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Cardiac Glycosides

Edited by G. Bodem and H. J. Dengler

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Introduction

In spite of old vintage and 200 years of clinical use, digitalis remains an interesting therapeutic agent, to clinicians as well as to the pharmacologist, the biochemist, and colleagues in other diciplines of theoretic medicine.

When a drug, however, has so many attractive facets, it seems proper and advisable for the success of a scientific meeting to focus on a number of well-defined aspects.

This symposium was devoted to pharmacokinetics, drug metabolism, analytic procedures, blood level determinations, and their interpretation both for therapeutic and toxic situations. Considerable progress has been made during the last years in this area of digitalis research. The time was suitable for a critical reappraisal of facts and theories and for future planning. Our main intention was to relate analytic data and biochemical findings to clinical problems and questions. Despite the undoubtedly basic character of clinical pharmacology, it is nevertheless an applied science which should help to develop the rational basis of therapeutics.

We are particularly grateful to the active participants who bore the burden of preparing presentations and — even worse — manuscripts. At the same time we are well-aware that many other active research groups would have been able to contribute in this way, but our program was limited because of the short time available. Their knowledge is included in the discussion parts of the meeting, so we hope a well-balanced description of the present state of affairs emerged in this volume.

Finally, we would like to express our gratitude to Boehringer Mannheim and their representatives who sponsored this meeting and were of great help as regards the organization.

The editors also acknowledge the secretarial work and assistance as a translator of Mrs. Ines Nandi.

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1 Evaluation of Different Methods for Determining Serum Concentrations of Cardiac Glycosides[1]

V. P. BUTLER, JR.[2]

Two centuries have passed since Withering first reported that digitalis "has a power over the motion of the heart, to a degree yet unobserved in any other medicine" (Withering, 1937). Although digitalis glycosides are now widely used in the therapy of congestive heart failure, the dosage of digitalis preparations must, as in Withering's day, be carefully adjusted to the needs of each individual patient in order that an optimal therapeutic effect may be achieved without the development of toxic side-effects because, as Withering noted, excessive digitalis "occasions sickness, vomiting. purging, giddiness, confused vision, objects appearing green or yellow . . . slow pulse . . . cold sweats, convulsions, syncope, death" (Withering, 1937). Developments in the first 6 decades of this century which have enhanced the physician's ability to adjust digitalis dosages to the specific needs of individual patients have included: the isolation of highly purified cardenolides, the recognition of the effects of digitalis on the electrocardiogram, a better definition of the effects of cardiac glycosides on myocardial contractility, conduction, and automaticity, and an appreciation of the role of electrolyte disturbances in facilitating the development of digitalis toxicity (Butler, 1972).

In the 1950s and 1960s, the use of radioactively labeled digitalis preparations made possible, for the first time, the direct measurement of cardiac glycosides and their metabolites in the blood, urine, and tissues of man and of experimental animals, thereby providing great insight into the pharmacokinetics of digitalis preparations (Okita et al., 1953; Doherty et al., 1961; Marcus et al., 1964; Marks et al., 1964; Doherty and Perkins, 1966; Doherty et al., 1967; Doherty, 1968; Ewy et al., 1969). Studies with tritiated digoxin, in particular, provided evidence that this drug is metabolized at a relatively slow rate and that its disappearance from the body is dependent, in large part, on its renal excretion (Doherty, 1968). These studies also provided evidence that there is a relationship between the serum level of this glycoside and its concentration in the myocardium and other tissues. (Doherty and Perkins, 1966; Doherty et al., 1967). Doherty et al. found myocardium-to-serum concentration ratios from 17:1 to 35:1 (mean 29:1) in man (Doherty, 1968) and pointed out that the relative constancy of these ratios in the

1 This work has been supported by research grants from the United States Public Health Service (HL 10608) and from the New York Heart Association.

2 Recipient of an Irma T. Hirschl Career Scientist Award.

face of large differences in total body digoxin stores "indicates that the serum-digoxin level is related to the cardiac-muscle digoxin level and that a serum digoxin determination . . . should be of definite value in clinical assessment of digoxin cardiac content" (Doherty et al., 1967). Under ordinary conditions, less than 1% of the total body digoxin is present in the vascular compartment (Doherty, 1968), and serum concentrations of digoxin and other cardiac glycosides are so low that it was not possible, until recently, to measure these concentrations. In the past decade, however, several new techniques (Table 1.1.) have been developed for the determination of serum or plasma concentrations of cardiac glycosides. As Doherty had predicted (Doherty et al., 1967; Doherty, 1968), these methods all have proved to be cf great value to the physician in determining the dosage of digitalis to be administered to patients requiring this drug (Butler, 1972; Smith and Haber, 1970; Butler, 1970; Smith, 1972; Bodem and Gilfrich, 1973; Duhme et al., 1974; Butler and Lindenbaum, 1975; Smith, 1975; Grosse-Brockhoff and Hausamen, 1975; Huffman et al., 1976). It is the purpose of this review to compare these methods for determining serum digitalis concentrations with special reference to their specificity, rapidity, and suitability for routine clinical use.

