International Journal of Pharmaceutics, 2 (1979) 127–144

Review Article

UTILISATION OF DIGITALIS GLYCOSIDES: THE RELEVANCE OF THEIR BIOTRANSFORMATION

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(Received June 1st, 1978)
(Accepted June 19th, 1978)

INTRODUCTION

The narrow therapeutic range of digoxin, 0.8–2.0 μg/litre (Ingelfinger and Goldman, 1976; Evered and Chapman, 1971), indicates that there is a need to measure accurately its plasma levels during therapy. The variation in bioavailability of differing pharmaceutical preparations (Goldfinger, 1971; Greenblatt et al., 1973; Huffman et al., 1974; Marcus et al., 1976) has led to the proposal that digoxin plasma levels should also be estimated as a measure of compliance with bioavailability standards (Kramer et al., 1977). For rapid evaluation in the clinical setting, radioimmunoassays (Oliver et al., 1968) and enzyme-immunoassays (Rosenthal et al., 1976) have proved valuable, as has the use of tritiated digoxin in clinical research. However, the metabolism of cardiac glycosides and its effect on the specificity of these assays is a cause for concern.

It has been reported that during pharmacokinetic evaluation of plasma levels following administration of tritiated digoxin, subsequent analysis of levels of radioactivity in the blood measured both unchanged and metabolized drug. Hence, the pharmacokinetic parameters obtained from such studies are questionable (Doherty et al., 1969, 1970; Dettli et al., 1972; Butler, 1975; Sumner et al., 1976).

Clark and Kalman (1974) reported on the complexity of interpreting the result of a radioimmunoassay as up to one-third of the cardioinactive dihydro form of digoxin (see Fig. 5) was detected together with the digoxin by their assay. During an investigation of problems associated with the development of radioimmunoassay for urinary digoxin, using a simple specific assay previously described for plasma, Haywood et al. (1975) found that the total urinary recovery was low, possibly due to high biliary excretion or, more likely, the excretion of significant concentrations of digoxin metabolites only poorly detected by the assay. More recently, Garrett and Hinderling (1977) stated that an 'a priori' assumption of radioimmunoassay equivalency of glycosides with their derivatives and their cardioactive metabolites is unwarranted. The fundamental basis of a radioimmunoassay depends on competition for binding sites, and minor functional changes in a molecule can modify the binding constants. Thus the extent to which the different metabolites of digoxin will be detected and estimated as digoxin by the radioimmuno-

assay is uncertain. Likewise, the contribution which these metabolites will make to the total pharmacodynamic effect is unknown. As the pharmacokinetic parameters (especially the half-life) of the metabolites will be different to those of digoxin, predictions of future plasma levels, and thereby therapeutic activity, based solely on radioimmunoassay may well be inaccurate.

Against this background, a review of the structure and metabolism of the cardiac glycosides is relevant.

CHEMICAL STRUCTURE OF THE GLYCOSIDES

Most currently used cardioactive preparations are derived from the leaves of *Digitalis purpurea* (e.g. digitoxin, gitalin, digitalis leaf) or *Digitalis lanata* (digoxin, lanatoside C, deslanoside). Exceptions include ouabain, which is derived from the seeds of *Strophanthus gratus*, and the glycosides from squill (*Urginea maritima*). There are, however, a large number of other botanical sources of similar glycosides, some of which are still used therapeutically in certain parts of the world.

Structurally, these glycosides consist of a hydroxylated steroidal backbone with an unsaturated lactone ring attached in the β-configuration to the C-17 position and a glycosidal link to a chain of sugar residues at the 3-β-hydroxyl group. Two main classes can be distinguished according to the nature of the lactone ring:

(a) the cardenolides, which include the digitalis and strophanthus glycosides and have a 5-membered α,β-unsaturated lactone ring, and

(b) the bufadienolides, which include the squill glycosides and some toad venoms and have a 6-membered α,β,γ,δ-double unsaturated lactone ring (see Fig. 1).

The steroid nucleus is unusual, in that rings C and D are in the cis configuration, rings B and C have the usual trans configuration and rings A and B are in the cis configuration. This has the effect of introducing a twist into the molecule, which is different from normal mammalian steroids. Fig. 2 shows a stereo model of digoxin to illustrate this point.

The position of the hydroxyl groups varies according to the genin, but the 14β-hydroxylation appears to be universal. In the digitalis glycosides, three major cardenolide genins (aglycones) occur; digitoxigenin, which has no additional hydroxyl groups; digoxi-

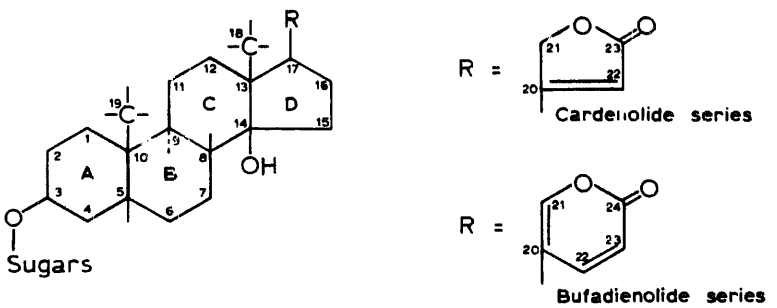

Fig. 1. Basic structure of the cardiac glycosides.

