Chapter II

The literatures review

Review of Digoxin

Nearly, two centuries have passed since Withering published his "Account of Foxglove" with its classic descriptions of both the benefits and risks of cardiac glycosides. Even then, the narrow therapeutic : toxic ratio of these drugs was all too clear indeed, in 1941. Withering wrote his monograph in part "a medicine of so much efficacy ___ be condemned and rejected as dangerous and unmanageable". Ever since, clinicians have sought objective means of taking the guesswork out of digitalis regimens.These efforts have produced valuable insights into the pharmacokinetics and pharmacodynamics of cardiac glycosides. In recent years, rapid and reliable assays of serum digoxin and digitoxin concentrations have been developed. Nevertheless, finding the appropriate digoxin dose and diagnosing toxicity in an individual patient remain common and challenging problems. (Lee and Smith, 1983)

The use of digitalis glycosides for the treatment of congestive heart failure has receive mixed reactions ranging from uncritical acceptance to withholding of therapy for fears of possible adverse effects.

Digoxin is the most frequently employed cardiac glycosides. Digoxin structure (in Figure 1) represents the combination of an aglycone, or genin, with three molecules of sugar. Pharmacological activity resides in the

aglycone, but the particular sugars attached to the aglycone modify water and lipid solubility, potency, and the pharmacokinetic properties.

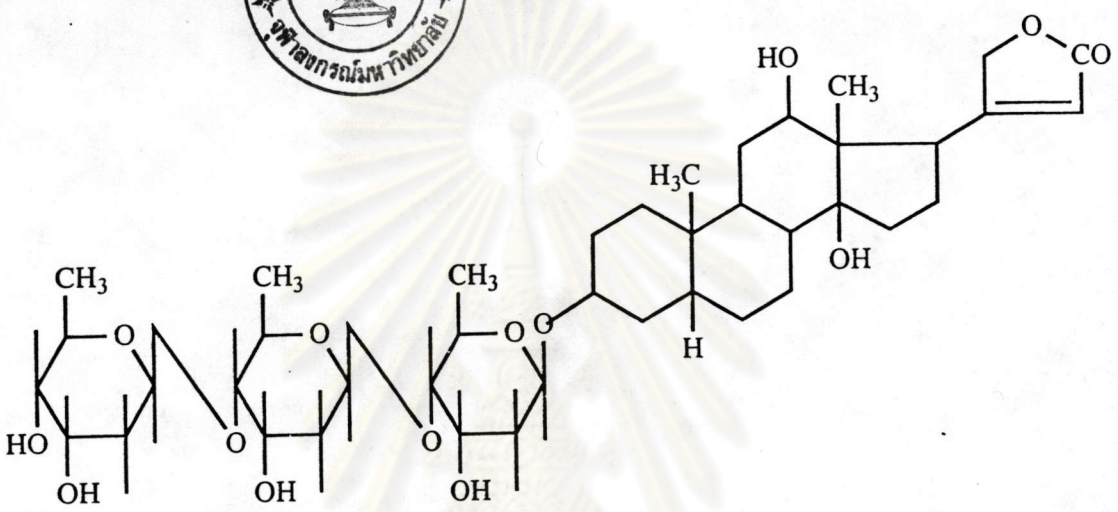

Figure 1 A diagram of molecular structure of digoxin

A. Pharmacological properties

Digoxin increases the contractility of cardiac muscle in a dose dependent manner - a positive inotropic effect. The effects are similar for both atrial and venticular muscle and are qualitatively the same for muscle obtained from either normal or failing hearts. The effects have also increased in stroke volume of the heart; as a consequence, end-systolic volume is reduced. Digoxin has an indirect effects through the autonomic nervous system, producing a negative chronotropic responses mediated indirect mechanism in vagal activity and via sympathetic withdrawal to prolong the effective refractory period and slow conduction through the atrioventricular (AV) node.

Digoxin binds to the extracellular side of Na^+/K^+-ATPases of cardiomyocytes and inhibits enzyme activity. Tha Na^+/K^+-ATPases operate to pump out Na^+ leaked into the cell and to retrieve K^+ leaked from the cell. When part of the enzyme is occupied and inhibited by digoxin, the unoccupied remainder can increase its level of activity and maintain Na^+ and K^+ transport. The effective stimulus is a small elevation of intracellular Na^+ concentration. Concomitantly, the amount of Ca^{2+} mobilized during systole and, thus, contractile force increases.

