$$

$$X_3 = \frac{-Dk_{13}(\alpha - E_2)}{(\alpha - \beta)(\alpha - \gamma)} e^{-\alpha t} + \frac{Dk_{13}(\beta - E_2)}{(\alpha - \beta)(\beta - \gamma)} e^{-\beta t} + \frac{-Dk_{13}(\gamma - E_2)}{(\beta - \gamma)(\alpha - \gamma)} e^{-\gamma t} \quad ...(3.5)$$

where,

$$E_1 = k_{12} + k_{13} + k_{10} \qquad E_2 = k_{21} \qquad E_3 = k_{31}$$

The equations for the first pass model were obtained from from the literature (Nagashima *et al.*,1968) and are as follows:

$$X_1 = D\left[\frac{(E_2 - \alpha)(E_3 - \alpha)}{(\beta - \alpha)(\gamma - \alpha)} e^{-\alpha t} + \frac{(E_2 - \beta)(E_3 - \beta)}{(\alpha - \beta)(\gamma - \beta)} e^{-\beta t} + \frac{(E_2 - \gamma)(E_3 - \gamma)}{(\alpha - \gamma)(\beta - \gamma)} e^{-\gamma t}\right] \quad ...(3.6)$$

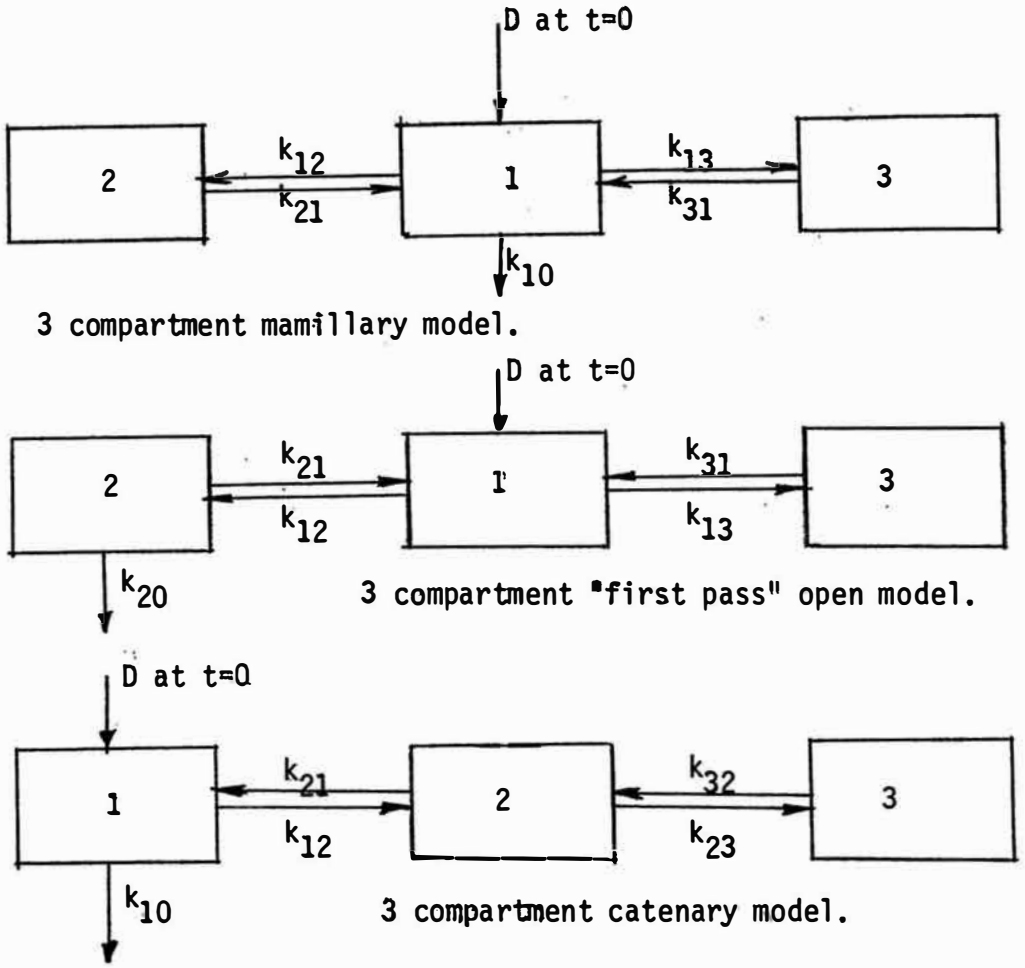

FIG. 3.4 Schematic representations of 3 compartment models investigated.

$$X_2 = k_{12}D\left[\frac{(E_3-\alpha)}{(\beta-\alpha)(\gamma-\alpha)}e^{-\alpha t} + \frac{(E_3-\beta)}{(\alpha-\beta)(\gamma-\beta)}e^{-\beta t} + \frac{(E_3-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t}\right] \quad ...(3.7)$$

$$X_3 = k_{13}D\left[\frac{(E_2-\alpha)}{(\beta-\alpha)(\gamma-\alpha)}e^{-\alpha t} + \frac{(E_2-\beta)}{(\alpha-\beta)(\gamma-\beta)}e^{-\beta t} + \frac{(E_2-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t}\right] \quad ...(3.8)$$

where,

$$E_1 = k_{12} + k_{13} \qquad E_2 = k_{21} + k_{20} \qquad E_3 = k_{31}$$

Equations for the 3-compartment catenary model could not be found in the literature and were derived. The derived equations are as follows:

$$X_1 = \frac{A\alpha^2 - B\alpha + C}{(\alpha-\gamma)(\alpha-\beta)}e^{-\alpha t} - \frac{A\beta^2 - B\beta + C}{(\alpha-\beta)(\beta-\gamma)}e^{-\beta t} + \frac{A\gamma^2 - B\gamma + C}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t} \quad ...(3.9)$$

$$X_2 = -k_{12}D\left[\frac{(\alpha-E_3)}{(\gamma-\alpha)(\alpha-\beta)}e^{-\alpha t} + \frac{(\beta-E_3)}{(\beta-\gamma)(\alpha-\beta)}e^{-\beta t} + \frac{(\gamma-E_3)}{(\beta-\gamma)(\gamma-\alpha)e^{-\gamma t}}\right] \quad ...(3.10)$$

$$X_3 = -k_{12}k_{23}D\left[\frac{e^{-\alpha t}}{(\gamma-\alpha)(\alpha-\beta)} + \frac{e^{-\beta t}}{(\beta-\gamma)(\alpha-\beta)} + \frac{e^{-\gamma t}}{(\beta-\gamma)(\gamma-\alpha)}\right] \quad ...(3.11)$$

where,

$$E_1 = k_{10} + k_{12} \qquad E_2 = k_{12} + k_{23} \qquad E_3 = k_{32}$$

A detailed derivation of the above equations can be found in appendix A.

a) The 3-compartment mammillary model

The micro rate constants for the 3-compartment mammillary model were obtained from the serum concentration time profile and using equations 3.3, 3.4 and 3.5 The amounts of digoxin in the shallow and deep compartments were simulated using the equations described before and parameters obtained from the tri-exponential fit of the data (*fig. 3.5*).

The simulations were confirmed by simulations using SPICE2. A detailed description of simulation methods using SPICE2 is given in a later part of the chapter. Results of linking the central, shallow and deep compartments for Study no.4 are given in *figs. 3.6, 3.7 and 3.8* respectively.

No apparent relationship is evident between the response and the simulated amounts of digoxin in the central and the deep compartment. However, there was a linear relationship (Corr. = 0.80) between the % change in heart rate and simulated concentrations of digoxin in the shallow compartment (*fig. 3.9*).

This implies that the biophase for the change in heart rate lies in the shallow pharmacokinetic compartment for the 3-compartment mammillary model for digoxin.

b) The 3-compartment "first pass" model.

The amounts of digoxin in the shallow and deep compartments were simulated using the equations 3.6, 3.7 and 3.8 (*fig. 3.10*).

The simulations were confirmed using SPICE2. Results of linking the central, shallow and deep compartments for Study no.4 are given in *figs. 3.11, 3.12, and 3.13* respectively.

No apparent relationship is evident between the response and the simulated amounts of digoxin in the central and the deep compartments. However, a linear

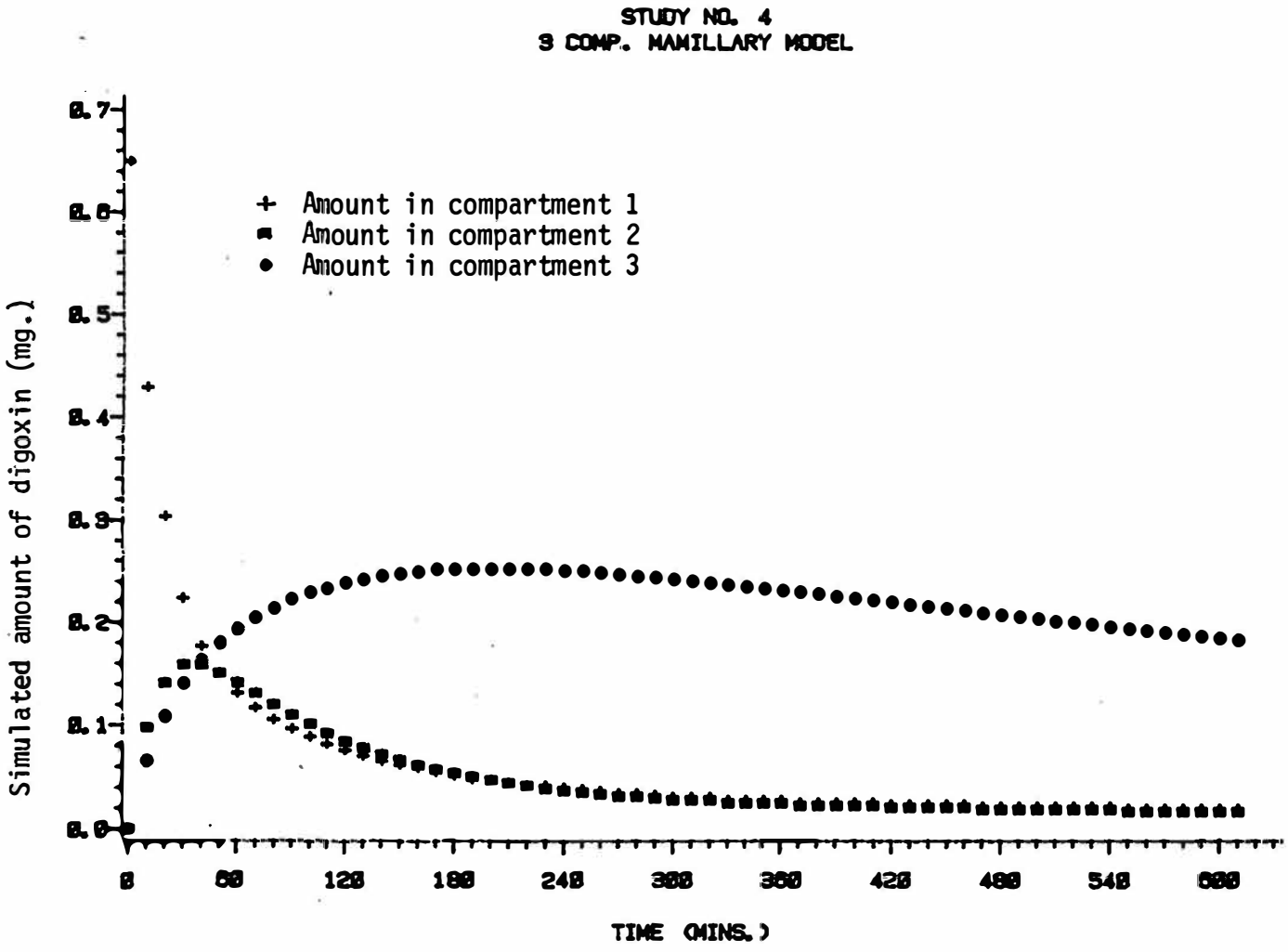

Fig. 3.5 Simulated amounts of digoxin in various compartments for a 3 compartment mamillary model. (Dose = 0.05 mg/kg administered to a normal dog i.v.)

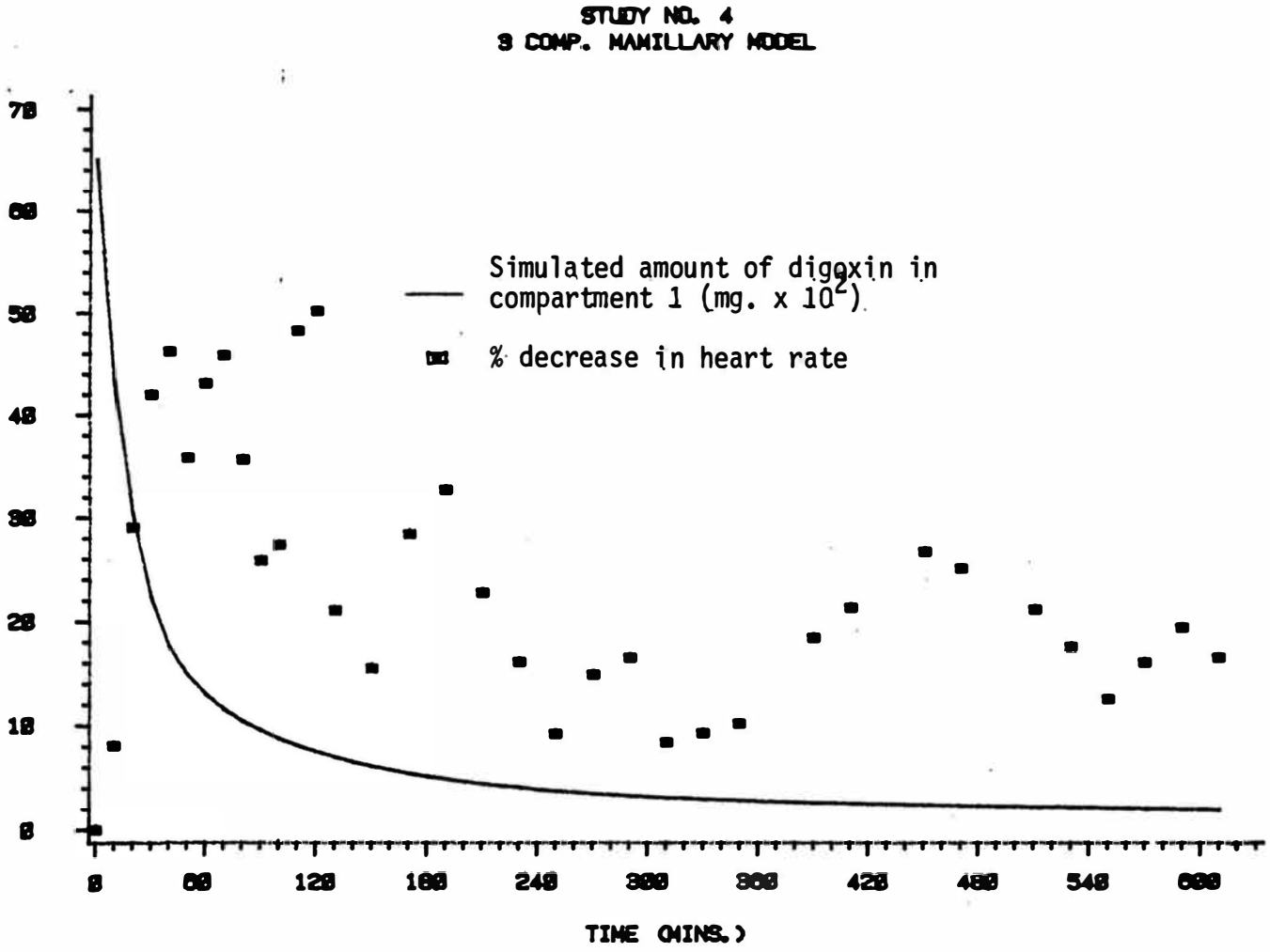

Fig. 3.6 Simulated amount of digoxin in compartment 1 of a 3 compartment mamillary model and the observed per cent decrease in heart rate, following administration of 0.05 mg/kg of digoxin i.v. (infused over 5 mins) to normal beagle dog.

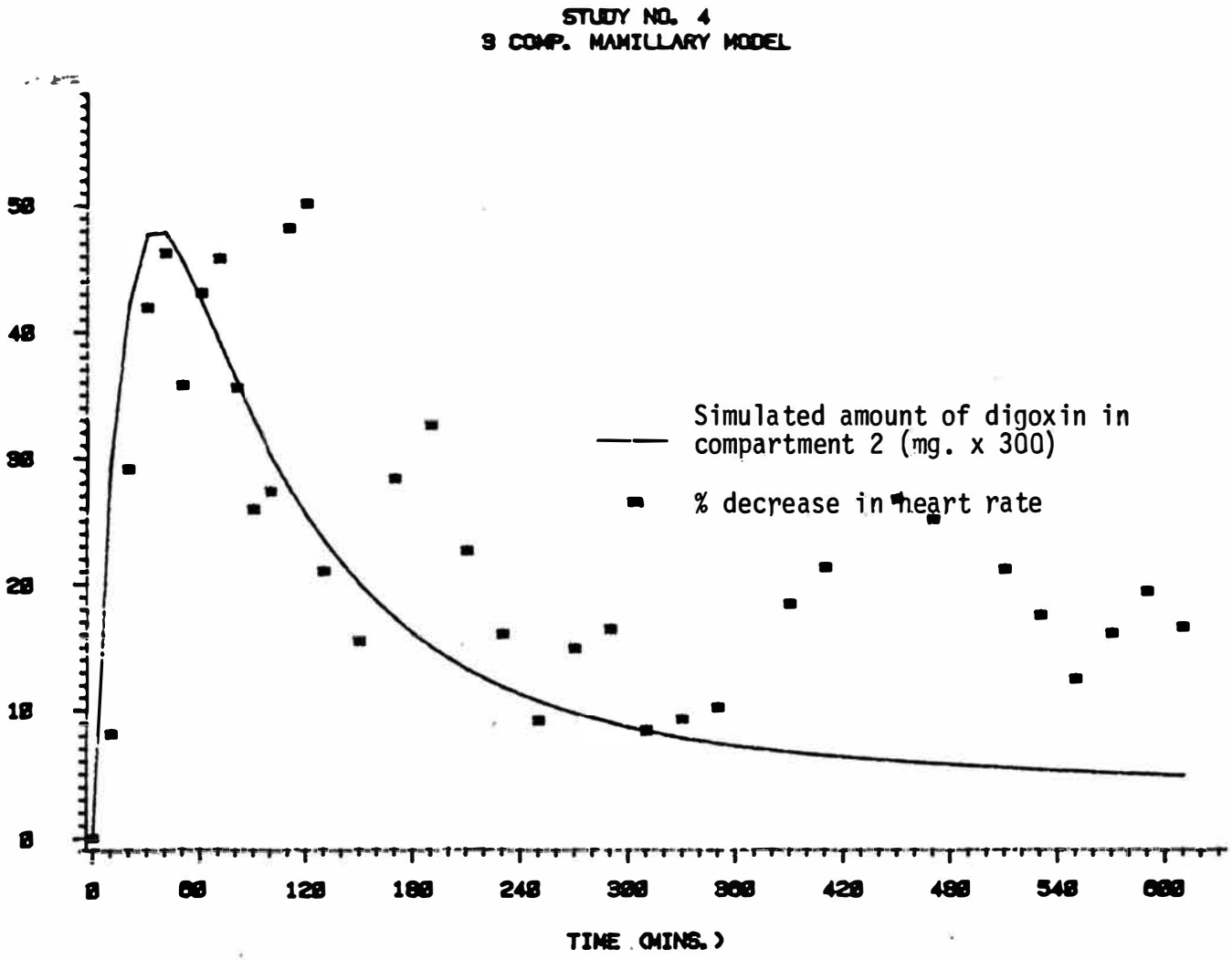

Fig. 3.7 Simulated amount of digoxin in the shallow compartment and the observed per cent decrease in heart rate following administration of 0.05 mg/kg. of digoxin i.v. (infused over 5 mins) to a normal beagle dog. Times are post-infusion.

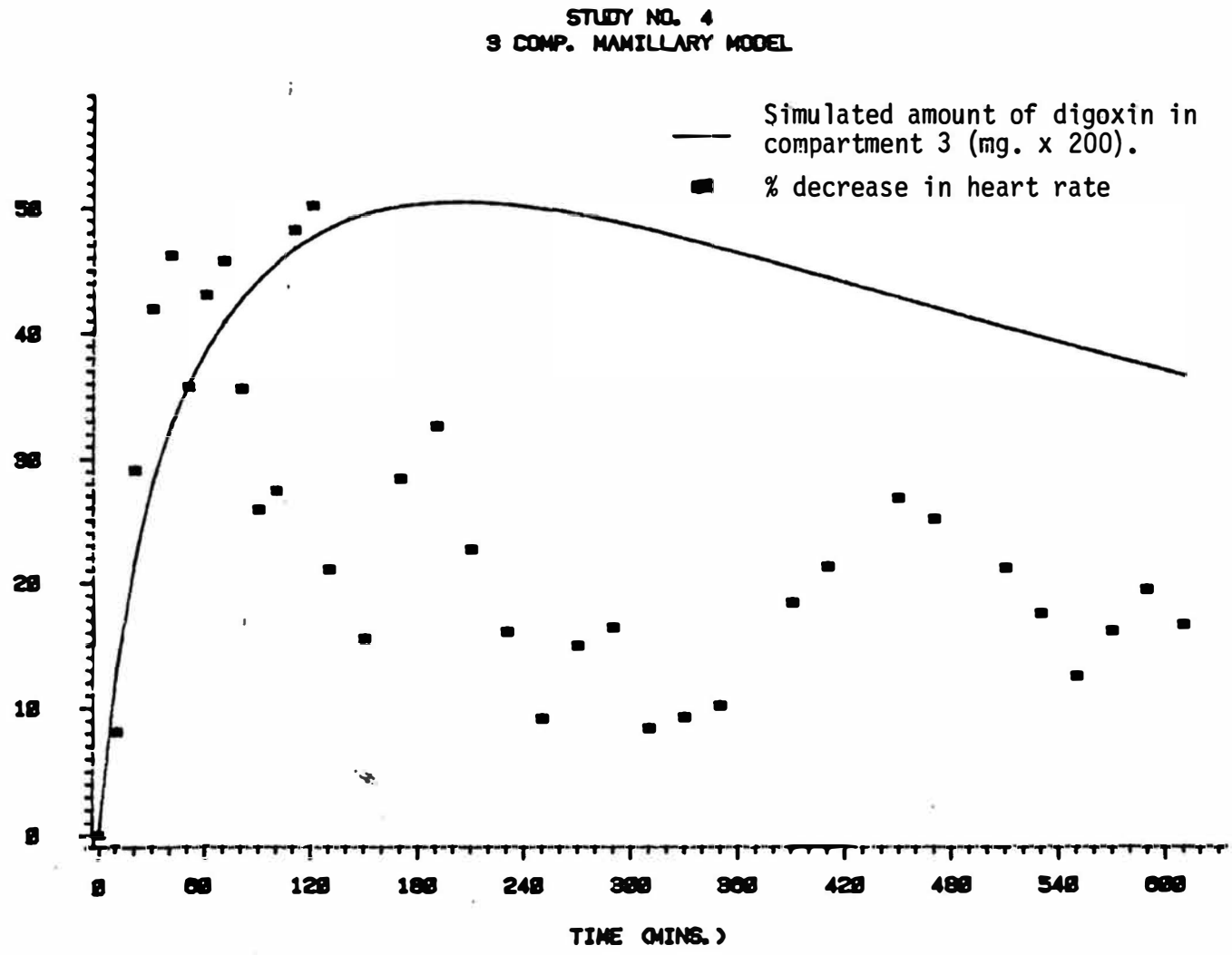

Fig. 3.8 Simulated amount of digoxin in the pharmacokinetic 'deep' compartment and the observed decrease in heart rate following administration of 0.05 mg/kg of digoxin i.v. (infused over 5 mins.) to a normal beagle dog. Times are post-infusion.

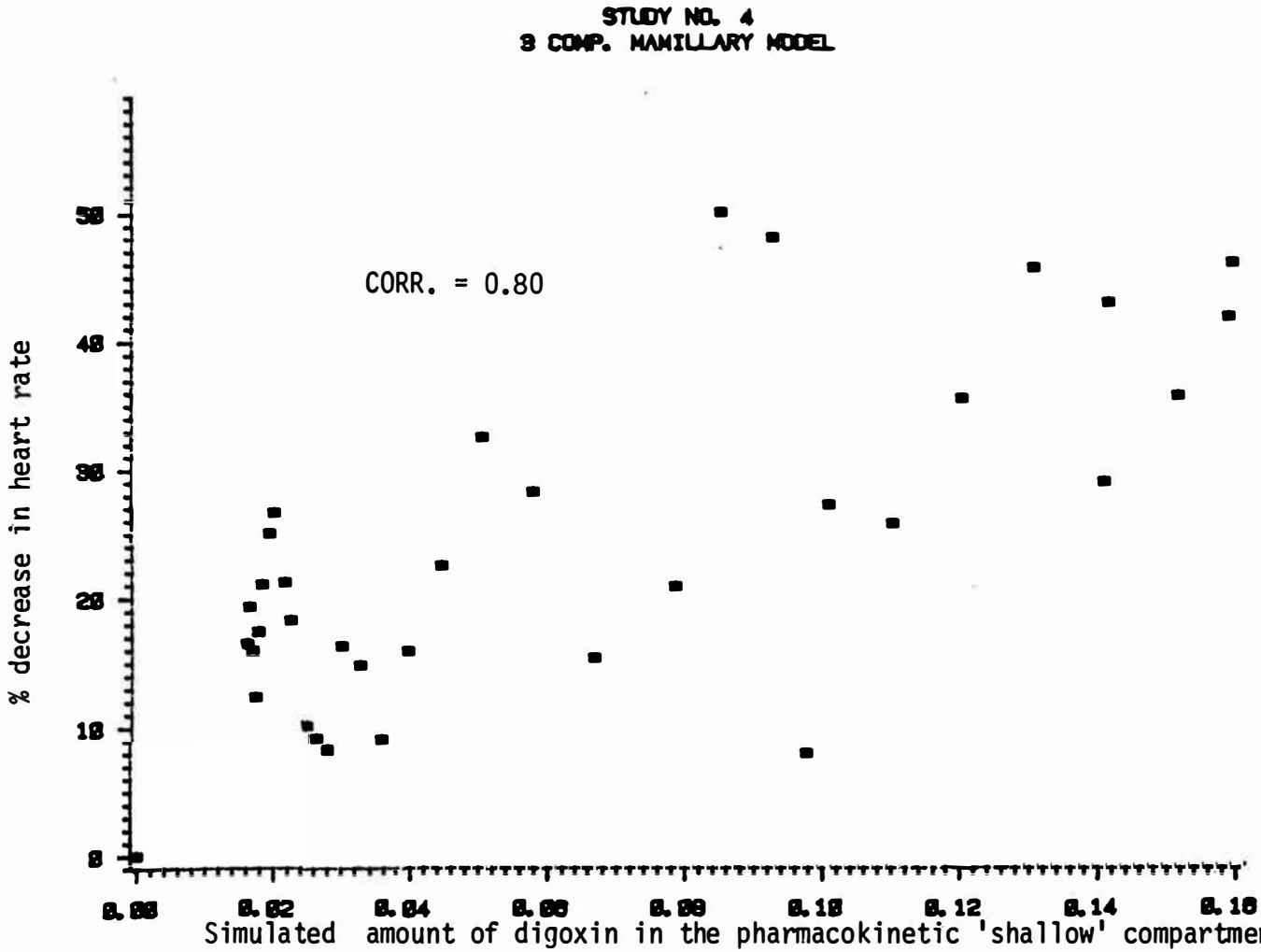

Fig. 3.9 Linear relationship between the simulated amount of digoxin in compartment 2 and the observed % decrease in heart rate following administration of 0.05 mg/kg of digoxin i.v. (infused over 5 mins.) to a normal beagle dog.

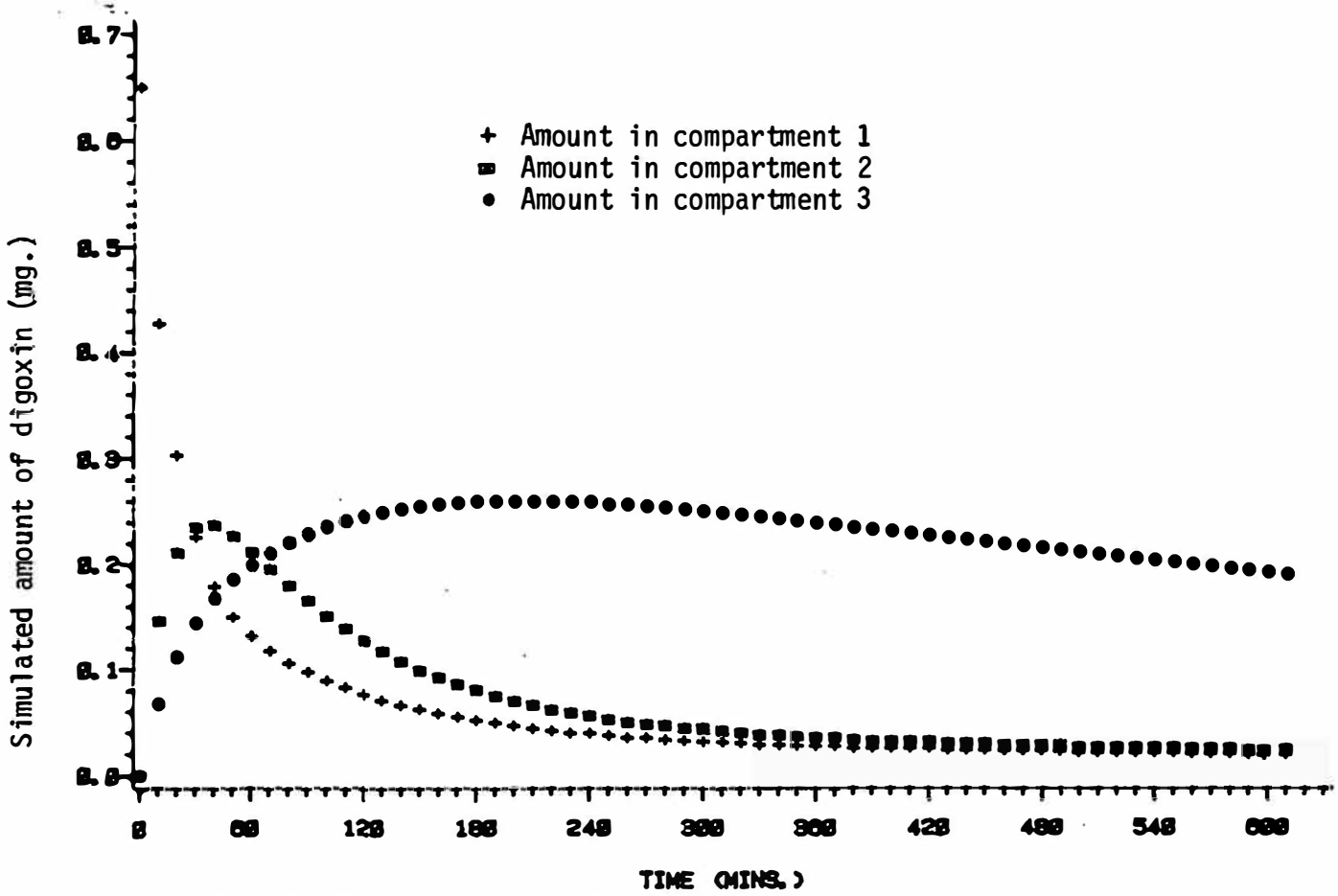

Fig. 3.10 Simulated amount of digoxin in various compartments of a 3 comp. first pass open model following administration of 0.05 mg/kg digoxin i.v. (infused over 5 mins) to a normal anesthetized beagle dog. Times are post-infusion.

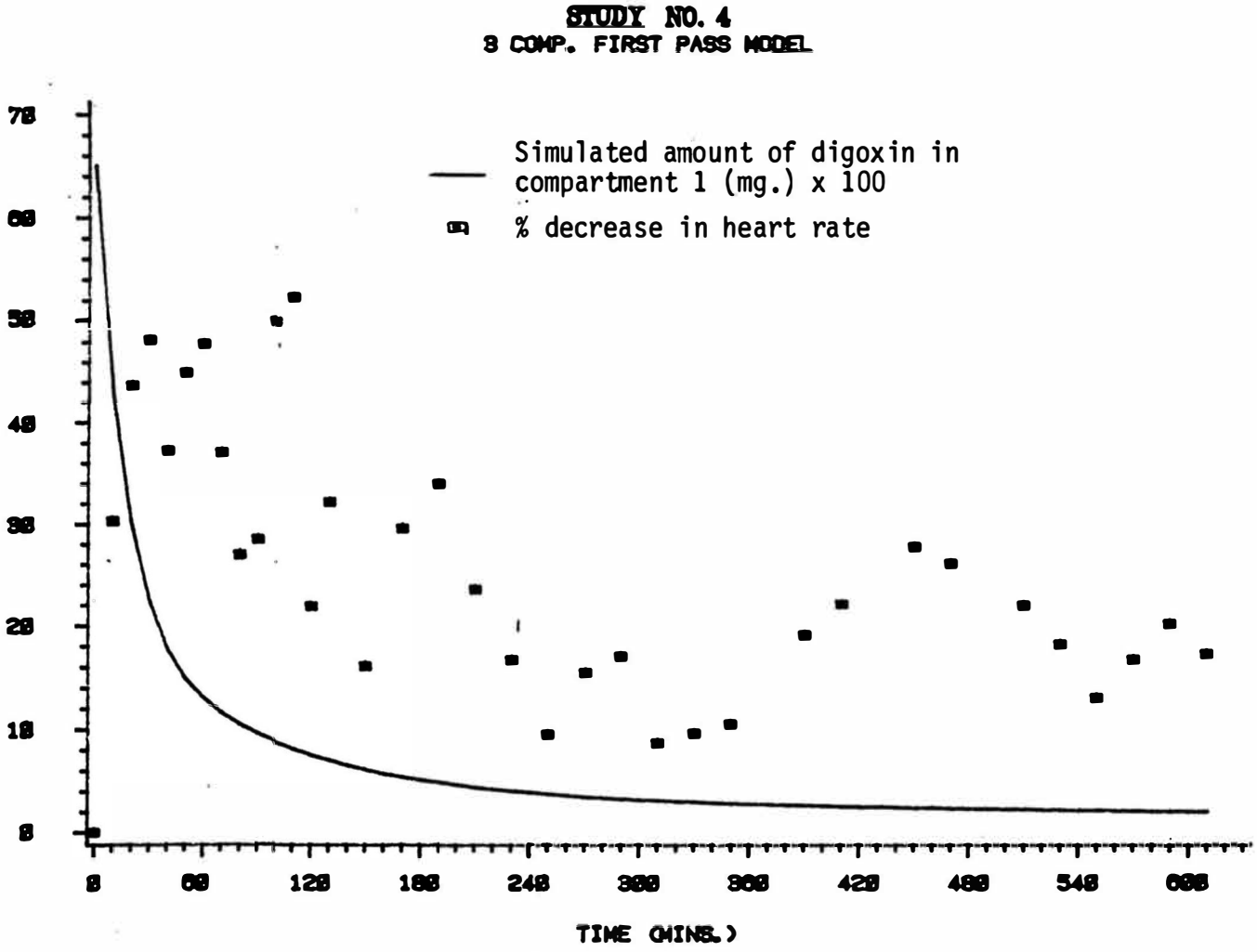

Fig. 3.11 Lack of correlation between simulated amounts of digoxin in compartment 1 and the observed % decrease in heart rate following administration of 0.05 mg/kg digoxin i.v. (infused over 5 mins) to an anesthetized normal beagle dog. Times are post infusion.

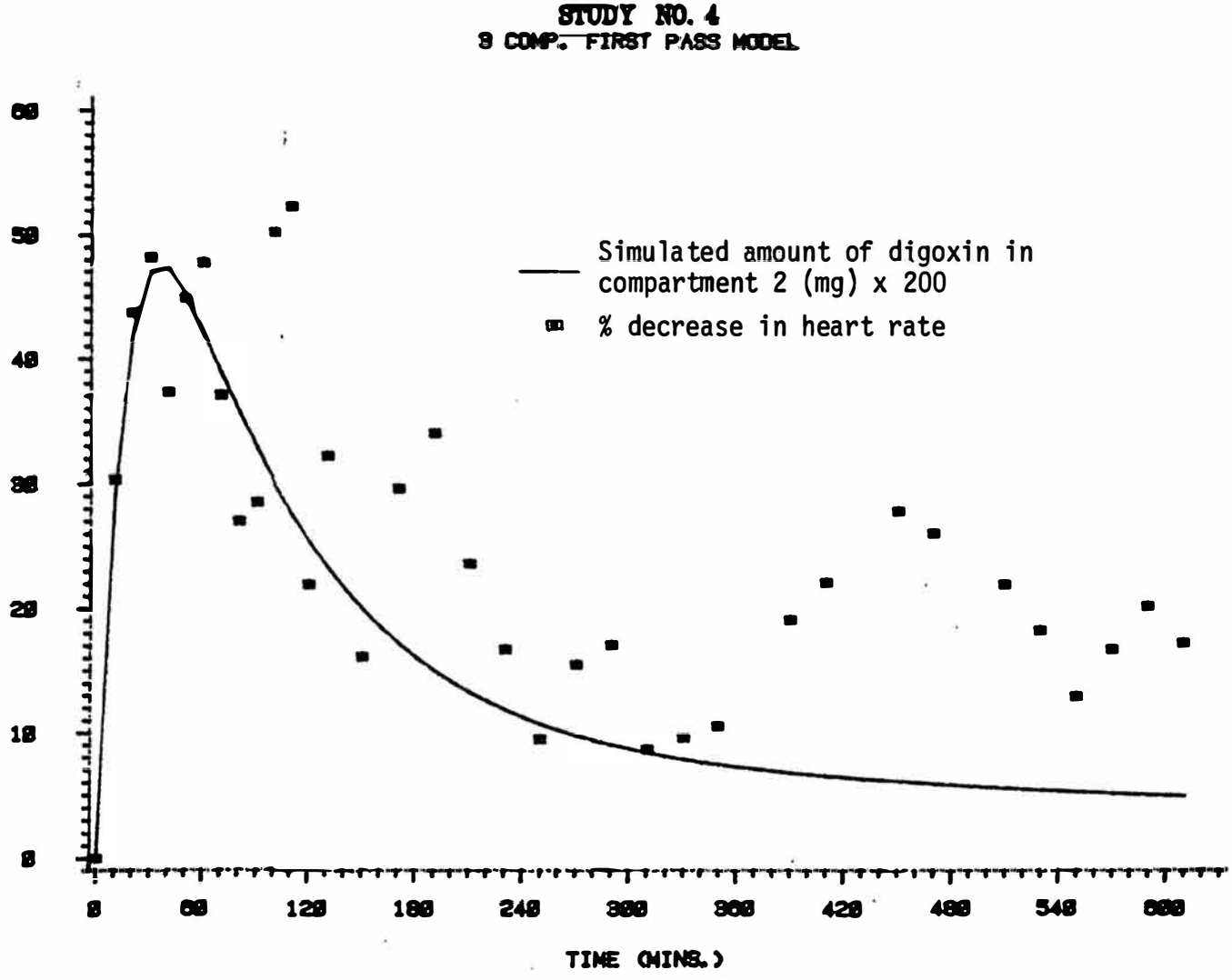

Fig. 3.12 Correlation between simulated amounts of digoxin in the pharmacokinetic 'shallow' compartment and the observed % decrease in heart rate following administration of 0.05 mg/kg digoxin i.v. (infused over 5 mins) to a anesthetized normal beagle dog.