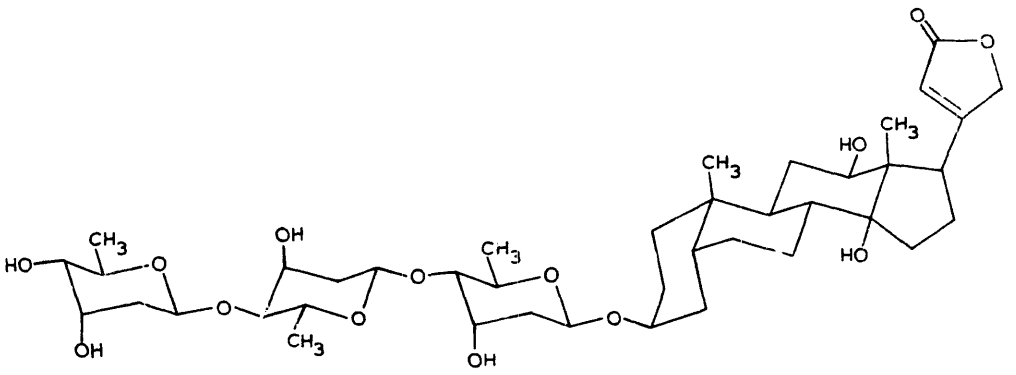

Fig. 2. Stereo structure of digoxin (digoxigenin-tris-digitoxoside).

genin, which is hydroxylated in the 12β-position; and gitoxigenin, which is hydroxylated in the 16β-position. The composition of the major digitalis glycosides listing the genin and sugars are given in Table 1.

Very little success has been achieved in elucidating the active centre of the glycoside molecule, due to the complicated chemical structures. Virtually all structural changes, however minor, seem to result in an almost complete loss of activity.

Using an in vitro test system, Repke (1972) showed a good correlation between the inhibition of the enzyme 'transport ATPase' in cardiac cell homogenates and the acute

TABLE 1

COMPOSITION OF DIGITALIS GLYCOSIDES INCLUDING SEMI-SYNTHETICS

D = D-digitoxose; AcD = 3-acetyl-D-digitoxose; MeD = 4-methyl-D-digitose; G = D-glucose; AcD * = 4-acetyl-D-digitoxose.

Glycoside	Genin (Aglycone)	Sugars at C-3				Molecular weight	Proprietary products (U.K.)
		1	2	3	4		
Purpurea glycoside A	Digitoxigenin	D	D	D	G	925	–
Digitoxin	Digitoxigenin	D	D	D	–	765	Digitaline Nativelle
Lanatoside A	Digitoxigenin	D	D	AcD	G	969	–
Acetyldigitoxin	Digitoxigenin	D	D	AcD	–	807	–
Purpurea glycoside B	Gitoxigenin	D	D	D	G	943	–
Digoxin	Gitoxigenin	D	D	D	–	781	–
Lanatoside B	Gitoxigenin	D	D	AcD	G	985	–
Acetylgitoxin	Gitoxigenin	D	D	AcD	–	823	–
Deslanoside	Digoxigenin	D	D	D	G	943	Cediland injection
Digoxin	Digoxigenin	D	D	D	–	781	Lanoxin, Diganox Nativelle
Lanatoside C	Digoxigenin	D	D	AcD	G	985	Cedilanid
α-Acetyldigoxin	Digoxigenin	D	D	AcD	–	823	–
β-Acetyldigoxin	Digoxigenin	D	D	AcD *	–	823	–
β-Methyldigoxin (Medigoxin)	Digoxigenin	D	D	MeD	–	795	Lanitrop

toxicity (presumably due to cardiac effects) of a series of glycosides and semi-synthetic compounds. He concluded that hydrogen bonding is essential to attach the glycoside to a specific receptor (probably 'transport ATPase') on the cell membrane and postulated that this occurs between the keto group of the cardenolide or bufadienolide ring and an -NH or -OH group of an amino acid within the membrane (Fig. 3). The molecular activity increases by a factor of about 10 from the cardanolides (20,22-dihydro-derivatives) to the cardenolides and again by 10 to the bufadienolides, among which are the most active glycosides. Not only is the strength of the hydrogen bonding important, but also the configuration of the rest of the molecule about the lactone ring, since this must now form a 'fit' on to the receptor surface. In this respect, there is a marked difference of about 50° in the angle between the hydrogen bond axis and the C-17 to C-20 bond from the cardenolide structure to the cardanolide structure. Fig. 4 shows a diagrammatic representation of the possible receptor surface showing a good fit by digitoxin, but poor fits by some other derivatives. Repke considered that short-acting Van der Waals forces hold the rest of the molecule in place on the receptor. A free rotation of the C-17 to C-20 bond is also necessary, since the introduction of a 14–21-epoxy bridge into the digitoxin molecule leads to a marked loss of activity. Likewise, fusion of the lactone ring to the D ring of the steroid backbone leads to complete loss of cardiac activity (Repke, 1972).