B. Clinical pharmacokinetic

Absorption

The principle part of digoxin absorption site is the proximal tubules in the small intestine. The intestinal absorption is a passive, non-saturable diffusion process for digoxin.

The average bioavailability of digoxin from tablets of fast dissolution rate (>70% in solution in 1 hour) is 67% and from elixir 80%. Digoxin is principally binded to albumin, ranging from 20 to 30%. A negligible amount is bound to lipoproteins. However, protein binding of digoxin is clinically unimportant, particularly because of its large apparent volume of distribution. (Aronson, 1980)

The absorption of digoxin varies between 40 and 100% depending on the type of preparation used, interindividual variability in absorption capacity and the presence or absence of gastrointestinal disease. (Mooradian, 1988)

Distribution

Digoxin is widely distributed throughout body tissues and has a high apparent volume of distribution. The plasma concentrations after an intravenous bolus dose of digoxin is biphasic. The drug distribution to the tissues which is the first phase takes 4 to 8 hours. The latter phase comprises the elimination of the drug from the body.

The distribution of digoxin to various tissues is : skeletal muscle 65%; liver 13%; heart 4%; brain 3%; kidneys 1.5%. There are variety of factors which can alter or interfere the volume of distribution. In patients with renal impairment, the apparent volume of distribution may be lowered. The reasons for this reduction may be related to altering tissue binding in the presence of diminished activity of membrane Na^+, K^+-ATPase. Furthermore, electrolyte imbalances such as hyperkalemia or hyponatremia can reduce digoxin binding to the myocardium tissues. At steady state digoxin are extensively bound to tissue, particularly myocardium, kidney, skeleton muscles and red blood cells (Doherty, 1968 and Jogestrand, 1980). Digoxin distributes poorly into adipose tissue and thus, lean body weight provides a better estimate of volume of distribution than does total body weight.

Metabolism

The metabolism of digoxin within the gastrointestinal tract may occure either by hydrolysis of digitoxose glycosidic moieties in the acidic environment of the stomach or by reduction of the lactone double bond by intestinal bacteria.

Although the GI tract plays a key role in both major metabolic pathways for digoxin, the liver and other organs also contribute.

The known pathways of digoxin metabolism in man are illustrated in Figure 2. One pathway consists of sequential hydrolysis of digitoxose sugar moieties attached to the 3-position of the steroid nucleus to form digoxigenin, bis-digitoxoside, digoxigenin mono-digitoxoside and digoxigenin. The latter two compounds may be further metabolized by conjugation. However, further metabolism of digoxigenin is mainly by oxidation to 3-keto-digoxigenin, which can subsequently be reduced to 3-alpha-digoxigenin (3-epi-digoxigenin). Subsequent conjugation of 3-epi-digoxigenin and conversion to other polar metabolites. The bis-and mono-digitoxosides are considered to be approximately as cardioactive as digoxin. Whereas the more polar metabolites - digoxigenin and subsequent are considered to be much less active.