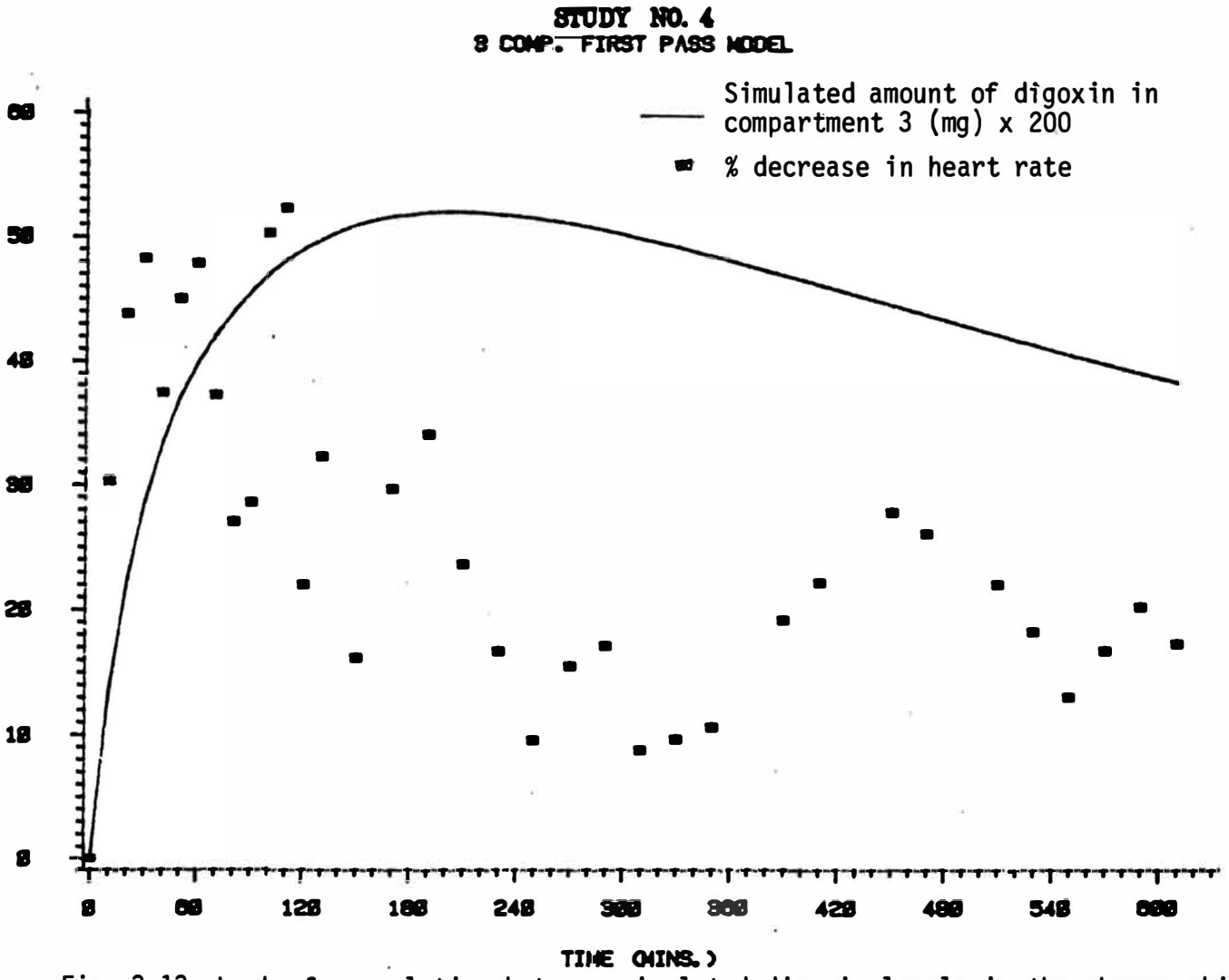

Fig. 3.13 Lack of correlation between simulated digoxin levels in the pharmacokinetic 'deep' compartment and the observed decrease in heart rate following administration of 0.05 mg/kg. digoxin i.v. (infused over 5 mins.) to an anesthetized normal beagle dog. Times are post-infusion.

relationship (Corr. = 0.80) is observed between the % change in heart rate and simulated concentrations of digoxin in the shallow compartment (*fig. 3.14*).

This again implies that the biophase for effecting the change in heart rate lies in the pharmacokinetic shallow compartment for the 3-compartment "first pass" model for digoxin.

c) The 3-compartment catenary model.

The amounts of digoxin in the shallow and deep compartments were simulated using equations 3.9, 3.10 and 3.11 (*fig. 3.15*).

The simulations were confirmed using SPICE2. Results of linking the central, shallow and deep compartments for Study no.4 are given in *figs. 3.16, 3.17, and 3.18* respectively.

No apparent relationship is evident between the response and the simulated amounts of digoxin in the central and the deep compartments. Again a linear relationship was observed between the % change in heart rate and simulated concentrations of digoxin in the shallow compartment (Corr. = 0.80). This again implies that the biophase for effecting the change in heart rate lies in the pharmacokinetic shallow compartment for the 3-compartment catenary model for digoxin.

Similar analyses were done on the data obtained using studies 7, 9 and 10. The results indicate that the biophase for the change in heart rate does not necessarily lie in the shallow pharmacokinetic compartment. This is evident from *figs. 3.19, 3.20, 3.21* where the response and the simulated amounts of digoxin in the shallow pharmacokinetic compartments for studies 7, 9 and 10 are plotted. Very little correlation is observed.

It also appears that the response does not necessarily correlate with the simulated levels in the shallow compartment for the same dog. A case in point is Dog no. 1. Study no. 4 and study no.7 were conducted in this same dog. The % change

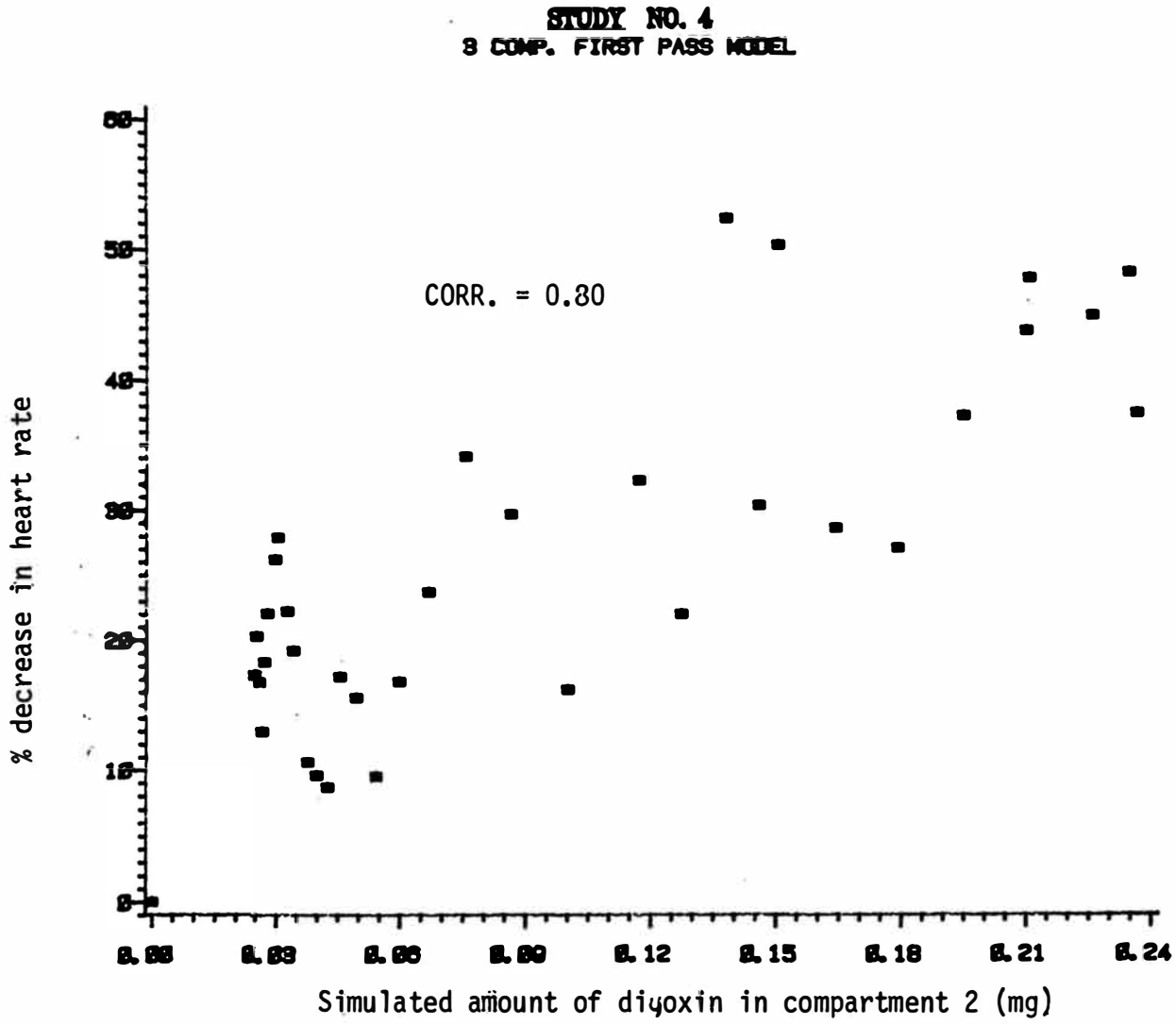

Fig. 3.14 Linear relationship between simulated amount of digoxin in the pharmacokinetic 'shallow' compartment and the observed % decrease in heart rate following administration of 0.05 mg/kg digoxin (infused over 5 mins.) to an anesthetized normal beagle dog.

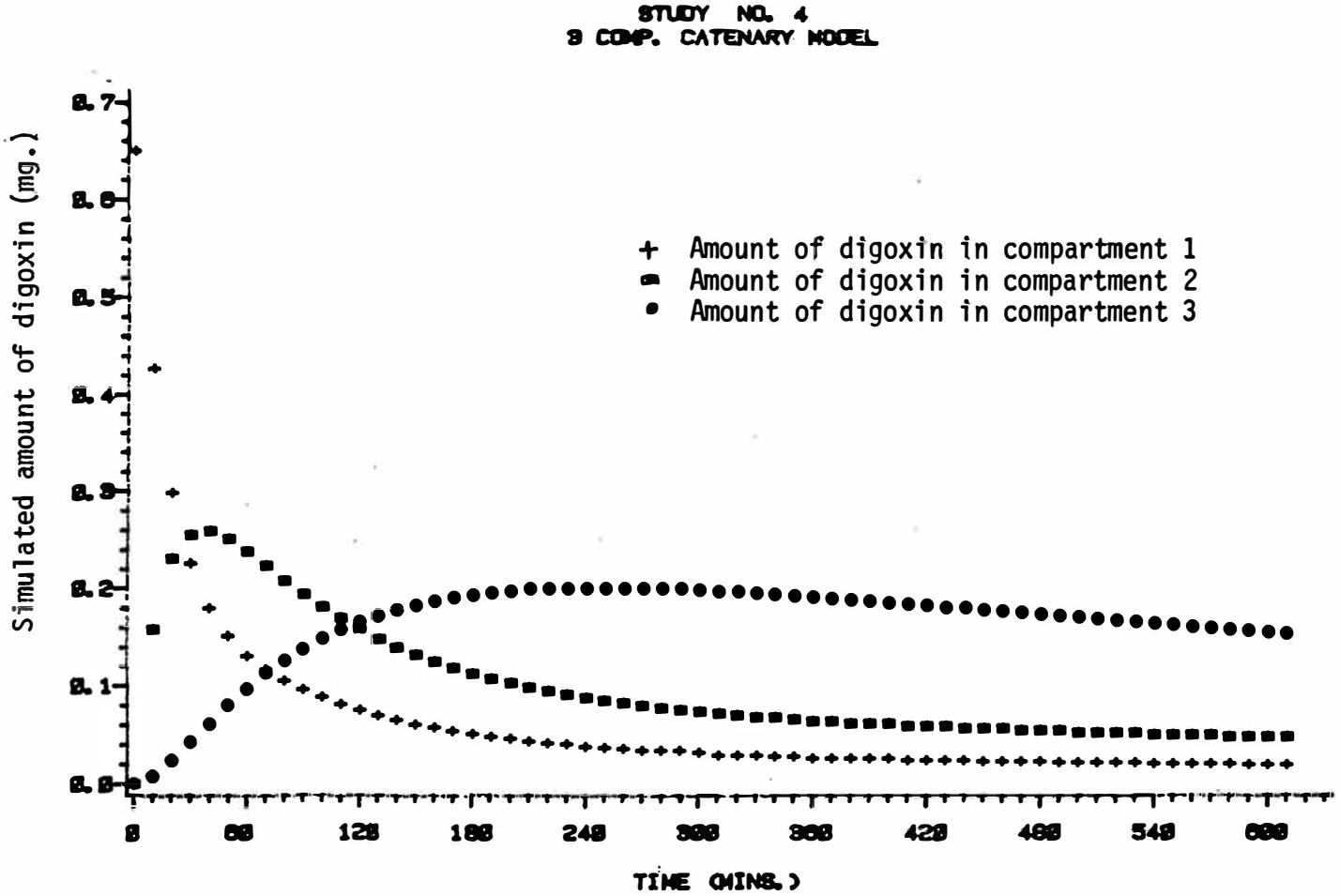

Fig. 3.15 Simulated amounts of digoxin in various compartments for a 3 compartment catenary model following administration of 0.05 mg/kg. digoxin i.v. (infused over 5 mins.) to an anesthetized normal beagle dog. Times are post-infusion.

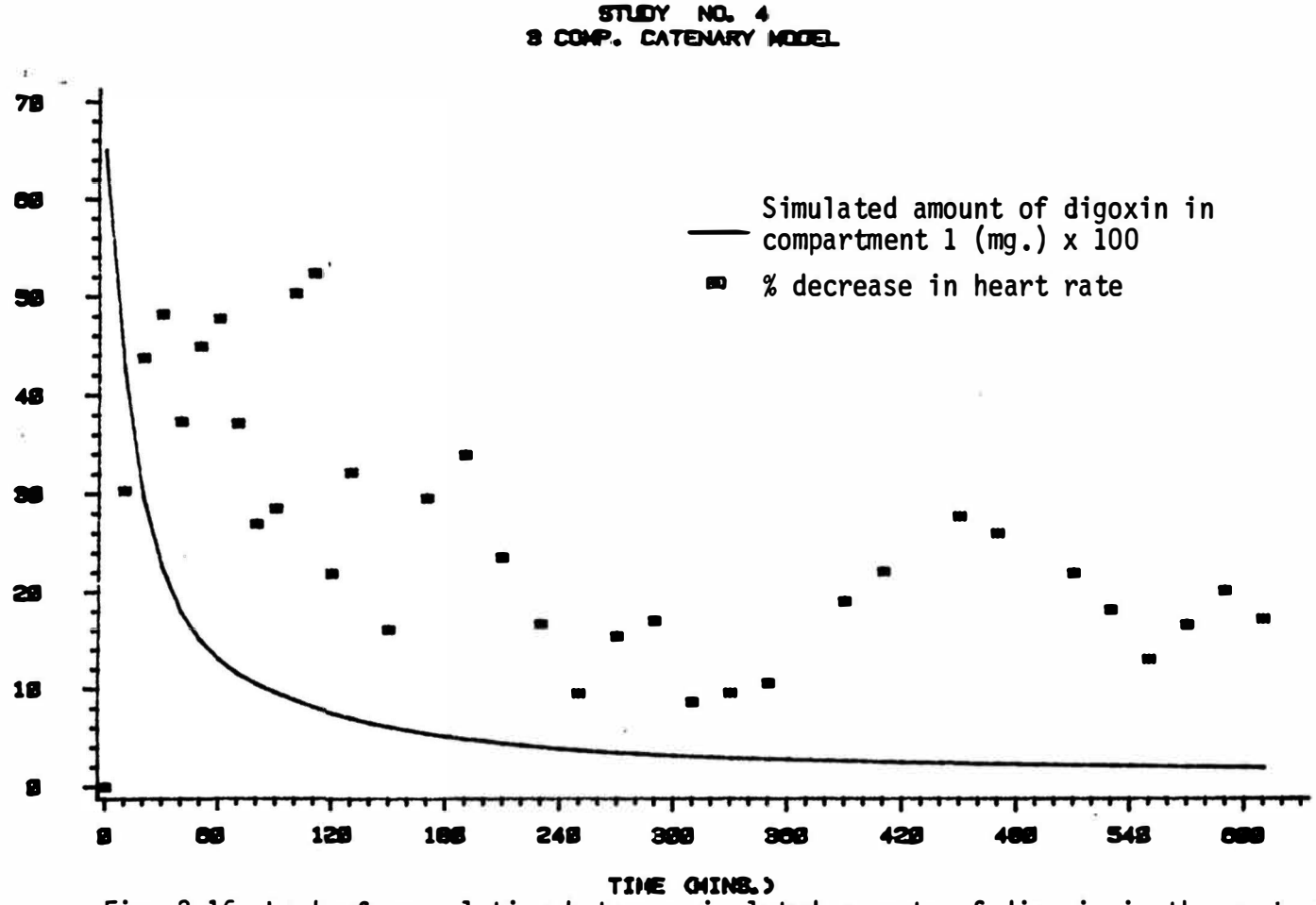

Fig. 3.16 Lack of correlation between simulated amounts of digoxin in the compartment 1 and the observed % decrease in heart rate following administration of 0.05 mg/kg. digoxin i.v. (infused over 5 mins.) to an anesthetized normal beagle dog. Times are post-infusion.

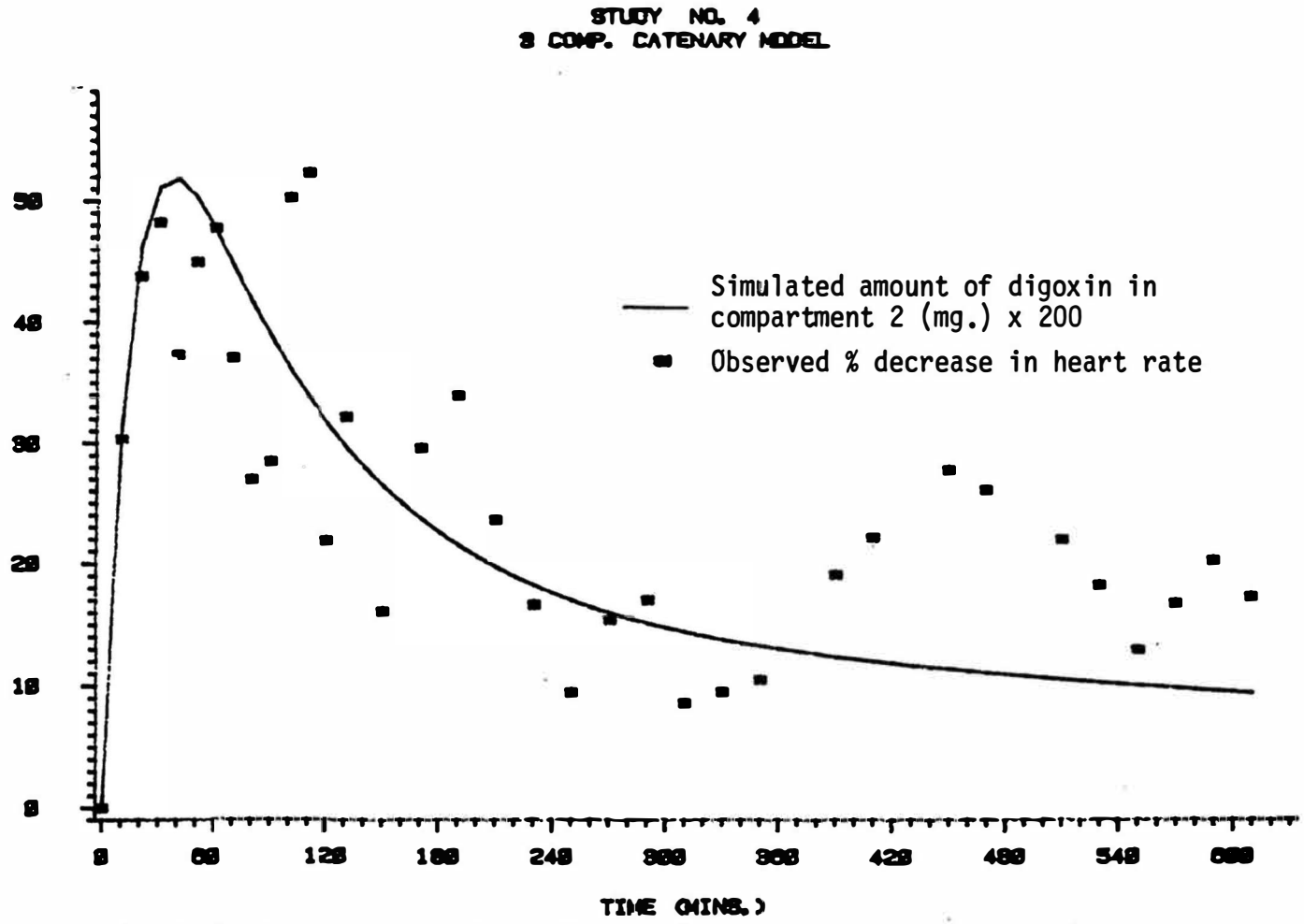

Fig. 3.17 Correlation between the simulated amounts of digoxin in the pharmacokinetic 'shallow' compartment and the observed % decrease in heart rate following administration of digoxin i.v. (0.05 mg/kg. infused over 5 mins.) to an anesthetized normal beagle dog. Times are post-infusion.

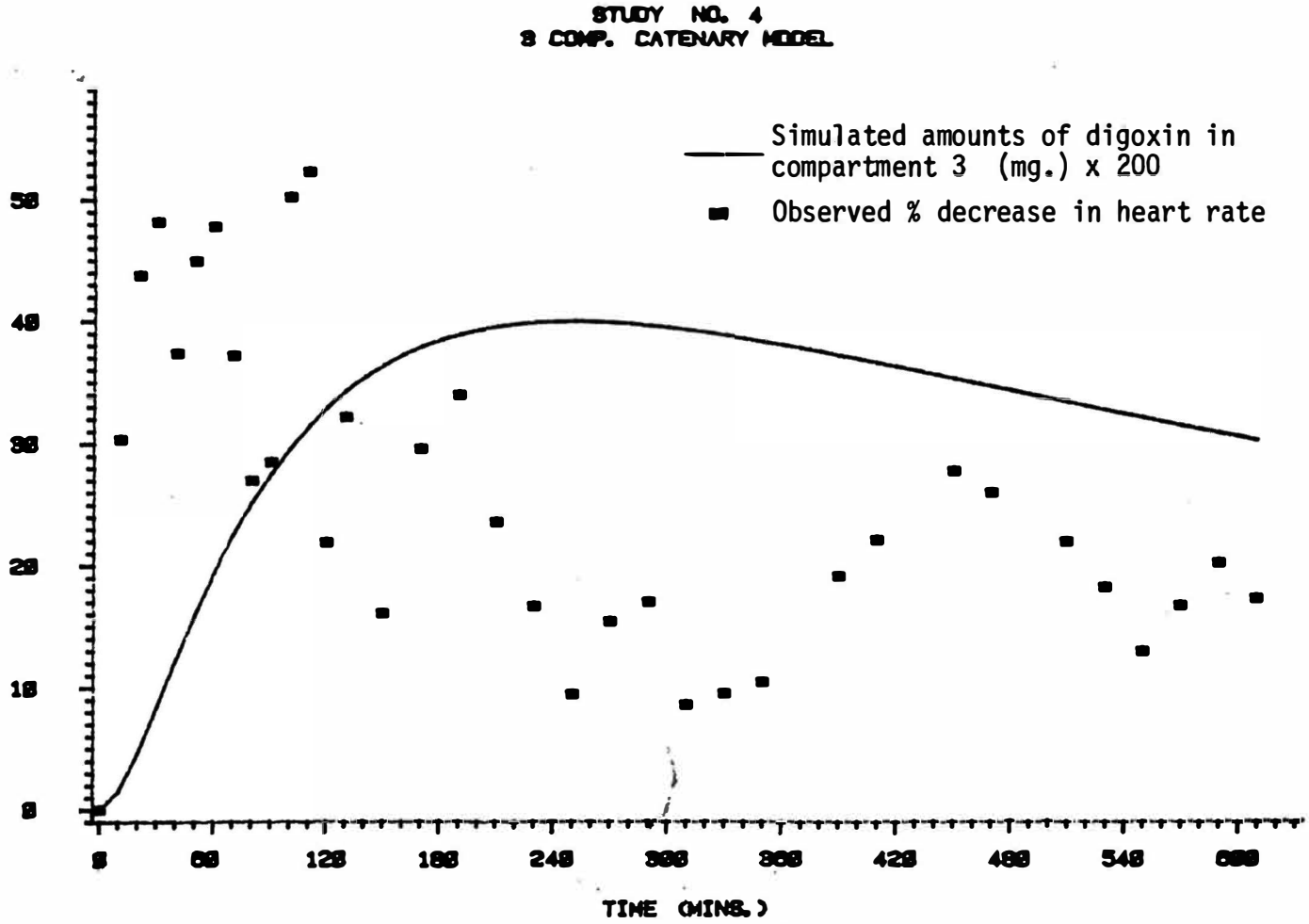

Fig. 3.18 Lack of correlation between simulated amounts of digoxin in the pharmacokinetic 'deep' compartment and the observed % decrease in heart rate following administration of 0.05 mg/kg digoxin i.v. (infused over 5 mins) to an anesthetized normal beagle dog. Times are post-infusion.

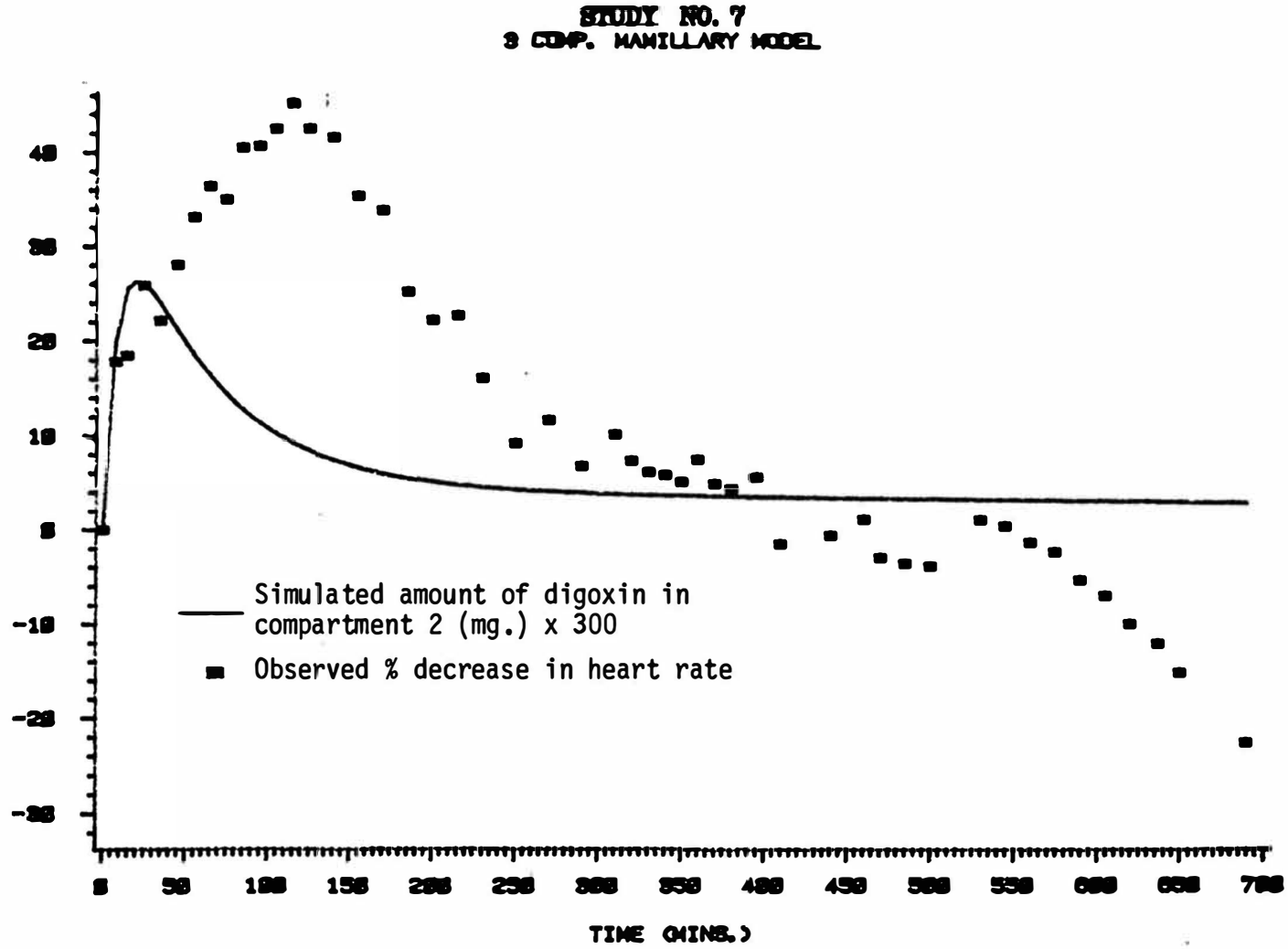

Fig. 3.19 Lack of correlation between simulated levels of digoxin in the pharmacokinetic 'shallow' compartment and the observed % decrease in heart rate following administration of 0.025 mg/kg. of digoxin i.v. (infused over 5 mins.) to an anesthetized normal beagle dog.

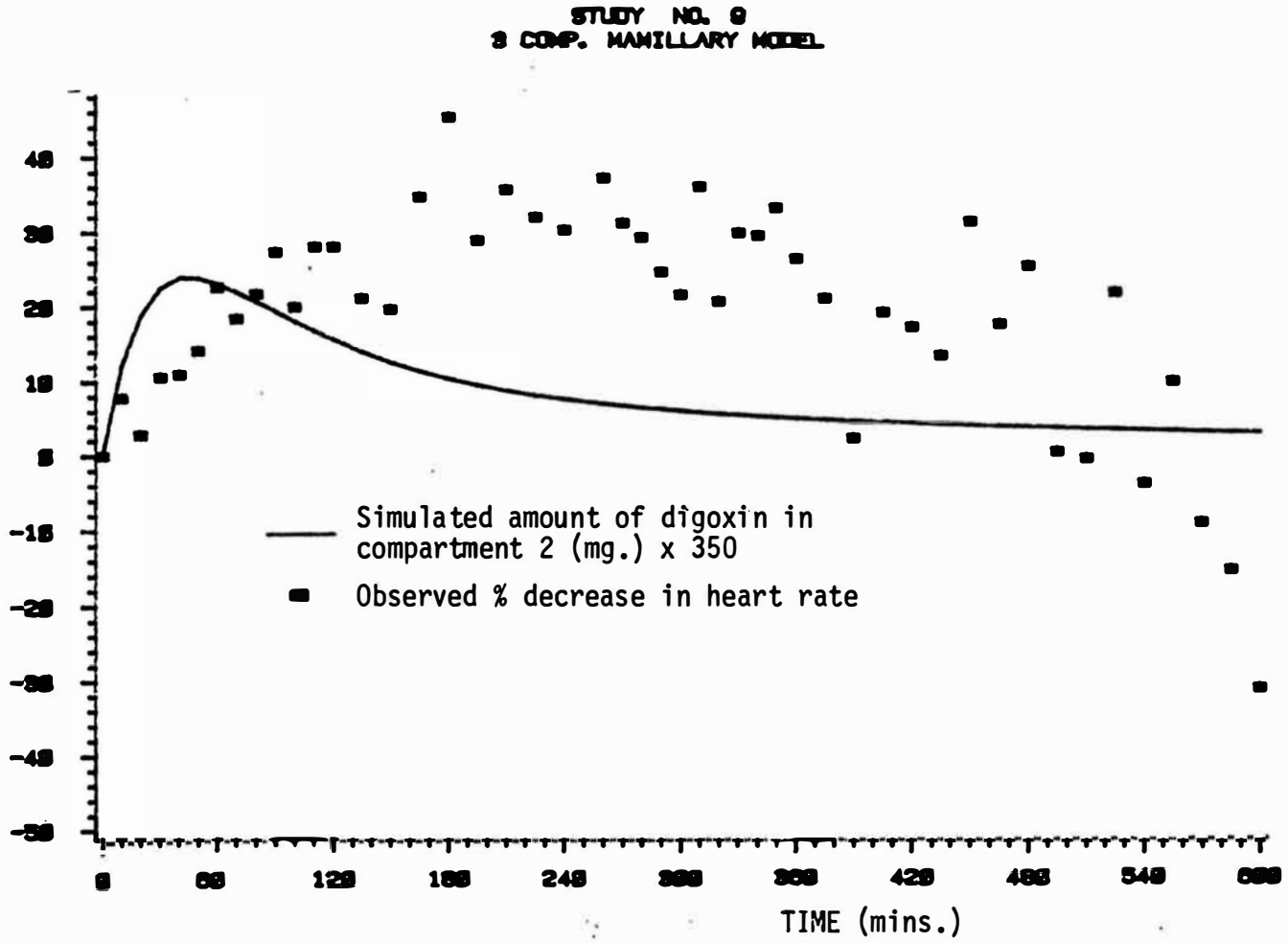

Fig. 3.20 Lack of correlation between simulated amounts of digoxin in the pharmacokinetic 'shallow' compartment and the observed % decrease in heart rate following administration of 0.05 mg/kg. of digoxin i.v. (infused over 5 mins.) to an anesthetized normal beagle dog. Times are post-infusion.

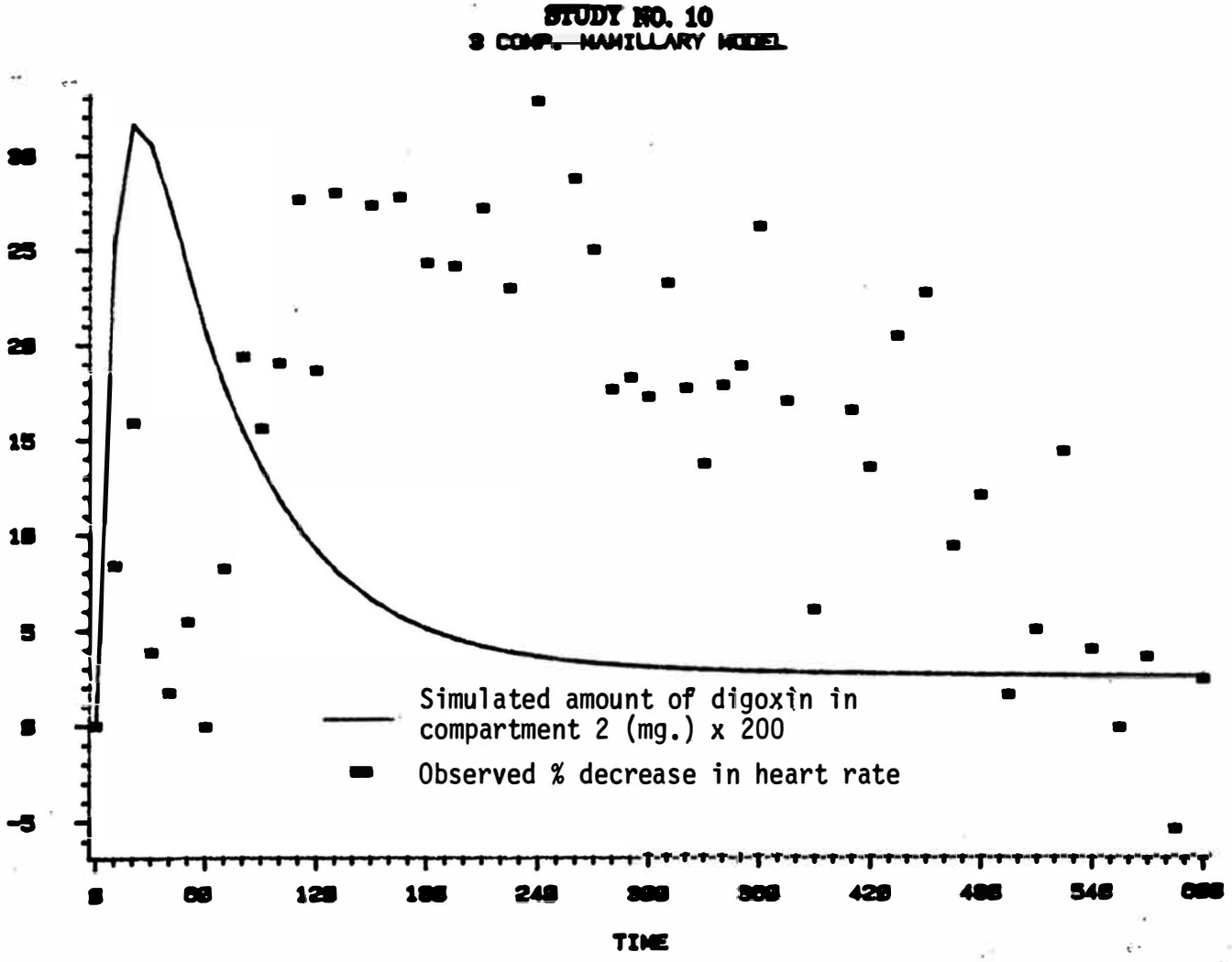

Fig. 3.21 Lack of correlation between simulated digoxin amounts in the pharmacokinetic 'shallow' compartment and the observed % decrease in heart rate following administration of 0.05 mg/kg. of digoxin i.v. (infused over 5 mins.) to an anesthetized normal beagle dog. Times are post-infusion.

in heart rate correlated with the simulated levels of digoxin in the shallow compartment in study no. 4 (fig. 3.9). However, no such relationship was observed in study no.7 (fig. 3.22).

Similarly simulated concentrations of digoxin in the shallow and deep compartments for studies 9 and 10 did not correlate with the observed change in heart rate. The results also indicate that the linear linking model may not be an adequate pharmacokinetic-pharmacodynamic model. A possible reason may be that the biophase for the bradycardic effect of digoxin may not be identical to any of the observed pharmacokinetic compartments. The amount of digoxin at the biophase may be insignificant as far as mass balance considerations. Hence this amount of drug would not be expected to contribute to the observed pharmacokinetic profile of digoxin although it would contribute to the pharmacodynamic response being monitored. Investigation of other models is called for.

The models proposed for investigation were:

1) The linear model.
2) The effect compartment model.
3) The physiologic pharmacokinetic model with a linear linking model.