From this concept of genin–receptor interaction, it is clear that the sugar molecules are not required for intrinsic activity. In fact, the genins themselves have a quantitatively similar, although extremely short, action on the heart compared to the complete glycosides (Kroneberg, 1959). The sugar residues, on the other hand, have a very marked effect on the overall polarity of the glycosides and hence determine their pharmacokinetic characteristics (Repke, 1972; Dwenger, 1973), although they have little effect on plasma protein binding (Storstein, 1976). A major function is to protect the vulnerable 3β-hydroxyl group from epimerization with the resultant loss of activity. In this respect, according to the results of Schmoldt (1976) (see later under Biotransformation of digitoxin), they may be even more successful than was considered earlier. The number and type of sugars also plays a decisive role in determining the amount of drug absorbed from the gastrointestinal tract. For instance, digoxin, which has three digitoxose molecules, is up to 80% absorbed,

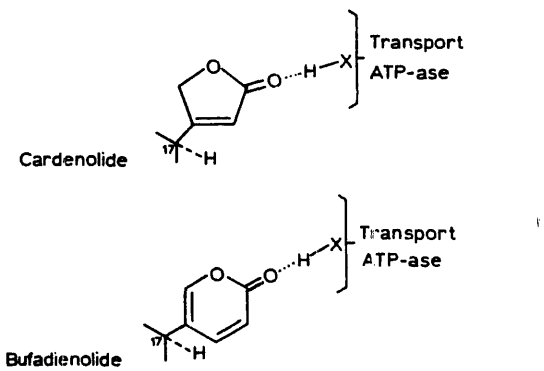

Fig. 3. Possible initial binding of the lactone ring to transport ATPase by hydrogen bonding (after Repke, 1972).

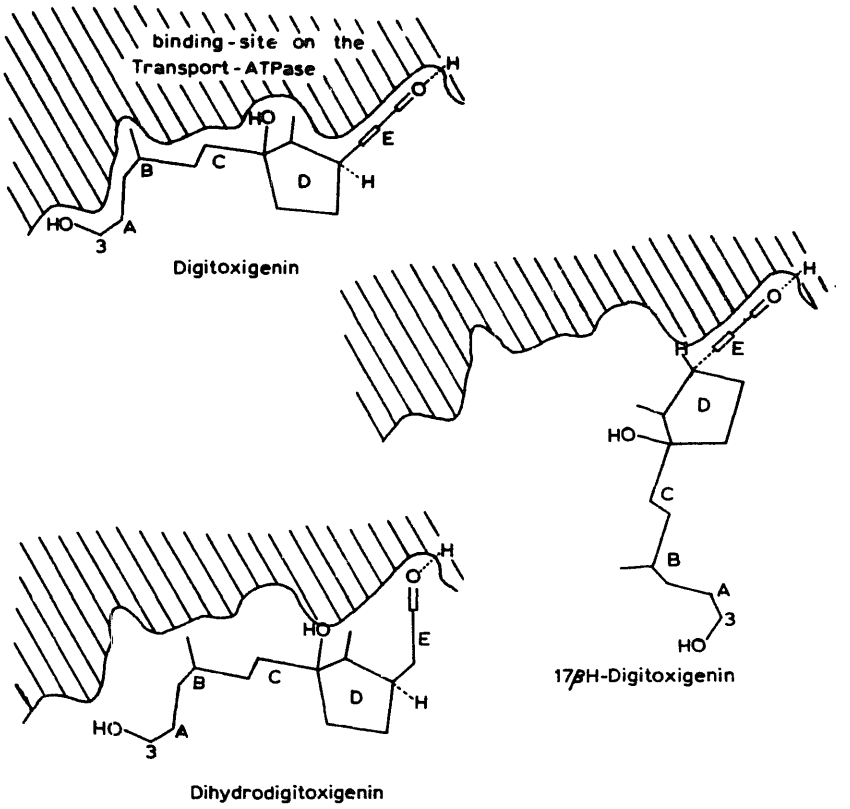

Fig. 4. Schematic representation of the hypothetical binding of digitoxigenin and related compounds to the receptor on transport ATPase (after Repke, 1972).

whereas lanatoside C, with an additional glucose molecule, has insufficient lipid solubility and is thus absorbed only to the extent of 1–5%. Alternatively, increasing the lipid solubility of digoxin by methylating the terminal sugar, as in β-methyldigoxin, results in complete absorption. The structure of the aglycone also has an effect on water solubility, such that gitoxin, a structural isomer of digoxin, is virtually insoluble in water and hence hardly absorbed at all. Through the incorporation of five acetyl groups, a semi-synthetic glycoside, penta-acetylgitoxin, with satisfactory oral absorption, has been synthesized.

The sugar groups not only dictate the rate and extent of absorption, but also play a significant role in the subsequent distribution throughout the organism. Repke (1963) has shown that approximately equal levels (3–5 nmol/g) of digitoxigenin and its metabolites are found in the heart and brain of the rat 10 min after an intravenous injection of digitoxigenin, whereas 10 min after an intravenous injection of digitoxin, less than 0.5 nmol/g was found in the brain and as much as 15 nmol/g in the heart. Thus part of the organ specificity of the cardiac glycosides is due to the sugar chain directing the organ distribution.

PROTEIN BINDING

The extent of cardiac glycoside protein binding may influence distribution, metabolism and excretion of that glycoside. For example, the markedly greater degree of binding of digitoxin to serum albumin, compared to digoxin in vivo, is reflected in higher plasma concentrations, lower rates of urinary excretion and longer half-life of the former. Lukas and De Martino (1969), in in vitro studies using pure human serum albumin, reported binding of the following glycosides:

Glycoside	Concentration (μg/ml)	% Binding
Digitoxin	0.01–12	97
Digitoxigenin	<5	94
Digitoxigenin	170	41
Digoxin	0.003	23
Digoxin	30	10
Digoxigenin	1	14

Sandberg et al. (1957) reported that the avidity of glycosides for albumin varies inversely with the number of polar functional groups they contain. The former workers (Lukas and De Martino, 1969), in an attempt to identify structural features of digitoxin critical to its binding, added digitoxose and β-angelica lactose (a compound with a similar lactone ring) to their in vitro system – this did not affect the binding of digitoxin. However, digitoxigenin-bis-digitoxoside, digitoxigenin-mono-digitoxoside, digoxin and digoxigenin in decreasing order did interfere with binding.