Immunoassay

Cardiac glycosides are relatively small molecules with molecular weights in the 500-1000 range and are too small to be immunogenic by themselves. To obtain antibodies to cardiac glycosides, it is necessary to conjugate these pharmacologic agents as haptens to antigenic protein carriers (Butler and Beiser, 1973; Beiser et al., 1976). For this purpose, periodate-oxidized glycosides have been coupled to albumin carriers by the method of Erlanger and Beiser to form synthetic glycoside-

Table 1.1. Digitalis Assay Methods

Biochemical methods
Immunoassay
Radioimmunoassay
Enzyme immunoassay
Competitive protein binding assay
Enzymatic isotope displacement assay
Inhibition of red Cell ^{86}Rb Uptake
Inhibition of $(Na^+ + K^+)$-ATPase
Chromatographic Methods
Double isotope dilution derivative assay
Gas chromatography
(High pressure liquid chromatography)

protein conjugates as shown in step 1 of Figure 1.1. (Butler and Chen, 1967); alternatively, the 3-0-succinyl derivative of digitoxigenin, the digitoxose-lacking cardioactive aglycone derived from digitoxin, has been conjugated to albumin carriers by the carbodiimide and mixed anhydride methods (Oliver et al., 1968). Rabbits immunized with these conjugates form antibodies to the albumin carriers, but more importantly, they also form antibodies capable of binding digoxin in the first instance (Fig. 1.1, step 2) and of binding digitoxin in the latter case. Antidigoxin antibodies from selected antisera possess a high affinity and great specificity for digoxin. For example, certain digoxin-specific antibodies have been shown to bind digoxin at least 20 times more effectively than they bind digitoxin or dihydrodigoxin, a digoxin metabolite (Butler and Chen, 1967; Smith et al., 1970; Butler et al., 1974), although digoxin differs structurally only slightly from these two closely related compounds (Fig. 1.2.).

The radioimmunoassay procedure is based on methods developed by Berson and Yalow for the assay of insulin and other peptide hormones (Yalow and Berson,

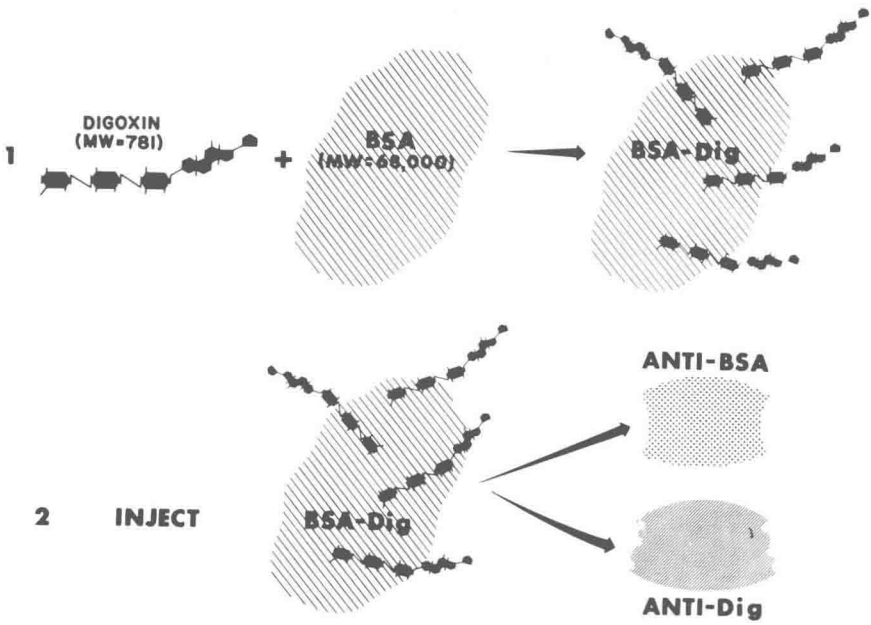

Fig. 1.1. Production of antibodies to digoxin. (Withering, 1937). Digoxin (Dig) is chemically conjugated as a hapten to a protein carrier such as bovine serum albumin (BSA) by the periodate oxidation method. (Butler, 1972). Rabbits immunized with BSA digoxin conjugates form antibodies that bind, but do not precipitate with, digoxin. The bivalent antidigoxin molecule shown here is capable of binding one digoxin molecule at each of its two binding sites. Animals form antibodies to the protein carrier, BSA, but such antibodies have no effect on most immunoassay systems. (Reproduced with permission from the New England Journal of Medicine (Butler, 1970)