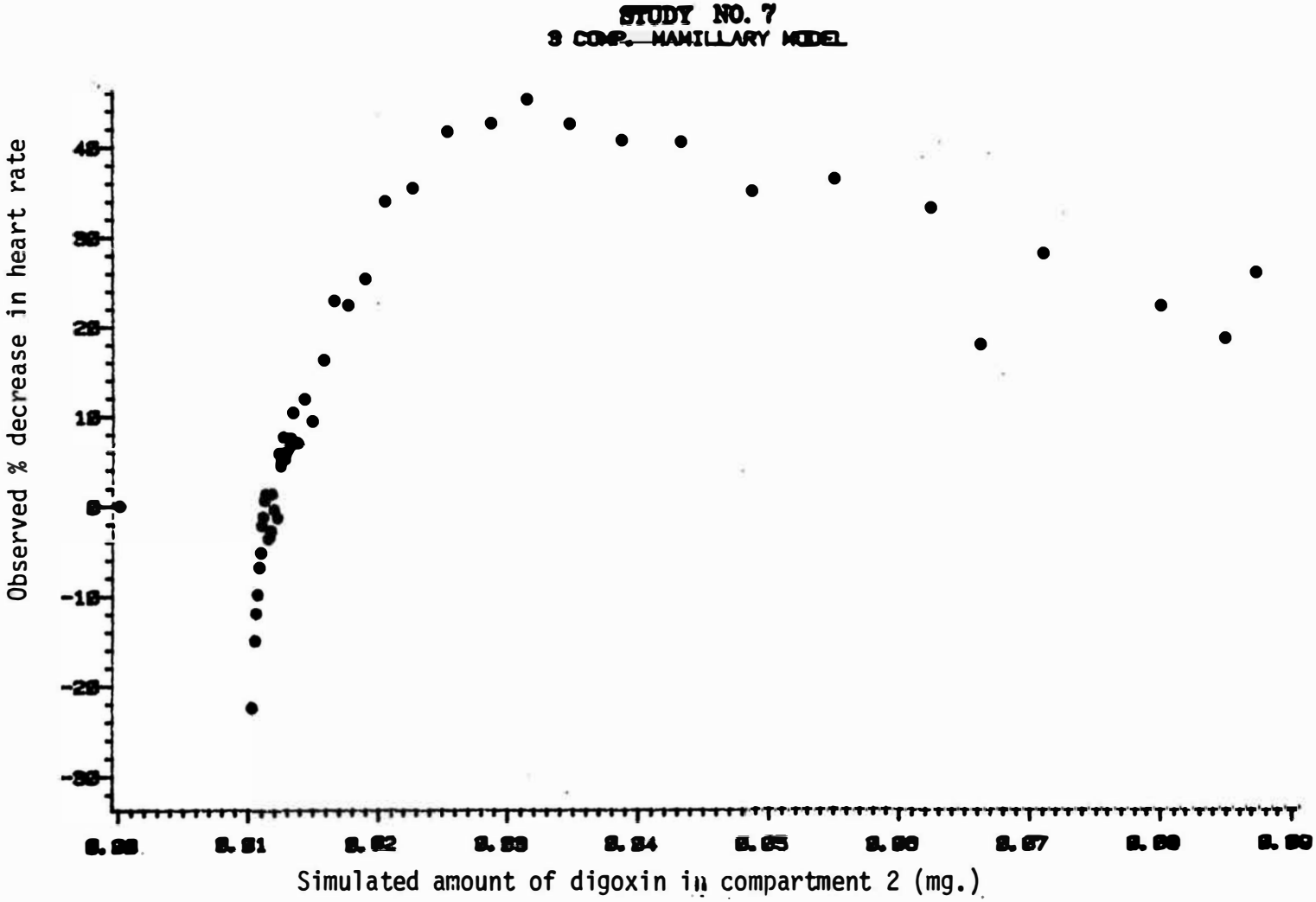

Fig. 3.22 Lack of correlation between simulated amounts of digoxin in the pharmacokinetic 'shallow' compartment and the observed % decrease in heart rate following administration of 0.025mg/kg. digoxin i.v. (infused over 5 mins) to an anesthetized normal beagle dog.

The Linear Model

A number of studies have used a linear pharmacodynamic model to simultaneously describe drug pharmacokinetics and pharmacodynamics. the model was proposed by Kelman and Whiting (1980) to model digoxin pharmacodynamics. They observed that digoxin kinetics was best explained by a tri-exponential equation. Therefore the model used was

$$R = a_0 + b \cdot XC + c \cdot XS + d \cdot XD \qquad ...(3.12)$$

where,

R = response

XC = amount of digoxin in the central compartment

XS = amount of digoxin in the shallow compartment

XD = amount of digoxin in the deep compartment

and, a_0, b and d are constants.

The amounts XC, XS and XD are simulated amounts in the respective pharmacokinetic compartments. Kelman and Whiting (1980) contended that such a model might be appropriate since the biophase did not necessarily have to be situated in a single pharmacokinetic compartment.

This model was initially investigated using the RSQUARE procedure (SAS User's Guide, 1982) to investigate the effect of various combinations of the terms in

eqn. 3.12, on the resulting R-square values obtained. The results of the RSQUARE procedure are given in Tables 3.17 and 3.18

The results indicate that for all studies the best R-square value is given by the use of all three terms in the equation (XC, XS, XD). It was decided to investigate this model further. The models were investigated with and without an intercept term i.e., for the model without an intercept a_0 (eqn. 3.12) was assigned a value of zero. Regression was conducted using the REG procedure (SAS User's Guide, 1982). The results of the analyses are given in tables 3.19 and 3.20

The results based on the residual sum of squares, the fit of the predicted and observed values and a plot of the residual vs. the observed response; indicate that the model fits the data well. Better fits were obtained without an intercept term in the equation than with an intercept term. This is also evident in the large standard deviation for the estimated intercept. The RSQUARE procedure further indicates that the response may be adequately described with only the XS and XD terms in the equation. Addition of a XC term results in an increase in the R-square value but is not appreciable. Large variability was observed in the coefficients a_0, b and c. While the data indicate that a pharmacodynamic model for digoxin may be adequately described by linear model the following points need to be noted:

1) The equation relating the amounts of digoxin in each of the pharmacokinetic compartments to the observed response implies the existence of receptors in each of these compartments. However a closer look at table 3.20 and the R-square values in tables 3.17 and 3.18 reveal that the coefficient b for the central compartment may be very close to zero and that its contribution to the overall response may be negligible.
2) The large variability in a_0, b, c and d imply receptors with varying sensitivity for the same evoked response. It is observed that some of the coefficients are negative. This indicates that the receptors in the same pharmacokinetic

TABLE 3.17

STUDY NO. 4

NUMBER IN MODEL	RSQUARE	VARIABLES IN MODEL
1	0.00495959	XC
1	0.01661176	XD
1	0.53207427	XS
2	0.02691761	XC XD
2	0.60540457	XS XD
2	0.63143736	XC XS
3	0.63219704	XC XS XD

STUDY NO. 7

NUMBER IN MODEL	RSQUARE	VARIABLES IN MODEL
1	0.04477467	XC
1	0.12494592	XD
1	0.72401900	XS
2	0.59086245	XC XD
2	0.95615120	XS XD
2	0.97381615	XC XS
3	0.97493637	XC XS XD

RSQUARE analysis for the linear model (with intercept) proposed by Kelman and Whiting (1980).

XC, XS and XD are simulated amounts of digoxin in the central, shallow and deep compartments for a three compartment mamillary model.

TABLE 3.18

STUDY NO. 9

NUMBER IN MODEL	RSQUARE	VARIABLES IN MODEL
1	0.00878101	XC
1	0.28335729	XD
1	0.37532803	XS
2	0.28509066	XC XD
2	0.41868876	XS XD
2	0.56372017	XC XS
3	0.59604948	XC XS XD

STUDY NO. 10

NUMBER IN MODEL	RSQUARE	VARIABLES IN MODEL
1	0.07996451	XC
1	0.08931063	XD
1	0.24114265	XS
2	0.08973375	XC XD
2	0.30735802	XS XD
2	0.63093475	XC XS
3	0.65528679	XC XS XD

RSQUARE analysis for the linear model (with intercept) proposed by Kelman and Whiting (1980).

XC, XS and XD are simulated amounts of digoxin in the central, shallow and deep compartments for a three compartment mamillary model.

TABLE 3.19
The Linear Model (with intercept)

	STUDY NO. 4	STUDY NO. 7	STUDY NO. 9	STUDY NO. 10
DOSE (mg/kg.)	0.05	0.025	0.05	0.05
Intercept (a_0)	-17.10 (14.11)	-193.10 (20.92)	-15.97 (7.84)	-117.64 (16.90)
b	-38.74 (25.78)	644.48 (74.64)	39.46 (22.33)	206.02 (32.42)
c	217.67 (30.48)	684.50 (59.34)	-387.21 (93.57)	-83.20 (49.49)
d	-14.95 (59.10)	1077.70 (109.47)	375.87 (68.60)	263.97 (32.58)
RESIDUAL (SSE)	2088.74	1382.54	1889.73	1106.70
TOTAL (Corr SSE)	5678.96	7798.54	4678.12	3210.48
RSQUARE	0.63	0.82	0.60	0.65
d.f.	34	35	42	43
N	34	35	42	44
P	4	4	4	4
AIC	275.55	268.34	332.40	316.40

Regression analysis of the linear model proposed by Kelman and Whiting (1980).

* numbers in brackets are standard deviations
N = no. of observations used
P = no. of parameters in the model

b, c and d are coefficients in the model

TABLE 3.20
The Linear Model (without intercept)

	STUDY NO. 4	STUDY NO. 7	STUDY NO. 9	STUDY NO. 10
DOSE (mg/kg.)	0.05	0.025	0.05	0.05
b	-10.56 (11.24)	-17.87 (38.75)	1.30 (12.62)	26.42 (28.83)
c	213.48 (30.50)	337.46 (86.53)	-259.66 (72.20)	-64.46 (72.58)
d	55.72 (9.72)	70.88 (17.67)	240.36 (17.34)	37.65 (3.07)
RESIDUAL (SSE)	2187.75	5063.92	2090.69	2446.84
TOTAL (Uncorr. SSE)	25738.68	22098.68	26100.57	15435.1
RSQUARE	0.91	0.76	0.91	0.84
d.f.	35	36	43	44
N	34	35	42	44
P	3	3	3	3
AIC	282.86	321.61	342.39	357.11

Regression anlysis of the linear model proposed by Kelman and Whiting (1980).

* numbers in brackets are standard deviations
N = no. of observations used
P = no. of parameters in the model

b, *c* and *d* are coefficients in the model

compartment may have a positive contribution to the response in one dog and a negative contribution in another. This contradiction casts doubts on the possibility of multireceptor sites and the applicability of such models.

THE EFFECT COMPARTMENT MODEL

The model was recently proposed by Sheiner *et al.*, (1979), and has been extensively used. A schematic representation of this model may be seen in fig. 1.2 A brief description of this model and a generalized derivation of the relevant equations follows has been presented in chapter 1. From equation (1.19) the effect compartment model may be modelled using the following two equations:

$$\frac{E}{E_{max}} = \frac{C_1^{\delta}}{C_1^{\delta} + Cp_{ss(50)}^{\delta}}$$

or,

$$E = E_0 - E_{max} \cdot \frac{C_1^{\delta}}{C_1^{\delta} + IC_{(50)}^{\delta}} \qquad ...(3.13)$$

where, IC_{50} is the concentration that effects a 50% inhibition of the maximal response (decrease in heart rate), and E is the response (heart rate). Eqn.(3.13) will be referred to as the "inhibitory model" and eqn.(1.19) as the "effect model". E_0 is usually the baseline value for the monitored response assuming that the baseline value has been reliably determined. E_{max} cannot usually be physiologically determined. In this specific case the maximum decrease in heart rate cannot be accurately determined without resulting non-reversible slowing of the heart. Therefore, E_{max} may be assigned a theoretical physiologic maximum (which is a 100% decrease in heart rate), the observed maximum response, or may be regressed as a variable with an upper constraint of the theoretical physiologic maximum. The response variable E is the percent decrease in the heart rate relative to the baseline value and is given by

$$E = \%\ decrease\ in\ heart\ rate = \frac{HR_0 - HR_{obs}}{HR_0} \cdot 100$$

where,

$$HR_0 = \textit{baseline heart rate}$$

$$\textit{and } HR_{obs} = \textit{the observed heart rate at any time.}$$

For our studies the plasma concentration vs. time profile was described by

$$C_1 = D\sum_{i=1}^{3} C_i e^{-\alpha_i t} \qquad ...(3.14)$$

and equation (1.18) is given by,

$$C_1 = k_{e0} \cdot D\sum_{i=1}^{3} \frac{X_i}{V_1(k_{e0} - \alpha_i)} \left[e^{-\alpha_i t} - e^{-k_{e0} t}\right] \qquad ...(3.15)$$

Generally, it is advisable to fit equations 3.14 and 1.19, or. 3.14 and 3.13 simultaneously. However, when the plasma concentration vs. time profile is well described by eqn.(3.14) or a similar expression, equation (1.19) and (3.14) may be regressed independently (Sheiner *et al.*, 1979). The plasma concentration vs. time profile for studies 4. 7. 9 and 10 were well described by equation 3.13 and as evident from table 3.16 had correlation coefficients and R-square values greater than or equal to 0.99 Therefore equations for the effect model and the inhibitory model were fitted without simultaneously regressing equation (3.14).

Equation (1.19) and (3.13) were regressed using the nonlinear regression program NLIN (SAS User's Guide, 1982). E_{max} was assigned a value equal to the observed maximum percent decrease in heart rate. regressed as a variable with the upper limit set at the theoretical physiologic maximum (100% decrease in heart rate), or assigned a value equal to the theoretical physiologic maximum (100%

decrease in heart rate). The results are tabulated in tables 3.21, 3.22 and 3.23 respectively.

The results indicate that the effect model adequately describes digoxin pharmacodynamics in the dogs with respect to its bradycardic effect. A wide variability is seen in the parameters describing the the model viz., K_{e0}, δ and $Cp_{ss(50)}$ in table 3.21 The variability is indicative of the wide variability in the bradycardic effect of digoxin in beagle dogs. There does not seem to be a large differences in the K_{e0} values for studies 7, 9 and 10 although there seems to be an appreciable difference in the values of the parameters obtained for studies 4 and 7 which were studies conducted in the same beagle dog at 0.05mg/kg. and 0.025 mg/kg. of digoxin respectively. Further studies will be necessary to determine whether these changes indicate saturation of the pharmacodynamic response measured. However, it is noted that the $Cp_{ss(50)}$ values for studies 4 and 7 are quite similar. It should also be noted that the δ values for studies 6 and 7 are similar whereas these values are not comparable to those obtained for study 4. Again, in studies 6 and 7 the dose was 0.025 mg/kg. whereas the dose was 0.05 mg/kg. in study 4. Studies 4, 6 and 7 were studies conducted in the same beagle dog.

The results of table 3.22 are those when E_{max} is regressed as a variable and has an upper limit of 100% decrease in heart rate. Except for studies 6 and 10 E_{max} attained a value of 100% after regression procedures. Again K_{e0} values for studies 7, 9 and 10 are not comparable to those observed for study 4 and are independent of E_{max}. However, in contrast to the values in table 3.21, the K_{e0} values for studies 4 and 6 seem to be quite similar. This confuses the picture since studies 4, 6 and 7 were conducted in the same dog. A fewer number of points were available for modelling the pharmacodynamics of study 6. This may detract from the reliability of the parameter values for study 6. The δ values for study 4 are again quite dissimilar to those for studies 6, 7, 9 and 10.

TABLE 3.21
The Effect Compartment Model

	STUDY NO. 4	STUDY NO. 6	STUDY NO. 7	STUDY NO. 9	STUDY NO. 10
DOSE (mg/kg.)	0.05	0.025	0.025	0.05	0.05
$K_{e0}(min._{-1})$	0.0415 (0.028)	0.04 (0.022)	0.0088 (0.00066)	0.0026 (0.00028)	0.0027 (0.0003)
$Cp_{ss(50)}$ (ng/ml.)	8.18 (1.21)	9.84 (0.18)	3.77 (0.10)	5.9 (0.27)	5.05 (0.23)
DELTA	0.92 (0.17)	7.15 (0.067)	5.16 (0.66)	5.85 (1.27)	7.13 (2.00)
E_{max} (%)	50.20	46.68	45.46	45.55	27.96
RESIDUAL (SSE)	2048.39	41.86	8107.53	1967.32	1116.14
TOTAL (Corr. SSE)	4839.91	5968.45	7389.95	4168.78	3419.90
RSQUARE	0.58	0.99	0.89	0.53	0.67
d.f.	31	14	32	39	41
N	34	17	35	42	44
P	3	3	3	3	3
AIC	265.24	69.48	228.27	324.54	314.77

Results of regression analysis for the effect compartment model.

* numbers in brackets are standard deviations
N = no. of observations used
P = no. of parameters in the model

E_{max} = observed maximum response

TABLE 3.22
The Effect Compartment Model

	STUDY NO. 4	STUDY NO. 6	STUDY NO. 7	STUDY NO. 9	STUDY NO. 10
DOSE (mg/kg.)	0.05	0.025	0.025	0.05	0.05
$K_{e0}(min._{-1})$	0.034 (0.023)	0.038 (0.0016)	0.0072 (0.00066)	0.0026 (0.00036)	0.0024 (0.00018)
$Cp_{ss(50)}$ (ng/ml.)	56.00 (613.87)	10.20 (0.19)	5.26 (0.25)	7.94 (1.56)	5.65 (1.40)
DELTA	0.60 (0.42)	7.16 (0.31)	3.77 (0.38)	4.033 (1.17)	7.12 (3.95)
E_{max} (%)	100.00 (---)	50.22 (1.26)	100.00 (---)	100.00 (—)	47.77 (42.01)
RESIDUAL (SSE)	1769.21	27.54	570.96	1838.57	1040.31
TOTAL (Corr. SSE)	4839.91	5968.45	7389.95	4168.78	3419.90
RSQUARE	0.63	0.99	0.92	0.56	0.70
d.f.	30	16	34	41	43
N	34	17	35	42	44
P	4	4	4	4	4
AIC	262.26	64.36	230.16	323.70	313.68

Results of regression analysis for the effect compartment model.

* numbers in brackets are standard deviations
N = no. of observations used
P = no. of parameters in the model

E_{max} was regressed as variable with an upper limit of 100%

TABLE 3.23
The Effect Compartment Model

	STUDY NO. 4	STUDY NO. 6	STUDY NO. 7	STUDY NO. 9	STUDY NO. 10
DOSE (mg/kg.)	0.05	0.025	0.025	0.05	0.05
$K_{e0}(min._{-1})$	0.033 (0.014)	0.041 (0.005)	0.007 (0.00004)	0.0026 (0.0002)	0.0023 (0.00016)
$Cp_{ss(50)}$ (ng/ml.)	54.56 (0.000016)	15.95 (1.3)	5.25 (0.25)	7.93 (0.67)	6.57 (0.49)
DELTA	0.61 (0.11)	2.42 (0.48)	3.78 (0.42)	4.03 (0.86)	6.34 (1.16)
E_{max} (%)	100.00	100.00	100.00	100.00	100.00
RESIDUAL (SSE)	1941.86	260.71	732.13	1907.84	1125.97
TOTAL (Corr. SSE)	4839.91	5968.45	7389.95	4168.78	3419.90
RSQUARE	0.60	0.96	0.90	0.54	0.67
d.f.	33	16	34	41	43
N	34	17	35	42	44
P	3	3	3	3	3
AIC	263.43	100.57	236.86	323.26	315.16

Results of regression analysis for the effect compartment model.

* numbers in brackets are standard deviations
N = no. of observations used
P = no. of parameters in the model

E_{max} was assigned a value of 100%

The results of table 3.21 are quite similar to those of tables 3.22 and 3.23 E_{max} was assigned a value of 100%. Treatment of E_{max} as a variable and assigning fixed values does not seem to cause any noticeable deviations in the values of the estimated parameters. However, the best model of the various models investigated above will be further discussed.

Results of modelling the inhibitory model described by eqn.(3.13) are given in table 3.24.

The values for the parameters obtained are comparable to those in table 3.22 The results indicate that significant differences may not exist when modelling is conducted using either of the equations (1.19 or 3.13) since mathematically they are but juxtaposed forms of each other.

TABLE 3.24
The IC_{50} Model

	STUDY NO. 4	STUDY NO. 6	STUDY NO. 7	STUDY NO. 9	STUDY NO. 10
DOSE (mg/kg.)	0.05	0.025	0.025	0.05	0.05
$K_{e0}(min._{-1})$	0.03377 (0.0144)	0.038 (0.0050)	0.0073 (0.00033)	0.0025 (0.0002)	0.0023 (0.00016)
IC_{50} (ng/ml.)	54.65 (41.17)	10.22 (0.57)	5.26 (0.55)	7.86 (0.68)	6.65 (0.83)
DELTA	0.61 (0.41)	7.16 (2.71)	3.77 (1.25)	4.03 (5.66)	5.96 (1.63)
E_{max} (bpm)	104.426 (—)	52.33 (5.32)	153.134 (—)	92.38 (—)	167.412 (—)
RESIDUAL (SSE)	1958.20	219.12	969.51	1575.53	2756.30
TOTAL (Corr. SSE)	5277.82	6494.13	17329.46	3557.66	8351.21
RSQUARE	0.63	0.97	0.94	0.56	0.67
d.f.	30	7	34	41	40
N	34	17	35	42	44
P	4	4	4	4	4

Results of regression analysis for the IC_{50} model.

* numbers in brackets are standard deviations
N = no. of observations used
P = no. of parameters in the model

E_{max} = variable with an upper limit as the basal heart rate.

COMPARISON OF VARIOUS MODELS

The various models investigated were compared to determine the best model based on certain statistical criteria. The computational procedures which formed the basis for the comparison were the F-value (Boxenbaum *et al.*, 1974) and the Akaike information criterion [AIC (Akaike H., 1973,1976; Yamaoka *et al.*, 1978)]. In general, a model A was considered to superior to model B if model A met one or more of the following criterion.

1) Residual sum of squares for A is less than the residual sum of squares for B.
2) Using the F-test, the weighted sum of squared deviations for model A is significantly lesser than that for model B.
3) AIC for model A is less than AIC for model B. It is assumed that the models A and B provide adequate visual fit and that a plot of the residual sum of squares vs. the observed values does not display any systematic deviations. The results of the comparison of the various models investigated are given in table 3.25

In the case of the linear models, the model without the intercept term was significantly better than the model with an intercept. The various models investigated were compared to the effect model with E_{max} as a variable. Overall the effect model with E_{max} as a variable was the best model based on the AIC, F-value and the residual sum of squares.

Very little difference was observed in the values of the parameters and the residual sum of squares obtained using either eqn.(1.19) or (3.13). However based on the criteria mentioned above effect model seems to be superior to the inhibitory model and use of the effect model with E_{max} as a variable may be a more desirable approach compared to use of the inhibitory model.

TABLE 3.25
COMPARISON OF VARIOUS MODELS

		LINEAR with intercept	LINEAR no intercept	E_{max} = variable	E_{max} = 100	E_{max} = max. observed	IC_{50} = variable
STUDY NO. 4	AIC	275.55	282.86	262.26	263.43	265.24	265.71
	F value	5.96**	3.90**	—	2.93**	4.73**	—
	RESIDUAL (SSE)	2088.74	2187.75	1769.21	1941.86	2048.39	1958.20
	d.f.	30	31	30	31	31	30
STUDY NO. 6	AIC	—	—	64.36	100.57	69.48	—
	F value	—	—	—	110.06**	6.76**	—
	RESIDUAL (SSE)	—	—	27.54	260.71	41.86	—
	d.f.	—	—	13	14	14	—
STUDY NO. 7	AIC	268.34	321.61	230.16	236.86	240.27	232.68
	F value	48.32**	133.77**	—	8.75**	12.82**	—
	RESIDUAL (SSE)	1382.54	5063.92	570.96	732.13	807.00	969.51
	d.f.	32	31	31	32	32	31
STUDY NO. 9	AIC	332.4	342.39	323.70	323.26	324.54	301.22
	F value	1.14	2.81	—	1.43	2.66	—
	RESIDUAL (SSE)	1889.73	2090.69	1838.59	1907.84	1967.32	1575.53
	d.f.	39	38	38	39	39	38
STUDY NO. 10	AIC	316.40	357.11	313.68	315.16	314.77	340.55
	F value	2.15	58.14**	—	3.29**	2.92**	—
	RESIDUAL (SSE)	1106.70	2446.84	1040.31	1125.97	1116.14	2756.3
	d.f.	41	40	40	41	41	40

Results of comparison of various models.

AIC = Akaike information criterion

** indicates statistical significance at $P < 0.01$

Physiologic Pharmacokinetic Models: Simulation Using SPICE2

Introduction:

Physiologic pharmacokinetic models have gained increasing acceptance since their application to drug disposition in the late 1960's by Bischoff and Brown (1966). Investigators have recognized the fact that the compartment volumes, rate constants and other parameters obtained by classical pharmacokinetics, i.e, by empirical curve fitting, provide no information on the actual physiological and anatomical mechanisms of the particular animal species being studied. In contrast, physiologic pharmacokinetic models (Bischoff, 1967; Dedrick and Bischoff, 1979; Chen and Gross, 1979) allow animal data to be extrapolated to humans, in cases where "scale-up" techniques (Dedrick. 1978; Boxenbaum, 1982) are applicable. Parameters obtained by classical pharmacokinetics in animals cannot usually be related to similar parameters in humans. Also, physiologic pharmacokinetic models provide information on the dispostion of drug in a specific region of the body such as the heart, brain or tumors. For these reasons, physiologic pharmacokinetic models are now being used to study the uptake and distribution of various drugs ranging from anesthetics (Mapleson, 1963; Saidman and Eger, 1973; Igari *et al.*, 1982; Bischoff and Dedrick, 1968; Price *et al.*, 1960) to anti-cancer drugs (Chen and Gross, 1979; Himmelstein and Bischoff, 1973; Chen and Coleman, 1978; Dedrick *et al.*, 1973; Dedrick *et al.*, 1972; Himmelstein and Gross, 1977; Harris and Gross. 1975; Chan *et al.*, 1978; Dedrick *et al.*, 1973; Bischoff *et al.*, 1970; Bischoff *et al.*, 1971). Specific drugs that have been studied include thiopental (Saidman and Eger, 1973; Igari *et al.*, 1982; Bischoff and Dedrick, 1968; Price *et al.*, 1960), lidocaine (Benowitz *et al.*, 1970),

sulfabromothalein (Montandon *et al.*, 1975), digoxin (Harrison and Gibaldi, 1977a, 1977b), salicylates (Chen *et al.*, 1978), Ara-C (Dedrick *et al.*, 1972, 1973), cyclocitidine (Himmelstein and Gross, 1977a, 1977b), adriamycin (Harris and Gross, 1975; Chan *et al.*, 1978), and methotrexate (Dedrick *et al.*, 1973; Bischoff *et al.*, 1970, 1971).

Although physiologic pharmacokinetic models have been in use over the past few years, their potential application to pharmacodynamics has not been exploited. Pharmacodynamic models have generally used conventional pharmacokinetic models with linear linking models (Kramer *et al.*, 1979; Kelman and Whiting 1980; Holford 1981; Eichelbaum 1980), the log linear model (Shephard 1979; Ishizaki 1980), the sigmoid model (Mitenko and Ogilvie 1973, Singh *et al.*, 1980) and the more recent effect compartment model (Sheiner *et al.*, 1979; Hennis *et al.*, 1985: Stanski *et al.*, 1984: Chiang and Barnett 1984). Therefore the primary aim was to develop and apply physiologic pharmacokinetic models using SPICE2 (Mikulecky, 1982, 1983; SPICE 1981; Thakker *et al.*, 1982) and to investigate their possible application to pharmacodynamics.

SPICE2 is a powerful integrated circuit simulation program, which was developed by electrical engineers at the Univeristy of California, Berkeley for the purpose of designing and testing complicated circuits. However, it has been recently realized that electrical elements and biological systems are both governed by principles that emphasize force-flow relationships and conservation of energy. It is an evolution of this understanding of common (thermodynamic) principles governing electrical and biological elements that led to network thermodynamics. Network thermodynamics uses elements that are distinguished by their thermodynamic properties, namely their manner of manipulating energy. The resistor is an energy dissipator, the capacitor an energy storage element of one kind, and the inductor the representative of intertial energy storage. The morphology of a system can therefore be translated

into a network with little effort. The compartments become nodes, the dissipative barriers (*e.g.* membranes) the branches between these compartmental nodes. Network thermodynamics may therefore be thought of as a collection of parts which allows us to investigate the 'whole system' by representing the topology of interactive systems by means of interconnected network elements which dissipate, store, supply or convert energy. It is this concept that allows us to use a circuit simulation program like SPICE2 to investigate the behavior of biological systems.

SPICE2 has been used extensively in simulating diverse and complex biological systems, but has been only recently introduced in the field of pharmacokinetics (Thakker *et al.*, 1982); and here has been only applied to classical pharmacokinetics. Here SPICE2 has been adapted for simulating a physiologic pharmacokinetic model with a linking linear pharmacodynamic model for the cardioactive drug digoxin, administered to the rat (Harrison and Gibaldi, 1977a), dog and human (Harrison and Gibaldi, 1977b). This model was developed so that simulated levels of digoxin in the heart may be obtained which could then be related to the pharmacologic responses in dogs.

The biophase (i.e., the compartment in which the pharmacologic effect for digoxin occurs) is in the heart and not readily accessible. Physiologic pharmacokinetic models using cardiac physiologic parameters and pharmacologic responses as an indirect measurement of heart concentrations will provide a new approach to understanding the relationship between digoxin plasma concentrations and biophasic concentrations. The developed models are applied to the results of our studies in dogs to illustrate the value of SPICE2 in modelling these data.

Generally, physiologic pharmacokinetic models are described by differential equations. Conventional computer programs like the Continous system modelling program (CSMP, 1967), General program for pharmacokinetic modelling (GPPM) (Dedrick *et al.*,). SAAM (CONSAM 1983) are utilized in solving these differential

equations. It was therefore of interest to compare these programs with SPICE2 which directly uses network topology rather than differential equations to describe and simulate complex systems.

Experimental:

A program was designed using SPICE2 to simulate the physiologic pharmacokinetic model for digoxin in the rat, dog and man described by Harrison and Gibaldi (1977a, 1977b). These models were simulated using SPICE2 and compared to those obtained using GPPM and CSMP. The data obtained from the initial pharmacokinetic studies (Tables 3.1-3.3) and from the pharmacodynamic studies (Tables 3.11, 3.13, 3.15 and 3.16) were used to verify the accuracy and validity of the simulations.

A general procedure developed for the simulation of physiologic pharmacokinetic models using SPICE2 is as follows:

a) The Physiologic Pharmacokinetic Model:

The physiologic pharmacokinetic model consists of blood (or plasma) flows in and out of compartments. In general a flow-limited model is used (whenever applicable) for simplification purposes. Fig. 3.23 is a simple representation of a physiologic model.

Using the "lumped compartment approach", anatomical regions of interest maybe lumped with other compartments of similar characteristics relative to drug disposition. Volumes for compartments, blood flows and other pertinent information are readily available in the literature. There have been several excellent reviews on creating physiologic pharmacokinetic models (Chen and Gross, 1979; Himmelstein and Lutz, 1979; Lutz, 1977: Gillette, 1982: Gerlowski and Jain, 1983).

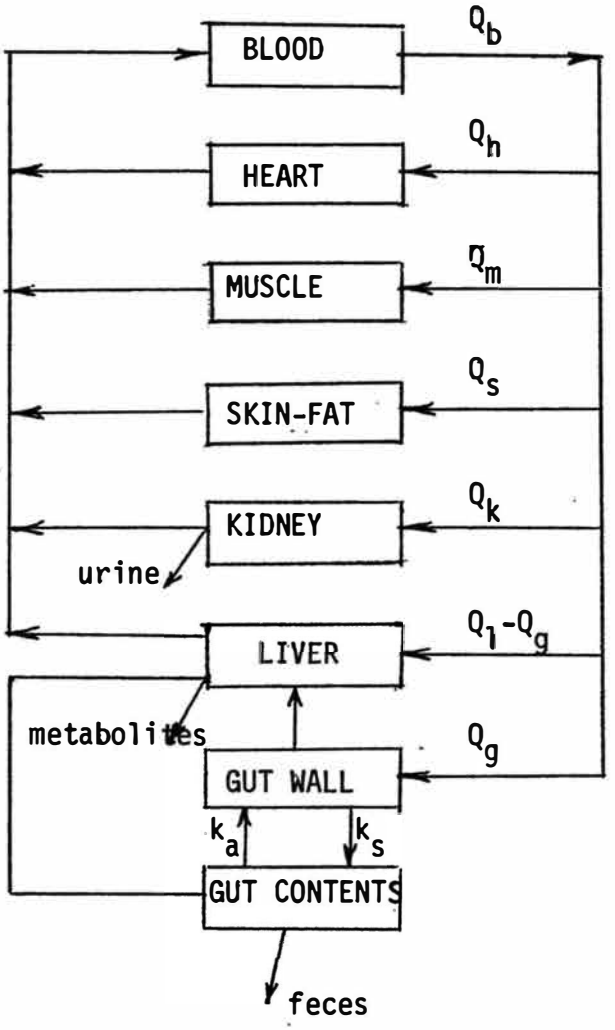

Fig. 3.23 A Physiologic Pharmacokinetic Model for digoxin.

b) Creating A Network:

Creating a network for a given physiologic pharmacokinetic model, requires that the information needed to describe a physiologic pharmacokinetic model be incorporated in that network. It may be described by the following three steps:

1) Constructing the blood flow network

2) Constructing the mass balance network

3) Link the blood flow and the mass balance networks

1) BLOOD FLOW NETWORK

Fig. 3.24 shows the network for blood flow corresponding to the physiologic model shown in Fig. 3.23

In the physiologic pharmacokinetic model the total plasma flow Q_p is distributed into flows into the various compartments. The relationship between the flows into the various compartments is as follows:

$$\frac{Q_g}{Q_l} - \frac{(Q_l - Q_g)}{Q_p} + \frac{Q_k}{Q_p} + \frac{Q_s}{Q_l} + \frac{Q_m}{Q_l} + \frac{Q_h}{Q_p} = 1$$

where, Q_p, Q_k, Q_m, Q_g, Q_s, Q_h, Q_l, are the plasma flows through the plasma, kidney, muscle, gutwall, skin, heart and liver compartments respectively. The fraction of the total flow (f_i) through each compartment is therefore,

$$f_i = \frac{Q_i}{Q_p}$$

The network is then constructed based on the above relationships. Resistances are used to partition the plasma-flow. The total plasma flow (cardiac output, Q_p) is

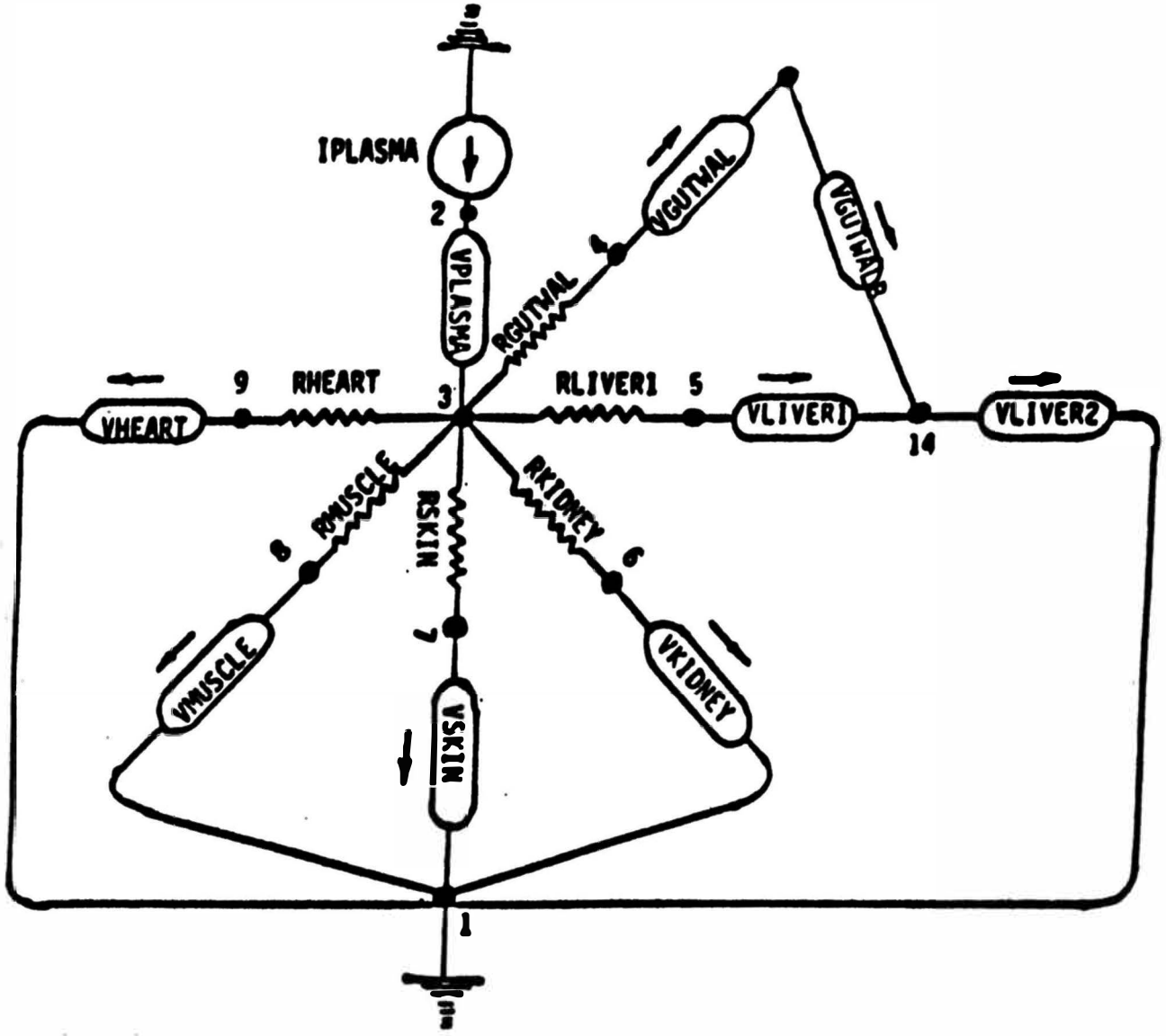

Fig. 3.24 Blood flow network for a physiologic pharmacokinetic model using SPICE2.

supplied by a constant current source termed IPLASMA. The relationship between plasma flows may be mimicked using a similar relationship between the resistances:

$$\frac{1}{RGUTWALL} + \frac{1}{RLIVER} + \frac{1}{RKIDNEY} + \frac{1}{RSKIN} + \frac{1}{RMUSCLE} + \frac{1}{RHEART} = 1$$

Hence, the resistances are assigned values as follows:

$$RGUTWALL = \frac{1}{f_g} = \frac{Q_p}{Q_g} \qquad RKIDNEY = \frac{1}{f_k} = \frac{Q_p}{Q_k} \quad etc.$$

(2) MASS BALANCE NETWORK

Rate constants and tissue to plasma partition coefficients have to be incorporated into the mass balance network. The network which incorporates mass balance is given in Fig. 3.25

The basic representation can be understood by the example in fig. 3.26

Flux through the compartment in fig. 3.26 is given by,

$$KC = J$$

where, K = *rate constant (equal to clearance in pharmacokinetic terms) or permeability constant (units of ml/min.)*

C = *concentration (mg/ml.). and*

J = *flux (mg/min.)*

The network for the above compartmental representation is also given in fig. 3.26 In this case,

C = *voltage at node* 1

J = *current flowing through the system*

K = *value given to GRATE*

(GRATE is a voltage controlled current source)

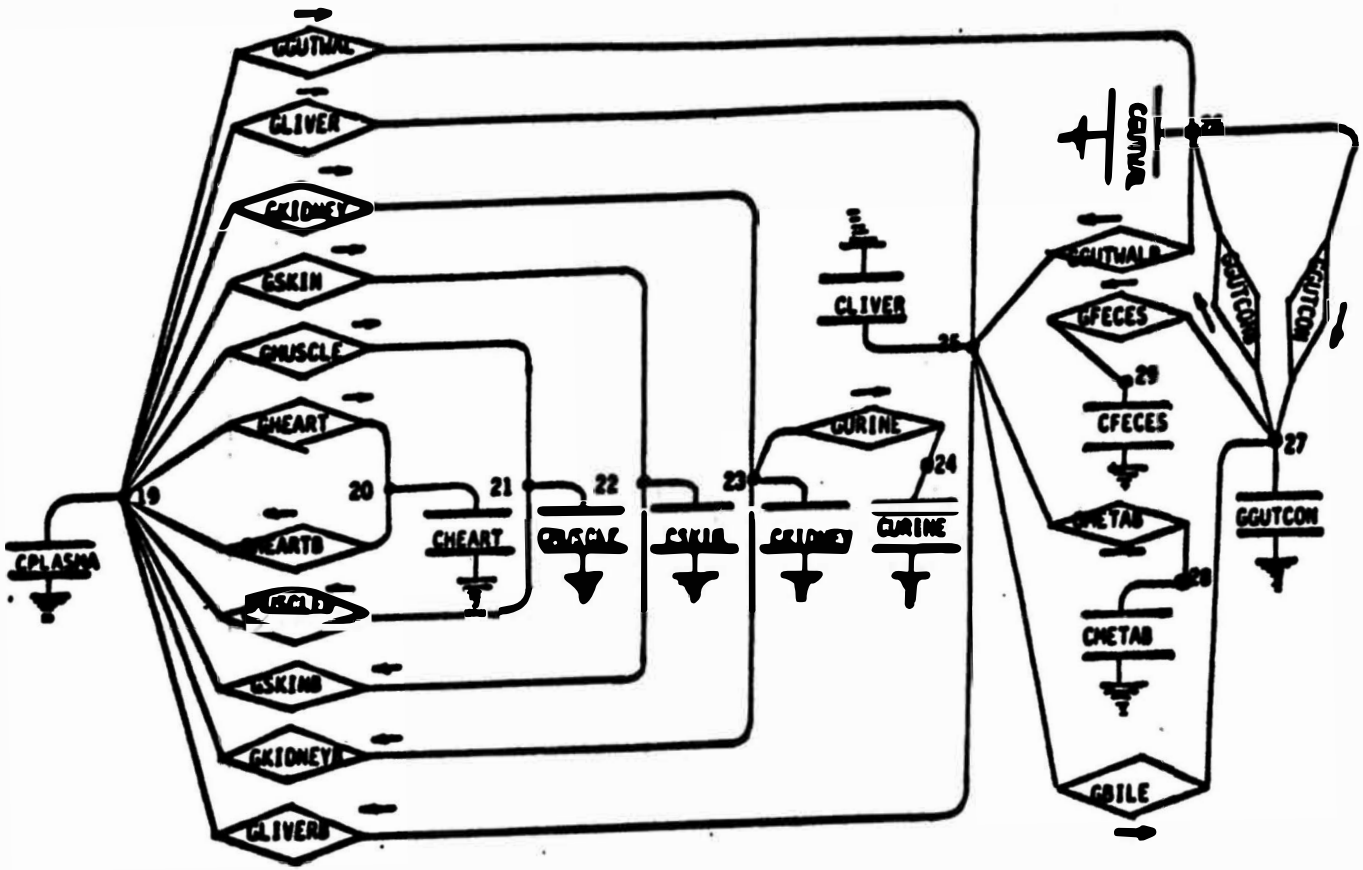

Fig. 3.25 Mass balance network for a physiologic pharmacokinetic model using SPICE2.