In contrast, Hinderling (1977) reported that protein binding of digoxin, dihydrodigoxin, digoxigenin-bis-digitoxoside and digoxigenin-mono-digitoxoside, which ranged between 22 and 27% and was concentration independent between 3 and 60 ng/ml, was unaltered by high concentrations of analogous compounds. While confirming that digoxin protein binding is concentration independent at high digoxin plasma levels (24%), Harron et al. (1978) have shown concentration-dependent binding (40–70%) within the normal therapeutic range of 0.8–2.0 ng/ml.

Storstein (1976), claiming to have used concentrations within the normal therapeutic range, reported albumin binding with digitoxin of 95.7%, digitoxigenin-bis-digitoxoside 96.5%, digitoxigenin mono-digitoxoside 98.7%, digitoxigenin 92.7%, digoxin 21.2%, digoxigenin-bis-digitoxoside 16.3%, digoxigenin-mono-digitoxoside 18.5% and digoxigenin 13.3%. Comparison of these data with those reported by Lukas and De Martino (1969) and Hinderling (1977) shows that the number of sugar residues in the molecule has little influence on the degree of protein binding.

BIOTRANSFORMATION

The metabolic pathways of cardiac glycosides include hydrolysis of sugar residues, epimerization, conjugation and hydroxylation. The most important site of drug metab-

olism appears to be in the endoplasmic reticulum of the liver, which contains the drug metabolizing microsomal enzymes. However, intestinal bacteria and stomach acid may play an important role in the breakdown of certain glycosides.

Digitoxin

Since the work of Repke and associates, it has been widely claimed that digitoxin is metabolized by stepwise cleavage of the sugars to give bis- and mono-digitoxosides of digitoxigenin and the free genin itself (Lauterbach and Repke, 1960). The exposed 3β-hydroxyl of the genin is then open to epimerization via the 3-keto derivative to the cardioinactive 3-α-hydroxygenin (Repke and Samuels, 1964). Conjugation of the hydroxyl group in either the α- or β-configuration with either sulphate or glucuronic acid occurs rapidly (Herrman and Repke, 1964). A further route of metabolism for digitoxin is via β-hydroxylation at C-12 to yield digoxin and its corresponding metabolites. This scheme of metabolism is illustrated in Fig. 5. However, it should be stressed that Repke's observations were limited to in vitro studies using rat liver slices. His in vivo studies revealed only mono- and bis-digitoxosides of digitoxigenin and digoxigenin as metabolites of digitoxin in the rat (Repke, 1959a, b). Lukas (1971) could not identify free or conjugated digitoxigenin or its epi-isomer as urinary metabolites of digitoxin in humans. Digitoxigenin-mono- and -bis-digitoxosides have subsequently been confirmed as the major metabolites in man (Vöhringer et al., 1973) and the guinea pig (Lüllman et al., 1971). During chronic maintenance therapy, 31% of daily digitoxin losses were by unchanged digitoxin; the remainder was by metabolism and/or elimination in the faeces (only 7.7% occurred through biotransformation to digoxin) (Jelliffe et al., 1970).

The hydroxylation of digitoxigenin and presumably its glycosides to the digoxin analogues is catalyzed by the liver mono-oxygenase system (Stohs et al., 1971; Talcott et al., 1972; Spratt, 1973). Further evidence for the involvement of cytochrome P-450 in this reaction was obtained by Talcott and Stohs (1972), who demonstrated type I spectral interaction between cytochrome P-450 and digitoxigenin, but no interaction with digoxigenin. Other positions of hydroxylation of digitoxigenin include 5β and 6β by rabbit liver homogenates (Bulger and Stohs, 1973; Bulger et al., 1974) and 1β, 7β and 11α by microorganisms (Scherrer-Gervai et al., 1969; Stohs et al., 1971).

More recently, some doubt has been cast on this scheme by the in vivo studies of Schmoldt (1976) in rats. He has demonstrated that the water-soluble metabolite fraction of the urine following digitoxin administration contains mainly the glucuronide and sulphate conjugates of digitoxigenin-mono-digitoxoside. The corresponding conjugates of the aglycone digitoxigenin could not be identified. Also, digitoxigenin, 3-epidigitoxigenin or digitoxigenone could not be found in the chloroform-soluble metabolite fraction.