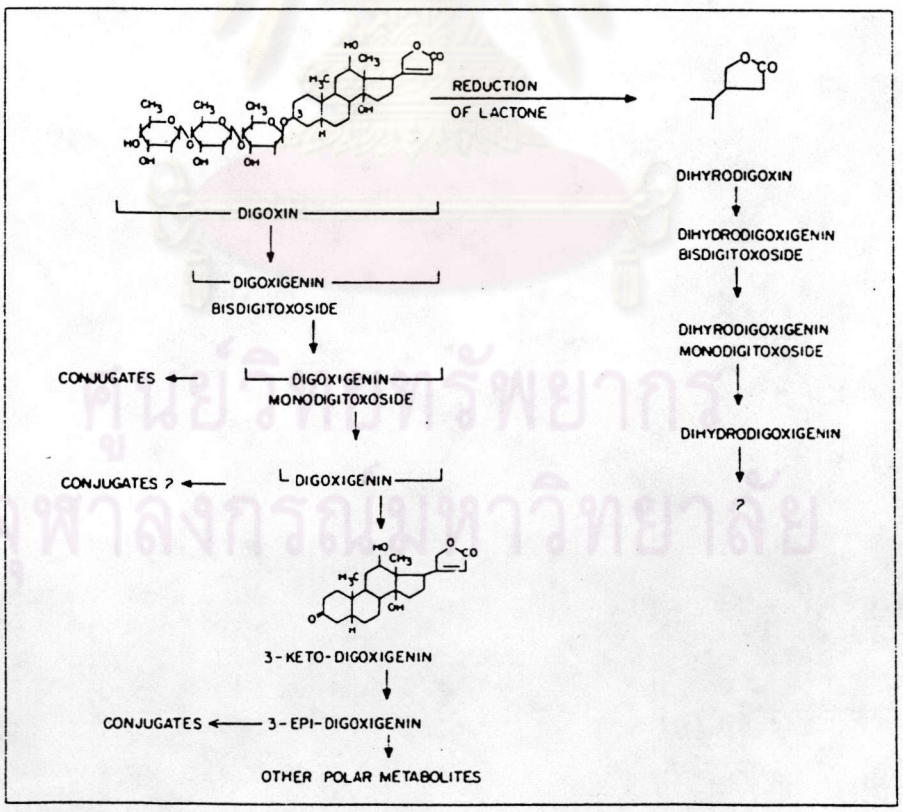

Figure 2 Digoxin metabolic pathway

The second pathway is the reduction of the double bond in the lactone ring of digoxin to form dihydrodigoxin, which is only slightly cardioactive. Dihydrodigoxin may be further metabolized to hydrolyzed reduction products.

Elimination

Digoxin is primarily eliminated or excreted through the renal route. Approximately 60 to 80% of bioavailable digoxin is excreted unchanged, by passive glomerular filtration and active tubular secretion There is also some reabsorption of digoxin from the tubular fluid. The remaining one-third (approximately) of the drug is eliminated by an extrarenal route. About 10% of patients digoxin reduction products constitute 30 to 40% of total urinary excretion of digoxin and its metabolites.

An acute increase in renal blood flow induced by vasodilators in congestive heart failure patients is associated with a 50% increase in renal digoxin clearance. Inactivation of digoxin by gut flora may substantially reduce the availability of oral digoxin tablets.I

The list of agents affecting or altering the overall pharmacokinetics; absorption, distribution, metabolism and elimination of serum digoxin concentration. showed in table I

Table I Agents affecting the pharmacokinetics of digoxin Pharmacokinetics of Digoxin in various Physiological or Pathological Conditions.

Alteraction	Agents
Decreased absorption	Activated charcoal, antacids, cholestyramine, colestipol, cytotoxicagents [cyclophos-phamide, doxorubicin (adriamycin)], dietary fibre, kaolin-pectin, metoclopramide, neomycin, sulphasalazine
Increased absorption	Antibiotics (by inhibiting gut flora), anticholinergics (propantheline)
Enhanced renal excretion	Hydralazine, levodopa, nitroprusside
Inhibition of renal tubular secretion	Quinidine, spironolactone, triamterene, trimethoprim, verapamil
Inhibition of extrarenal clearance	Diltiaze m quinidine, verapamil
Decreased volume of distribution	Quinidine
Increased serum digoxin concentrations (mechanism unknown)	Amiodarone, aspirin, diltiazem, flecainide, ibuprofen, indomethacin, nifedipine, nicardipine, nisoldipine, nitrendipine.

The biological half life (t 1/2) of digoxin is normally about 1.6 days for healthy, normal renal and hepatic function. The altered function of renal can cause changes in the half life time for each patients.

Effect of Pregnancy

Digoxin distributes across the placental barrier. In general, digoxin clearance is increased with the pregnancy-related increase in creatine clearance.

Effect of Age

Age influences digoxin distribution. The elderly may have a reduced apparent volume of distribution of digoxin and should therefore be given lower loading doses.

In children, myocardial tissue concentrations of digoxin are higher than in adults and the elderly. Also in infants which the steady-state serum concentrations are higher than in adults.

Renal Diseases

The apparent volume of distribution (Vd) for digoxin decreases in chronic renal failure. Renal dysfunction may also cause decreased binding of digoxin to tissue. It could be wise to reduce the digoxin dose in patients with severe renal impairment.