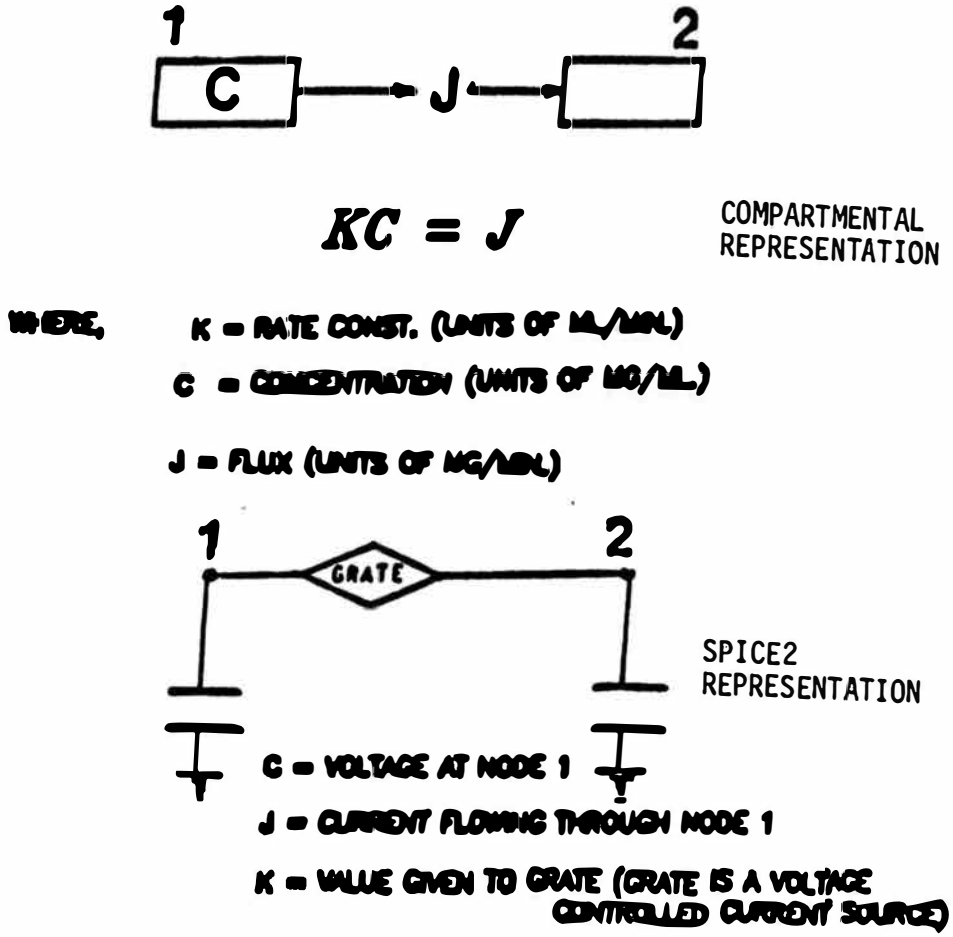

Fig. 3.26 Comparison between compartmental and SPICE2 representations.

For an explanation of the network given in fig. 3.25, one can consider the mass balance for drug in the heart compartment in fig. 3.25, where,

$$Amount\ \ in = Q_h C_p$$

$$Amount\ \ out = \frac{Q_h C_p}{R_h}$$

$$= \frac{Amount\ \ in}{R_h}$$

Hence, in fig. 3.26 we assign a

$$value = 1\ \ for\ \ GHEART\ \ and\ \ a$$

$$value = \frac{1}{R_h}\ \ for\ \ GHEARTB\ \ (GHEART\ \ back)$$

where, C_p is the concentration in the plasma at any time and R_h is the tissue to plasma concentration ratio for the heart.

(3) LINKING THE BLOOD FLOW AND MASS BALANCE NETWORKS:

To combine the blood flow and mass balance networks the SPICE2 program provides (a) F elements and (b) Polynomial G elements.

The G elements, as mentioned before, are voltage controlled current sources and as the name implies, are voltage controlled elements. But, in fig. 3.24 the plasma flows are represented by current flows. Therefore, to use G elements for mass transfer, current flowing through the nodes in fig. 3.24 have to be converted to voltages. To accomplish this, the network shown in fig. 3.27 is used.

The network in fig. 3.27 is composed of F elements which are current controlled current sources, and are defined as follows:

$$F******\ \ N^+\ \ N^-\ \ VNAM\ \ value$$

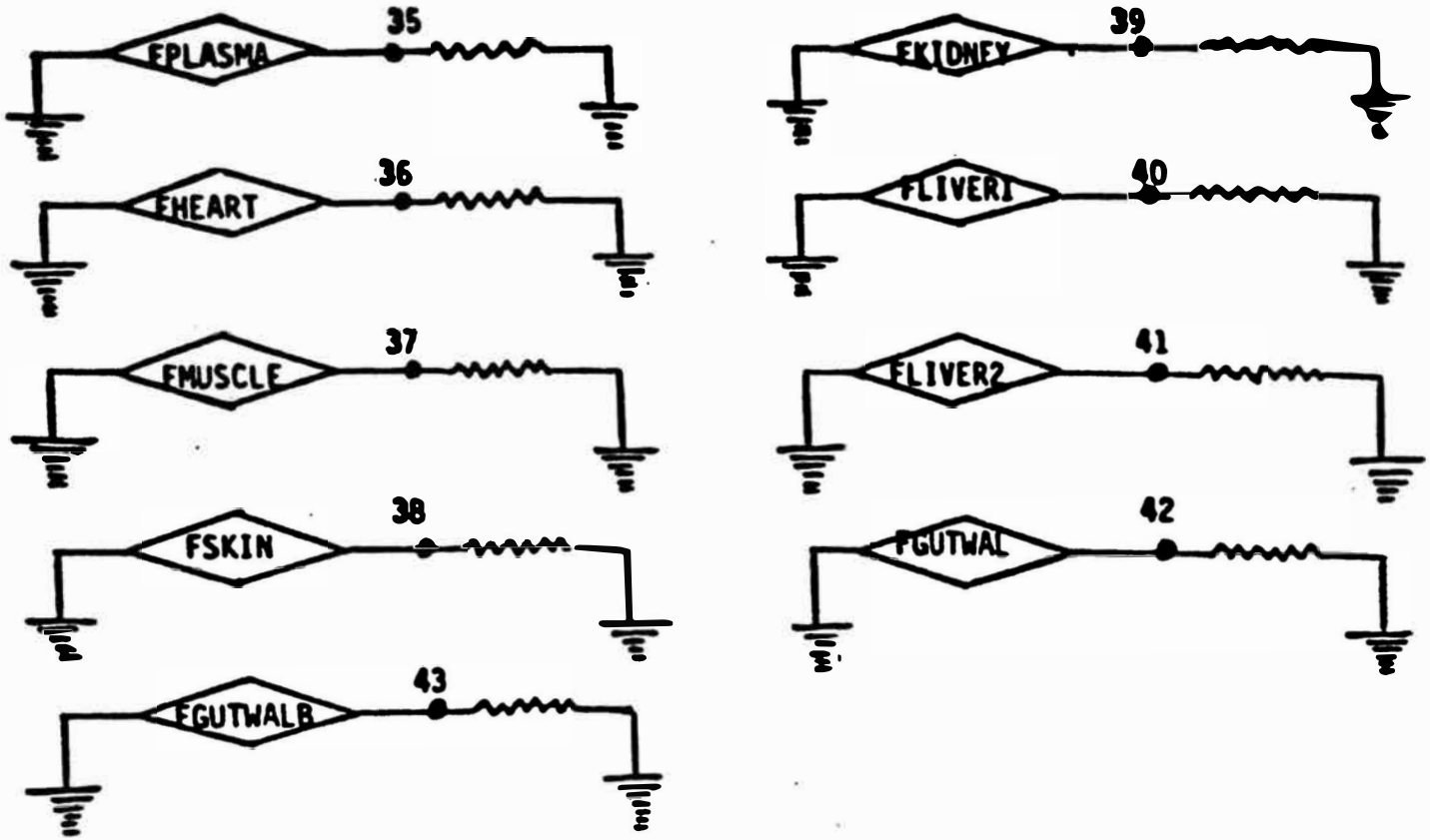

Fig. 3.27 SPICE2 network linking the blood flow and mass balance networks.

where, F******* represents the name assigned to the element, N^+ and N^- are the positive and negative nodes which describe the location of the F element. VNAM = name of the voltage source through which the controlling current flows and *value* = current gain.

Since $V = I \cdot R$, the F elements are assigned a *value* = 1, and multiplied by a *Resistance* = 1 in order to convert the current flow to a voltage. This technique has been used to convert the currents flowing in fig. 3.24 to voltages in fig. 3.27 at nodes 35, 36, 37, 38, 39, 40, 41, 42 and 43. For example, in the circuit given fig. 3.27; FHEART is defined as.

$$FHEART \quad 0 \quad 36 \quad VHEART \quad 1$$

This auxiliary circuit converts the current flowing through the dummy ammeter VHEART, to a voltage at node 36; by multiplying it by a resistance of value 1.

In fig. 3.23. the net flux into or out of any compartment is a product of:

a) plasma flow through the compartment (Q)

b) Concentration in the compartment (C)

Specifically, the rate of change of drug in the heart is given by:

$$V_h(\frac{dC_h}{dt}) = Q_h[C_p - (\frac{C_h}{R_h})]$$

where, V_h is the volume of the heart, C_h and C_p are the concentrations of drug in the heart and plasma respectively, Q_h is the plasma flow through the heart and R_h is the tissue to plasma partition coefficient. Hence, to obtain the product of Q and C, polynomial G elements are used. A polynomial G element is described as follows:

$$G******* \quad POLY(n)$$

In fig. 3.25, GHEART is described as,

$$GHEART \;\; 19 \;\; 20 \;\; POLY(2) \;\; 36 \;\; 0 \;\; 19 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 1$$

Here, 19 20 describes the location of the element. 36 0 and 19 0 are the flow and concentration controlling nodes. The polynomial is given by:

$$a_0 + a_1 x + a_2 y + a_3 x^2 + a_4 xy$$

if, x = plasma flow and y = concentration, flux = xy

Therefore, GHEARTB is defined as,

$$GHEARTB = 20 \;\; 19 \;\; POLY(2) \;\; 36 \;\; 0 \;\; 19 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; \frac{1}{R_h}$$

Here,

$$a_0, a_1, a_2, a_3 = 0$$

$$a_4 xy = \frac{1}{R_h} \cdot x \cdot y$$

$$hence, \;\; a_4 = \frac{1}{R_h}$$

A computer listing of the program is given in Table 3.26.

Results:

SPICE2 SIMULATIONS AND COMPARISONS TO CSMP AND GPPM

The physiologic pharmacokinetic model for digoxin in the rat (Harrison and Gibaldi, 1977a), dog and human (Harrison and Gibaldi, 1977b), was simulated using SPICE2 and using the programs, CSMP and GPPM, in order to validate and identify comparative advantages and disadvantages of the programs. Values for flows, compartment volumes, and tissue to plasma partition coefficients were obtained from Harrison and Gibaldi (1977a, 1977b).

TABLE 3.26

```
PHYSIOLOGIC PHARMACOKINETIC MODEL FOR DIGOXIN IN DOGS
IPLASMA  0  2  DC 63
R101  1  0  1N
R115  15  0  1E25
VPLASMA  2  3  DC  0
RGUTVAL 3  4  3.5
RLIVER  3  5  10.5
RKIDNEY  3  6  5.25
RSKIN  3  7  5.25
RMUSCLE  3  8  5.25
RHEART  3  9  21
VGUTVAL  4  15  DC  0
VGUTVALB  15  14  DC  0
VLIVER1 5  14  DC  0
VKIDNEY  6  1  DC  0
VSKIN  7  1  DC  0
VMUSCLE  8  1  DC  0
VHEART  9  1  DC 0
VLIVER2  14  1  DC  0
.IC  V(1)-0  V(2)-63  V(3)-63  V(4)-0  V(5)-0  V(6)-0  V(7)-0
+  V(8)-0 V(9)-0 V(14)-0 V(15)-0 V(19)-1000  V(23)-1N  V(25)-1N
+  V(26)-1N V(27)-1E-5 V(24)-1E-5 V(28)-1E-5 V(29)-1U  V(20)-1N
+  V(21)-1N  V(22)-1N
CPLASMA 19  0  0.5  IC-1000
CHEART  20  0  0.05 IC-1N
GHEART 19  20  POLY (2) 36  0  19  0  0  0  0  0  1
GHEARTB  20  19  POLY (2)  36  0  20  0  0  0  0  0  0.025
R36  20  0  1E25
CMUSCLE  21  0  5  IC-1N
GMUSCLE  19  21  POLY (2)  37  0  19  0  0  0  0  0  1
GMUSCLEB  21  19  POLY (2)  37  0  21  0  0  0  0  0  0.111
R37  21  0  1E25
CSKIN  22  0  3.5 IC-1N
GSKIN  19  22  POLY (2) 38  0  19  0  0  0  0  0  1
GSKINB  22  19  POLY (2)  38  0  22  0  0  0  0  0  0.111
R38  22  0  1E25
GKIDNEY  19  23  POLY (2)  39  0  19  0  0  0  0  0  1
CKIDNEY  23  0  0.05 IC-1N
GKIDNEYB  23  19  POLY (2)  39  0  23  0  0  0  0  0  0.005
 .PRINT TRAN V(19) V(20)
R35  19  0  1E25
R39  23  0  1E25
GURINE  23  24  23  0  0.012
CURINE  24  0  1  IC-1E-5
R40  24  0  1E25
GLIVER  19  25  POLY (2) 40  0  19  0  0  0  0  0  1
CLIVER  25  0  0.25  IC-1N
GLIVERB  25  19  POLY (2)  41  0  25  0  0  0  0  0  0.0667
GMETAB  25  28  25  0  0.0534
CMETAB  28  0  1  IC-1E-4
R44  28  0  1E25
R41  25  0  1E25
GGUTVAL  19  26  POLY (2)  42  0  19  0  0  0  0  0  1
GGUTVALB  26  25 POLY (2)  43  0  26  0  0  0  0  0  0.0333
R42  26  0  1E25
CGUTVAL  26  0  0.24  IC-1N
CGUTCON  27  0  0.42  IC-1E-5
GGUTCON  26  27  26  0  0.106
GGUTCONB  27  26  27  0  0.0924
GFECES  27  29  27  0  0.04
CFECES  29  0  1  IC-1U
R45  29  0  1E25
GBILE  25  27  25  0  0.0333
```

TABLE 3.26 contd.

```
R43 27  0  1E25
.TRAN 0.16667  60.16667   UIC
FPLASMA  0  35  VPLASMA 1
FHEART   0  36  VHEART  1
FMUSCLE  0  37  VMUSCLE 1
FSKIN  0  38  VSKIN  1
FKIDNEY  0  39  VKIDNEY  1
FLIVER1  0  40  VLIVER1  1
FLIVER2  0  41  VLIVER2  1
FGUTWAL  0  42  VGUTWAL  1
FGUTWALB  0  43  VGUTWALB  1
R8  35  0  1
R9  36  0  1
R10  37  0  1
R11  38  0  1
R12  39  0  1
R13  40  0  1
R14  41  0  1
R15  42  0  1
R16  1  0  1E25
R17  2  0  1E25
R18  3  0  1E25
R19  4  0  1E25
R20  5  0  1E25
R21  6  0  1E25
R22  7  0  1E25
R23  8  0  1E25
R24  9  0  1E25
R29  14  0  1E25
R46  35  0  1E25
R47  36  0  1E25
R48  37  0  1E25
R49  38  0  1E25
R50  39  0  1E25
R51  40  0  1E25
R52  41  0  1E25
R53  42  0  1E25
R54  43  0  1E25
R55  43  0  1
.OPTIONS ITL1=500 ABSTOL=1F VNTOL=1N RELTOL=1U NODE LIST NOPAGE
.OP
.END
```

Typical program listing for SPICE2. The program listing is for a physiologic pharmacokinetic model for digoxin in dogs (Gibaldi and Harrison, 1977).

Fig. 3.28 gives the SPICE2 simulated concentrations of digoxin in the plasma of a 10 kg. dog given a 0.05 mg/kg. dose of digoxin i.v.

The three sets of data points (denoted by 0's, 1's and 2's) are the actual serum levels that were obtained experimentally in three dogs administered 0.05 mg/kg. i.v. There is good agreement between the actual observed values and those predicted by SPICE2 using the digoxin model.

Statistical comparisons were made on the simulations obtained using SPICE2 and those obtained using GPPM and CSMP. The simulated values obtained from all three programs were very much similar to each other as evident from the correlation tests and regression procedures (table 3.27).

Regression of SPICE2 simulated values against simulated values obtained using CSMP and GPPM resulted in slopes of one and intercepts very close to zero indicating the similarity of the values obtained.

Application to pharmacodynamics

It was intended to simulate digoxin levels in the heart using the physiologic model and to relate it to the bradycardic effect observed in studies 4, 7, 9 and 10. Simulations of digoxin plasma levels using the model of Harrison and Gibaldi (1977b), did not agree with the plasma levels of digoxin observed in studies 4, 6, 9 and 10. However, the same model was useful in predicting plasma levels of digoxin in the initial pharmacokinetic studies (fig. 3.28). A possible explanation is that the plasma to tissue partition coefficients used in the physiologic pharmacokinetic model by Harrison and Gibaldi (1977b) are obtained at equilibrium. Hence the model does not accurately predict the levels of digoxin in the distributive phase i.e. the first 6 hours (which comprise most of the data from studies 4, 7, 9 and 10). The model was therefore modified to simulate the levels observed in the above studies. This involved altering the plasma to tissue partition coefficients. The elimination rate constants were assigned values obtained from pharmacokinetic analysis of the data.

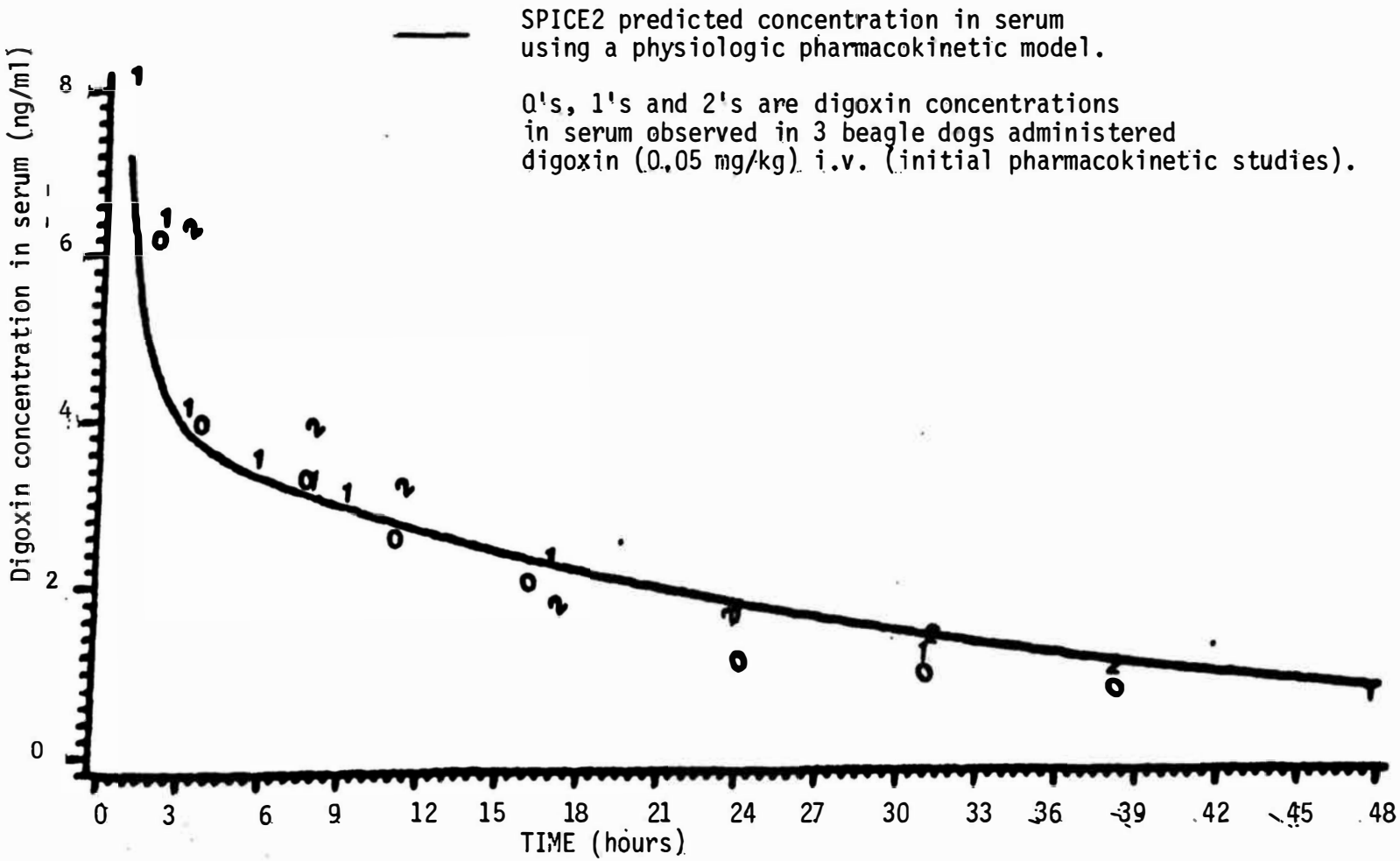

Fig. 3.28 Digoxin concentrations in serum predicted by SPICE2 using a physiologic pharmacokinetic model, following administration of 0.05 mg/kg of digoxin i.v. bolus.

SPICE2 vs. GPPM	SPEARMAN, KENDALL AND PEARSON CORR. COEFF.	REGRESSION MODEL	R^2
1) PLASMA CONC. (SPICE2) vs. PLASMA CONC. (GPPM)	1	PLASMA CONC. (SPICE2) = PLASMA CONC. (GPPM) - 0.0025	1
2) HEART CONC. (SPICE2) vs. HEART CONC. (GPPM)	1	HEART CONC. (SPICE2) = HEART CONC. (GPPM) - 9.55×10^{-5}	1
SPICE2 vs. CSMP			
1) PLASMA CONC. (SPICE2) vs. PLASMA CONC. (CSMP)	1	PLASMA CONC. (SPICE2) = PLASMA CONC. (CSMP) + 4.1×10^{-5}	1
2) HEART CONC. (SPICE2) vs. HEART CONC. (CSMP)	1	HEART CONC. (SPICE2) = HEART CONC. (CSMP) + 9.3×10^{-5}	1

TABLE 3.27 Table comparing concentrations in the plasma and in the heart as simulated by SPICE2 to those obtained using CSMP and GPPM. Concentrations were simulated for a 10 kg. dog administered 0.05 mg/kg of digoxin i.v.

The model developed was found to predict the observed concentrations extremely well. The simulations are given in figs. 3.29, 3.30 and 3.31

The levels in the heart were then simulated. The results of the simulation may be seen in figs. 3.32 and 3.33

It is observed that the bradycardic effect does not correlate well with the simulated levels in the heart. In fig. 3.32 and 3.33 the peak levels of digoxin in the heart occur much earlier than the peak bradycardic effect. This implies that the biophase for the bradycardic effect for digoxin may be a subcompartment of the heart whose diffusion characteristics are different from those of the highly perfused regions. However efforts at modelling the biophase as a subcompartment of the heart were not successful. This may indicate that the biophase for the bradycardic effect of digoxin may lie in a compartment other than the heart. This is possible since the heart rate is strongly influenced by the sympathetic and parasympathetic controls on the heart. The biophase then could be the nodose ganglion or the carotid sinus (Chai *et al.*, 1967; Higgins *et al.*, 1973; Abiko 1963; Eliakim *et al.*, 1961). The tissue to plasma partition coefficients used to fit the plasma concentration time profile obtained in the pharmacodynamic studies (studies 4, 7, 9 and 10) are arbitrary values. Therefore, the simulated concentrations in the heart obtained from this model may not be predictive of the true concentrations observed in the heart. This could be a possible explanation for the lack of correlation between simulated concentrations of digoxin in the heart and the observed bradycardic response.

Discussion

Pharmacokineticists have long recognized the fact that the compartments in conventional models, cannot usually be translated directly into the physiologic or anatomic regions that they represent. Hence, the concentrations of drug in specific organs of the body, where the toxic or therapeutic responses may be elicited, cannot

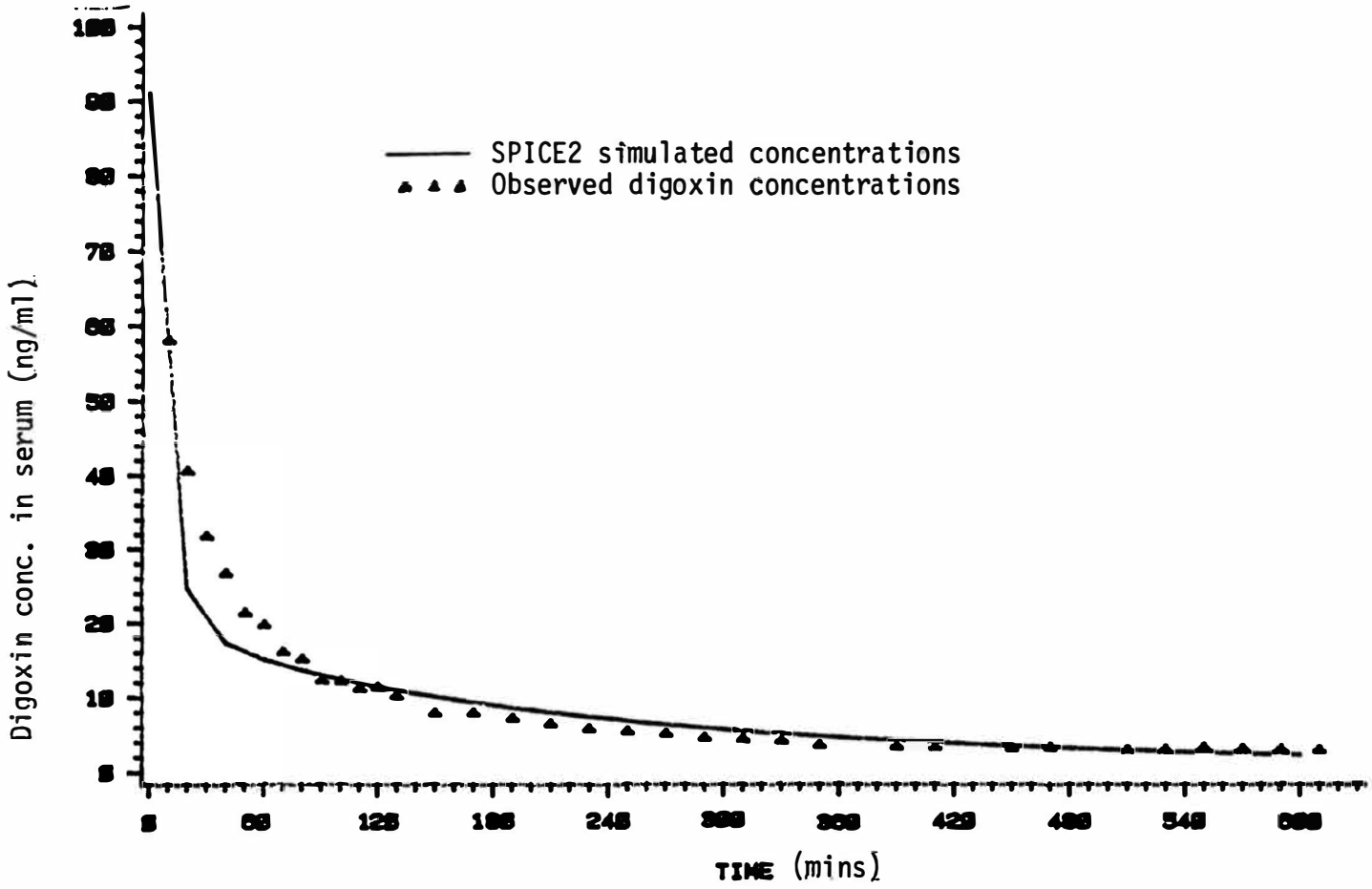

Fig. 3.29 Digoxin concentrations after administration of 0.05 mg/kg digoxin i.v. to a normal beagle dog. SPICE2 simulated concentrations are obtained using a physiologic pharmacokinetic model.

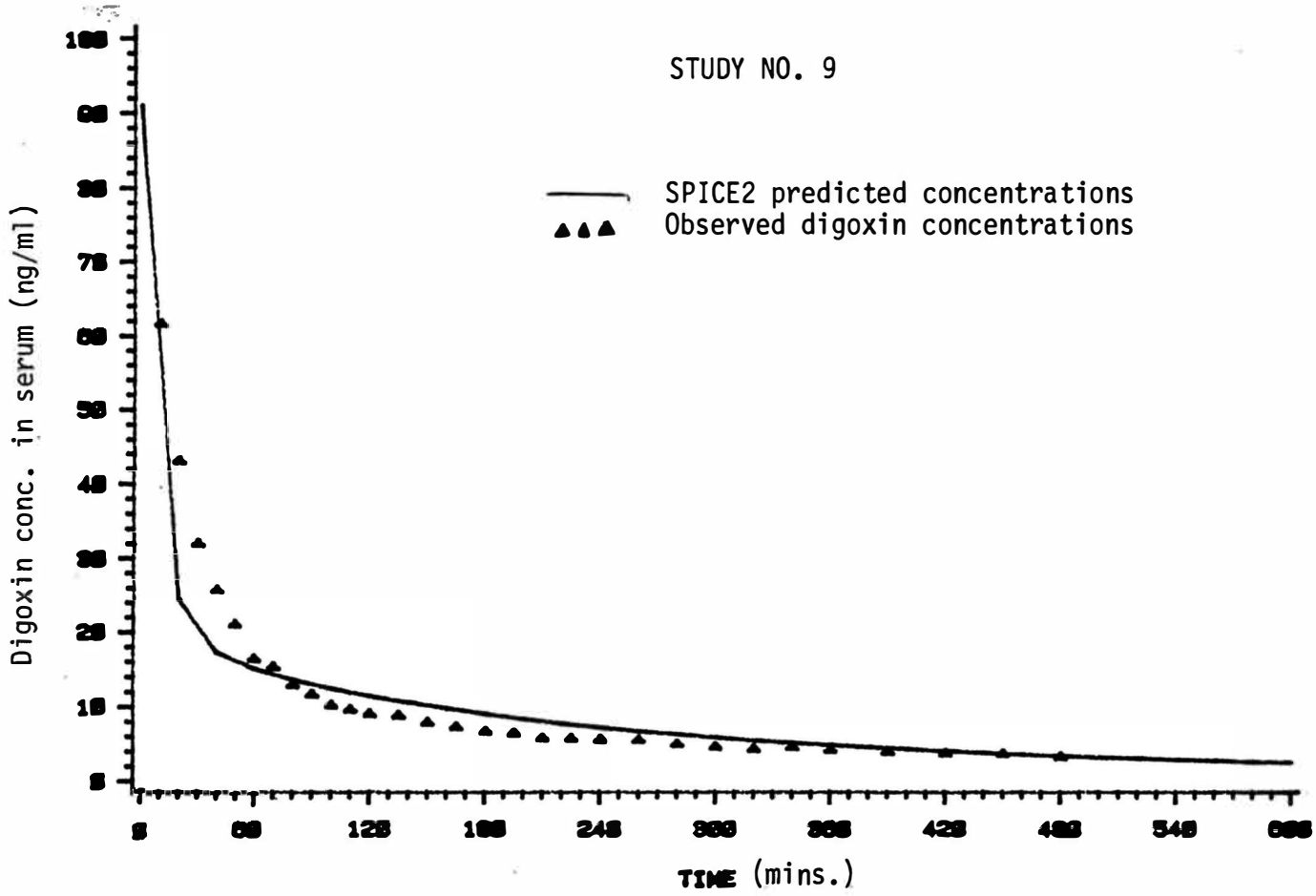

Fig. 3.30 Digoxin concentrations in serum following administration of 0.05 mg/kg digoxin i.v. to a normal beagle dog. SPICE2 simulated values are obtained using a physiologic pharmacokinetic model.

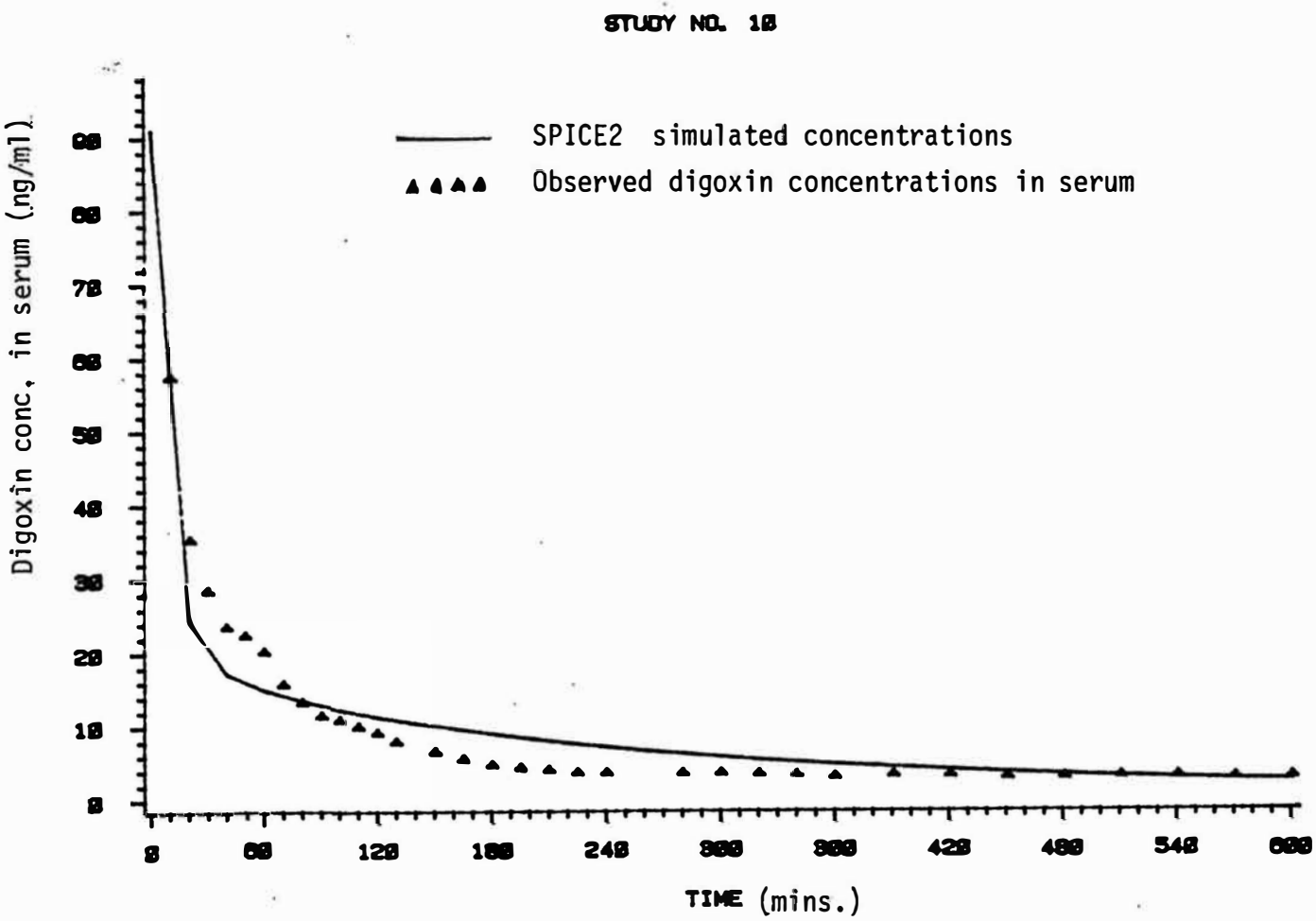

Fig. 3.31 Digoxin concentrations in serum following administration of 0.05 mg/kg of digoxin i.v. to a normal beagle dog. SPICE2 simulated concentrations are obtained using a physiologic pharmacokinetic model.