Züllich et al. (1975) have also reported the absence of the aglycone and its metabolites in the bile of rats treated with digitoxin. They also suggested that it was the digitoxigenin-mono-digitoxoside which was conjugated and formed the major biliary metabolite. Similar results were found by Castle and Lage (1973a), who later showed that of the three digitoxosides of digitoxigenin and the genin itself, the mono-glycoside was clearly the preferred substrate for glucuronidation by rat and rabbit liver homogenates (Richards et al., 1977). In retrospect, it is interesting to note that in 1966, Katzung and

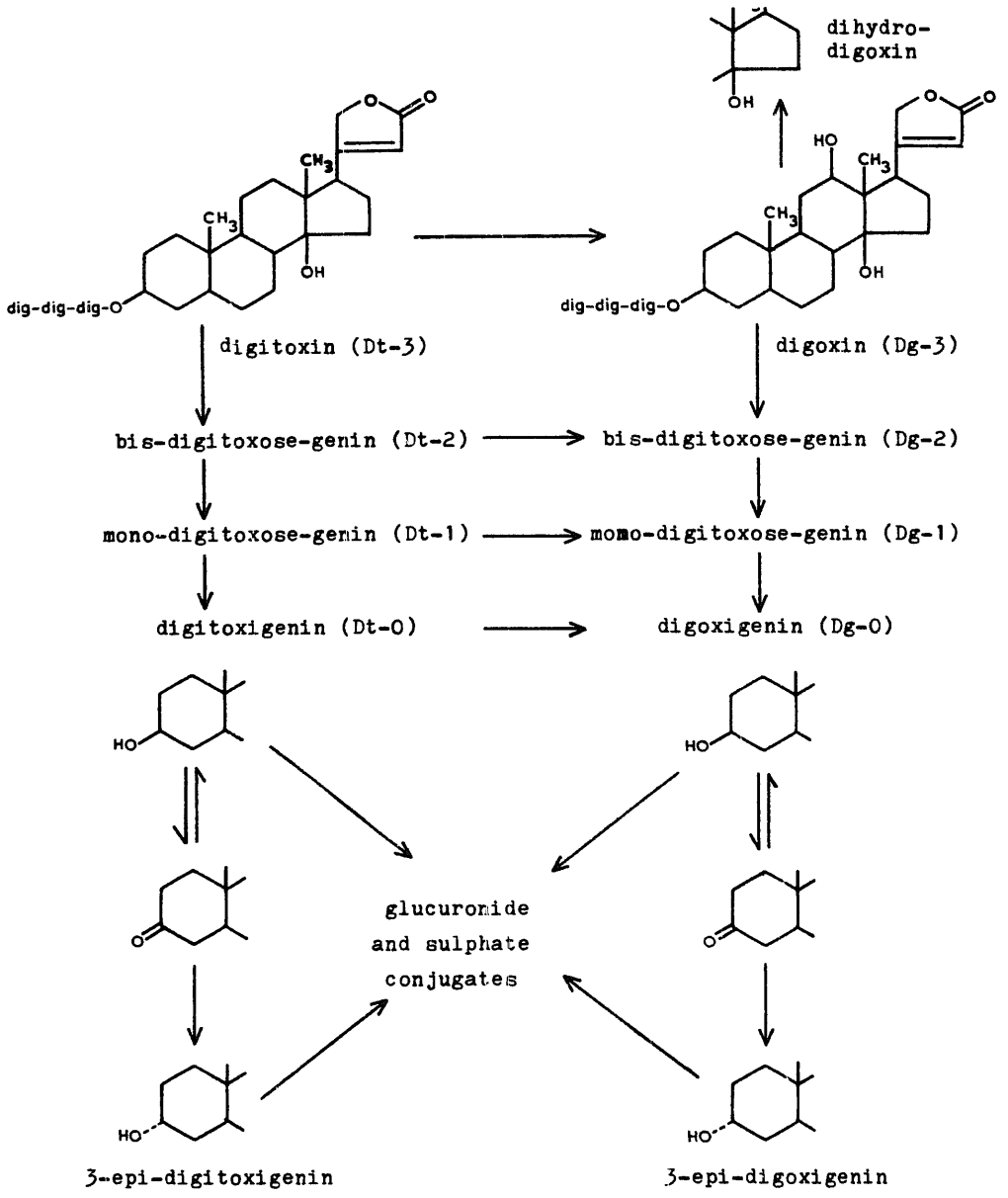

Fig. 5. Traditional interpretation of digitoxin metabolism.

Meyers had postulated a water-soluble metabolite of digitoxin isolated from dog bile to be a conjugate of the parent glycoside. Further evidence doubting the original concept of digitoxin metabolism comes from the demonstration that the liver homogenates are unable to cleave the digitoxose sugars from the steroid nucleus (Lage and Spratt, 1965). However, in their original studies, Lauterbach and Repke (1960) found very little cleavage of any sugars using liver homogenates, although liver slices were quite efficient at this reaction.

Schmoldt (1976, 1977) also found several highly non-polar metabolites, which were probably oxidation products of the ultimate sugar of digitoxigenin-digitoxosides. These have probably previously been recognized as digitoxigenin, its ketone and epi-derivatives. Several of these have subsequently been chemically synthesized, identified as dehydro-digitoxosides and shown to be identical with the metabolites (Schmoldt and Rohloff, 1978). They are stable under neutral and weakly acidic conditions, but at pH 10 they break down by cleavage of the ultimate sugar (Fig. 6).

It is possible that these dehydro-derivatives play a role in the metabolic cleavage of the sugars, since Schmoldt has shown from in vitro experiments that the enzymic activities for both this cleavage and sugar oxidation reside in the microsomal fraction of the liver homogenates. Both require the presence of NADPH and oxygen and can be inhibited by carbon monoxide or SKF 525 A, thus implicating cytochrome P-450 in both reactions (Schmoldt et al., 1975). His scheme for the metabolism of digitoxin is shown in Fig. 7.

A further water-soluble metabolite found in rat urine was postulated by Schmoldt (1976) to be 'digitoxin acid', formed by hydrolysis and ring opening of the cardenolide ring. At pH 3 this ring is reclosed, whence the metabolite can be extracted with chloroform. Final proof of the metabolic route is still lacking (Schmoldt, 1978).