Thyroid Disease

There are pharmacokinetic diferences in variety states of thyroid dysfunction. In hyperthyroidism, the increasing of renal function which leads to an increased clearance of digoxin provides lower plasma digoxin

concentrations. On the other hand, plasma digoxin concentrations has been found to be higher in patients when hypothyroid than when euthyroid.

Drug Interactions

The interaction of digoxin with other drugs can cause alternation in any phase of pharmacokinetic scheme. The reviewed papers about this have extensively been studied. The list of commonly used drugs that interfere or change the usual pharmacokinetics were summarized in table II..

C. Dose of digoxin

Loading Dose

The need for a loading dose of digoxin requires careful consideration. Loading doses of digoxin were commonly used to slow down ventricular response in patients with symptomatic supraventricular tachyarrhythmias

Digitalizing doses for each age group are given below. Administer loading dose in several portions ,give roughly half the total as the first dose . Give additional fraction of total dose at 6 to 8 hours intervals (oral) or 4 to 8 hours intervals (parenteral) . Carefully assess clinical response before each additional dose .

Age	Digitalizing dose (mcg / kg)	
	Oral	Intravenous
Premature	20-30	15-25
Full term	25-35	20-30
1-24 months	35-60	30-50
2-5 years	30-40	25-35
5-10 years	20-35	15-30
Over 10 years	10-15	8-12

Maintenance Dose

If loading dose of digoxin are well tolerated, or if a loading dose is unnescessary, maintenance therapy can be initiated at a dose of 0.25 mg/day in adult patients with creatinine clearances greater than 20 ml/min.

In patient with creatinine clearance less than 20 ml/min, maintenance doses of 0.125 mg/day of digoxin are recommended. Actual dosing decisions must be based on a careful evaluation, ranging of patients renal and hepatic function. Also clinical condition is involved in deciding the digoxin administration. Lower maintenance doses, compared to the usual doses, may be appropriate for the elderly.

D. Adverse drug reactions

Digoxin toxicity was determined by the presence of cardiac arrhythmias consistent with digoxin toxicity (premature ventricular contraction, bigeminy, AV junctional rhythms, AV dissociation or block with a ventricular rate <50), with or without nausea, anorexia, sedation and vomiting.

The digoxin serum concentrations considered to be within the therapeutic range are 0.8 to 2 mcg/L (ng/ml). (Brodie, 1986) Patients who have the higher or lower serum concentrations as above are consider to show the side effects or the continuing of the symtoms. But the variability of digoxin serum concentrations associated with effective and toxic responses is due to the multiple factors which influence individual response. Of these, the one most frequently encountered clinically is alteration of serum electrolyte concentrations. Serum potassium, magnesium, and calcium are known to influence the response to digoxin. Hypokalemia, hypomagnesemia, hypercalcemia may enhance in the potentiation of digoxin toxicities. The toxic

response to digoxin is also influenced by the nature and severity of the underlying diseases of patients.

Table II also shows the adverse effect of digoxin and other drugs including comments and recommendations.

Table II The drug interactions of digoxin and adverse effects.

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Alcohol	Possible decreased digitoxin effect (competition for common enzyme)	*In vitro* studies; monitor digitoxin concentration in alcoholics
Aminogluthetimide	Possible decreased digitoxin effect (increased metabolism)	Monitor digitoxin concentrations
Amphotericin B	Possible increased digitoxin toxicity (hypokalemia)	Monitor potassium concentration
Barbiturates	Decreased digitoxin effect (increased metabolism)	Use digoxin in epileptic patients

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Cholestyramine	Decreased digitoxin effect (binding in intestine)	Give digitoxin 1 1/2 hours before cholestyramine and monitor digitoxin concentration
Cimetidine	Possible digitoxin toxicity (decreased metabolism)	Monitor digitoxin concentration
Diltiazem	Possible digitoxin toxicity (mechanism not established)	Monitor digitoxin concentration; not observed with nifedipine
Ethacrynic acid	Increased digitoxin toxicity (potassium and magnesium depletion)	Monitor potassium and magnesium concentrations
Furosemide	Increased digotoxin toxicity (potassium and magnesium depletion)	Monitor potassium and magnesium concentration
Neuromuscular blocking agents	Increased incidence of arrhythmias (mechanism not established)	Fewer arrhythmias with succinylcholine than with pancuronium; treat resulting arrhythmias with tubocurarine