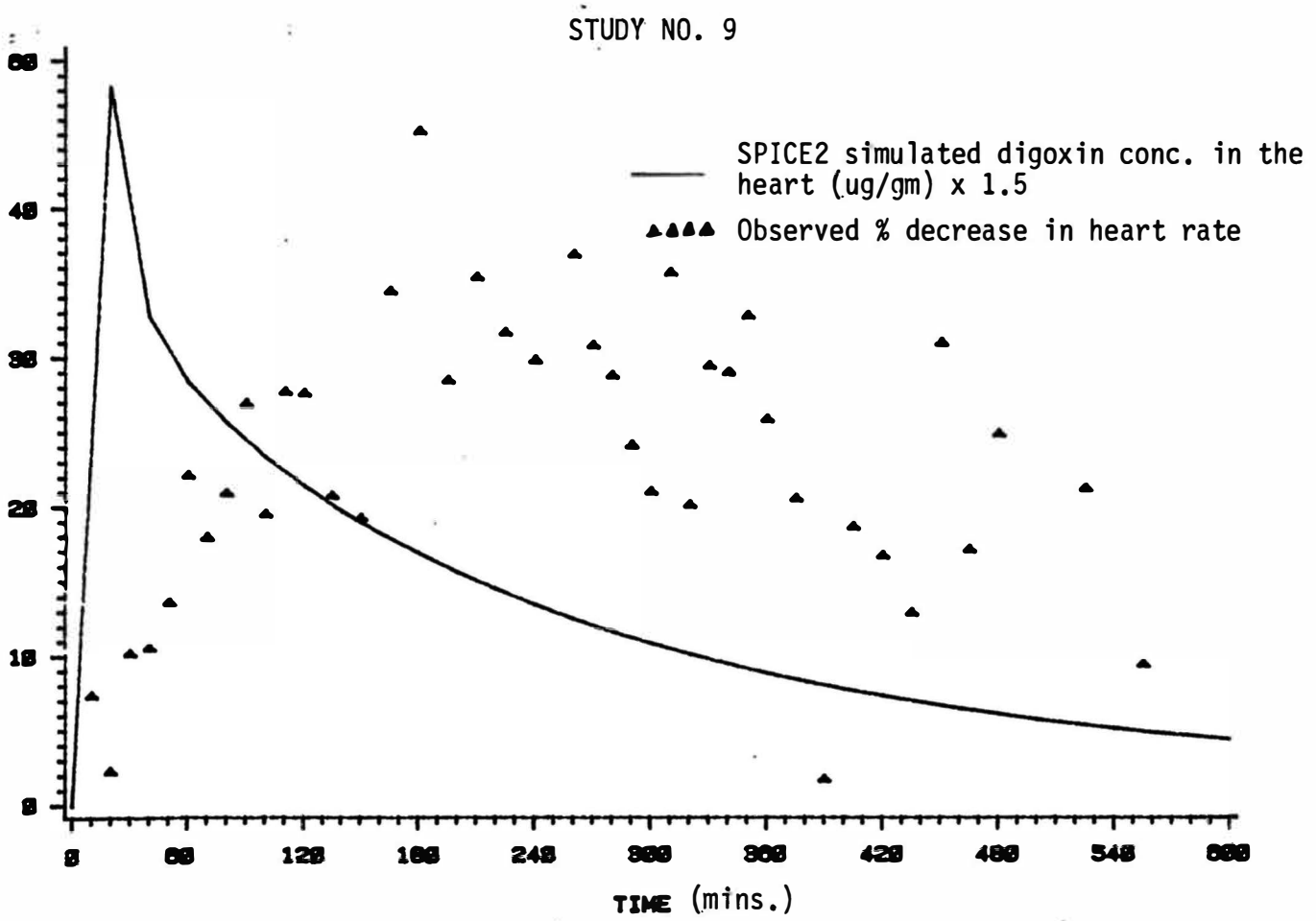

Fig. 3.32 Lack of correlation between simulated digoxin concentrations in the heart and the observed % decrease in heart rate following administration of 0.05 mg/kg. of digoxin i.v. to a normal beagle dog. SPICE2 simulated concentrations are obtained using a physiologic pharmacokinetic model.

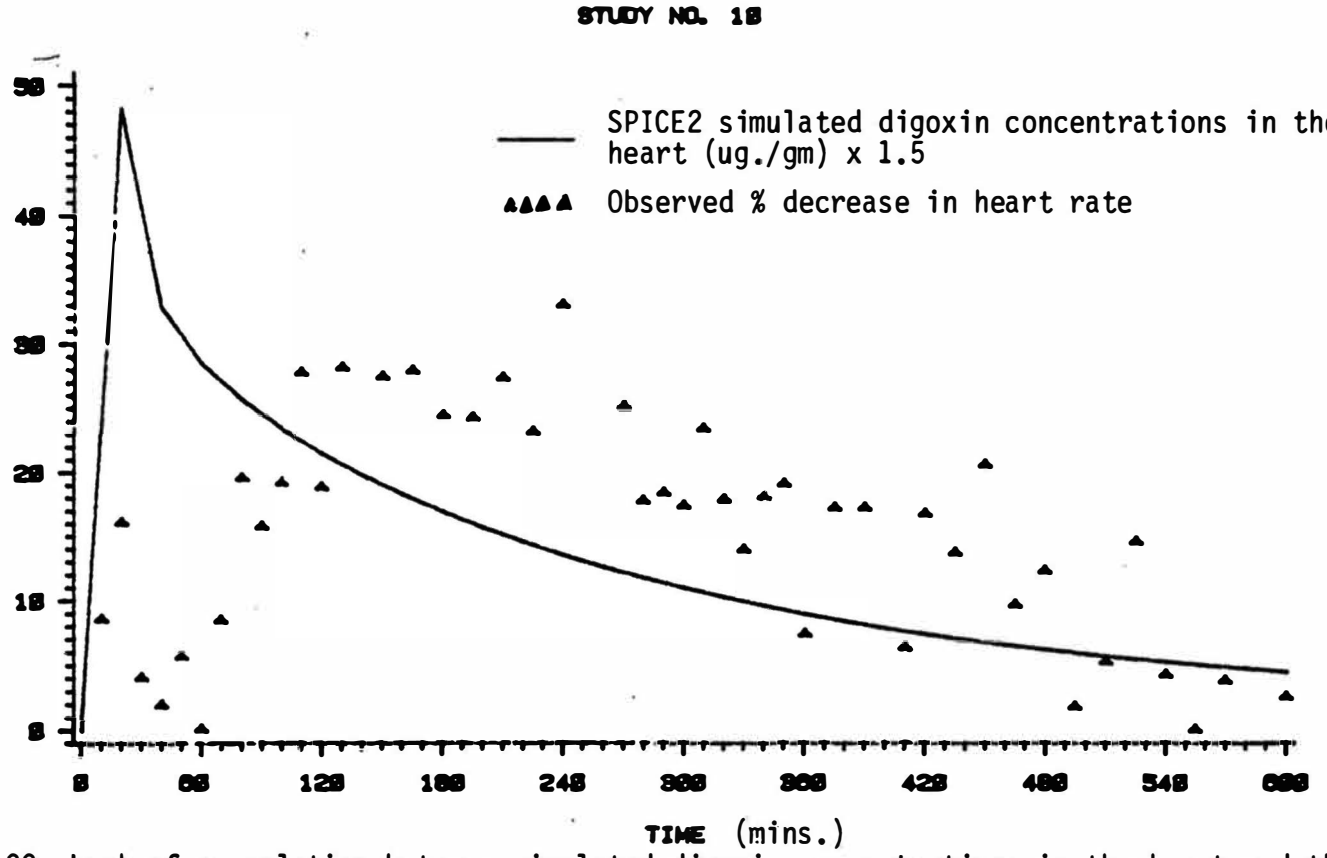

Fig. 3.33 Lack of correlation between simulated digoxin concentrations in the heart and the observed % decrease in heart rate following administration of 0.05 mg/kg digoxin i.v. to a normal beagle dog. SPICE2 simulated concentrations are obtained using a physiologic pharmacokinetic model.

be obtained from conventional models. A few examples where such information is needed are:

1) concentrations in the heart (cardioactive drugs) e.g., digoxin.

2) concentrations in the brain for monitoring CNS effects, e.g., side effects, of digoxin, and therapeutic effects of antipsychotic drugs.

3) concentrations in the CNS, e.g., (anesthetics) lidocaine, thiopental.

4) concentrations in tumors of anti-cancer drugs, e.g., methotrexate, adriamycin.

Since regions such as brain, tumor or heart are not accessible in clinical practice, models that will simulate the levels of drugs in these compartments are neeeded. Such models can also be used to design dosage regimens to achieve desired drug levels in a target organ. The interest in physiologic pharmacokinetic models has also been partly fueled by the renewed interest in pharmacodynamics. This is because in order to completely define a drug effect we must know not only its kinetics, but also the relationship between levels in the various tissues and the observed therapeutic and adverse responses. Comprehensive, descriptive models and techniques are thus needed to simulate the levels of the drug at the biophase which may then be related to the observed pharmacodynamic response(s). SPICE2 would appear to fulfill these requirements. It is a simple, yet powerful, simulation program that can simulate complex systems by using network thermodynamics to describe the physiologic pharmacokinetic models.

SPICE2 provides several advantages over conventional simulation techniques, including:

1) It requires no knowledge of computer languages such as FORTRAN. SPICE2 is a tool that one can use without having to master complex computer languages.

2) SPICE2 programs were found to be more robust than the CSMP and GPPM programs. The GPPM program has the drawback of a fixed step size for computational and print-out routines. This led to abnormal termination of the program

when using small step sizes. Tightening the error limits could also led to abnormal termination of the program.

3) Multiple dosing is very easily simulated using PULSE or PIECEWISE LINEAR functions. This can be accomplished by connecting an independent current source IDOSE to the plasma compartment (node 19 in fig. 3.25). The PULSE function is then defined as follows:

$$IDOSE \ N^- \ N^- \ PULSE \ (V_1 \ V_2 \ TD \ TR \ TF \ PW \ PER)$$

where,

$V_1 = initial \ \ value$

$V_2 = pulsed \ \ value$

$TD = delay \ \ time$

$TR = rise \ \ time$

$TF = fall \ \ time$

$PW = pulse \ \ width$

$PER = period$

The period (PER) above can be used as the dosing interval. The pulse width (PW) is assigned small or large values depending upon whether an intravenous bolus dose, or a slow i.v. infusion is required. Oral dosing can be similarly simulated by connecting the source IDOSE to a G element, which in turn is connected to the plasma compartment. The value assigned to the G element would be the rate constant for the process of absorption of drug in to the plasma compartment. Thus by introducing only two additional cards describing IDOSE and the PULSE specifications, the sophistication of simulating multiple dosing can be added to an

existing model. The original program requires no reinitialization, since the dose is directly introduced into the desired compartment in the fashion desired.

Conventional programs (including GPPM and CSMP) require the original program to be drastically changed to convert an existing model for oral dosing, to a model for multiple dosing, or, vice-versa. Also, conventional programs require reinitialization steps after every dose administered

4) In most cases, extra compartments or additional sophistication can be introduced into the main SPICE2 program by introducing additional cards. These cards can be introduced anywhere in the existing program. Conventional programs do not offer this advantage, since the main program or subroutines would have to be re-written.

5) One of the salient advantages that SPICE2 offers is the ease with existing programs can be modified. Ex., deleting one card describing GBILE in fig. 3.25 gives a model of a bile-ligated rat (dog or human, depending upon the model in use). Similarly, deleting the card describing GURINE converts the model to that of a ureter-ligated rat.

6) The SPICE2 program can also simulate sophisticated physiologic pharmacokinetic models which additionally specify the residence times of the drug in the various compartments. SPICE2 can also simulate with considerable ease, physiologic phenomena like gastric emptying (Thakker *et al.*, 1982), urine flow, and drug responses such as increasing cardiac output with time.

This can be a very important feature with cardioactive drugs (e.g., digoxin) which increase cardiac output as a function of time, thus altering drug kinetics. For the example given in fig. 3.25, the cardiac output can be changed over time by assigning the independent current source, IPLASMA, a time-dependent value(s) or by using the independent source functions: PULSE. PIECEWISE LINEAR. Another

example of physiologic response that can be varied is the increase in splanchnic flow in response to various stimuli (e.g., food).

Conclusion:

It has been demonstrated that SPICE2 can be used to simulate physiologic pharmacokinetic models and that the physiologic pharmacokinetic model may be extended to model drug pharmacodynamics. SPICE2 can be used as a powerful simulation tool by clinicians, physiologists and pharmacokineticists, due to the relative ease with which it can simulate complex systems. SPICE2 possesses distinct advantages over conventional simulation programs such as CSMP and GPPM. It can be used to simulate physiologic pharmacokinetic models, to obtain simulated concentrations of drugs in the various physiological compartments in order to relate them to their observed therapeutic and non-therapeutic responses. Using SPICE2 and physiologic pharmacokinetic models, we thus have the capability to obtain simulated drug concentrations in the various organs in the body. This information can also be used to design dosage regimens, to give desired concentrations in the target organ, or to avoid undesired side effects due to accumulation of drug or its metabolite(s) in organs other than the target organ.

CHAPTER 4

DISCUSSION

The primary objective of the research was to develop and evaluate analytical, pharmacokinetic and pharmacodynamic methods for the study of digoxin.

Plasma digoxin samples obtained from studies conducted in normal beagle dogs were assayed using the HPLC-RIA and RIA procedures. It was observed that there existed little difference between the values obtained using the HPLC-RIA and RIA methods. This implies that upon digoxin administration, significant amounts of metabolites are not formed by the normal beagle dog. Since plasma samples of digoxin were obtained frequently in the initial distributive phase as well as over a period of 72 hours (post digoxin administration). it seems apparent that significant amounts of metabolites are not formed or do not accumulate in the normal dog. This correlates well with the information available on humans. Gibson and Nelson (1979, 1980) reported that the values obtained were essentially identical in patients with glomerular filtration rates greater than 40 ml min. They suggested that the differences in the values may be significant in patients with renal failure presumably due to accumulation of cross-reacting metabolites. The specific HPLC-RIA assay developed could be of clinical value in determining digoxin levels in patients with poor renal function. The assay would also be of value in studies with digoxin in dogs with induced renal failure or altered renal function. Based on these studies it is possible to conclude that for studies in dogs with normal renal function. the greater specificity achieved with the use of specific HPLC-RIA assay is not necessary and the conventional RIA would generally be sufficient.

Having developed a specific method for assaying digoxin, the next objective was to develop and evaluate pharmacokinetic and pharmacodynamic methods for studies in dogs. Conceptually, the relationship between pharmacokinetics and pharmacodynamics may be depicted as in figure 4.1

It is necessary to simultaneously monitor both the plasma levels of drug and the response over time. to characterize the pharmacokinetic-pharmacodynamic model. There have been very few studies that have investigated digoxin pharmacokinetics in dogs. Hence. the initial studies conducted were primarily designed to elucidate pharmacokinetics of digoxin in beagle dogs. Results of the compartmental analysis conducted (tables 3.5 and 3.6) indicate that digoxin disposition in dogs is best described by a tri-exponential equation. The terminal half-life for the initial pharmacokinetic studies was 57.5 hrs. and 46.2 hrs. for dogs 2 and 3. However a half-life of 216.56 hrs. was estimated for dog 1. This enormously large half-life for dog no. 1 is probably an artifact arising from fitting the data to a tri-exponential equation and the weighting function used. Another probable reason is that the changes in concentration observed during the terminal elimination phase border on the lower limits of the sensitivity of the assay. The large half-life for digoxin observed for dog no. 1 on using a tri-exponential equation could possibly be an artifact since a half-life of 46.2 hrs. was estimated using a bi-exponential equation to describe the data. This is supported by observed half-lives of 49.5 hrs. for both dogs 2 and 3 on using a bi-exponential equation to describe the data. However, statistical analysis of the plasma concentration *vs.* time data from initial pharmacokinetic studies and the pharmacodynamic studies indicate a tri-exponential equation to be superior to a bi-exponential equation.

Pharmacodynamic studies using digoxin in dogs revealed linear relationships between LVET, QS_2 and PR-intervals vs. HR. Similar relationships have been established for humans by Weissler *et al.,* (1966, 1968). These relationships have

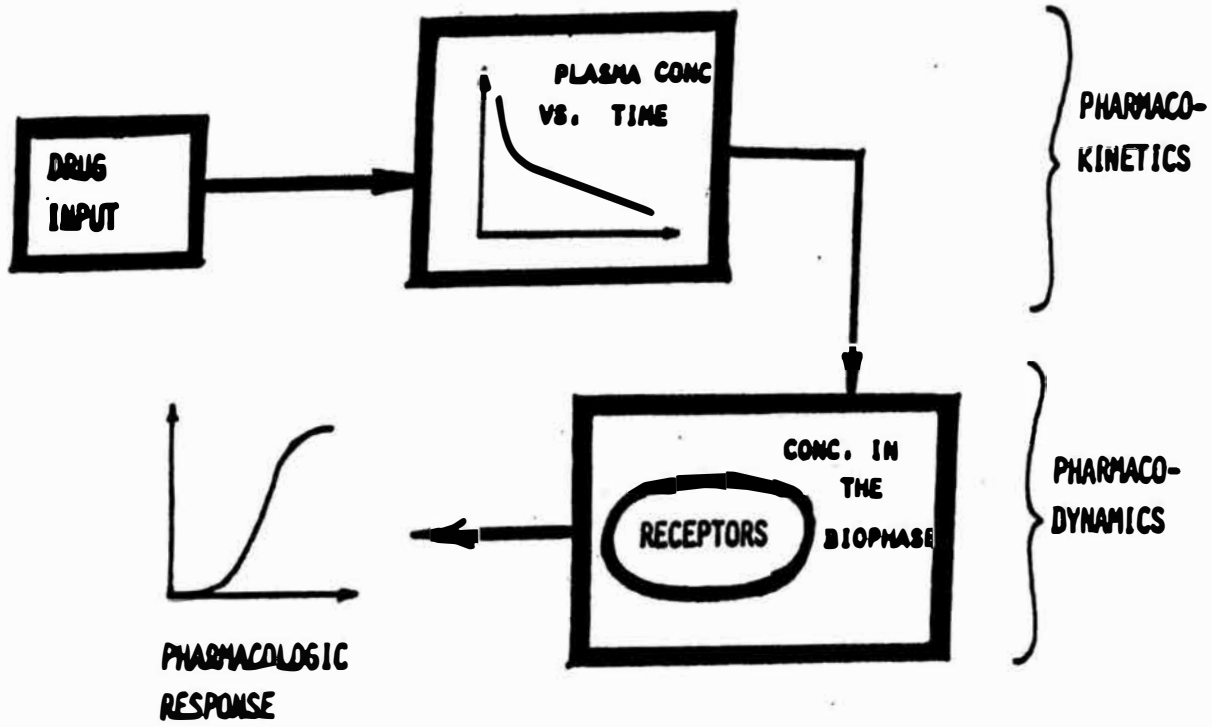

Fig. 4.1 Relationship between pharmacokinetics and pharmacodynamics.

not been established before in dogs. Further there have been no studies in which LVET has been non-invasively determined in dogs. The exteriorization of the carotid artery in the dog provides a method of non-invasively measuring LVET (post surgery) in the dog. This represents an important contribution to the use of the dog as a model for investigating digoxin pharmacodynamics. It should be noted that although linear relationships exist between STI and HR, the slopes and the intercepts for the relationship obtained in dogs is not identical to those observed in humans. It is observed in figs. 3.1, 3.2 and 3.3 that the dog no. 5 (study no. 1B), does not obey the same linear relationship. As mentioned before dog no. 5 was different from the other dogs in that dog no. 5 was a mongrel whereas the dogs used in the remaining studies were beagles. Dog no. 5 also had a much lower basal HR compared to the other dogs. Further dog no. 5 was not under anesthesia, whereas the studies in beagles were conducted under anesthesia. The serum potassium levels and the thyroid function for all the dogs were normal. Any one or more of the above mentioned factors could be contributory to the different linear relationship observed in the case of dog no. 5. This is indicative of the differences in the observed pharmacodynamic relationship that may arise from differences in experimental conditions or selection of experimental animals.

Similarities in the relationship between HR and STI in dogs and in humans indicate the adequacy of the dog as a model for investigative pharmacodynamic studies. Further 2 or 3 compartment models have also been used to describe digoxin disposition in humans (Sumner *et al.*, 1976, Shenfield *et al.*, 1977, Koup *et al.*, 1975). The half-life for digoxin in humans and in dogs is similar. Also, the plasma concentration *vs.* time profile following the administration of a single dose of digoxin may be described by a tri-exponential equation in both dogs and humans indicating the dog to be an adequate model for investigational pharmacokinetic studies using

digoxin. These studies therefore indicate that the dog is an adequate model for investigative pharmacokinetic-pharmacodynamic studies with digoxin.

Pharmacodynamic modelling of the STI was investigated. The STI viz., P-R interval, LVET, QS_2 and PEP, as a function of time after administration of a intravenous dose of digoxin, is given in figures 4.2-4.9 The data did not lend itself well to pharmacodynamic modelling. Also, as mentioned before, the STI were related to the the HR. Further, the bradycardic response to digoxin was readily monitored and lent itself to pharmacodynamic modelling. For the the above reasons the bradycardic response to digoxin was primarily investigated for the purposes of pharmacodynamic modelling.

Quest and Gillis (1972), suggest that the rapid initial fall in HR after digoxin is probably due to sensitization of the carotid sinus and aortic arch baroreceptors. A direct effect of digitalis on the sinus node could not be demonstrated (Ten Eick and Hoffman, 1969). Pace *et al.*, (1974), also suggest that the most important mechanism involved in the effect of digoxin on the sinus and AV-node may be reflex inhibition of the nervous system.

Abiko *et al.*, (1963), suggest the vagus nerves which contain both afferent and efferent fibres and the sympathetic nervous system as pathways through which the bradycardic response to digoxin may be mediated. These two systems are independent of each other in producing cardiac slowing. Impulses sent to the central nervous system through the sinus nerves are suggested to exert an inhibitory effect on the sympathetic center. This effect may be relayed to the heart through the cervical cord and the stellate or nodose ganglia. Yet another mechanism that has been suggested is that cardiac glycosides stimulate chemoreceptors in the body. It was suggested that the impulses originate in the chemoreceptors in the carotid body, pass through the cervical cord and the stellate ganglia to the heart to exert an

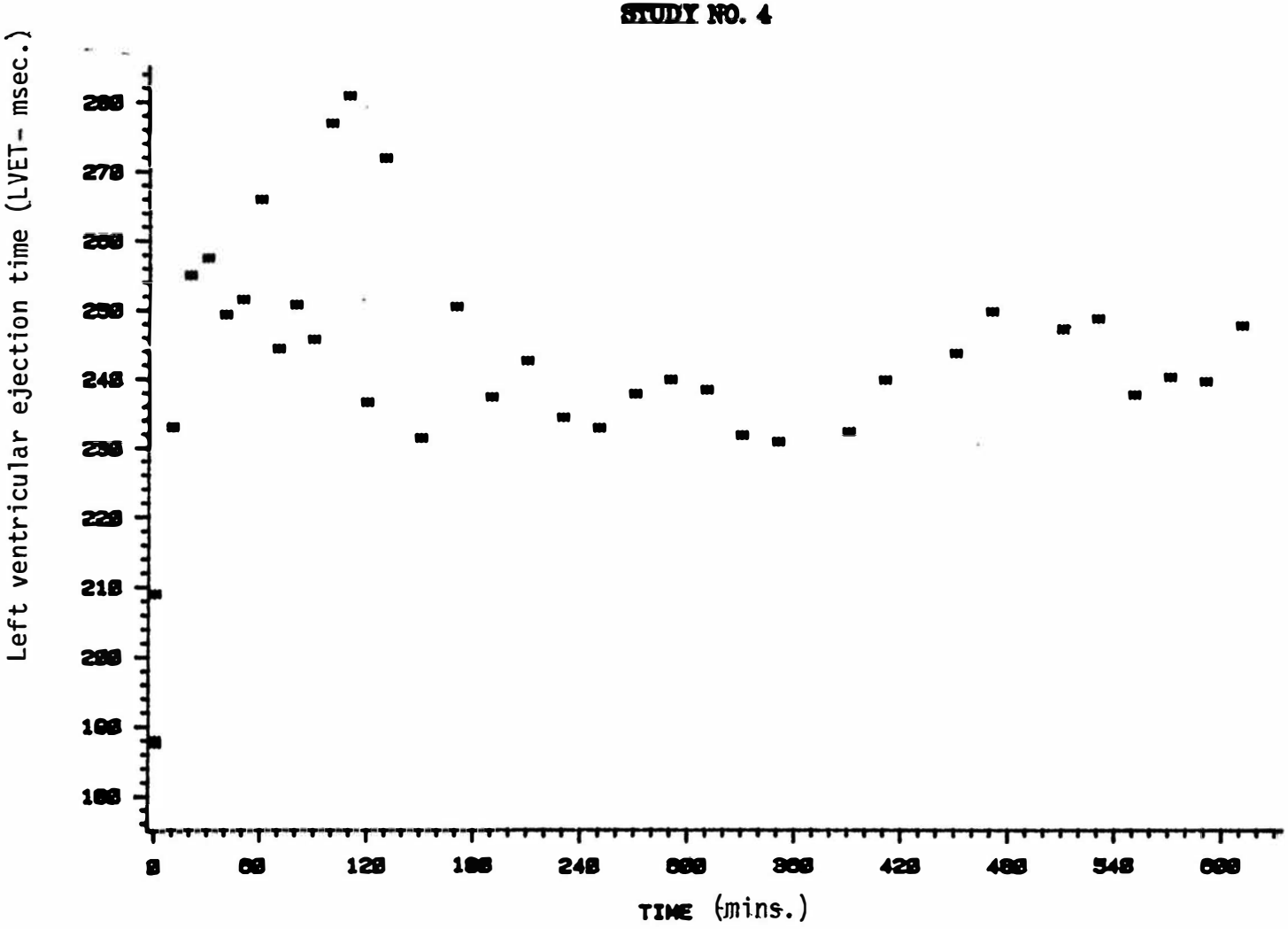

Fig. 4.2 Left ventricular ejection time vs. time following administration of 0.05 mg/kg. digoxin i.v. to a normal anesthetized beagle dog.

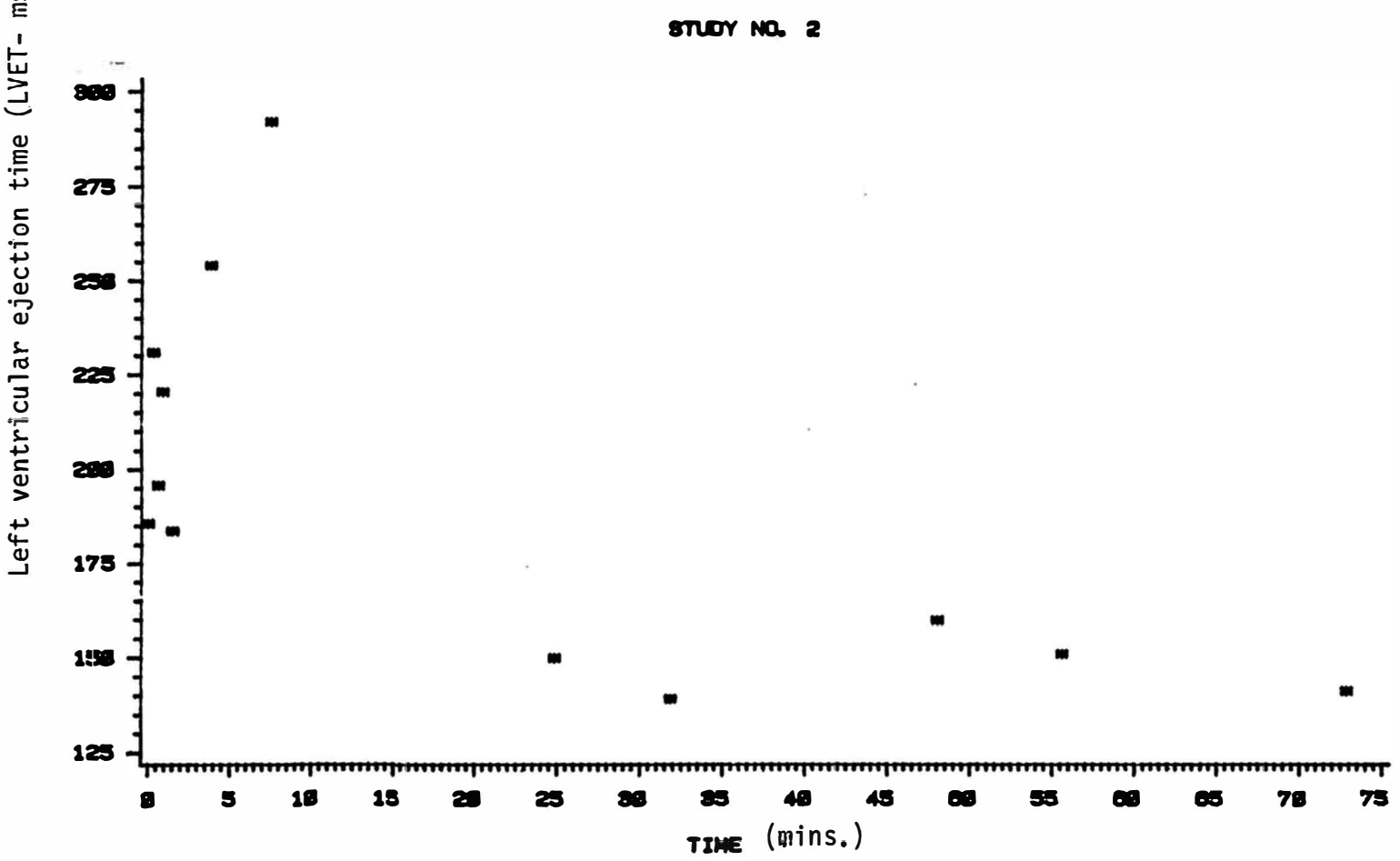

Fig. 4.3 Left ventricular ejection time vs. time following administration of 0.05 mg/kg. digoxin i.v. to a normal anesthetized beagle dog.

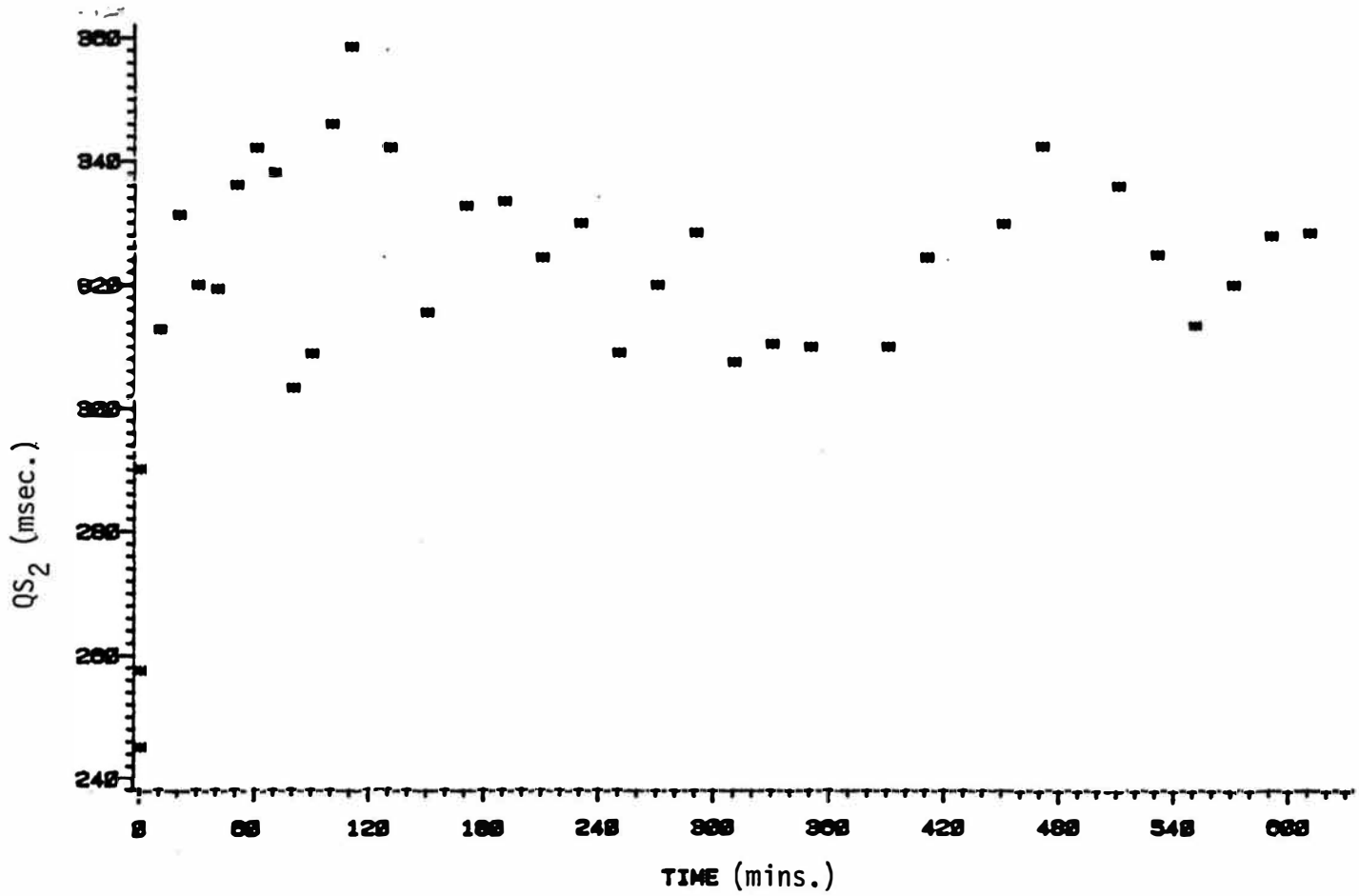

Fig. 4.4 Duration of total electromechanical systole vs. time after administration of 0.05 mg/kg. digoxin i.v. to a normal anesthetized beagle dog.

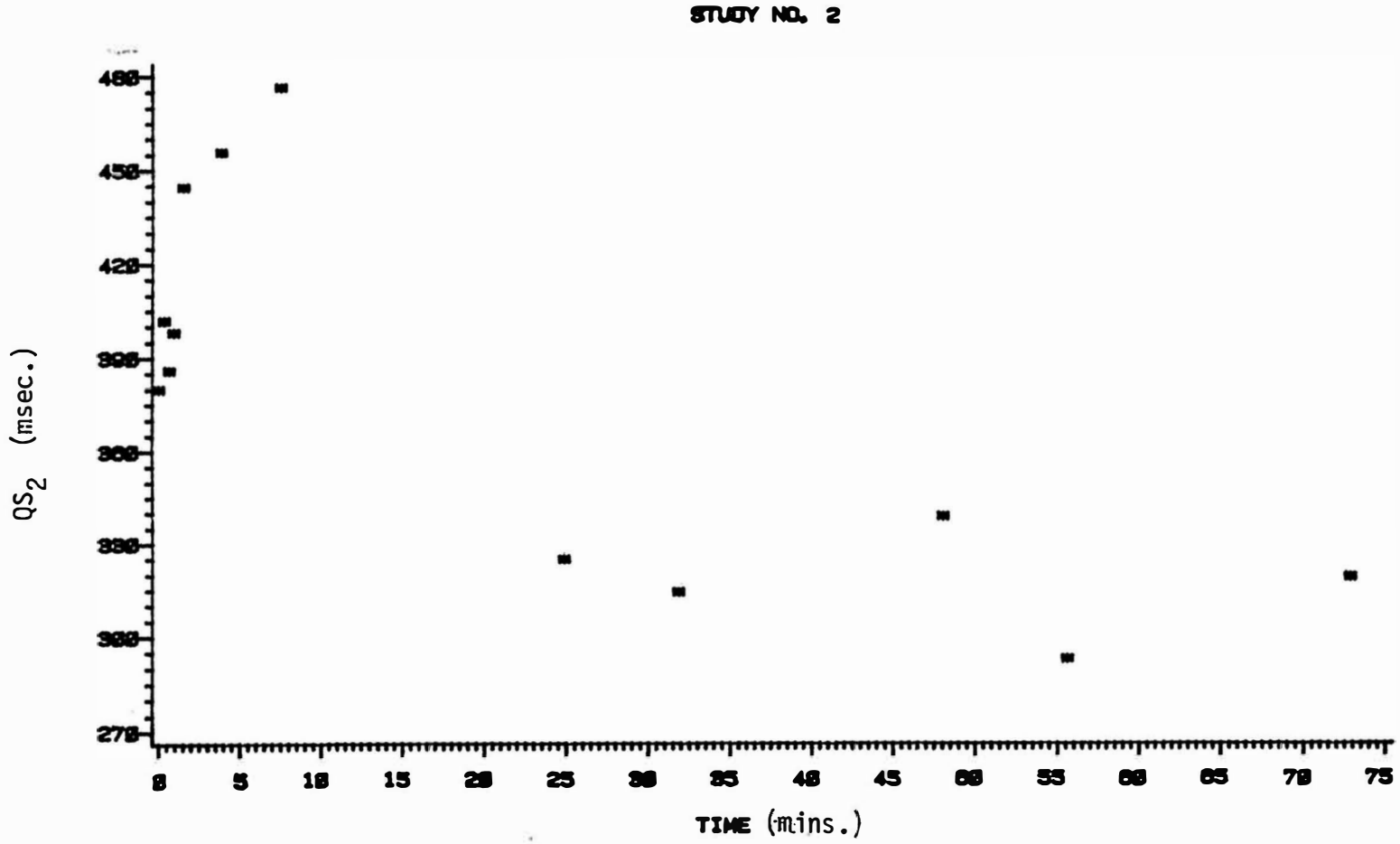

Fig. 4.5 Duration of total electromechanical systole vs. time following administration of 0.05 mg/kg. digoxin i.v. to a normal anesthetized beagle dog.

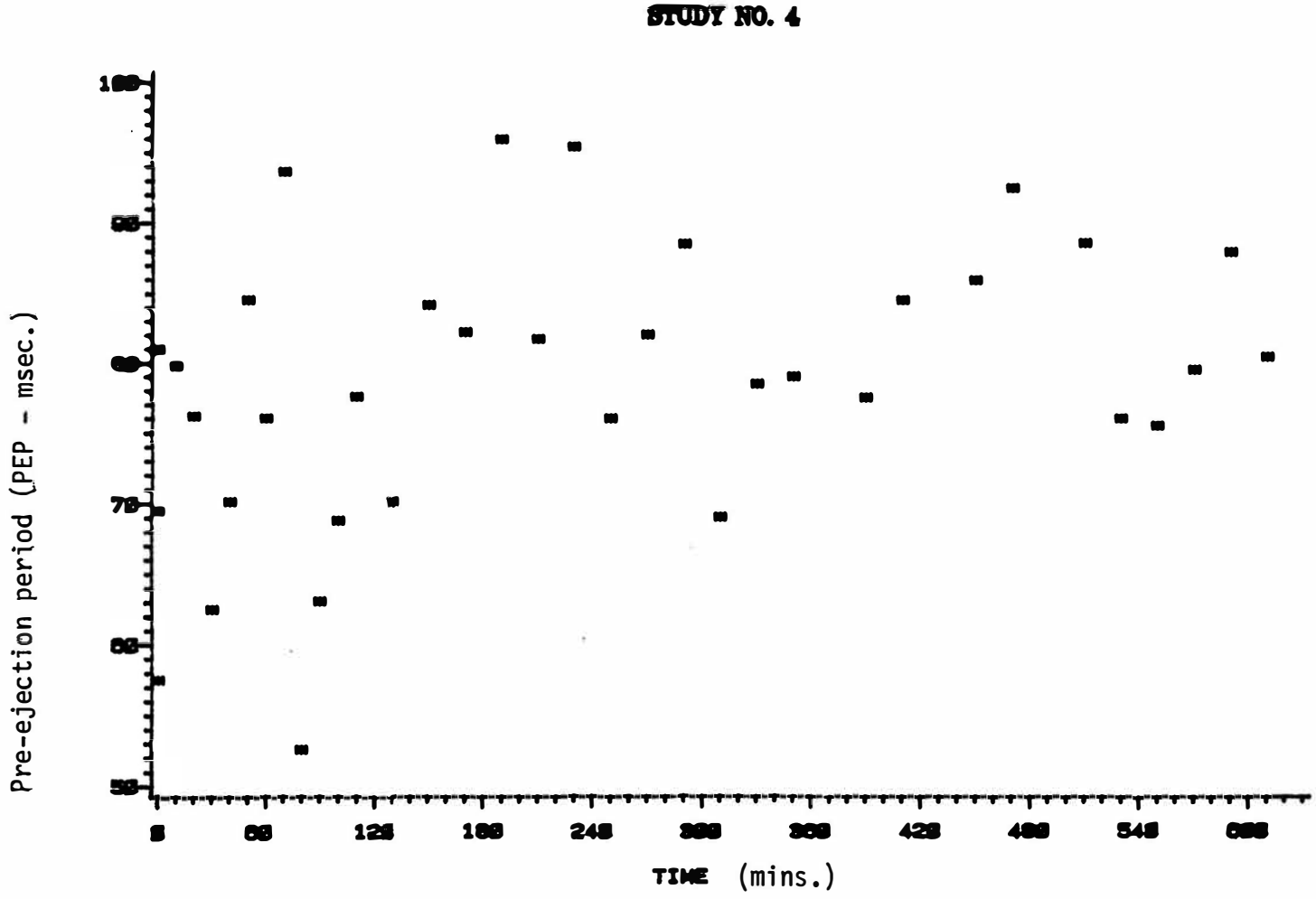

Fig. 4.6 Pre-ejection period of the heart vs. time following administration of 0.05 mg/kg. digoxin i.v. (infused uniformly over 5 mins.) to a normal anesthetized beagle dog.