Digoxin

Wong and Spratt (1963) reported the in vitro formation of mono- and bis-digitoxosides from digoxin by liver tissue (species unspecified). Harrison and Gibaldi (1975)

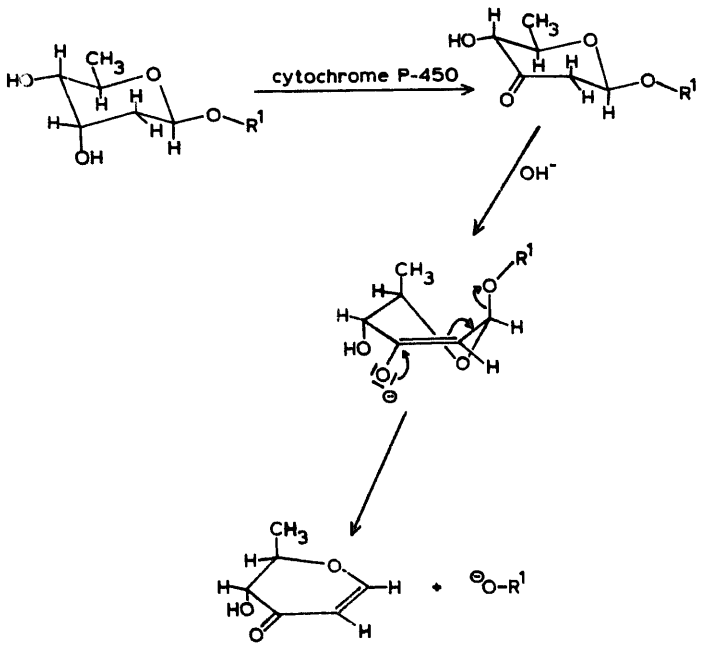

Fig. 6. Proposed mechanism for cleavage of ultimate sugar involving cytochrome P-450.

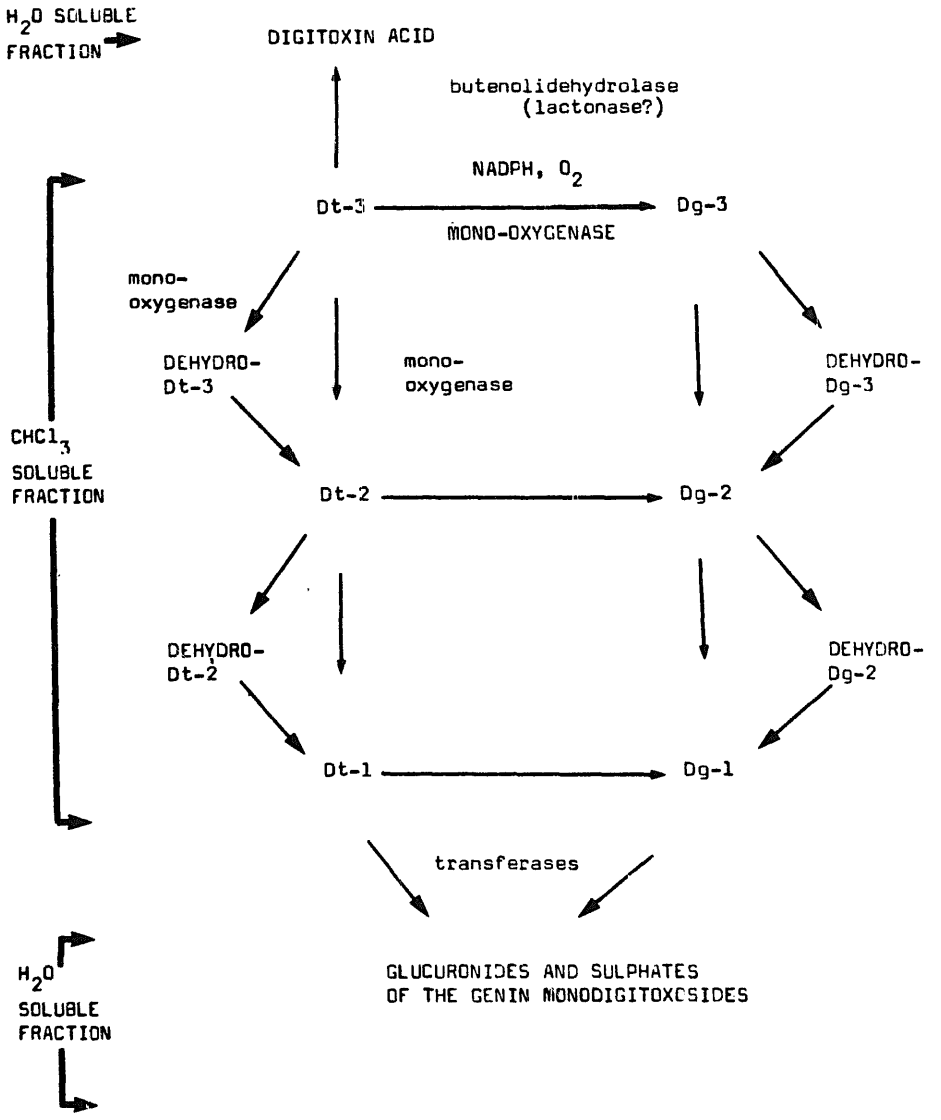

Fig. 7. Digitoxin metabolism according to Schmoldt (1976).

demonstrated the metabolism of digoxin in vivo to digoxigenin-bis-digitoxoside in the rat. The formation of the mono- and bis-digitoxosides has also been observed in man by Marcus et al. (1964). Jelliffe (1967) reported the urinary excretion of digoxin and mono- and bis-digitoxosides of digoxigenin to be 59% of the daily digoxin losses in man. However, Lüllman et al. (1971), although showing the excretion of some conjugates of digoxin in guinea pig bile, human bile and urine, could not identify the mono- and bis-digitoxosides.