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Quinidine	Possible digitoxin toxicity (mechanism not established)	Monitor digitoxin concentration
Flecainide	Possible digoxin toxicity (possibly decreased metabolism)	Small effect; might be clinically significant at upper end of digoxin dosage range
Furosemide	Increased digoxin toxicity (potassium and magnesium depletion)	Monitor potassium and magnesium concentrations
Hydralazine	Decreased digoxin effect with IV hydralazine (increased renal excretion)	Monitor digoxin concentration
Hydroxychloroquine	Possible digoxin toxicity (mechanism not established)	Monitor digoxin concentration
Hypoglycemics, sulfonylurea	Possible increased digoxin toxicity (mechanism not established)	Monitor digoxin concentration

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Isradipine	Possible digoxin toxicity (possibly decreased metabolism)	Clinical significance not established; based on studies in healthy men
Itraconazole	Digoxin toxicity (mechanism not established)	Single case report (1992); monitor digoxin concentration
Kaolin-pectin	Decreased digoxin effect (decreased absorption)	Use digoxin capsules rather than tablets and monitor digoxin concentration
Methyldopa	Sinus bradycardia (additive)	Monitor heart rate
Metoclopramide	Possible decreased effect of digoxin tablets (decreased absorption)	Use digoxin capsules, which contain solution
Neuromuscular blocking agent	Increased incidence of arrhythmias (mechanism not established)	Fewer arrhythmias with succinylcholine than with pancuronium treatment resulting arrhythmias with tubocurarine

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Nifedipine	Possibility of both increased and decreased digoxin effect has been reported (mechanism not established)	Clinical significance not established; monitor digoxin concentration
Nitrendipine	Digoxin toxicity (probably decreased metabolism)	Based on studies in healthy volunteers, one of whom developed toxicity
Nitroprusside	Decreased digoxin effect (increased renal excretion)	Monitor digoxin concentration
Nonsteroidal anti-inflammatory drugs	Possible digoxin toxicity with indomethacin in preterm infants and neonates (decreased renal excretion)	Decrease digoxin dosage by 50% and monitor digoxin concentration; clinical significance in adults not established
Omeprazole	Possible digoxin toxicity (increased absorption)	Based on study in healthy young men
Penicillamine	Decreased digoxin effect (mechanism not established)	Monitor digoxin concentration

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Phenytoin	Decreased digoxin effect (increased metabolism)	Monitor digoxin concentration
Prazosin	Possible digoxin toxicity (mechanism not established)	Based on study in hypertensive patients
Procarbazine	Decreased digoxin effect (decreased intestinal absorption)	Clinical significance not established; monitor digoxin concentration
Propafenone	Possible increased digoxin toxicity (mechanism not established)	Monitor digoxin concentration
Quinidine	Possible digoxin toxicity (altered excretion and tissue binding)	Monitor for signs of digoxin toxicity; digoxin concentration may not correlate with cardiac effect
	Possible digoxin toxicity (altered excretion and tissue binding)	Quinidine and verapamil may be synergistic in their effect on digoxin

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Quinidine		Rifampin can increase the metabolism of both digoxin and quinidine, and either prevent or lessen the interaction or cause a decreased digoxin effect Patients receiving barbiturates may not be affected unless the drugs are with drawn, since barbiturates increase quinidine metabolism The interaction may be potentiated by cimetidine, which inhibits quinidine metabolism The effects of quinidine and spironolactone on digoxin may be additive

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Quinine	Possible digoxin toxicity (mechanism not established)	Monitor digoxin concentration
Rifampin	Decreased digoxin effect (increased metabolism)	Probably rare; effect on digoxin may be increased in patients on dialysis
Spironolactone	Possible digoin toxicity (decreased renal clearance and possibly decreased metabolism)	Monitor digoxin concentration; effects of spironolactone and quinidine on digoxin may be additive
Sucralfate	Decreased digoxin effect (possibly decreased absorption)	Single case report (1991); medications were taken 2 hours apart
Sulfonamides	Possible decreased digoxin effect with sulfasalazine (decreased absorption)	Monitor digoxin concentration