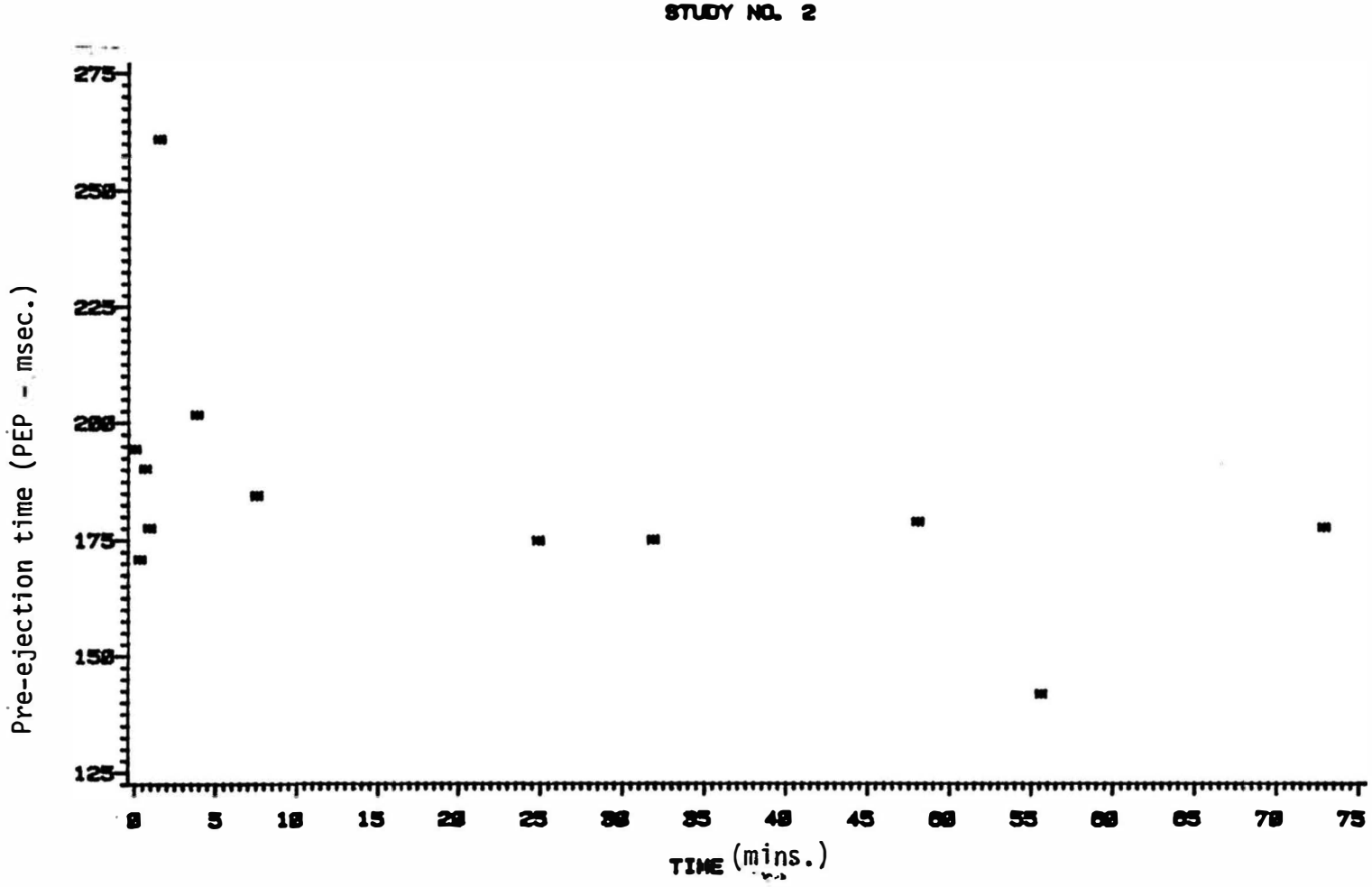

Fig. 4.7 Pre-ejection time of the heart vs. time following administration of 0.05 mg/kg. digoxin i.v. (infused uniformly over 5 mins.) to a normal anesthetized beagle dog.

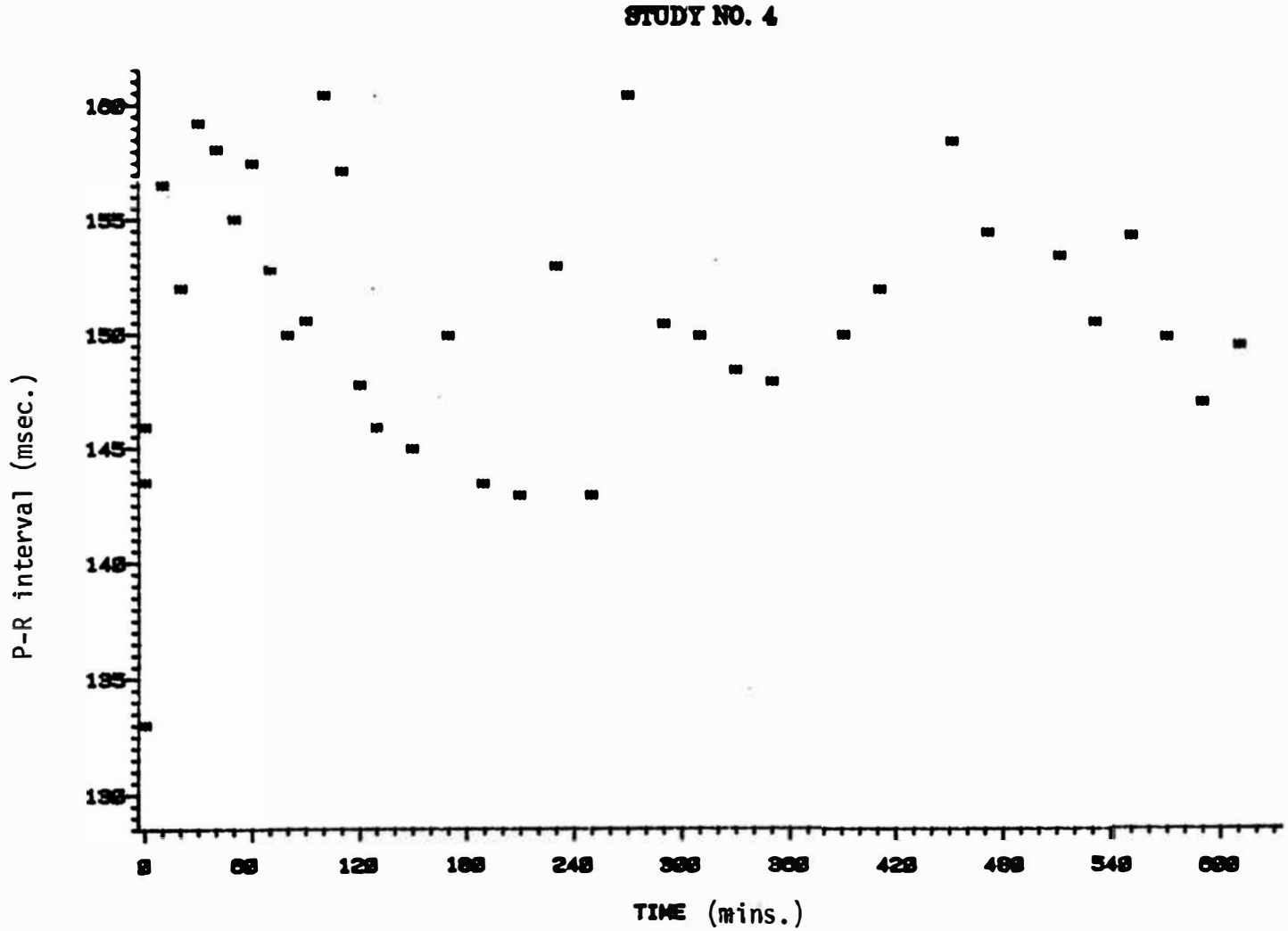

Fig. 4.8 P-R interval vs. time following administration of 0.05 mg/kg. digoxin i.v. (infused uniformly over 5 mins.) to a normal anesthetized beagle dog.

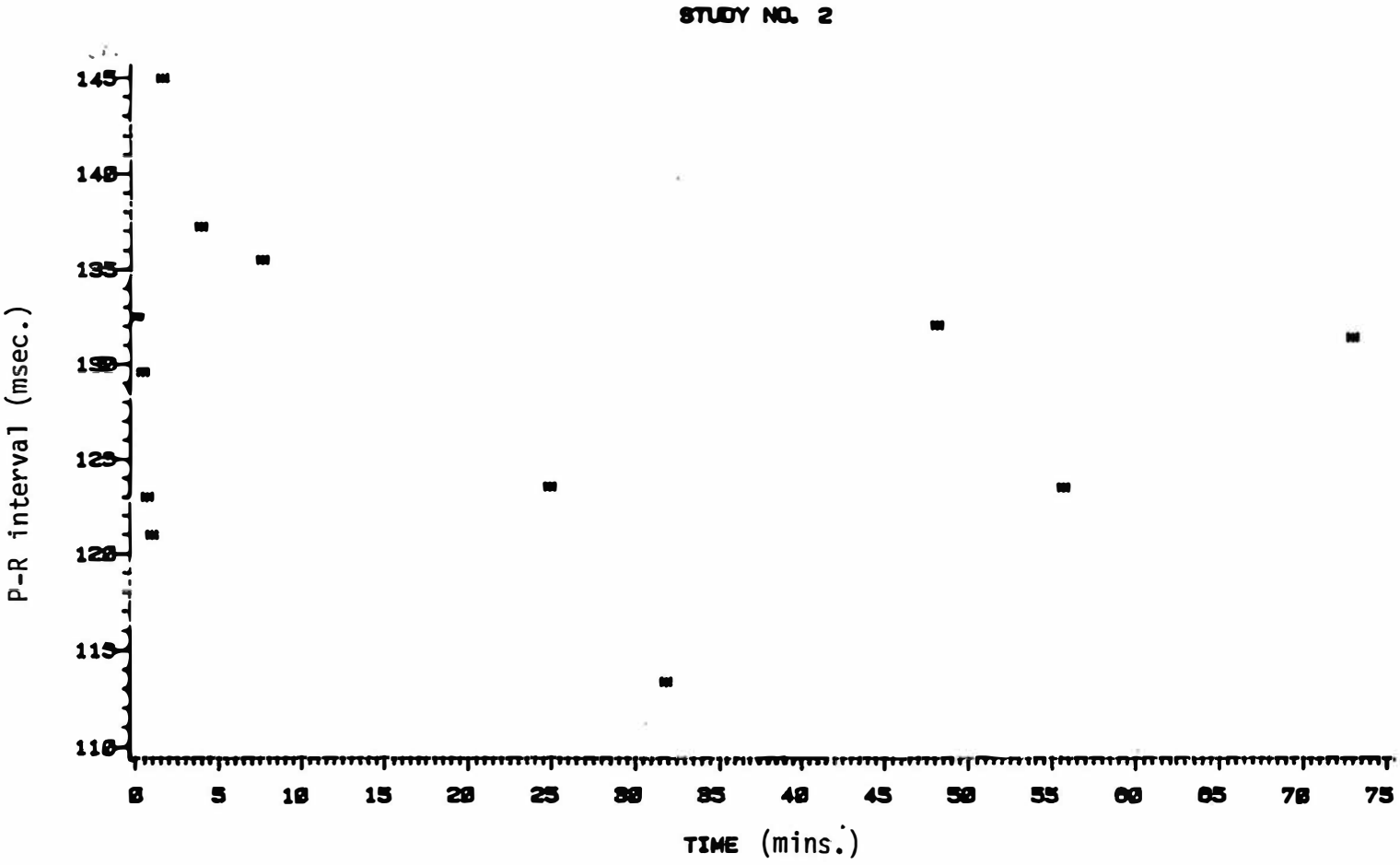

Fig. 4.9 P-R interval vs. time following administration of 0.05 mg/kg. digoxin i.v. (infused uniformly over 5 mins.) to a normal anesthetized beagle dog.

inhibitory effect on the heart rate. It is probably due to these two co-existing pathways that, administration of either atropine or propanolol alone, does not abolish the bradycardic response to digoxin in animals (Higgins *et al.*, 1973).

Having conducted pharmacodynamic studies in dogs, the next objective was the modelling of digoxin induced bradycardia as a pharmacodynamic response. It has been noted that simultaneous fitting of pharmacokinetic and pharmacodynamic data is perhaps more desirable that individually fitting the data (Sheiner *et al.*, 1979). However, he noted that little harm is done in fitting the pharmacodynamic data alone when the pharmacokinetic data is well defined by the fitted equation. Kramer *et al.*, (1979). however, suggest that in attempting to establish a relationship between drug levels and response it is important that the relationship not be "forced" by the simultaneous fitting of both drug level-time and response-time data. As discussed earlier, the pharmacokinetic data in all the studies conducted were very well described by a tri-exponential equation, with correlation coefficients and R-square values of greater than 0.90 The pharmacodynamic models investigated were therefore fitted to the response-time data alone, instead of a simultaneous fitting of response-time and concentration-time data.

Linear Model:

The linear model used was essentially that of Kelman and Whiting (1980). The model did not adequately describe the data in all the studies. The model suffers from several drawbacks. The model cannot be used to extrapolate or to predict pharmacodynamics when different doses are used. The model is related to simulated levels in the central, shallow and deep compartments. This implies the presence of multiple receptors of varying sensitivity. Also, the negative coefficients obtained upon regression (tables 3.19 and 3.20) imply the existence of receptors with opposing functions. This has detracted from the acceptance of this model by

previous investigators. The implication of multiple receptors may be unacceptable in the case of many drugs since it implies a non-specific effect site or multiple effect sites. However for the bradycardic effect of digoxin the existence of multiple receptors may be reconciled by the observation that the HR is controlled by both sympathetic and parasympathetic controls which have opposing functions. The feedback mechanisms that control the HR may therefore account for the negative coefficients observed. Comparison of tables 3.19 and 3.20 reveal that better correlations are obtained using a model without an intercept term as compared to using an intercept term. The inclusion of an intercept term is also physiologically unappealing since it implies the existence of an effect (a change in heart rate) when no drug has been administered. For study no. 7 table 3.20 shows values of -17.87, 337.46 and 70.88 for '*b*', '*c*' and '*d*'; the coefficients relating the observed effect to the simulated amounts in the central shallow and deep compartments respectively. The values are associated with relatively large standard deviations. Although fairly good R-square values and correlation coefficients are obtained using the model, a plot of the predicted and the observed response against time reveals the inadequacy of the model (Fig. 4.10 and 4.11).

It is apparent from the figures that the model does not adequately explain the pharmacodynamics of the bradycardic effect of digoxin. Although not accepted by some investigators, the existence of multiple receptors sites is reasonable since the pharmacokinetic compartments may include various subcompartments at the physiologic effector site viz., heart. Further, negative coefficients are physiologically meaningful since they may be indicative of the co-existence of physiological feedback mechanisms at the effector site. The observed negative or positive coefficients would then be dictated by the dominance of a particular type of receptors over the other. The model is simple and shows promise, but has not yet been shown to be applicable to a specific drug.

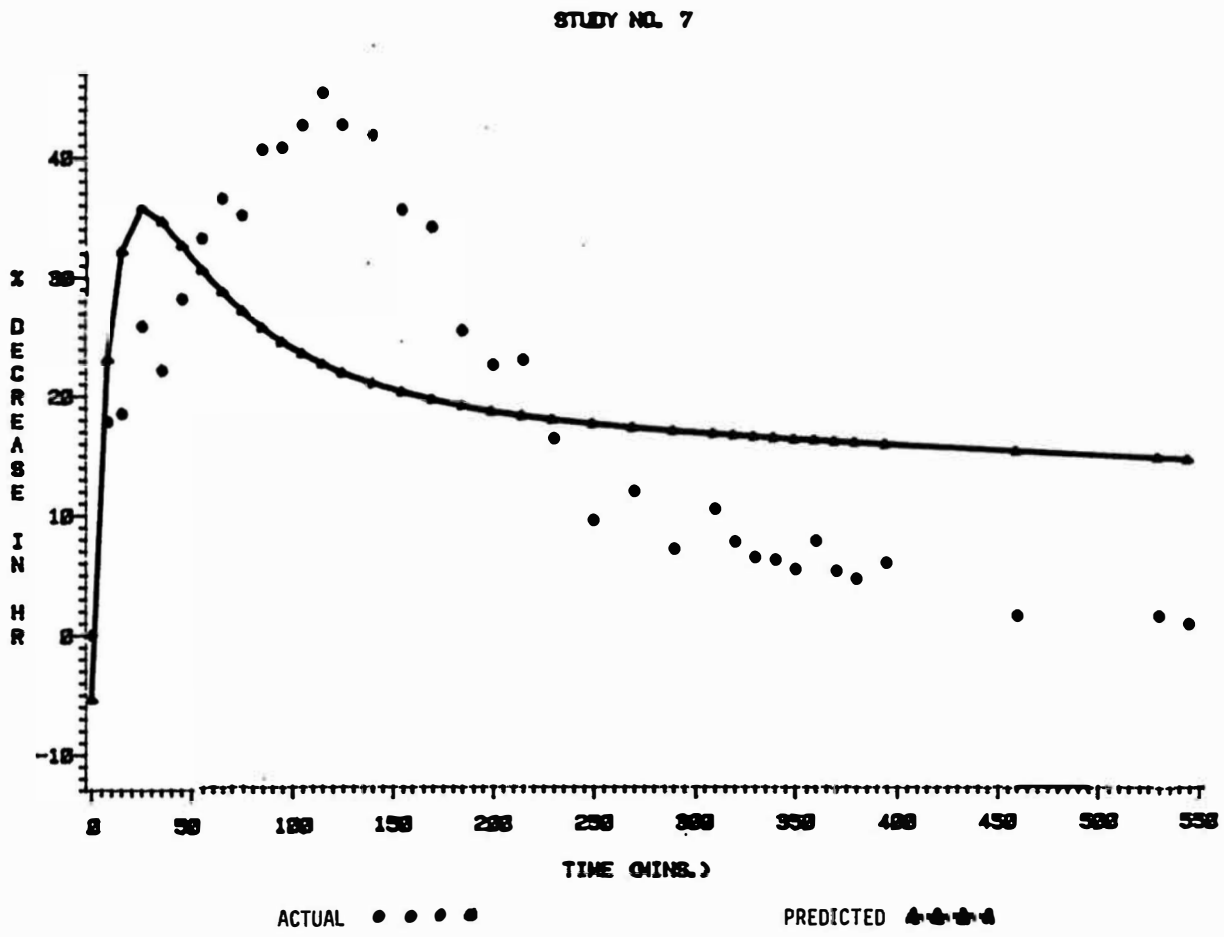

Fig. 4.10 Inadequacy of the linear model (Kelman and Whiting) in predicting the bradycardic response to digoxin following administration of 0.025mg/kg digoxin i.v. (infused uniformly over 5 mins.) to a normal anesthetized beagle dog.

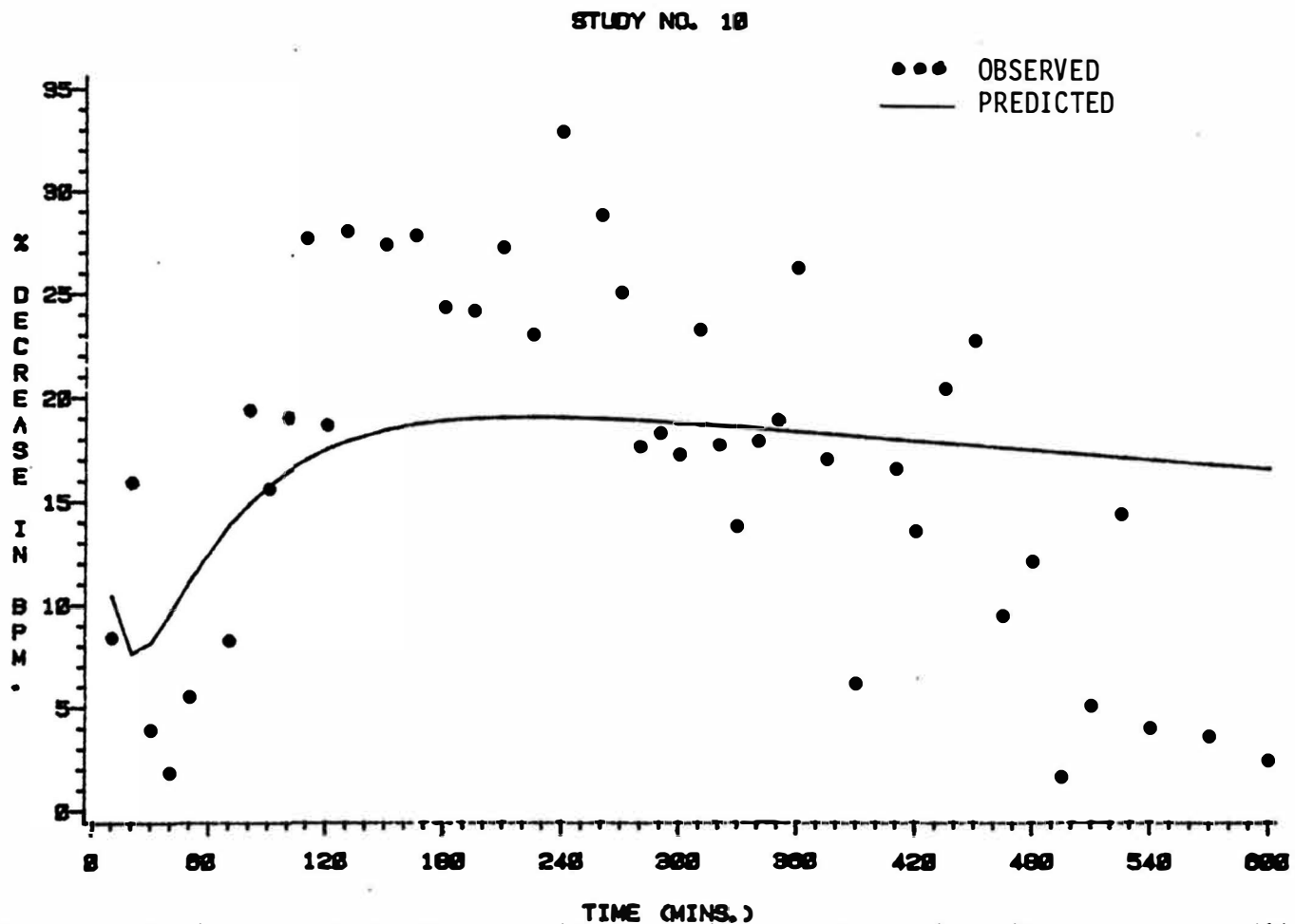

Fig. 4.11 Inadequacy of the linear model in predicting the bradycardic response to digoxin following administration of 0.05 mg/kg. i.v. (infused uniformly over 5 mins.) to a normal anesthetized beagle dog.

The Linking Model.

Another model investigated was the linking model. The simulated levels in each of the pharmacokinetic compartments was linked to the observed response (% decrease in bradycardic effect) through a linear relationship. The simulated amounts of drug in the various pharmacokinetic compartments was related to the response. This relationship may be expressed as follows:

$$E = S \cdot X_i \qquad \ldots(4.1)$$

where E = measured response i.e., % decrease in HR

S = sensitivity constant

X_i = simulated amount of drug in ith compartment.

The measured response was plotted against the simulated amounts of drug in the various pharmacokinetic compartments to determine whether such a relationship was visually discernible. The pharmacokinetic models investigated were the three compartment mammillary, catenary and first pass models. For study no. 4 the biophase was identified to be in the pharmacokinetic shallow compartment for all three models. However for the remaining studies the biophase could not be identified with any of the pharmacokinetic compartments. The implications of this finding are noteworthy. It indicates that:

1) The biophase may be identifiable with the pharmacokinetic compartments only when the amount of drug in the biophase i.e. at the receptor site is large enough to contribute to the mass balance of the drug, and therefore contributes appreciably to the observed pharmacokinetics of the drug.

2) In consideration of the above, when the biophase is not readily identifiable with any of the pharmacokinetic compartments, the conventional compartmental approach of describing drug kinetics cannot be extended to describe the pharmacodynamics of a drug.

For study no. 4 the biophase for all three models (three compartment mammillary, three compartment catenary and the three compartment first pass) was identified with the pharmacokinetic shallow compartment, indicating that the three models are unidentifiable based purely on the pharmacokinetics of the drug in the central compartment and based on its pharmacodynamics. Supportive reasoning for this observation is supplied below:

For a three compartment first pass model, the amount of drug in the pharmacokinetic shallow compartment (X_2) is given by eqn.(3.7). viz.,

$$X_2 = k_{12}D\left[\frac{(E_3-\alpha)}{(\beta-\alpha)(\gamma-\alpha)}e^{-\alpha t} + \frac{(E_3-\beta)}{(\alpha-\beta)(\gamma-\beta)}e^{-\beta t} + \frac{(E_3-\gamma)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t}\right]$$

The amount of drug in the pharmacokinetic shallow compartment for the 3 compartment mammillary and catenary models is given by equations 3.4 and 3.10 respectively. Equations 3.4, 3.7 and 3.10 appear to be identical. However, these equations are not identical since, although the numerical values for α, β, and γ are identical the numerical values obtained for E_3 is disimilar in each of the models considered. For the 3 compartment mammillary model and the 3 compartment first pass model $E_3 = k_{31}$ and for the 3 compartment catenary model $E_3 = k_{32}$. Also α and β are much larger than γ, therefore the amount in the shallow compartment during the distributive phase, is largely dictated by the first two terms in equations 3.4, 3.7 and 3.10 And, since α and β are much greater than E_3, similar profiles for the amount of digoxin in the shallow compartment as function of time, are obtained using equations 3.4, 3.7 and 3.10 And since, the biophase is identifiable with the shallow pharmacokinetic compartment, the 3 models investigated are non-identifiable. Based on the results arrived at, the inadequacy of a pharmacokinetic

model with a linear linking model in describing digoxin induced braydcardia is evident.

Physiologic-pharmacokinetic model with a linear linking model.

The physiologic-pharmacokinetic model with a linear linking model was investigated. The model is quite complex and incorporates a large number of parameters viz., blood flows to the various compartments, tissue to plasma partition coefficients for the various compartments, volumes of the compartments etc. These may be used as constants if accurately determined. The physiologic-pharmacokinetic model proposed by Harrison and Gibaldi (1977), did not accurately predict digoxin concentration-time profile in the first 6 hours, although the model was quite adequate in predicting the concentration-time profile in the post-distributive phase. The physiologic-pharmacokinetic model was therefore modified by substituting various values for the tissue to plasma partition coefficients, until a good visual fit was obtained between the observed and the model predicted concentration-time profiles (fig. 3.29-3.31). Since, the tissue to plasma partition coefficients used in (modifying the model) were not experimentally determined, they may not reflect the actual tissue to plasma partition coefficients that exist among the various compartments. This physiologic-pharmacokinetic model was used to simulate the levels of digoxin in the heart. It was attempted to relate the levels of digoxin in the heart to the response using a linear linking model. The model did not adequately predict the bradycardic response observed.

The tissue to plasma partition coefficients used could possibly be a contributory factor for the observed inadequacy of the model. However, the physiologic pharmacokinetic model shows promise as a powerful investigative tool. It has the capability to predict the concentrations at the physiologic site of action. Further

since it incorporates the blood flows it can account for changes in the pharmacokinetics and pharmacodynamics of a drug due to local or systemic changes in blood flow. This could be a very important factor particularly with a drug like digoxin since it increases cardiac output in patients with congestive heart failure. The pharmacodynamics of digoxin could therefore be constantly changing with an improvement in cardiac function.

The physiologic-pharmacokinetic model was simulated using SPICE2. SPICE2 is a computer program that uses network thermodynamics to describe physiological systems. A detailed description of SPICE2 and its capabilities have been described in the previous chapter.

The Effect Compartment Model

Another model evaluated was the 'effect compartment' model. The model proved quite satisfactory in describing the pharmacodynamic data obtained in all the studies. This was evidenced in the low residual sum of squares, the R-square values, correlation coefficients and from plots of the predicted and observed response against time (Fig. 4.12 and 4.13).

However, a large variability is observed in the parameters describing the model. Large differences in the K_{e0} are not observed for studies 7, 9 and 10 in which the dogs were dosed at 0.05 mg/kg. However apreciable differences are seen in the K_{e0} values for study 4 and 7 which were studies conducted in the same dog. The dose of digoxin administered was 0.025 and 0.05 mg/kg. for studies 4 and 7 respectively. The K_{e0} for study no. 7 (0.0088 min^{-1}) is much smaller than the value obtained in study no. 4 (0.042 min^{-1}). The longer half-life for the decline of response with time, for the lower dose in for study no. 7, seems to suggest that saturation of the bradycardic effect in dogs does not occur even at a dose of 0.05 mg/kg. of digoxin. The value of $Cp_{ss(50)}$ ranged from 3.77 ng/ml to 9.84 ng/ml. This is the predicted steady state

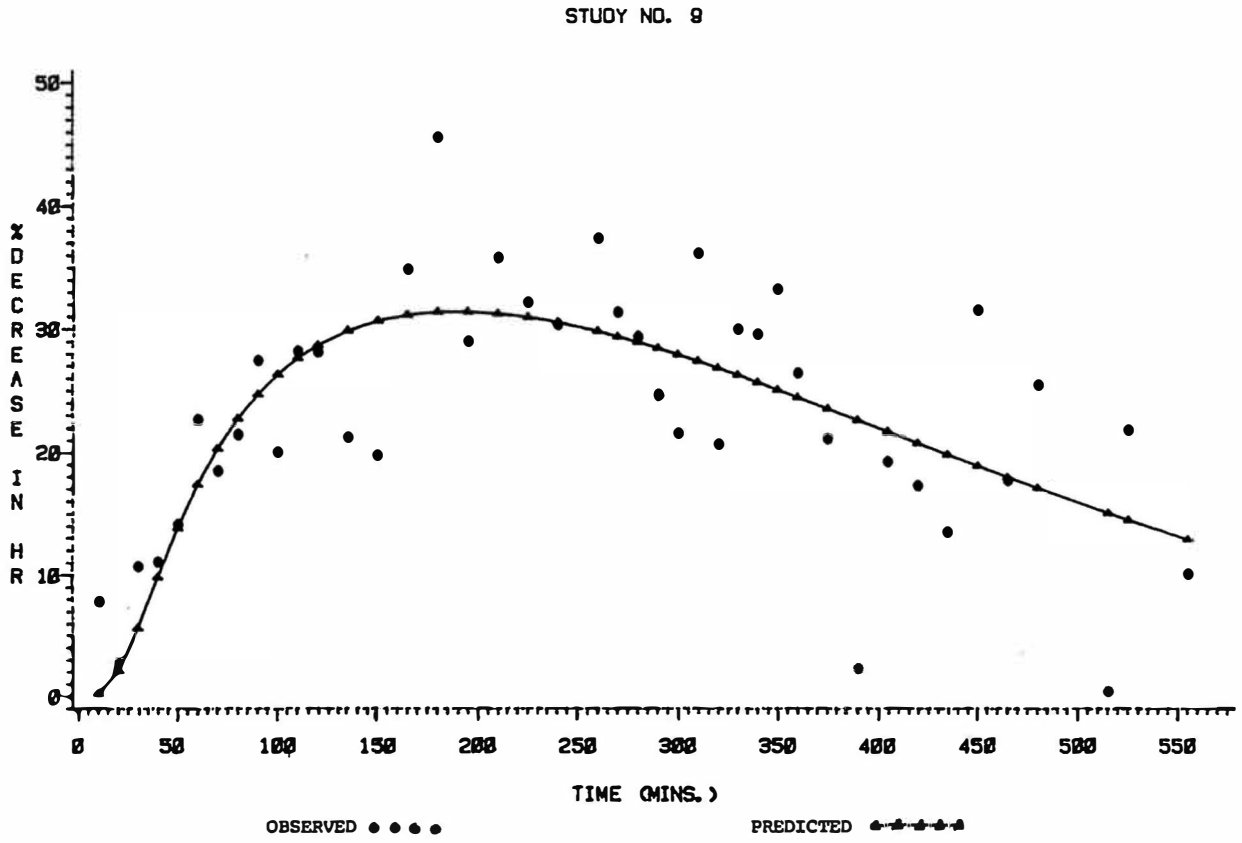

Fig. 4.12 Bradycardic response to digoxin predicted by the effect compartment model. The response is the observed response on administration of 0.05 mg/kg. digoxin i.v. (infused uniformly over 5 mins.) to a normal beagle dog.

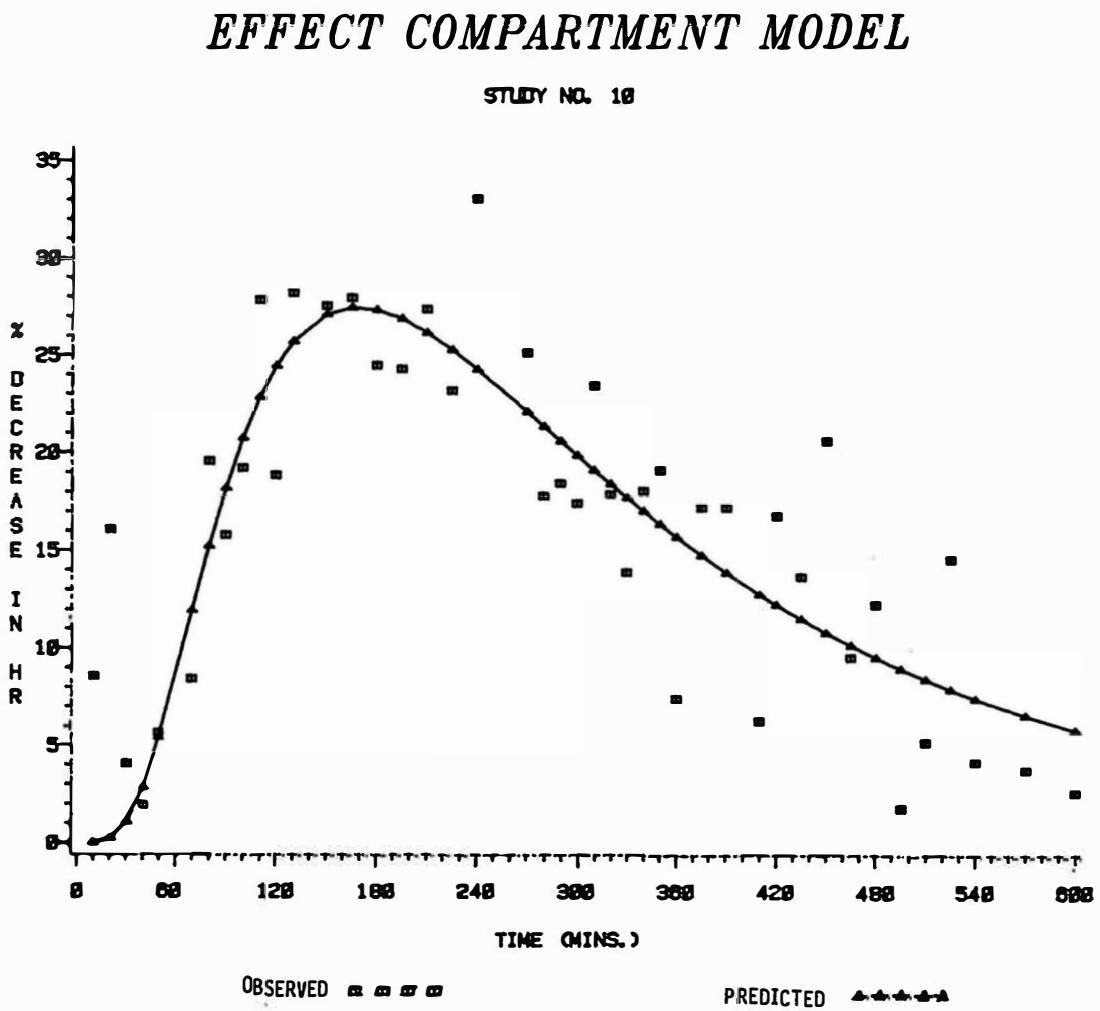

Fig. 4.13 Bradycardic response to digoxin as predicted by the effect compartment model. The bradycardic response is the response observed on the administration of 0.05 mg/kg. digoxin i.v. (infused uniformly over 5 mins.) to a normal anesthetized beagle dog.

plasma concentration of digoxin that will effect 50% of the maximal response. One of the drawbacks of using a sigmoid relationship to describe a pharmacodynamic response is the need to determine E_{max}, the maximal response. This may be used as a constant if it can be accurately determined. However some *in vivo* systems do not permit an accurate determination of E_{max} since the maximal response may be associated with concomitant non-reversible changes or death and hence would be of very little or no clinical value. The effect compartment model was therefore modeled with E_{max} assigned a value of 100 (i.e. a 100% decrease in HR), as a variable or assigned a value equal to the observed maximum response. A model also investigated was one in which the response modelled was not the per cent decrease in HR, but HR itself (IC_{50} model). All of the models adequately described the data. DELTA (δ) values ranged from 0.60 to 7.12 These values have been previously proposed as an indication of the number of drug molecules interacting with the receptor molecule. However it has also been noted that such an interpretation is not often applicable since the δ values obtained are often non-integers. These values do however indicate the steepness of the response-concentration relationship. This may be visualized in fig. 4.14, where the effect of changing δ values is demonstrated ($Cp_{ss(50)}$ is held constant at 7.94 ng/ml.).

Comparison of various models

Comparison of the various models based on their adequacy of describing the data, SSE, F-tests and AIC revealed the the effect compartment model with E_{max} as a variable to be the best model. The model adequately described the data obtained from all of the pharmacodynamic studies conducted (studies 4-10). After the model has been identified, and the parameters describing the model have been

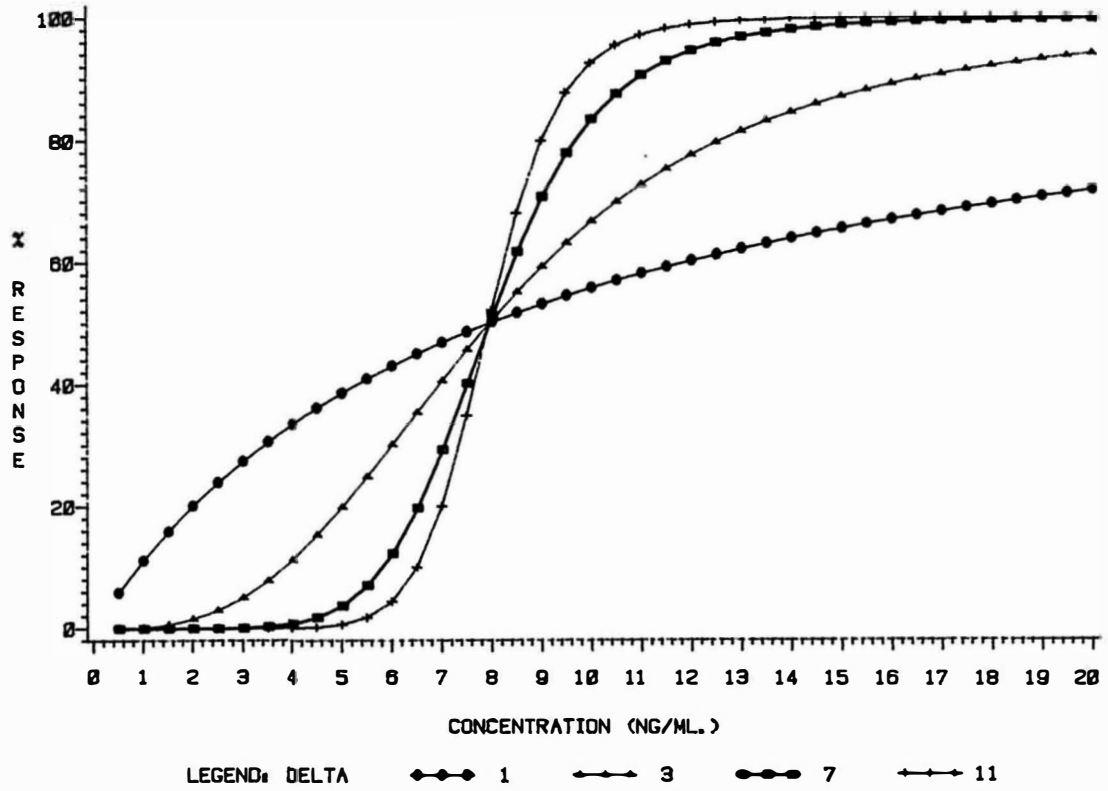

Fig. 4.14 Simulations showing the change in the concentration-response curve with changing delta values as predicted by the sigmoid effect equation.

determined ($Cp_{ss(50)}$ and δ), the model may be used to predict the dose-response curve for steady state concentrations of drug using eqn.(4.2).

$$E(\%) = \frac{Cp_{ss}^{\delta}}{Cp_{ss(50)} + Cp_{ss}^{\delta}} \qquad ...(4.2)$$

where Cp_{ss} is the concentration in the blood or plasma compartment at steady state, and $E(\%)$ is the response observed as a percent of the maximum response. The model can also be used to predict the respone-time profile for any known concentration-time profile. The effect compartment model is model-independent in its approach since it does not presume a pharmacokinetic model but utilizes an exponential equation that best describes the concentration-time profile.