Dihydrodigoxin has for some years been recognized as an important urinary metabolite both in dogs (Able et al., 1965) and man (Luchi and Gruber, 1968). Recently, using a sensitive and specific gas chromatography/mass-spectrometry procedure, this metabolite

has been identified in the plasma of 3 out of 150 patients investigated by Watson et al. (1973). This suggested the possibility of a genetic difference leading to a high production of this metabolite, which accounted for about one-third of the plasma digoxin levels in these patients. Subsequently, the same group (Clark and Kalman, 1974) have positively identified this metabolite in the urine of 48 out of 50 patients receiving digoxin. The cardioactivity of this metabolite is considered to be 1/7th to 1/20th of that of digoxin using normal laboratory testing procedures (Bach and Reiter, 1964; Lage and Spratt, 1966; Okarma et al., 1972).

Double maxima in the plasma level curves have been observed after oral digoxin (Shaw et al., 1972), the second of which may be due to absorption of dihydrodigoxin (wrongly identified as digoxin) formed by bacteria in the lower intestine (Beerman, 1973). It may well be that the supposed genetic difference in the formation of the dihydro metabolite referred to above is due to differences in the gastrointestinal flora of individuals. This has previously been shown dramatically in the metabolism of cyclamate to cyclohexylamine by human gut bacteria (Drasar et al., 1972; Renwick and Williams, 1972).

Other glycosides

Voigtländer et al. (1972) reported that β-methyldigoxin is unmetabolized in guinea pigs. The only significant metabolite observed in plasma was digoxin, although glucuronides and sulphates of β-methyldigoxin, digoxin and digoxigenin were observed in urine (Hinderling et al., 1977). In humans, 144-hour total excretion was 92%, of which 47% was unchanged, 35% digoxin and 5.8% water-soluble metabolites in the urine, 13% was eliminated via the faeces.

After i.v. administration of lanatoside C to man, most of the glycoside is excreted unchanged in the urine using tritiated, chromatographic or radioimmunoassay techniques (Aldous et al., 1972a; Beerman, 1972). However, metabolism occurs on incubation of lanatoside C with fresh faeces (but not with boiled faeces) to digoxin and acetyldigoxin (desglucolanatoside C). Due to the poor absorption following oral administration, this intestinal bacterial metabolism is reflected in the urinary excretion pattern (Beerman, 1972). Aldous et al. (1972a), and several other authors have observed a double peak in the plasma glycoside levels following oral administration of lanatoside C. In a recent paper, Aldous and Thomas (1977) suggest that lanatoside C itself is not normally absorbed, but is first hydrolyzed to digoxin partially by the stomach acid (Aldous et al., 1972b) and subsequently by the gut bacteria in the ileum. The absorption of the digoxin thus gives rise to the observed double peak, which consists of digoxin and its metabolites and not lanatoside C.

Chemical breakdown in the gut

Experiments concerning the stability of digoxin and its derivatives in buffer solutions indicate sugar side-chain cleavage of all glycosides at low pH (Kuhlman et al., 1973). Hydrolysis is dependent upon the pH of the incubation fluid and time of incubation, and differs from one glycoside to the other (Brücke, 1936 and Svec, 1937). Thus chemical

hydrolysis can take place in the stomach acid, the extent of which depends on the acidity of the stomach fluid and the rate of stomach emptying. Following this, it is clear that not only will digoxin be absorbed, but also its hydrolysis products. Evidence for this can be taken from the differences in the elimination half-life of tritium following i.v. and p.o. administration of labelled digoxin to man, that were observed by Sumner et al. (1976).

BILIARY EXCRETION

Smith (1971) indicated that a marked species difference exists in the extent and rate of biliary elimination of low-molecular weight molecules, although the variation becomes less with larger polar molecules. Clark et al. (1971) have shown that compounds with a molecular weight less than 300 are rapidly re-absorbed from the biliary tract of the rat, whereas larger molecules are not. They suggest that preferential biliary elimination of high molecular weight compounds may be partially a function of limited re-absorption rather than secretion.

The effect of polarity on biliary excretion is well illustrated by ouabain, digitoxin and digoxin. Ouabain, with five free hydroxyl groups in its aglycone, is extensively excreted in rat bile unchanged (85%); the relatively non-polar digitoxin, with only one free hydroxyl group in its aglycone, is poorly excreted (10%) in rat bile; whereas digoxin of intermediate polarity, having two hydroxyl groups in its aglycone, is about 40% biliary excreted by the rat (Cox and Wright, 1959; Cox et al., 1959).

There is little reported work on direct estimation of biliary excretion of cardiac glycosides in man. Evidence for biliary excretion of digitoxin and its metabolites has been obtained from post-mortem material (Okita et al., 1955). By observing patients with biliary fistulae, ouabain (2–8% in 24 h) has been shown to be poorly excreted by this route (Marks et al., 1964). Using a similar technique, Doherty et al. (1970) found approximately 8% biliary excretion of digoxin. However, Caldwell and Cline (1976), using an intestinal perfusion method, measured 30% of an i.v. dose of [^{3}H]digoxin in the small intestine of healthy volunteers. The extent to which this reflects biliary excretion is unclear.