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Sympathomimetic amines	Increased tendency to cardiac arrhythmias (additive)	Theoretical, but best to avoid concurrent use
Sympathomimetic bronchodilators	Possible digoxin toxicity with oral or IV albuterol (hypokalemia and possibly increase in intramyocardial digoxin concentration)	Monitor cardiovascular status; digoxin concentration may be normal or low; based on studies in healthy men
Tetracyclines	Possible digoxin toxicity (decreased gut metabolism and increased absorption)	Monitor digoxin concentration
Thiazide diuretics	Increased digoxin toxicity (potassium and magnesium depletion)	Monitor potassium and magnesium concentrations
Trazodone	Increased digoxin toxicity (mechanism not established)	Single case report (1984)

Table II (Continuing)

Interacting drugs	Adverse effects (Probable mechanism)	Comments and recommendations
Trimethoprim	Possible digoxin toxicity (decreased renal excretion and possibly decreased metabolism)	Monitor for signs of digoxin toxicity; digoxin concentration may not correlate with cardiac effects
Vancomycin	Possible decreased digoxin effect (possibly decreased absorption)	Monitor digoxin concentration
Verapamil	Increased digoxin toxicity (possibly decreased renal excretion and biliary excretion; possibly decreased metabolism)	Monitor digoxin concentration; quinidine and verapamil may be synergistic in their effect on digoxin; cirrhosis may increase likelihood of toxicity
Vincristine	Decreased digoxin effect (decreased intestinal absorption)	Clinical significance not established; monitor digoxin concentration

Review of therapeutic drug monitoring

"Therapeutic drug monitoring" aims to promote optimum treatment by ensuring that plasma concentrations lie within a therapeutic range, above which toxicity occurs and below which the drug is ineffective. Clinical pharmacokinetics embraces not only therapeutic drug monitoring but also an assessment of the clinical and pathological factors which modify the absorption, distribution, metabolism, and excretion of drugs in individual patients. Therapeutic drug monitoring is most useful for compounds with a narrow therapeutic index and shows a good relation between concentration and efficacy or toxicity. Douglas Ried and et al have integrated from the meta-analysis of the studies which have been published since 1972 using on-line medical literature databases that therapeutic drug monitoring can reduce toxic drug reactions. Therapeutic drug monitoring appeared to be most beneficial for patients taking theophylline or digoxin.

Monitoring serum digoxin concentrations

Circulating concentrations of digoxin are unrepresentative of tissue content during absorption and distribution, so samples should always be taken at least six hours after the last dose. Indications for monitoring include assessing compliance, confirming clinical toxicity, and suspected drug interactions. Occasionally measurement will be useful when there is a poor initial response to treatment, when the drug history is uncertain, or when the clinical state is fluctuating. Serum digoxin concentration monitoring is a useful diagnostic tool if used with good clinical judgement based on patient age, clinical status, electrocardiograms, and serum electrolyte and arterial blood gas measurements.

Lee and Smith (1983) offer the guidelines for the use of serum digoxin in summary :-

1. Asessment of the timing and magnitude of digoxin doses, renal function and body mass will in most instances allow a satisfactory estimate of total body digoxin stores. In conjuction with information routinely available in the clinical setting.

2. If a digitalised patient develops unusal fatique, anorexia, navsea, vomitting, visual changes, or certain arrhythmias, toxicity should be suspected. Taken together with other clinical data, serum concentrations are often helpful in arriving at proper adjustments in the therapeutic regimen.

3. Since metabolic and other parameters may predispose to digitalis toxicity, a serum concentration within the range conventionally regarded as ' therapeutic' should not be considered proof that toxicity is not present.

4. Conversely, in the absence of signs or symptoms of toxicity, a concentration of 2 to 3 ng/ml should not dictate withholding digoxin if such levels are required to adequatedly control the ventricular response to supraventricular tachyarrhythmias.

5. Finally, serum cardiac glycoside concentrations should be used as a supplement to, not as a substitute for clinical judgement. An isolated value should rarely if ever be used as the sole criterion for assessment of efficacy or toxicity. The determination of concentration of drugs in plasma is progressively becoming a standard procedure for the monitoring of drug treatment, espectially for drugs whose range of therapeutic and toxic concentrations is very narrow. Laboratories which measure drugs in serum or plasma usually follow a protocol published in the literature, with minor or major modifications. Major considerations in the selection of an assay are cost and availability of instrumentation, along with the inherent limits of accuracy and specificity of the method.