Variability in the monitored response

Results of studying and modelling the bradycardic effect of digoxin reveal a large variability in pharmacodynamic response. Further, as mentioned before the variability in the response prevented modelling of STI as the pharmacodynamic response. The inherent variability and the oscillatory nature of the STI and HR require these responses to be monitored freqently (or continously, if possible) to completely characterize the response-time profile. The results of studies 4 to 10 indicate that the observed bradycardic effect may also be related to the basal heart rate prior to administration of digoxin and also to the levels of sympathetic and parasympathetic simulation that exist. This may also be responsible for the oscillatory nature of the data.

Kramer *et al.*, (1979), also reported that the variability inherent in the response measurement, both intrasubject and intersubject. limited their quantitative treatment of ΔQS_2I data, in their pharmacodynamic studies with digoxin in 12 normal volunteers. They found that although a relationship could be established

for averaged data from 12 subjects, the same relationship could not be established for individual subjects. The results of their study suggested that further efforts designed to develop more reproducible measurements for digoxin response would be desirable.

Applications of the effect compartment model

The pharmacodynamic models investigated reveal that the pharmacokinetic model with linking pharmacodynamic models and the linear model may not be adequate in describing the pharmacodynamics of a drug. The effect compartment model on the other hand is predictive of pharmacodynamic effects at steady state and hence is of clinical value in this respect. Also, it adequately describes data even in situations where the drug in the biophase may not be present in amounts large enough to contribute to the observed mass balance of the system.

1) Duration of effect:

The model will enable estimation of the cumulative effect, since the overall drug effect may be given by

$$\overline{E} = E_{max} \int \frac{Cp^{\delta}}{Cp_{ss(50)} + Cp^{\delta}} \cdot dt. \qquad ...(4.3)$$

where $\overline{E}$ is the cumulative effect over a certain time period. The model will also enable better understanding of the relationship of drug levels to response during the different phases of drug disposition in the body e.g., distribution, elimination. When concentrations are much larger than the $Cp_{ss(50)}$ the effect will be close to the maximal effect and will show little change for large changes in concentration. For concentrations between 20% - 80% of the $Cp_{ss(50)}$ the effect will change in proportion to the change in concentration, although the proportionality constant to this change is related to the δ value for the model.

2) Influence of dosing regimen

The overall drug effect is given by eqn. (4.3). Cp is a constant at steady state following continous infusion, but varies over the dosing interval if the dosing is intermittent. Equation (4.3) enables mathematical estimation of the differences in clinical benefit that may result due to changes in the overall effect arising from differences in dosing. This could be important e.g., if the peak levels of drug are associated with toxicity and the trough levels with therapeutic benefit.

3) Investigative pharmacodynamic studies

The effect model for the bradycardic response to digoxin may be incorporated in pharmacodynamic studies measuring cardiac response so that these responses may be corrected for contributions made by concomitantly occuring changes in heart rate. Similar models may be derived in clinical situations. These models would then afford the capability of accurately predicting heart rate and cardiac function in clinical situations. This could be important when patients are administered more than one drug that contributes to or affects cardiac function. The δ values and $Cp_{ss(50)}$ values derived for a patients would then help individualize therapy and drug effect monitoring.

4) As a predictor of drug levels

The usefulness of the effect model in predicting the response for a known drug concentration is the body has been discussed earlier. Conversely, provided the time and route of administration of drug are known, the effect model may be used to predict the concentration of drug in the body any time (t) for a given measured response at time t. These models could therefore reduce the frequency of venupuncture associated with drawing blood samples for drug level monitoring. Such models could also

offer an immediate reliable estimate of drug levels in cases of accidental poisoning or over-ingestion of drug until a more accurate 'laboratory value' is obtained.

SUMMARY

1. An HPLC-RIA assay specific for digoxin in the presence of its major metabolites viz., digoxigenin mono- digitoxoside, digoxigenin bis- digitoxoside and digoxigenin, was developed.
2. Methodology for non-invasive measurement of LVET and other STI in dogs was developed. However, the STI measured did not lend itself to pharmacodynamic modelling.
3. Relationships between the STI and HR in the presence of digoxin were established in dogs.
4. The bradycardiac response to digoxin was investigated in beagle dogs, and pharmacodynamic modelling conducted.
5. The pharmacodynamic models investigated and evaluated were the pharmacokinetic model with a direct linear link, the linear model, the physiologic-pharmacokinetic model with direct linear link and the effect compartment model.
6. The phsyiologic pharmacokinetic model was investigated using a computer program SPICE2, which uses a thermodynamic network to simulate biological systems. The model simulated using SPICE2 was compared to the same model simulated using two other computer programs viz., CSMP and GPPM.
7. Criterion for selecting the best model are discussed including evaluation of 'fits'. F-values, AIC's and the weighted residual sum of squares.
8. The effect compartment model was demonstrated to be the best model.
9. The implications and applications of pharmacodynamic models in general and specifically of the pharmacodynamic model for the bradycardiac response to digoxin are discussed.

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CHAPTER 5

APPENDIX

APPENDIX A - MATERIALS

Analytical:

All solvents used for the extraction or chromatography were HPLC grade and were obtained from Fisher Scientific Co. (Pittsburgh, PA, USA). Digoxin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Digoxigenin, digoxigenin mono-digitoxoside and digoxigenin bis-digitoxoside were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN, USA). Dihydrodigoxin was a gift from H. Hull (Burroughs Wellcome, Research Triangle, N.C., USA). RIA was performed using the Digoxin RIA kit (Diagnostic Products Corp. LA, CA, USA). The HPLC system used was Waters M-6000 A solvent delivery system (Waters Associates, Milford, Mass.). The column was Lichosorb SI-100 (Hewlett Packard). A C_{18} μBondapak reverse phase column (Waters Associates, Milford, Mass.) was also used during assay development. The UV detector used was a Gilson variable wavelength spectrophotometer. (model: HM Holochrome UV monitor, Gilson Medical Electronics, Middleton, WI.) The injector used was Rheodyne syringe loading sampling injector (model 7125, Rheodyne Inc., Berkeley, CA.). The refractive index (RI) detector used during assay development was a Differential Refractometer R401 (Waters Associates, Milford, Mass.) and the liquid chromatography electrochemical (LCEC) detector used was a LC-4B/17 with a glassy carbon electrode (Bioanalytical Systems, West Lafayette, Indiana). Flourimetric measurements were made using

an Aminco-Bowman spectroflourometer (American Instrument Co., Silver Springs, MD.).

Dog studies:

Digoxin administered to the dogs was Lanoxin (0.25 mg/ml., Burroughs Wellcome Co., Research Triangle, N.C.). Heparin locks were Butterfly ($21\frac{3}{4}$, 12 inch tubing infusion set, Abbott Hospitals, N. Chicago, N. IL.). Normal saline used for intravenous administration flushing the heparin locks and for diluting heparin. Heparin (Heparin Sodium Injection, USP 10,000 units/ml, Lypho-Med Inc., Chicago, IL.) diluted with normal saline to give a concentration of 100 U/ml.) was used to flush the heparin locks to keep them patent. Blood samples were drawn with disposable sterile syringes (Becton-Dickinson, Rutherford, N.J.) into 10 ml. red-top sterile Vacutainers$^{(R)}$ (Becton-Dickinson). After centrifugation the serum was transferred using disposable glass pipettes (Fisher Scientific Co. Pittsburgh, PA) into polystyrene tubes (Falcon, Fisher Scientific Co.) and frozen. Electrocardiography was done using a VS4 Electrocardiograph (Cambridge Instruments, N.Y.). The microphone pick-up unit for recording the heart sounds was the Cambridge Instruments microphone transducer head (Part no. 03040500) and the pressure transducer used to record the carotid pressure tracing was the Cambridge Instruments pressure transducer head (Part no. 03040000) connected to a funnel-shaped pickup unit (diameter 1.9 cm, volume 0.94 cc) attached to a tubing (internal dia. 0.4 cm, volume 0.79 cc). For invasive measurement of the ventricular pressure tracing (Study 1A) a pigtail catheter (5 fr.) was connected to a strain gauge pressure transducer through a dome (Disposable dome no. 1295 A, Hewlett Packard, Palo Alto, CA). The carotid pressure tracing was also non-invasively obtained after exteriorization of the carotid artery. The exteriorized artery was retained in position using 2-prolene, blue monofilament, polypropylene retaining sutures (Ethicon,

Somerville, N.J.). The suture used for closure of the incision was 00 black silk. The dogs were anesthetized using pentobarbital (60 mg/ml., Barber Veterinary Supply Co. Inc. Richmond, VA). The endotracheal tube used was a sterile tracheal tube (American Hospital Supply, McGaw Park, IL, USA). The respirator used was an animal respirator (Model 616, Harvard Apparatus Co. Inc, Millis, Mass., USA).

Computer analysis:

All computer analysis was done on the combined computer facilites of Virginia Commonwealth University East and West campuses. Pharmacokinetic analysis using the nonlinear regression program NONLIN (NONLIN 1972) was done on the IBM 3081-D (IBM Corporation) . Statistical analyses were done using the statistical package SAS (SAS 1982, SAS Institute, Cary, NC). Graphics was accomplished using the SAS graphics package and an HP7221B plotter (Hewlett Packard, Palo Alto, CA.). All statistical analyses, editing and graphics was done using the mentioned packages on a VAX/VMS 11/780 (Digital Equipment Corporation, Maynard, Mass., operating system version 4.1).

APPENDIX B

ASSAY DEVELOPMENT

Objective:

To develop a specific. sensitive assay for digoxin in the presence of its major metabolites viz., digoxigenin mono- digitoxoside, digoxigenin bis-digitoxoside, digoxigenin and dihydro-digoxin.

Introduction:

Recent HPLC Assays

Initial studies measured digoxin concentrations by administration of ^{3}H-digoxin to patients (Beall et. al. 1963; Doherty *et al.* 1961; Doherty et al. 1969; Hernandez *et al.* 1963). Most of the subsequent studies have used RIA for measuring digoxin concentrations (Biddle *et al.* 1978; Coltart *et al.* 1974; Gorodischer *et al.* 1976; Gullner *et al.* 1974; Jusko *et al.* 1974; Karjalainem *et al.* 1974; Krasula *et al.* 1974; Park *et al.* 1982).

It has been demonstrated that the digoxin metabolites: digoxigenin, digoxigenin mono-digitoxoside, digoxigenin bis-digitoxoside; cross-react extensively using RIA (Stoll *et al.* 1972). Kramer *et al.* (1976), showed that dihydrodigoxin also cross-reacts.

Recent methods have separated digoxin from its metabolites by HPLC. If the digoxin fraction at the end of the HPLC column is collected, then, by either applying RIA or measuring radioactivity (when radiolabeled digoxin is used), specific assay methods for digoxin have been made available (Eriksson *et al.* 1981; Loo *et al.* 1977, 1981; Morais *et al.* 1981; Nelson *et al.* 1979).

It was therefore decided to follow a similar procedure i.e., to separate digoxin from its metabolites using HPLC and to quantitate the digoxin fraction after chromatography, by conventional RIA.

Structure:

Digoxin is a cardiac glycoside which represents the combination of an aglycone, or genin, with 3 digitoxose sugar molecules. The basic structure of the genin is a cyclopentanoperhydrophenanthrene nucleus to which is attached an unsaturated lactone ring at C 17. The sugar moieties are attached at C 3. (Fig. 1.1).

Molecular weight: 781.0

Solubility:

Almost insoluble in water, ethanol, ether and chloroform; soluble 1 in 22 of 80% ethanol and 1 in 4 of pyridine.

UV Absorption spectrum:

Digoxin in sulfuric acid, maxima at 230 mμ (E1%, 1 cm. 260).

320 mμ (E 1%, 1 cm. 225).

390 mμ (E1%, 1 cm. 305).

490 mμ (E 1%, 1 cm. 2100).

Dihydrodigoxin absorbs under UV light very poorly. A 125 μg/ml. solution in ethanol absorbed only to the extent of 0.01 AUFS. It therefore seemed unlikely that quantities in the low nanogram range could be detected using a UV detector. This is in agreement with Eriksson and Tekenbergs (1981), who indicated that dihydrodigoxin (DH) absorbs approximately 1000 times less than digoxin. Therefore it may be neccessary to inject mg. quantities of dihydro-digoxin to determine its retention time on the column.

EXPERIMENT 1

Objective:

To separate digoxin from its metabolites using HPLC.

Introduction:

The intended procedure involved separation of digoxin and metabolites using HPLC and collection of digoxin fraction to be assayed by RIA. Since the fractions were to be evaporated for RIA it was decided to use a normal phase HPLC procedure, so that organic solvents constituting the collected eluant could be easily evaporated. The assay selected for modification after review of the literature was that by Loo et al., (1977).

Procedure:

Column: Lichosorb SI-60 (5μm.)

length = 25 cm.

internal diameter = 4.0 mm.

Mobile phase: Hexane : Methylene chloride : Ethanol

λ_{max} = 230 nm.

Flow rates and the concentrations of hexane, methylene chloride and ethanol were varied to achieve optimal separation.

Results:

The following chromatographic conditions were found optimal for the separation of digoxin and its metabolites.

Flow rate = 2.5 ml/min.

Mobile phase: Hexane : Methylene Chloride : Ethanol (50:40:10)

Retention times were:

Digoxin	14.2 min.
Digoxigenin bis-digitoxoside	12.0 min.
Digoxigenin mono-digitoxoside	9.2 min.
Digoxigenin	6.8 min.

A peak for dihydrodigoxin was not observed even after injection of 400 μg. on to the column. This is probably due to the low UV absorption of dihydrodigoxin.

Conclusion:

Dihydrodigoxin cannot be detected using a UV detector. Detection using a UV detector in conjuction with HPLC procedures may require mg. quantities to be injected onto the column. The peak for dihydrodigoxin detected in such cases may not reflect the true retention time for digoxin due to "column loading". It was therefore decided to determine whether an electro-chemical detector may be used to detect digoxin. It was also decided to investigate the refractive index detector for detection of dihydrodigoxin since the refractive index detector is a non-specific detector.

EXPERIMENT 2

Objective:

To determine whether dihydrodigoxin can be detected in HPLC eluant using an electrochemical detector.

Introduction:

Reverse phase HPLC systems are generally used in conjuction with an electrochemical detector (ECD), since the ECD requires a polar medium in which the calomel electrodes are immersed and to facilitate electrical conduction. Therefore the HPLC procedure used in Experiment 1. was not suitable for the present needs.

Review of the literature revealed a reverse phase HPLC procedure for chromatographing dihydrodigoxin (Eriksson and Tekenbergs, 1981). The chromatographic procedure used was a modification of their procedure.

Procedure:

Column : Spherisorb RP-8 (reverse phase)

Mobile Phase : MeOH : Phosphate buffer (0.1 M) pH=6.3 (60 : 40)

Flow rate = 1 ml/min.

Digoxin had a retention time of 6.0 mins. 100 μg. of digoxin was injected on to the column and it was attempted to detect digoxin with the ECD at 0.95v. The injection was repeated with the ECD at -0.95v. The procedure was repeated using 500 μg. of dihydrodigoxin.

Results:

No peaks for digoxin or for dihydrodigoxin were detected using an ECD, although peaks for digoxin were detected using an UV detector.

Conclusion:

Since digoxin and dihydrodigoxin could not be detected using an ECD it was decided to investigate the detection of dihydrodigoxin using a refractive index detector.

EXPERIMENT 3

Objective:

To determine whether the refractive index detector may be used for the detection of dihydrodigoxin.

Introduction:

Morais *et al.* (1981), indicated that digoxin could be separated from dihydrodigoxin using the following chromatographic procedure :

Column : C18, Reverse phase, μBondapak (3.9 mm. i.d. x 30 cm.)

Mobile phase : Acetonitrile : water (29:71 v/v)

Flow rate : 3 ml./min.

It was therefore decided to use the above system for separation and detection of digoxin and dihydrodigoxin.

Procedure:

A chromatographic system similar to that described by Morais *et al.* (1981). was used and the refractive index detector attached in series to an UV detector, was used to detect dihydrodigoxin in the post column eluate. Various amounts of dihydrodigoxin or digoxin alone were injected on to the column.

Results:

100 μg. of digoxin and dihydrodigoxin both demonstrated peaks using using the refractive index detector. Both had identical retention times. It was also observed that injection of digoxin gave rise to one large peak and one other much smaller peak. This may indicate possible hydrolysis of digoxin on the column since the digoxin was found to be chromatographically pure.

Conclusion:

The reverse phase HPLC procedure proposed by Morais *et al.* (1981), did not meet the chromatographic needs because of the following reasons:

1. Dihydrodigoxin may be detected using a refractive index detector only when large amounts (100 μg. or more) are injected on an HPLC column.
2. Identical retention times were observed for digoxin and dihydrodigoxin i.e. separation could not be achieved.
3. Possible hydrolysis of digoxin: Although not conclusively proven; results indicated possible hydrolysis of digoxin in the presence of an aqueous mobile phase associated with reverse phase HPLC procedures.

It was therefore decided to attempt separation of dihydrodigoxin and digoxin using a silica-gel column and a R.I. detector.

EXPERIMENT 4

Objective:

To attempt separation of dihydrodigoxin and digoxin using a silica- gel column coated with silver nitrate.

Introduction:

Review of the literature indicated that positional and geometrical isomers of some alkenes and unsaturated fatty acid esters have been separated by HPLC on silver nitrate coated silica-gel columns (Mikés *et al.* 1973, Heath *et al.* 1975, 1977; Lam and Grushka 1977, Aigner *et al.* 1976, Ozcimder and Hammers 1980). It was therefore decided to attempt separation of digoxin and dihydrodigoxin using a similar column. It was felt that possible attraction of Ag^{+} to the π electrons in the unsaturated lactone ring of the digoxin molecule would lead to separation, since the lactone ring in the dihydrodigoxin molecule is saturated. It was also decided to use the refractive index detector for detection purposes in light of previous success with the refractive index detector (Experiment 3) in detecting dihydrodigoxin.

Procedure :

Column : Lichosorb SI-60 coated with silver nitrate

Mobile phase : Hexane : Methylene chloride : Ethanol

(various ratios were attempted)

Flow rate : flow rates ranging from 1-3 ml./min. were used

The procedure for the preparation of the column was similar to that of Yamauchi *et al.* (1980). 3 gms. of Lichosorb SI-60 (5 μ) and 0.3 gm. of silver nitrate were mixed together in 50 ml. of acetonitrile. This was evaporated to

dryness under a vacuum on a rotary evaporator at 40 ^{0}C. A slurry was made in 1-hexanol (freshly distilled) saturated with silver nitrate and the column was packed at 6000 psi using 1-hexanol as the flushing solvent.

The output end of the column served as the input of eluant mobile phase into the UV detector, for the detection of digoxin; and the outlet end of this detector served as the inlet into the refractive index detector for the detection of dihydrodigoxin and digoxin (Fig. 67).

Results :

On passage of the mobile phase a large amount of the silver nitrate was washed off the column. The column failed to separate digoxin and dihydrodigoxin. This indicates that a very non-polar mobile phase needs to be used to prevent silver nitrate from being washed off of the column. It was felt that use of a very non-ploar mobile phase would result in excessive tailing and prohibitively large retention times for the digoxin and dihydrodigoxin peaks.

Conclusion:

The silver nitrate coated silica-gel column was found to be impractical for separation of dihydrodigoxin and digoxin. It was therefore decided to pursue an ordinary silica-gel column to study the separation of digoxin and dihydrodigoxin.

EXPERIMENT 5

Objective:

To attempt separation of digoxin and dihydrodigoxin using a silica-gel column and a normal phase HPLC procedure.

Introduction:

Previous experiments (1 to 4), had determined that the RI detector could be used to detect dihydrodigoxin. However certain reservations exist to the use of the RI detector viz.,

1) μg. quantities of both dihydrodigoxin and digoxin have to be injected on to the HPLC column so that they may be detected by the RI detector. It is felt that the retention times evidenced by these injections may not neccessarily reflect the retention times upon injection of ng. quantities (which are the levels expected in pharmacokinetic-pharmacodynamic studies).

2) The RI detector has an extremely noisy baseline and is suceptible to minor changes in the mobile phase, temperature and flow rate.

It was therefore decided that other means of detection were to be investigated.

Review of the literature showed that digoxin could be detected fluorimetrically (Wells *et al.* 1961; Jensen 1952).The fluorimetric procedure for digoxin was a non-specific procedure for cardiac glycosides, and involved oxidation of the steroid nucleus. It was therefore deduced that the same procedure may be utilized for the detection of dihydrodigoxin.

Procedure:

Column : Lichosorb Sl-60 4 mm. i.d. Length 25 cm.

Mobile phase : Hexane : Methylene chloride : Ethanol (various ratios were attempted)

Flow rate : 3 ml/min. (other flow rates from 1 - 3 ml/min. were also used)

Detection:

Digoxin was detected using a UV detector. Digoxin (DIG) and dihydrodigoxin (DH), were also quantitated using the fluorimetric procedure as follows:

The eluant was collected at regular intervals. These fractions were evaporated to dryness and assayed for digoxin using the fluorometric method of Wells et al., (1961). To the dry residue was added 2 ml. of methanol containing 2 mg. ascorbic acid, 3 ml. concentrated hydrochloric acid and 0.2 ml. of 0.003 M aqueous hydrogen peroxide. The tubes were immediately vortexed to mix the contents and are then allowed to stand at room temperature. After 20 - 40 mins. the samples and a reagent blank were then read in a Aminco- Bowman spectroflourometer (American Instrument Company, Silver Springs, MD.). The amounts were then obtained from a standard curve obtained using standard solutions of digoxin and dihydrodigoxin of 0, 0.25, 1.0 and 3.0 μg/ml. The standard curves for both digoxin and dihydrodigoxin were linear. An excitation wavelength was 360 nm. and an emission wavelength of 490 nm were used for digoxin. and an excitation wavelength of 360 nm. and an emission wavelength of 420 nm. was used for dihydrodigoxin.

Results :

The nature and the retention time of the digoxin peak were determined. Under identical conditions dihydrodigoxin was injected on the column was injected on the column and fractions 6 to 2 mins. before the onset of the digoxin peak (A), 2 to 0 mins. before the onset of the digoxin peak (B), the fraction corresponding to the digoxin peak (C) and the fraction corresponding to a duration of 2 mins.

TABLE 5.1

Fractions (%) of the amount of dihydrodigoxin injected on column.

INJ.	A	B	C	D
1	47.0	21.3	28.6	3.1
2	35.8	23.1	37.3	3.8
3	55.7	26.8	11.3	6.2

after the digoxin peak (D) were collected, evaporated to dryness and assayed for dihydrodigoxin. The results are given in Table 5.1

The results indicate that an average of 25.7% of the dihydrodigoxin appears under the digoxin peak. The mobile phase was Hexane : Methylene chloride : Ethanol (50 : 42 :8). The retention time for digoxin was 34.2 mins. - 37.2 mins. This was considered prohibitively too large a retention time for regular batch analysis of samples. During development of the chromatographic procedure it was observed that the degree of separation between digoxin and its metabolites was largely influenced by the ethanol concentration in the mobile phase; a reduction in the ethanol concentration leading to greater separation. However reduction of the ethanol concentration to 5% of the mobile phase resulted in retention times greater than 1 hr. for digoxin along with an unacceptable level of tailing of the digoxin peak. It therefore seemed unlikely that a separation of digoxin and dihydrodigoxin could be arrived at by HPLC procedures.

Conclusion:

It was concluded that due to the minor differences in structure between digoxin and dihydrodigoxin (a double bond in the lactone ring) separation using conventional HPLC systems was unlikely. Hence it was decided to investigate radioimmunoassay procedures for digoxin that did not cross-react with dihydrodigoxin.

Evaluation of Immunoasays Specific for Digoxin in the Presence of Dihydrodigoxin.

Introduction:

Kramer et al., (1976), reported that commercial radioimmunoassay kits cross-react with dihydrodigoxin. Specifications on commercially available kits either revealed no information regarding its cross-reactivity to dihydrodigoxin or revealed that the kits did cross-react to an appreciable extent with dihydrodigoxin. Hence a few commercially available kits were selected for evaluation of their cross-reactivity to dihydrodigoxin.

EXPERIMENT 6

Objective:

To evaluate the TDx procedure for assaying, for specificity to digoxin in the presence of dihydrodigoxin.

Introduction:

Review of the specifications on the TDx (Abott Laboratories, Diagnostics Division, Irving , Texas, USA) procedure revealed no information about its cross-reactivity to dihydrodigoxin. It was therefore decided to investigate this assay due to its advantage of automation.

Procedure:

9 serum samples were spiked with digoxin, dihydrodigoxin or both were assayed in a blinded manner (by a technician) using the automated TDx system at the toxicology

laboratory of the McGuire Veterans Administration Hospital, Richmond, VA. The results are given in Table 5.2

Results:

Appreciable cross-reactivity with dihydrodigoxin is noticed in the prescence and absence of digoxin.

Conclusion:

The TDx sustem for assaying digoxin is not specific for digoxin in the presence of dihydrodigoxin.

TABLE 5.2

SAMPLE NO.	ACTUAL CONC. (ng/ml.)		ASSAYED CONC. (ng/ml.)
	DIGOXIN	DIHYDRODIG.	DIGOXIN EQUIVALENT
1	1.0	0.0	0.9
2	1.0	1.0	1.1
3	1.0	2.0	1.2
4	1.0	4.0	1.6
5	0.0	0.0	0.0
6	0.0	1.0	0.3
7	0.0	2.0	0.4
8	0.0	4.0	0.7
9	1.0	0.0	1.0

Cross-reactivity of the antibody (used in the TDx system for assaying digoxin) to dihydrodigoxin is noticed in the presence and absence of digoxin.

EXPERIMENT 7

Objective:

To evaluate the digoxin RIA kit by Diagnostic Products Corporation, Los Angeles, CA; for specificity for digoxin in the presence of dihydrodigoxin.

Introduction:

Specifications on the digoxin RIA kit (Diagnostic Products Corporation, LA, CA) indicated a cross-reactivity for dihydrodigoxin of only 1.4% corresponding to 50% binding of the antiserum. Specifications also indicated that a digoxin equivalent of only 0.1 ng/ml. would be observed on assaying a serum sample of 5 ng/ml. of dihydrodigoxin (implying 2% cross-reactivity). Experiments were conducted to validate this lack of cross-reactivity.

Procedure:

Samples of dihydrodigoxin were assayed in duplicate using RIA. The results are listed in Table 5.3

TABLE 5.3

ANALYTE	CONC. (ng/ml.)	OBSERVED DIGOXIN EQUIVALENT (ng/ml.)
Dihydrodigoxin	0.98	0.10
	3.92	0.14
	5.88	0.175

For the calibration curve for digoxin Corr. = 0.998

The results indicate that dihydrodigoxin cross-reacts to a minimal extent with the RIA antiserum.

Conclusion:

It was found that the RIA kit by Diagnostic Products Corporation (DPC), cross-reacts with dihydrodigoxin to a minimal extent. Further studies need to be conducted to validate this lack of cross-reactivity to dihydrodigoxin.

EXPERIMENT 8

Objective:

To further evaluate the specificity of the DPC RIA kit for digoxin in the presence of dihydrodigoxin.

Introduction:

This experiment is similar to Experiment 7

Procedure:

Similar to Experiment 7 In this study plasma samples containing known amounts of both digoxin and dihydrodigoxin were assayed in duplicate for their digoxin content.

Results:

The results are listed in Table 5.4

For the calibration curve for digoxin Corr. = 0.998

The results indicate a trend towards cross-reactivity when high concentrations of dihydrodigoxin (approx. 8.0 ng/ml.) are present concomitantly with low concentrations of digoxin (0.5 ng/ml.). The observed trend towards cross reactivity could also be variability inherent in the assay. A larger number of samples need to be assayed to determine if the cross-reactivity is a significant phenomenon.

Conclusion

As seen in experiment 7 a certain trend to cross-reactivity is observed at higher concentrations of dihydrodigoxin concomitant with low concentrations of digoxin. It is proposed to evaluate this trend thorougly so that we may assess whether significant cross-reactivity exists. It was also decided to verify the purity of digoxin and dihydrodigoxin by TLC.

TABLE 5.4

ANALYTE	CONC. (ng/ml.)	DIGOXIN EQUIVALENT OBSERVED (ng/ml.)
DIG +	0.50	0.49
DH	0.00	
DIG +	0.50	0.60
DH	0.98	0.60
DIG +	0.50	0.67
DH	3.92	
DIG +	0.50	0.72
DH	7.84	

For the calibration curve for digoxin Corr. = 0.998

Digoxin equivalent observed on assaying serum containing known amounts of digoxin and dihydrodigoxin using RIA (Diagnostic Products Corp., LA, CA, USA)

DIG = digoxin

DH = dihydrodigoxin

EXPERIMENT 9

Objective:

To verify purity of digoxin and dihydrodigoxin by TLC.

Introduction:

Since a trend towards cross-reactivity of dihydrodigoxin to the RIA kit was ob-

served in the previous experiments (7 and 8), it was decided to verify that the dihydrodigoxin was pure.

Procedure:

The TLC procedure selected was that of Carvalhas and Figueira (1973). The purity of digoxin and dihydrodigoxin was verified using TLC on silica-gel plates (F254, E. Merck). The plates were heat activated at 100 degrees for 1 hour. They were then spotted with 10 μg. of digoxin and dihydrodigoxin. The solvent system used was Chloroform : Methanol : Acetic acid (9 : 0.9 : 0.1). After chromatography the plate was treated with a solution of chloramine T and trichloroacetic acid in ethanol and viewed under long wave UV light for visualisation of digoxin and dihydrodigoxin.

Results:

Digoxin and dihydrodigoxin had Rf values of 0.348 and 0.383 respectively. Single symmetric spots were observed for both compounds.

Conclusion:

Dihydrodigoxin and digoxin are both chromatographically pure. Specifications obtained from Sigma Chemical Company (for digoxin), and Burroughs Wellcome Company (for dihydrodigoxin) also indicated that the compounds were 99.9% pure. Since digoxin and dihydrodigoxin are both pure, the cross-reactivity of dihydrodigoxin to the antiserum is not due to the presence of impurities present in the compounds, but is due to interaction of dihydrodigoxin with the RIA antiserum. Further studies were conducted to verify this cross-reactivity.

EXPERIMENT 10

Objective:

To determine whether dihydrodigoxin in serum samples cross-reacts significantly with the RIA antiserum of the digoxin RIA kit by DPC. Also to determine whether addition of dihydrodigoxin to the antiserum minimizes this interaction.

Introduction:

It was observed in previous experiments that dihydrodigoxin possibly cross-reacts with the digoxin antiserum. Consultation with the R & D personnel at Diagnostic Products Corporation (manufacturers of the digoxin RIA kit) yielded a suggestion to minimize the error for digoxin in the prescence of dihydrodigoxin. This involved adding a small amount of dihydrodigoxin to the antiserum prior to its use in the assay. The objective therefore, was to determine the extent of the dihydrodigoxin – antiserum interaction and to determine whether addition of dihydrodigoxin to the antiserum minimizes this interaction.

Procedure:

Studies were conducted with various amounts of dihydrodigoxin in the prescence of digoxin to study the effect of dihydrodigoxin cross-reactivity to the RIA antiserum. Studies were also conducted with various amounts of spiked dihydrodigoxin in the antiserum (prior to the assay) to assess the effect of minimizing the cross-reactivity of dihydrodigoxin to the antiserum using kits from 3 different lots. Blank serum was spiked with varying amounts of digoxin, dihydrodigoxin or both. These samples containing known amounts of digoxin, dihydrodigoxin or both digoxin and dihydrodigoxin were assayed by RIA using antiserum spiked with 0, 4.0, 6.0, 8.0, 12.0, 16.0 or 32.0 ng/ml. of dihydrodigoxin. The error upon assaying the samples was computed as a percentage of the deviation from the known or actual digoxin

concentration. Statistical tests were then carried out on the data to determine whether 1) the contribution of dihydrodigoxin (DH) to the error is significant. 2) the interaction between dihydrodigoxin and digoxin (DH*DIG) contributes significantly to the error of the assay 3) whether dihydrodigoxin in the antiserum (DHA) contributes significantly towards minimizing the error 4) whether the contribution of the DH*DHA interaction is significant, i.e., whether the addition of dihydrodigoxin to the antiserum results in a reduction of the error contributed to the assay, by dihydrodigoxin in the serum samples.

Results:

The data from all of the samples assayed are given in tables 5.5-5.10

TABLE 5.5

DIGOXIN CONC. ACTUAL (ng/ml.)	DIGOXIN CONC. OBSERVED (ng/ml.)	ERROR (%)
0.5	0.47	-6.0
0.5	0.30	-40.0
1.01	0.93	-7.9
1.01	0.94	-6.0
1.01	1.14	14.0
2.01	1.97	-2.0
2.01	2.16	8.0
2.01	2.14	6.5
4.02	4.26	6.0
4.02	4.06	1.5
4.02	4.34	8.0
8.05	8.22	2.1
8.05	8.38	4.1
8.05	7.71	-3.6

Serum containing known amounts of digoxin assayed by RIA (Diagnostic Products, LA, CA, USA).

TABLE 5.6

DIG ng/ml.	DH ng/ml.	DIG. EQUIVALENT ng/ml.	% ERROR
0.0	0.0	0.12	12.0
0.50	0.0	0.42	-16.0
0.50	0.0	0.70	40.0
0.50	0.0	0.66	32.0
0.52	0.0	0.36	-30.8
1.04	0.0	0.98	-5.8
2.07	0.0	1.99	-3.8
4.00	0.0	4.25	6.2
4.00	0.0	3.84	-4.0
4.00	0.0	4.20	5.0
4.14	0.0	3.76	-9.2
8.28	0.0	6.56	-20.8
0.0	0.49	0.20	20.0
0.0	0.98	0.32	32.0
0.0	1.97	0.22	22.0
0.0	3.84	0.32	32.0
0.0	7.88	0.38	38.0
0.52	0.49	0.51	-1.9
0.52	0.98	0.55	5.8
0.52	1.94	0.59	13.4
0.52	7.88	0.72	38.4
1.04	0.49	1.14	9.6
1.04	0.98	1.06	1.9
1.04	1.97	1.12	7.6
1.04	3.84	1.17	12.5
1.04	7.88	1.30	25.0
2.07	0.49	2.14	3.4
2.07	0.98	2.20	6.2
2.07	1.97	1.99	-3.9
2.07	3.84	2.09	0.97
2.07	7.88	2.35	13.5
4.14	0.49	3.80	-8.2
4.14	0.98	3.86	-6.8
4.14	1.97	4.32	4.3
4.14	3.84	4.45	7.5
4.14	7.88	4.33	6.4

TABLE 5.6 (contd.)

DIG ng/ml.	DH ng/ml.	DIG. EQUIVALENT ng/ml.	% ERROR
8.28	0.49	7.88	-4.8
8.28	0.98	7.22	-12.8
8.28	1.97	7.44	-10.1
8.28	3.84	7.64	-7.7
8.28	7.88	7.86	-5.1

Digoxin equivalent observed on assaying serum containing known amounts of digoxin (DIG) and dihydrodigoxin (DH) using RIA (Diagnostic Products Corp., LA, CA, USA).

TABLE 5.7

Lot NO. 141

DIG ng/ml	DH_s ng/ml	DH_a ng/ml	ERROR %	MEAN (SD) %
0.5	0	8	-10	-4 (8.48)
0.5	0	8	2	
0.5	0.5	8	-16	-13 (4.24)
0.5	0.5	8	-10	
0.5	2.0	8	-8	-2 (8.48)
0.5	2.0	8	4	
0.5	8.0	8	14	12 (2.82)
0.5	8.0	8	10	
1.0	0	8	-11	-5.5 (7.8)
1.0	0	8	0	
1.0	0.5	8	-6	-6 (0)
1.0	0.5	8	-6	
1.0	2.0	8	-9	5.5 (20.5)
1.0	2.0	8	20	
1.0	8.0	8	16	14.5 (2.12)
1.0	8.0	8	13	
2.0	0	8	0	0 (0)
2.0	0	8	0	
2.0	0.5	8	0	4.5 (6.4)
2.0	0.5	8	9	
2.0	2.0	8	1	-5.75 (9.5)
2.0	2.0	8	-12.5	
2.0	8.0	8	9	15.5 (9.19)
2.0	8.0	8	22	

Error observed in assaying serum containing known amounts of digoxin and dihydrodigoxin using RIA (Diagnostic Products Corp., LA, CA, USA). The antiserum used in the RIA was spiked to result in a final concentration of 8 ng/ml. of dihydrodigoxin. DIG = digoxin

DH_s = digoxin in serum

DH_a = digoxin in antiserum

TABLE 5.8

NO. 141

DIG ng/ml	DH_s ng/ml	DH_a ng/ml	ERROR %	MEAN ± (s.d.) %
0.5	0	0	-7.0	-3.5 (4.9)
0.5	0	0	0	
0.5	0.5	0	26	36.0 (14.1)
0.5	0.5	0	46	
0.5	2.0	0	74	67 (9.9)
0.5	2.0	0	60	
0.5	8.0	0	88	88 (0)
0.5	8.0	0	88	
1.0	0	0	-9	-11 (2.8)
1.0	0	0	-13	
1.0	0.5	0	20	18 (2.8)
1.0	0.5	0	16	
1.0	2.0	0	17	19.5 (3.5)
1.0	2.0	0	22	
1.0	8.0	0	52	52.5 (0.7)
1.0	8.0	0	53	
2.0	0	0	11.5	13.75 (3.2)
2.0	0	0	16	
2.0	0.5	0	7	15.75 (12.4)
2.0	0.5	0	24.5	
2.0	2.0	0	12	19.25 (10.2)
2.0	2.0	0	26.5	
2.0	8.0	0	39.5	40.25 (1.1)
2.0	8.0	0	41	

Error associated with assaying serum containing known amounts of digoxin and dihydrodigoxin using RIA (Diagnostic Products Corp., LA, CA, USA).

DIG = digoxin, DH_s = dihydrodigoxin in serum, DH_a = dihydrodigoxin in antiserum.