RELEVANT DRUG INTERACTIONS

Certain interactions of drugs with the digitalis glycosides may influence the plasma levels of metabolites and hence further complicate the interpretation of the measured plasma concentrations of the glycosides. Some of these are discussed below.

Magnesium peroxide causes decomposition of digoxin by hydrogen peroxide, which is liberated from the magnesium perhydrol by gastric juice (Van der Vijgh et al., 1976). Other reports of decreased bioavailability of digoxin with antacids (Khalil, 1974; Brown and Juhl, 1976) may be due to decreased absorption, a point which has recently been demonstrated in vitro (McElnay et al., 1978). This could lead to increased bacterial degradation in the intestine with the formation of inactive dihydrodigoxin (see Fig. 5).

Solomon et al. (1971a, b) have reported that phenobarbital, phenytoin and phenylbutazone each reduce the biological half-life of digitoxin, presumably by enzyme induction. However, Nevasaari et al. (1976a), observing a similar interaction between phenobar-

bital and digoxin, suggested that it was possibly due to an increase in bile flow.

The elimination of digitoxin is accelerated by cholestyramine, presumably owing to the interruption of the digitoxin enterohepatic circulation (Caldwell et al., 1971; Thompson, 1973). Decreased urinary excretion and greatly increased biliary excretion due to spironolactone have also been observed with [^{3}H]digitoxin (Castle and Lage, 1973a, b). These authors (1972, 1973b) have also shown that spironolactone pretreatment markedly increases the metabolism and/or excretion of digitoxin and that it apparently stimulated enzymes that were unaffected by pretreatment with phenobarbital. Using isolated perfused rat livers, Nevasaari et al. (1976a) have shown that spironolactone increased biliary excretion of digoxin, although it did not increase bile flow.

Ejvinsson (1978) has observed an increase in digoxin blood levels following concurrent administration of quinidine. The mechanism is under investigation, but could be due to a competition for binding within the tissues.

Acetylcholine in the presence of physostigmine has an inhibitory action on bile flow and biliary elimination of digoxin in the perfused rat liver, whereas physostigmine alone has no effect (Nevasaari et al., 1976b). They also reported that atropine enhanced the biliary elimination of [^{3}H]digoxin.

Rifampicin alone and in combination with isoniazid and ethambutol, when administered to tuberculous patients, caused a decrease in plasma levels of digitoxin and a decrease in its half-life. This was considered to be caused by an increased 12β-hydroxylation of digitoxin (Peters et al., 1974).

CONCLUSION

From the literature, it can be seen that there are widely differing opinions on the metabolism and excretion of the cardiac glycosides. At present, classical ideas are being challenged. Herrmann and Repke's 1964 paper on digitoxin metabolism and the stepwise cleavage of the sugars down to the free genin in now susperseded by the work of Schmoldt (1976), which indicates that the final metabolic products are the glucuronides and sulphate conjugates of digitoxigenin-mono-digitoxoside. Likewise, digoxin, the most commonly used cardiac glycoside, was once regarded as being eliminated in the urine essentially unchanged, but it is now known that up to 30% can be excreted as the cardioinactive metabolite 20,22-dihydrodigoxin (Clark and Kalman, 1974).

The basic problem, however, still remains and that is an accurate prediction of therapeutic activity within the clinical setting. Radioimmunoassay has its own problems with variation in sensitivity (Kubasik et al., 1974a, b; Kubasik et al., 1976; Lister, 1977). However, it is the specificity which is of chief concern here. Most commercially available kits claim to be 'specific', but in fact considerable cross-reactivity with other glycosides and metabolites is known to occur. For instance, Stoll et al. (1972) reported that the mono- and bis-digitoxosides of digoxin were capable of binding to the antiserum of a tritiated digoxin kit to approximately the same extent as digoxin itself. Digoxigenin had a somewhat lower affinity for the same antiserum. In support of the suggestion that the sugar side-chain is of little relevance to the antibody binding capacity, Garrett and Hinderling (1977) have shown that a standard digoxin kit can be used to measure plasma levels of β-methyldigoxin. However, compared to measurement of tritium, the kit under-

estimated plasma levels by between 12 and 38% depending on the individual plasma sample. Likewise, lanatoside C has been found to cross-react identically with digoxin (Lader et al., 1972). In one of the earliest papers on digoxin-specific antibodies as the basis for an assay method, Butler and Chen (1967) showed about 10% immunological activity of digitoxin and a somewhat weaker effect of ouabain. Edmonds et al. (1972) have confirmed the cross-reactivity between digoxin and digitoxin in their respective immunoassys. Although this may be irrelevant for digoxin therapy, it is critical to digitoxin measurements, since digoxin is a major metabolite. More serious for digoxin analysis is the report from Clark and Kalman (1974), that the immunoassay will detect varying amounts up to one-third of the cardioinactive 20,22-dihydrometabolite present.

The reported increased toxicity of ouabain and digoxin in hypoxic patients (Fishman, 1975) serves to illustrate the lack of understanding of the correlation between plasma levels and inotropic effect in disease states. Until there are sensitive methods available to distinguish and estimate glycosides and their metabolites and more is known about their pharmacodynamic as well as their pharmacokinetic parameters, these problems are likely to remain.

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