Creager M.A. (1990) found that treatment for patients with congestive heart failure is primarily directed at reducing symptoms and improving functional capacity. In patients with moderate to severe heart failure, therapeutic interventions incoperating diuretics, digoxin and selected vasodilators, specificially angiotensin-converting enzyme (ACE) inhibitors, are designed to correct pathophysiological mechanisms such as left ventricular dysfunction excessive vasoconstriction and renal reabsorption of sodium and water .

Diuretic control the symptoms of pulmonary congestion and peripheral edema. Angiotensin converting enzyme (ACE) inhibition and digoxin may be used in the management of heart failure. Digoxin increases myocardial contractility and has a modest but durable beneficial effect in congestive heart failure due to impaired left ventricular systolic function. ACE inhibitors have clear beneficial effects in all grades of heart failure and, in addition, modify the natural history and reduce mortality. (Crozier and Ikran , 1992)

Well-designed randomized trials have shown that digoxin improves dyspnea on exertion, left ventricular function, exercise capacity and resting hemodynamics. The benefits are greatest in patients with more severe congestive heart failure and have been demonstrated in clinical studies to last as long as three months with continued use and to be additive to the effects of diuretics and vasodilators. (Creger , 1990)

ACE inhibition is indicated, in conjunction with diuretic therapy, for all grades of heart failure. Digoxin is best reserved for patients with atrial fibrillation and a rapid ventricular response, and for those whose heart failure is not controlled with ACE inhibitor plus a diuretic.

Nitrates and angiotensin converting enzyme inhibitors, such as captopril, lisinopril and enalapril, have been shown in randomized placebo-controlled trials to improve hemodynamics, exercise capacity or both in patients with congestive heart failure.

No studies have been reached about the optimal therapy for congestive heart failure, but it seems reasonable to use a triple-drug regimen (a diuretic, digoxin and a vasodilator) for treating any patient with moderate to severe symptoms. Combining a vasodilator and an inotropic agent with a diuretic yields additive improvement in cardiac output and produces a decrease in pulmonary capillary wedge pressure. The loop diuretics are very effective for treating dyspnea and edema.

The addition of digoxin will improve the hemodynamic function and exercise capacity of a significant number of patients, but currently no practical way exists for determining which patients will respond. It is therefore recommended that digoxin be administered in doses that produce a serum level of 1.0 to 2.0 ng per ml and that serum digoxin and potassium levels be measured during digitalization three to four times each year during maintenance therapy. (Vine., 1990)

Digoxin is measured by various methods. Methods based on physicochemical seperation of digoxin and its metabolites are highly specific. Method using high performance liquid chromatography followed by radioimmunoassay is highly sensitive and reproducible, but is still not widely available for clinical use. Methods based on Na^+-K^+-ATPase inhibition, such as rubidium (radiolabelled or non-radioactive) uptake measurement are also not suitable for clinical labaratory use. Methods based on competitive protein

binding, such as the various types of immunoassays, have been extensively used.

Automatic immunoassays that either use fluorescence or enzyme linked are now available; examples include : fluorescence energy transfer immunoassy (FETI), fluorescence polarization immunoassay (FPIA) and enzyme linked immunoassay.

The fluorescence polarization immunoassay (FPIA) is a seperation free competitive assay developed by colbert and others in 1984 for the detection of low-molecular-mass haptens of less than 20,000 daltons. This method monitors the reaction between hapten and antibody using a fluorescent label conjugated to a hapten. In the assay, test sample is added to the fluorescent conjugated hapten, and an initial fluorescent reading to compensate for sample quenching or background variation is made using a fluorometer equipped with a polarized light source. To the extent that the test sample contains the substances of interest, competition occurs between unlabeled and labeled hapten for a limited number of antibody-binding sites. After incubation, the polarized fluorescence is measured again to determine the amount of hapten present.

The Abbott TDX® System is based on FPIA technique. Fluorescence polarization measurements can be made very accurately, and they are less affected by variations in fluorescence intensity than are standard fluorescence measurements. Thus, precision on the order of 1% or greater of measurement is readily achieved, which translates into more precise assay measurements. A disadvantage is that FPIA is limited to measurements that can be performed with fluorescent compounds. The instrumentation required for performing

fluorescence polarization measurements is specialized and may only measure fluorescence intensity of polarization.