TABLE 5.9

LOT NO. 142

DIG ng/ml	DH_s ng/ml	DH_a ng/ml	ERROR %	MEAN ± s.d. %
0.5	0	0	4.4	3.5 ± 1.3
0.5	0	0	2.6	
0.5	0	1	15.8	13.3 ± 3.5
0.5	0	1	10.8	
0.5	0	4	-6.4	-6.7 ± 0.4
0.5	0	4	-7.0	
0.5	0	8	-3.6	2.4 ± 8.5
0.5	0	8	8.4	
0.5	0	16	-20.0	-21.0 ± 1.4
0.5	0	16	-22.2	
0.5	8	0	60.0	62.7 ± 3.8
0.5	8	0	65.4	
0.5	8	1	46.0	52.0 ± 8.5
0.5	8	1	58.0	
0.5	8	4	40.0	32.8 ± 10.2
0.5	8	4	25.6	
0.5	8	8	62.0	57.0 ± 7.1
0.5	8	8	52.0	
0.5	8	16	12.8	14.2 ± 3.8
0.5	8	16	15.6	

Error associated with assaying digoxin in serum containing known amounts of digoxin and dihydrodgioxin using RIA (Diagnostic Products Corp., LA, CA, USA). The antiserum used in the RIA was spiked with dihydrodigoxin in varying (known) amounts.

DIG = digoxin

DH_s = dihydrodigoxin in serum

DH_a = dihydrodigoxin in antiserum

TABLE 5.10

Lot NO. 146

DIG ng/ml	DH_s ng/ml	DH_a ng/ml	ERROR %	MEAN (SD) %
0.5	0	0	4.8	
0.5	0	0	5.2	3.0 (3.5)
0.5	0	0	-1.0	
0.5	0	4	4.2	
0.5	0	4	22	8.5 (11.9)
0.5	0	4	-0.6	
0.5	0	6	6.4	
0.5	0	6	7.4	8.6 (3.0)
0.5	0	6	12.0	
0.5	0	8	27.4	
0.5	0	8	22	24.3 (2.8)
0.5	0	8	23.6	
0.5	0	10	14.2	
0.5	0	10	12.0	14.9 (3.2)
0.5	0	10	18.4	
0.5	2	0	17.2	
0.5	2	0	25.0	23.4 (5.6)
0.5	2	0	28.0	
0.5	2	4	11.2	
0.5	2	4	8.6	12.3 (4.4)
0.5	2	4	17.2	
0.5	2	6	22.6	
0.5	2	6	11.4	16.6 (5.6)
0.5	2	6	15.8	
0.5	2	8	25.4	
0.5	2	8	17.6	18.2 (6.9)
0.5	2	8	11.6	

contd....

TABLE 5.10 (contd.)

DIG ng/ml	DH_s ng/ml	DH_a ng/ml	ERROR %	MEAN (SD) %
2.0	0	0	-4	-3 (1.4)
2.0	0	0	-2.0	
2.0	0	4	-7.0	4 (15.5)
2.0	0	4	15	1.25 (1.8)
2.0	0	8	2.5	1.25 (1.8)
2.0	0	8	0	
2.0	0	12	5	-2 (9.9)
2.0	0	12	-9	
2.0	0	16	-7	-0.75 (8.8)
2.0	0	16	5.5	
2.0	8	0	8	19 (15.6)
2.0	8	0	30	
2.0	8	4	22	24.5 (3.5)
2.0	8	4	27	
2.0	8	8	16	14 (2.82)
2.0	8	8	12	
2.0	8	12	18.5	14.75 (5.3)
2.0	8	12	11	
2.0	8	16	18	18 (0)
2.0	8	16	18	

Error associated with assaying digoxin in serum containing known amounts of digoxin and dihydrodigoxin using RIA (Diagnostic Products Corp., LA, CA, USA). The antiserum used in the RIA was spiked with varying (known) amounts of dihydrodigoxin.

DIG = digoxin

DH_s = dihydrodigoxin in serum

DH_a = dihydrodigoxin in antiserum

TABLE 5.11

Lot no. 146

DIG (ng/ml.)	DH_s (ng.ml.)	DH_a (ng/ml.)	ERROR %	ERROR % MEAN ± s.d.
0.5	0.0	0.0	-3.6	
0.5	0.0	0.0	8.7	2.0
0.5	0.0	0.0	-2.2	±
0.5	0.0	0.0	5.1	5.9
0.5	0.0	4.0	1.8	
0.5	0.0	4.0	11.3	2.4
0.5	0.0	4.0	-3.4	±
0.5	0.0	4.0	0.0	6.3
0.5	0.0	6.0	14.0	9.5
0.5	0.0	6.0	6.4	±
0.5	0.0	6.0	8.2	4.0
0.5	0.0	8.0	6.7	
0.5	0.0	8.0	-4.9	2.8
0.5	0.0	8.0	-4.0	±
0.5	0.0	8.0	13.6	8.9
0.5	0.0	10.0	15.3	
0.5	0.0	10.0	6.2	10.9
0.5	0.0	10.0	22.2	±
0.5	0.0	10.0	0.0	9.8
0.5	0.0	12.0	9.1	12.0
0.5	0.0	12.0	17.1	±
0.5	0.0	12.0	0.9	8.9
0.5	0.0	16.0	11.1	
0.5	0.0	16.0	8.4	8.8
0.5	0.0	16.0	13.1	±
0.5	0.0	16.0	2.7	4.5
0.5	0.0	32.0	9.1	
0.5	0.0	32.0	9.1	6.8
0.5	0.0	32.0	9.1	±
0.5	0.0	32.0	0.0	4.6

contd....

TABLE 5.11 (contd.)

DIG (ng/ml.)	DH_s (ng.ml.)	DH_a (ng/ml.)	ERROR %	ERROR % MEAN ± s.d.
0.5	8.0	0.0	30.7	
0.5	8.0	0.0	32.5	
0.5	8.0	0.0	29.9	24.8
0.5	8.0	0.0	25.4	±
0.5	8.0	0.0	17.1	7.9
0.5	8.0	0.0	13.1	
0.5	8.0	4.0	20.4	
0.5	8.0	4.0	16.5	
0.5	8.0	4.0	4.5	14.4
0.5	8.0	4.0	13.8	±
0.5	8.0	4.0	10.0	6.6
0.5	8.0	4.0	22.0	
0.5	8.0	6.0	5.4	
0.5	8.0	6.0	10.7	
0.5	8.0	6.0	5.2	6.26
0.5	8.0	6.0	10.0	±
0.5	8.0	6.0	0.0	2.5
0.5	8.0	6.0	36.0	
0.5	8.0	8.0	21.3	
0.5	8.0	8.0	19.6	
0.5	8.0	8.0	19.5	13.3
0.5	8.0	8.0	2.0	±
0.5	8.0	8.0	17.4 9.6	
0.5	8.0	8.0	0.0	
0.5	8.0	10.0	0.0	
0.5	8.0	10.0	14.0	
0.5	8.0	10.0	9.1	7.1
0.5	8.0	10.0	2.0	±
0.5	8.0	10.0	10.4 5.3	
0.5	8.0	10.0	7.1	
0.5	8.0	12.0	16.4	
0.5	8.0	12.0	12.4	
0.5	8.0	12.0	12.9 13.8	
0.5	8.0	12.0	14.4	±
0.5	8.0	12.0	4.4	5.8
0.5	8.0	12.0	22.0	

contd....

TABLE 5.11 (contd.)

DIG (ng/ml.)	DH_s (ng.ml.)	DH_a (ng/ml.)	ERROR %	ERROR % MEAN ± s.d.
0.5	8.0	16.0	16.4	
0.5	8.0	16.0	12.4	
0.5	8.0	16.0	12.9	8.0
0.5	8.0	16.0	14.4	±
0.5	8.0	16.0	4.4	10.0
0.5	8.0	16.0	22.0	
0.5	8.0	32.0	0.0	
0.5	8.0	32.0	-5.4	0.62
0.5	8.0	32.0	0.0	±
0.5	8.0	32.0	-5.6	6.5
0.5	8.0	32.0	2.5	
0.5	8.0	32.0	12.2	

Error associated with assaying digoxin in serum containing known amounts of digoxin and dihydrodigoxin using RIA (Diagnostic Products Corp., LA, CA, USA). The antiserum used in the RIA was spiked with varying (known) amounts of dihydrodigoxin.
DIG = digoxin
DH_s = dihydrodigoxin in serum
DH_a = dihydrodigoxin in antiserum

The results of the statistical analysis are given in tables 5.12 and 5.13.

When all the data are included in the analysis the results indicate that contribution of DH is significant. This therefore implies that there exists a definite cross-reactivity of DH to the digoxin antiserum. The results also show that the DH*DIG interaction is significant. This indicates that the presence of dihydrodigoxin contributes significantly to the error in the measurement of digoxin concentrations.

However, re-analysis of the data after deletion of data containing dihydrodigoxin (DH) concentrations of 8 ng/ml. (in serum), indicate different results. In this case. the contribution of DH to the error is significant, indicating again that the DH cross-reacts with the digoxin antiserum. However, the DIG*DH interaction is

Table 5.12

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DIG	3	1 2 0.5
DH	4	0 2 8 0.5
ANTISER	9	0 1 4 6 8 10 12 16 32

NUMBER OF OBSERVATIONS IN DATA SET = 197

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: ERROR

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	32	34377.53704704	1074.29803272	6.62
ERROR	164	26610.82985651	162.26115766	PR > F
CORRECTED TOTAL	196	60988.36690355		0.0001

R-SQUARE	C.V.	ROOT MSE	ERROR MEAN
0.563674	96.8290	12.73817717	13.15532995

SOURCE	DF	TYPE I SS	F VALUE	PR > F
ANTISER	8	9441.49354839	7.27	0.0001
DIG	2	1062.73806051	3.27	0.0403
DH	3	11140.52710597	22.89	0.0001
DIG*DH	6	2310.20159729	2.37	0.0317
DH*ANTISER	13	10422.57673487	4.94	0.0001

SOURCE	DF	TYPE III SS	F VALUE	PR > F
ANTISER	8	11451.41963014	8.82	0.0001
DIG	2	947.94222063	2.92	0.0567
DH	3	3246.45847404	6.67	0.0003
DIG*DH	6	1095.64447076	1.13	0.3498
DH*ANTISER	13	10422.57673487	4.94	0.0001

The table indicates that Dihydrodigoxin (DH) and the DIG*DH interaction contribute significantly to the error when digoxin is assayed using RIA in the presence of dihydrodigoxin.

Table 5.13

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
DIG	3	1 2 0.5
DH	3	0 2 0.5
ANTISER	9	0 1 4 6 8 10 12 16 32

NUMBER OF OBSERVATIONS IN DATA SET = 117

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: ERROR

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	21	12691.56492395	604.36023447	5.14
ERROR	95	11171.90806750	117.59903229	PR > F
CORRECTED TOTAL	116	23863.47299145		0.0001

R-SQUARE	C.V.	ROOT MSE	ERROR MEAN
0.531841	131.0321	10.84430875	8.27606838

SOURCE	DF	TYPE I SS	F VALUE	PR > F
ANTISER	8	3582.98809364	3.81	0.0006
DIG	2	752.78747942	3.20	0.0452
DH	2	3818.69167393	16.24	0.0001
DIG*DH	4	515.19446650	1.10	0.3635
DH*ANTISER	5	4021.90321046	6.84	0.0001

SOURCE	DF	TYPE III SS	F VALUE	PR > F
ANTISER	8	4970.80828837	5.28	0.0001
DIG	2	1043.37017061	4.44	0.0144
DH	2	564.23084966	2.40	0.0963
DIG*DH	4	704.30497093	1.50	0.2092
DH*ANTISER	5	4021.90321046	6.84	0.0001

Table indicates that DIG*DH interaction is not significant when digoxin is assayed using RIA in the presence of dihydrodigoxin (DH).

not significant. This indicates that although, DH cross-reacts with the antiserum, DH (at concentrations less than 8 ng/ml.) in the presence of DIG does not add significantly to the error associated with assaying digoxin alone. In both analyses the DH*DHA interaction was significant, indicating that dihydrodigoxin in the antiserum minimizes the cross-rectivity of DH to the digoxin antiserum.

The results therefore indicate that dihydrodigoxin does cross-react with digoxin to a maximum of 20% when dihydrodigoxin concentrations are 4 times greater than the digoxin concentrations. However, this would not be expected to contribute to error in clinical samples since dihydrodigoxin has a much shorter half-life than digoxin.The results also indicate that the RIA kit by DPC may be used for the assaying of samples collected off the HPLC column in our assay, which may contain DH along with the digoxin being collected, provided the DH levels are expected to be less than 8 ng/ml.

Conclusion:

1) Provided dihydrodigoxin concentrations are less than 8 ng/ml., DH in the presence of digoxin does not significantly increase the error normally associated with the assaying of digoxin alone. 2) The addition of DH to the antiserum significantly reduces the cross-reactivity of the DH to the digoxin antiserum.

EXPERIMENT 8.0

Objective:

To confirm that the presence of DH does not contribute significantly to the error associated with assaying digoxin levels in serum samples obtained from pharmacokinetic studies in beagle dogs.

Introduction:

Results of the previous experiment (experiment 7.3), indicated that DH in the presence of digoxin did not contribute significantly to the error associated with assaying digoxin provided DH concentrations were less than 8 ng/ml.

To confirm this result, the samples from a representative pharmacokinetic study were assayed in the presence and absence of added dihydrodigoxin.

Procedure:

Samples from study no. 1 (refer to the experimental section) were used for the assays. The samples were divided into two parts A and B. The samples in Part B were spiked with dihydrodigoxin such that the samples had a final concentration of dihydrodigoxin of 4 ng/ml. Also, the antiserum in the RIA kit was divided into two halves. One half of the antiserum was spiked with dihydrodigoxin to result in a dihydrodigoxin concentration of 8 ng/ml. Samples from both part A and B were assayed by RIA using the unaltered antiserum and the antiserum spiked with dihydrodigoxin. Univariate analyses were conducted to determine whether the samples of part A (labeled "ONE" and "TWO" in table 5.14) and part B (labeled "THREE"and "FOUR" in table 5.14) were statistically different and whether addition of DH to the serum or to the antiserum produced any statistical differences in the observed concentrations.

Results:

The results are listed in table 5.14.

TABLE 5.14

TIME	DH_a = 0		DH_a = 8	
(hrs)	PART A ONE (DIG)	PART B TWO (DIG ÷ DH)	PART A THREE (DIG)	PART B FOUR (Dig ÷ DH)
0.33	33.28	31.7	30.00	32.6
1.33	7.65	8.1	7.88	7.5
1.83	5.36	6.0	6.12	5.8
3.08	3.76	4.1	3.64	4.2
4.92	3.30	3.11	3.8	3.6
7.0	3.1	2.85	3.0	3.3
23.33	1.7	1.5	1.6	1.8
30.83	2.0	2.2	2.0	2.1
48.00	1.2	1.4	1.4	1.3
54.75	0.91	0.89	0.93	1.0
72.42	0.71	0.68	0.70	0.70
78.5	0.41	0.54	0.56	0.57

DIG = digoxin

DH = dihydrodigoxin

DH_a = dihydrodigoxin in the antiserum

The statistical analyses were conducted as follows: If the addition of DH to the serum does not produce any statistical differences in the measured concentrations of digoxin, then, the variable "E"(E = ONE - TWO) should have a mean of zero provided it has a normal distribution. The t-statistic for the hypothesis that the mean of E = 0. was calculated. Also, if ONE is not statistically significant from TWO then the variable "I" (I = ONE/TWO - 1) should also have a mean of zero provided it has a normal distribution. A test of normality was also conducted on the generated variables. The generated variables were:

E = ONE - TWO

F = ONE - FOUR

G = TWO - FOUR

H = ONE - THREE

I = (ONE/TWO - 1)

J = (ONE/FOUR - 1)

K = (TWO/FOUR - 1)

L = (ONE/THREE - 1)

The results of the statistical analyses are given in table 5.15

Variables E, F, H and J do not have a normal distribution. Hence variables I, K, L and G were used in the analyses. The t-statistic and the rank values both indicate that the variables I, K, L and G all have a mean of zero and are normally distributed. This indicates that the addition of DH to the serum or the antiserum does not produce any difference in the measured concentration of digoxin.

Conclusion:

The results indicate that addition of DH to the serum or the antiserum does not produce any significant differences in the measured concentrations of digoxin. This is in agreement with the findings of Malini *et al.*, (1982) who after evaluating several radioimmunoassays kits found that the RIA kit by the Diagonostic Products Corp. was specific for digoxin in the presence of dihydrodigoxin. It was therefore expected that serum samples of digoxin in our studies that may contain dihydrodigoxin (in concentrations of < 4 ng/ml.) would not contribute to a "false positive" in the measured digoxin concentration.

Table 5.15

UNIVARIATE

VARIABLE=E

MOMENTS

N	12	SUM WGTS	12
MEAN	0.0341667	SUM	0.41
STD DEV	0.585964	VARIANCE	0.343354
SKEWNESS	1.75541	KURTOSIS	4.09385
USS	3.7909	CSS	3.77689
CV	1715.02	STD MEAN	0.169153
T:MEAN=0	0.201986	PROB>|T|	0.843614
SGN RANK	-4	PROB>|S|	0.783652
NUM ^= 0	12		
W:NORMAL	0.851843	PROB<W	0.043

QUANTILES(DEF=4)

100% MAX	1.58	99%	1.58
75% Q3	0.235	95%	1.58
50% MED	-0.055	90%	1.256
25% Q1	-0.385	10%	-0.583
0% MIN	-0.64	5%	-0.64
		1%	-0.64
RANGE	2.22		
Q3-Q1	0.62		
MODE	-0.64		

EXTREMES

LOWEST	HIGHEST
-0.64	0.03
-0.45	0.19
-0.4	0.25
-0.34	0.5
-0.2	1.58

VARIABLE=F

MOMENTS

N	12	SUM WGTS	12
MEAN	-0.0908333	SUM	-1.09
STD DEV	0.296478	VARIANCE	0.0878992
SKEWNESS	1.57853	KURTOSIS	3.88655
USS	1.0659	CSS	0.966892
CV	-326.4	STD MEAN	0.0855858
T:MEAN=0	-1.0613	PROB>|T|	0.311294
SGN RANK	-20	PROB>|S|	0.125361
NUM ^= 0	12		
W:NORMAL	0.847276	PROB<W	0.039

QUANTILES(DEF=4)

100% MAX	0.68	99%	0.68
75% Q3	-0.015	95%	0.68
50% MED	-0.1	90%	0.521
25% Q1	-0.275	10%	-0.44
0% MIN	-0.44	5%	-0.44
		1%	-0.44
RANGE	1.12		
Q3-Q1	0.26		
MODE	-0.1		

EXTREMES

LOWEST	HIGHEST
-0.44	-0.1
-0.44	-0.09
-0.3	0.01
-0.2	0.15
-0.16	0.68

Table 5.15 (contd.)

UNIVARIATE

VARIABLE=G

MOMENTS

N	12	SUM WGTS	12
MEAN	-0.125	SUM	-1.5
STD DEV	0.420876	VARIANCE	0.177136
SKEWNESS	-0.219514	KURTOSIS	-0.1996
USS	2.136	CSS	1.9485
CV	-336.7	STD MEAN	0.121496
T:MEAN=0	-1.0288	PROB>|T|	0.325648
SGN RANK	-12	PROB>|S|	0.366617
NUM ^= 0	12		
W:NORMAL	0.977498	PROB<W	0.937

QUANTILES(DEF=4)

100% MAX	0.6	99%	0.6
75% Q3	0.175	95%	0.6
50% MED	-0.065	90%	0.51
25% Q1	-0.48	10%	-0.81
0% MIN	-0.9	5%	-0.9
		1%	-0.9
RANGE	1.5		
Q3-Q1	0.655		
MODE	-0.9		

EXTREMES

LOWEST	HIGHEST
-0.9	-0.02
-0.6	0.1
-0.49	0.2
-0.45	0.3
-0.11	0.6

VARIABLE=H

MOMENTS

N	12	SUM WGTS	12
MEAN	0.145833	SUM	1.75
STD DEV	1.02163	VARIANCE	1.04374
SKEWNESS	3.03273	KURTOSIS	10.0527
USS	11.7363	CSS	11.4811
CV	700.549	STD MEAN	0.29492
T:MEAN=0	0.494484	PROB>|T|	0.630691
SGN RANK	-9	PROB>|S|	0.44958
NUM ^= 0	11		
W:NORMAL	0.570121	PROB<W	<.01

QUANTILES(DEF=4)

100% MAX	3.28	99%	3.28
75% Q3	0.1	95%	3.28
50% MED	-0.01	90%	2.332
25% Q1	-0.2225	10%	-0.682
0% MIN	-0.76	5%	-0.76
		1%	-0.76
RANGE	4.04		
Q3-Q1	0.3225		
MODE	0.1		

EXTREMES

LOWEST	HIGHEST
-0.76	0.01
-0.5	0.1
-0.23	0.1
-0.2	0.12
-0.15	3.28

Table 5.15 (contd.)

UNIVARIATE

VARIABLE=I

MOMENTS

N	12	SUM WGTS	12
MEAN	-0.0183871	SUM	-0.220645
STD DEV	0.153565	VARIANCE	0.0235821
SKEWNESS	0.807651	KURTOSIS	1.39321
USS	0.26346	CSS	0.259403
CV	-835.18	STD MEAN	0.0443303
T:MEAN=0	-0.414775	PROB>|T|	0.686281
SGN RANK	-9	PROB>|S|	0.504903
NUM ^= 0	12		
W:NORMAL	0.939133	PROB<W	0.474

QUANTILES(DEF=4)

100% MAX	0.333333	99%	0.333333
75% Q3	0.0582805	95%	0.333333
50% MED	-0.0165418	90%	0.259649
25% Q1	-0.13381	10%	-0.225661
0% MIN	-0.240741	5%	-0.240741
		1%	-0.240741
RANGE	0.574074		
Q3-Q1	0.19209		
MODE	-0.240741		

EXTREMES

LOWEST	HIGHEST
-0.240741	0.0441176
-0.190476	0.0498423
-0.142857	0.0610932
-0.106667	0.0877193
-0.0829268	0.333333

VARIABLE=J

MOMENTS

N	12	SUM WGTS	12
MEAN	-0.0683515	SUM	-0.820218
STD DEV	0.0800502	VARIANCE	0.00640803
SKEWNESS	-1.6296	KURTOSIS	4.43397
USS	0.126551	CSS	0.0704883
CV	-117.12	STD MEAN	0.0231085
T:MEAN=0	-2.9579	PROB>|T|	0.0130252
SGN RANK	-33	PROB>|S|	0.0107874
NUM ^= 0	12		
W:NORMAL	0.811449	PROB<W	0.013

QUANTILES(DEF=4)

100% MAX	0.0208589	99%	0.0208589
75% Q3	-.00119048	95%	0.0208589
50% MED	-0.0682341	90%	0.0206012
25% Q1	-0.0883333	10%	-0.22792
0% MIN	-0.280702	5%	-0.280702
		1%	-0.280702
RANGE	0.301561		
Q3-Q1	0.0871429		
MODE	-0.280702		

EXTREMES

LOWEST	HIGHEST
-0.280702	-0.0555556
-0.104762	-0.047619
-0.09	0.0142857
-0.0833333	0.02
-0.0769231	0.0208589

Table 5.15 (contd.)

UNIVARIATE

VARIABLE=K

MOMENTS

N	12	SUM WGTS	12
MEAN	-0.0368947	SUM	-0.442736
STD DEV	0.120875	VARIANCE	0.0146108
SKEWNESS	-0.363585	KURTOSIS	0.509203
USS	0.177053	CSS	0.160719
CV	-327.62	STD MEAN	0.0348936
T:MEAN=0	-1.0573	PROB>|T|	0.313021
SGN RANK	-11	PROB>|S|	0.410117
NUM ^= 0	12		
W:NORMAL	0.971878	PROB<W	0.885

QUANTILES(DEF=4)

100% MAX	0.166667	99%	0.166667
75% Q3	0.066313	95%	0.166667
50% MED	-0.0280894	90%	0.140667
25% Q1	-0.129583	10%	-0.240909
0% MIN	-0.285714	5%	-0.285714
		1%	-0.285714
RANGE	0.452381		
Q3-Q1	0.195896		
MODE	-0.285714		

EXTREMES

LOWEST	HIGHEST
-0.285714	-0.0238095
-0.136364	0.0344828
-0.136111	0.0769231
-0.11	0.08
-0.0526316	0.166667

VARIABLE=L

MOMENTS

N	12	SUM WGTS	12
MEAN	-0.0387292	SUM	-0.46475
STD DEV	0.107224	VARIANCE	0.011497
SKEWNESS	-0.826844	KURTOSIS	0.318688
USS	0.144467	CSS	0.126467
CV	-276.86	STD MEAN	0.0309529
T:MEAN=0	-1.2512	PROB>|T|	0.236808
SGN RANK	-10	PROB>|S|	0.398305
NUM ^= 0	11		
W:NORMAL	0.930717	PROB<W	0.418

QUANTILES(DEF=4)

100% MAX	0.109333	99%	0.109333
75% Q3	0.0332418	95%	0.109333
50% MED	-0.0107527	90%	0.0952833
25% Q1	-0.12973	10%	-0.230357
0% MIN	-0.267857	5%	-0.267857
		1%	-0.267857
RANGE	0.37719		
Q3-Q1	0.162972		
MODE	-0.267857		

EXTREMES

LOWEST	HIGHEST
-0.267857	0.0142857
-0.142857	0.032967
-0.131579	0.0333333
-0.124183	0.0625
-0.0291878	0.109333

APPENDIX C

Although equations to obtain the micro- rate constants for a three compartment mammillary and "first pass" model may be found in the literature, equations to obtain the micro- rate constants for a three compartment catenary model could not be found in the literature. The equations were therefore derived.

The following equations are derived for a three compartment catenary model. In the following derivation

$$E_1 = (k_{12} + k_{10})$$

$$E_2 = (k_{23} + k_{21})$$

$$E_3 = k_{32}$$

For an i.v. bolus dose (D) administered in to the sampling compartment (compartment 1),

$$\begin{aligned} \frac{dX_1}{dt} &= -k_{12}X_1 - k_{10}X_1 + k_{21}X_2 \\ &= -(k_{12} + k_{10})X_1 + k_{21}X_2 \end{aligned} \qquad \text{...(5.1)}$$

Taking laplace transforms of eqn.(5.1)

$$s\overline{X_1} - D = -(k_{12} + k_{10})\overline{X_1} + k_{21}\overline{X_2}$$

therefore,

$$\overline{X_1} = \frac{k_{21}\overline{X_2} + D}{(s + k_{12} + k_{10})} \qquad \text{...(5.2)}$$

For compartment 2,

$$\frac{dX_2}{dt} = k_{12}X_1 + k_{32}X_3 - k_{21}X_2 - k_{23}X_2$$
$$= -X_2(k_{21} + k_{23}) + k_{12}X_1 + k_{32}X_3 \qquad \ldots(5.3)$$

Taking laplace transform of eqn.(5.3)

$$s\overline{X_2} = -(k_{21} + k_{23})\overline{X_2} + k_{12}\overline{X_1} + k_{32}\overline{X_3} \qquad \ldots(5.4)$$

Therefore,

$$\overline{X_2}(s + k_{21} + k_{23}) = k_{12}\overline{X_1} + k_{32}\overline{X_3} \qquad \ldots(5.5)$$

From eqn.(5.2) and (5.5),

$$\overline{X_2}(s + k_{21} + k_{23}) = \frac{k_{12}(k_{21}\overline{X_2} + D)}{(s + k_{12} + k_{10})} + k_{32}\overline{X_3} \qquad \ldots(5.6)$$

$$\overline{X_2}(s + k_{21} + k_{23}) = \frac{k_{12}(k_{21}\overline{X_2} + D)}{(s - E_1)} - k_{32}\overline{X_3}$$

$$\overline{X_2}\left[(s + E_1)(s + E_2) - k_{12}k_{21}\right] = k_{12}D + k_{32}\overline{X_3}(s + E_1) \qquad \ldots(5.7)$$

$$\frac{dX_3}{dt} = -k_{32}X_3 + k_{23}X_2$$

Taking laplace transforms,

$$s\overline{X_3} = -k_{32}\overline{X_3} + k_{23}\overline{X_2}$$

Therefore,

$$\overline{X_3} = \frac{k_{23}}{(s + k_{32})}\overline{X_2} \qquad \ldots(5.8)$$

Substituting for $\overline{X_3}$ in eqn.(5.7) and on rearrangement,

$$\overline{X_2} = \frac{sk_{12}D + k_{12}E_3D}{(s + E_1)(s + E_2)(s + E_3) - k_{12}k_{21}(s + E_3) - E_3k_{23}(s + E_1)}$$

This is of the form

$$\frac{As^2 + Bs + C}{(s + \alpha)(s + \beta)(s + \gamma)}$$

where,

$A = 0 \qquad B = k_{12}D \qquad C = k_{12}E_3D$

Taking inverse laplace transforms,

$$X_2 = \frac{-1}{(\beta - \gamma)(\gamma - \alpha)(\alpha - \beta)}\left[(\beta - \gamma)(-k_{12}D\alpha + k_{12}E_3D)e^{-\alpha t}\right.$$
$$+ (\gamma - \alpha)(-k_{12}D\beta + k_{12}E_3D)e^{-\beta t}$$
$$\left. + (\alpha - \beta)(-k_{12}D\gamma + k_{12}E_3D)e^{-\gamma t}\right]$$

Therefore.

$$X_2 = -k_{12}D\left[\frac{(\alpha - E_3)}{(\gamma - \alpha)(\alpha - \beta)}e^{-\alpha t} + \frac{(\beta - E_3)}{(\beta - \gamma)(\alpha - \beta)}e^{-\beta t} + \frac{(\gamma - E_3)}{(\beta - \gamma)(\gamma - \alpha)}e^{-\gamma t}\right]$$

$\ldots(5.9)$

From eqn.(5.7) and (5.8),

$$\frac{\overline{X_3}(s + E_3)}{k_{23}} = \frac{k_{12}D + E_3(s + E_1)\overline{X_3}}{(s + E_1)(s + E_2) - k_{12}k_{21}}$$

Therefore,

$$\overline{X_3} = \frac{k_{12}k_{23}D}{(s+E_1)(s+E_2)(s+E_3) - k_{12}k_{21}(s+E_3) - k_{23}E_3(s+E_1)}$$

After expansion of the denominator and rearrangement the above equation may be represented as

$$\overline{X_3} = \frac{k_{12}k_{23}D}{(s+\alpha)(s+\beta)(s+\gamma)}$$

Taking inverse laplace transforms,

$$X_3 = \frac{-1}{(\beta-\gamma)(\gamma-\alpha)(\alpha-\beta)}\left[(\beta-\gamma)(k_{12}k_{23}D)e^{-\alpha t} + (\gamma-\alpha)(k_{12}k_{23}D)e^{-\beta t} + (\alpha-\beta)(k_{12}k_{23}D)e^{-\gamma t}\right]$$

The equation may be rearranged to give

$$X_3 = -k_{12}k_{23}D\left[\frac{e^{-\alpha t}}{(\gamma-\alpha)(\alpha-\beta)} + \frac{e^{-\beta t}}{(\beta-\gamma)(\alpha-\beta)} + \frac{e^{-\gamma t}}{(\beta-\gamma)(\gamma-\alpha)}\right]$$

where,

$$\alpha+\beta+\gamma = E_1+E_2+E_3 = k_{12}+k_{10}+k_{21}+k_{23}+k_{32}$$

$$\alpha\beta+\beta\gamma+\gamma\alpha = E_1E_2+E_1E_3+E_2E_3-k_{12}k_{21}-k_{23}E_3$$

$$\alpha\beta\gamma = E_1E_2E_3 - k_{12}k_{21}E_3 - k_{23}E_1E_3$$

From eqn.(5.2)

$$\overline{X_1} = \frac{k_{21}}{(s+E_1)}\overline{X_2} + \frac{D}{(s+E_1)} \qquad \ldots(5.10)$$

Rearrangement of eqn.(5.5) gives,

$$\overline{X_2} = \frac{k_{12}\overline{X_1} + E_3\overline{X}3}{(s + E_2)} \qquad ...(5.11)$$

From eqn.(5.8) and (5.11),

$$\overline{X_2} = \frac{k_{12}\overline{X_1}}{(s + E_2)} + \frac{E_3 k_{23}\overline{X_2}}{(s + E_2)(s + E_3)} \qquad ...(5.12)$$

Therefore,

$$\overline{X_2} = \frac{k_{12}(s + E_3)\overline{X_1}}{(s + E_2)(s + E_3) - E_3 k_{23}} \qquad ...(5.13)$$

Substituting for $\overline{X_2}$ from eqn.(5.13) in to eqn.(5.10) gives,

$$\overline{X_1} = \frac{k_{21}k_{12}(s + E_3)\overline{X_1}}{(s + E_1)\left[(s + E_2)(s + E_3) - k_{23}k_{32}\right]} + \frac{D}{(s + E_1)} \qquad ...(5.14)$$

Rearrangement of the above equation gives.

$$\overline{X_1} = \frac{D\left[(s + E_2)(s + E_3) - k_{23}k_{32}\right]}{(s + E_1)(s + E_2)(s + E_3) - k_{23}k_{32}(s + E_1) - k_{12}k_{21}(s + E_3)}$$

On expansion and rearrangement the above equation is of the form:

$$\frac{As^2 + Bs + C}{(s + \alpha)(s + \beta)(s + \gamma)}$$

where,

$$A = D$$

$$B = D(E_2 + E_3)$$

$$C = D(E_2E_3 - k_{23}E_3)$$

and

$$\alpha + \beta + \gamma = E_1 + E_2 + E_3$$

$$\alpha\beta + \beta\gamma + \gamma\alpha = E_1E_2 + E_2E_3 + E_3E_1 - k_{23}E_3 - k_{12}k_{21}$$

$$\alpha\beta\gamma = E_1E_2E_3 - k_{23}E_3E_1 - k_{12}k_{21}E_3$$

Therefore, after taking inverse laplace transforms

$$X_1 = \frac{-1}{(\beta-\gamma)(\gamma-\alpha)(\alpha-\beta)}\left[(\beta-\gamma)(A\alpha^2 - B\alpha + C)e^{-\alpha t}\right.$$
$$+ (\gamma-\alpha)(A\beta^2 - B\beta + C)e^{-\beta t}$$
$$\left. + (\alpha-\beta)(A\gamma^2 - B\gamma + C)e^{-\gamma t}\right]$$

$$X_1 = \frac{(A\alpha^2 - B\alpha + C)}{(\alpha-\gamma)(\alpha-\beta)}e^{-\alpha t} - \frac{(A\beta^2 - B\beta + C)}{(\alpha-\beta)(\beta-\gamma)}e^{-\beta t} + \frac{(A\gamma^2 - B\gamma + C)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t} \quad \ldots(5.15)$$

The concentration in compartment 1 is then given by,

$$C_1 = \frac{1}{V_1}\left[\frac{(A\alpha^2 - B\alpha + C)}{(\alpha-\gamma)(\alpha-\beta)}e^{-\alpha t} - \frac{(A\beta^2 - B\beta + C)}{(\alpha-\beta)(\beta-\gamma)}e^{-\beta t} + \frac{(A\gamma^2 - B\gamma + C)}{(\alpha-\gamma)(\beta-\gamma)}e^{-\gamma t}\right] \quad \ldots(5.16)$$

where V_1 is the volume of distribution of compartment 1.

Determination of the micro- rate constants

If the concentration time profile is described by a tri-exponential expressed as,

$$C_1 = Xe^{-\alpha t} + Ye^{-\beta t} + Ze^{-\gamma t} \quad \ldots(5.17)$$

then, X, Y and Z and α, β, γ may be obtained by linear regression on the concentration time data.

Comparison of eqn.(5.16) and (5.17) yields,

$$X = \frac{1}{V_1}\left[\frac{(A\alpha^2 - B\alpha + C)}{(\alpha-\gamma)(\alpha-\beta)}\right] \quad \ldots(5.18)$$

$$Y = \frac{-1}{V_1}\left[\frac{(A\beta^2 - B\beta + C)}{(\alpha - \beta)(\beta - \gamma)}\right] \qquad ...(5.19)$$

$$Z = \frac{1}{V_1}\left[\frac{(A\gamma^2 - B\gamma + C)}{(\alpha - \gamma)(\beta - \gamma)}\right] \qquad ...(5.20)$$

From eqn.(5.18),

$$C = XV_1(\alpha - \gamma)(\alpha - \beta) - A\alpha^2 + B\alpha \qquad ...(5.21)$$

From eqn.(5.19),

$$C = -YV_1(\alpha - \beta)(\beta - \gamma) - A\beta^2 + B\beta \qquad ...(5.22)$$

From eqn.(5.20),

$$C = ZV_1(\alpha - \gamma)(\beta - \gamma) - A\gamma^2 + B\gamma \qquad ...(5.23)$$

From eqn.(5.21) and (5.22),

$$-B = XV_1(\alpha - \gamma) + YV_1(\beta - \gamma) - A(\alpha + \beta) \qquad ...(5.24)$$

At time = 0, $X_1 = D$ Therefore from eqn.(5.15) and (5.16),

$$V_1 = \frac{D}{X + Y - Z}$$

Now a numerical value for B may be obtained in eqn.(5.24), by substituting the known values. Having determined B. a numerical value for C may be obtained from eqn.(5.21).

As noted before.

$$\begin{aligned} C &= D(E_2E_3 - k_{23}E_3) \\ &= DE_3(E_2 - k_{23}) \\ &= DE_3k_{21} \end{aligned} \qquad ...(5.25)$$

Also,

$$\begin{aligned}\alpha\beta\gamma &= E_1E_2E_3 - k_{23}E_3E_1 - k_{12}k_{21}E_3 \\ &= E_1E_3k_{21} - k_{12}k_{21}E_3 \\ &= E_3k_{21}(E_1 - k_{12}) \\ &= k_{10}k_{21}E_3\end{aligned} \qquad \ldots(5.26)$$

From eqn.(5.25) and (5.26),

$$\alpha\beta\gamma = k_{10}\frac{C}{D}$$

Therefore,

$$k_{10} = \frac{\alpha\beta\gamma \cdot D}{C} \qquad \ldots(5.27)$$

Since,

$$\alpha + \beta + \gamma = E_1 + E_2 + E_3$$

and

$$\begin{aligned}B &= D(E_2 + E_3) \\ E_1 &= \alpha + \beta + \gamma - E_2 - E_3 \\ &= \alpha + \beta + \gamma - \frac{B}{D}\end{aligned} \qquad \ldots(5.28)$$

E_1 may therefore be determined. By definition $E_1 = k_{10} + k_{12}$ Therefore, from eqn.(5.27) and (5.28),

$$k_{12} = E_1 - k_{10} \qquad \ldots(5.29)$$

and a numerical value may be determined.

As noted before,

$$\begin{aligned}C &= DE_3(E_2 - k_{23}) \\ &= DE_3k_{21}\end{aligned}$$

therefore,

$$E_3 k_{21} = \frac{C}{D}$$

Also,

$$\alpha\beta + \beta\gamma + \gamma\alpha = E_1(E_2 + E_3) + E_3(E_2 - k_{23}) - k_{12}k_{21}$$

$$= E_1(E_2 + E_3) + E_3 k_{21} - k_{12}k_{21}$$

$$= E_1(E_2 + E_3) + \frac{C}{D} - k_{12}k_{21}$$

Therefore,

$$k_{21} = \frac{DE_1(E_2 + E_3) + C - D(\alpha\beta + \beta\gamma + \gamma\alpha)}{k_{12}D} \qquad ...(5.30)$$

k_{21} is the only unknown constant in the above equation and may be determined by substituting the values for the other constants.

Since,

$$C = DE_3 k_{21}, \qquad E_3 = \frac{C}{Dk_{21}}$$

and,

$$\frac{B}{D} = E_2 + E_3$$

$$Therefore, \quad E_2 = \frac{B}{D} - E_3 = \frac{B}{D} - \frac{C}{Dk_{21}}$$

$$Also \quad E_2 = k_{21} + k_{23}$$

$$= \frac{B}{D} - \frac{C}{Dk_{21}}$$

Therefore,

$$k_{23} = \frac{B}{D} - \frac{C}{Dk_{21}} - k_{21} \qquad ...(5.31)$$

From eqn.(5.30) and (5.31) k_{23} may now be determined.

Having determined k_{23} (eqn. 5.31), k_{32} may now be determined, since,

$$k_{32} = \frac{C}{Dk_{21}}$$

Vita