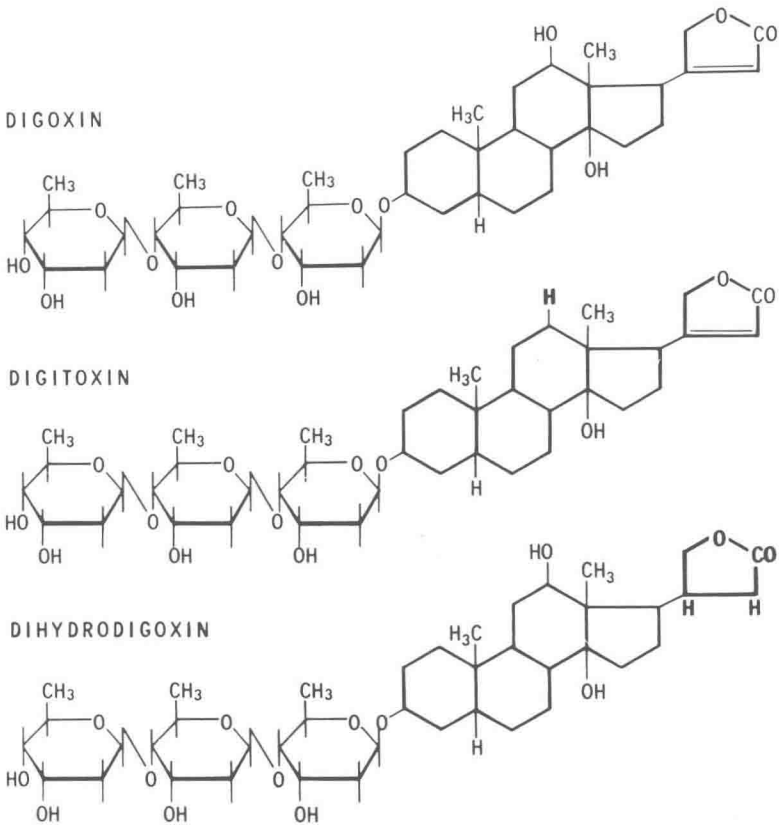

Fig. 1.2. Structural formulas of digoxin, digitoxin, and dihydrodigoxin. All three glycosides consist of aglycones (steroidal portion and lactone ring) shown at right and glycosidic portions (consisting of three digitoxose sugar molecules) shown at left. Digitoxin differs from digoxin only in that it lacks the hydroxyl group at the C-12 position in the aglycone portion of the molecule. Dihydrodigoxin differs from digoxin only in that its lactone ring is saturated (Reproduced with permission from the Annals of the New York Academy of Sciences (Butler et al., 1974)

1964; Berson and Yalow, 1967). The underlying principle is that nonradioactive glycoside (in known standard solutions or in patients sera) will compete with radioactively labeled glycoside for combining sites on antidigitalis antibody. If one mixes varying quantities of unlabeled digitalis with a standard amount of radiolabeled glycoside, the amount of radioactivity bound by a standard amount of antibody will decrease as increasing amounts of unlabeled glycoside are added. A standard curve can then be constructed (Fig. 1.3) from which the concentration of digitalis in a given patient's serum can be determined on the basis of the decrease it causes in the binding of radioactive glycoside by specific antibody (Oliver et al., 1968; Smith et al., 1969).

A large number of radioimmunoassay procedures has now been described, differing principally in the method by which antibody-bound labeled glycoside is physicochemically separated from unbound ("free") labeled glycoside. In the dextran-coated charcoal method (Herbert et al., 1965), sera to be tested and standard glycoside reference solutions are added to test tubes. Tritiated glycoside (Smith et al., 1969) or a radioiodinated digitalis derivative (Oliver et al., 1968) is then added. After mixing, a small volume of dilute antiserum is added, followed, after a brief incubation period, by the addition of a suspension of dextran-coated charcoal. Essentially all nonantibody-bound radioactivity is rapidly adsorbed by the charcoal. The charcoal and nonantibody-bound labeled glycoside are removed from suspension by centrifugation, and the supernatant solution, containing the antibody-bound radiolabeled digitalis, is removed and assayed for radioactivity (Smith et al., 1969; Smith, 1970). More recently described methods have employed antibodies coupled to a solid matrix or support; in these solid-phase methods, the unbound radiolabeled digitalis remains in solution and is measured as an indicator of the extent of antibody-binding in each tube (Line et al., 1973). Radioimmunoassay methods have been described to date for several cardiac glycosides and related compounds, including digoxin (Smith et al., 1969), digitoxin (Oliver et al., 1968; Smith, 1970), ouabain (Selden and Smith, 1972), gitoxin (Lesne, 1972), gitaloxin (Lesne, 1972), proscillaridin (Belz et al., 1973), acetyl strophanthidin (Selden et al., 1973), and β-methyl-digoxin (Härtel et al., 1973; Haasis et al., 1975).

Because the immunoassay procedures reported to date have employed antisera with specificity for the aglycone portion (Fig. 1.2) of the digitalis molecule (Butler and Chen, 1967; Oliver et al., 1968; Smith et al., 1970), metabolic breakdown products of cardiac glycosides containing the intact aglycone (e.g., digoxigenin and its mono- and bisdigitoxosides (Doherty, 1968) will react significantly in these procedures. Metabolites in which the aglycone has been altered may also react but to a lesser extent. Because these closely related compounds react in digitalis immunoassay procedures (Smith et al., 1970), the term "immunoreactive" might be more accurate in describing results. The fact that immunoassay methods will detect metabolites of cardiac glycosides constitutes a theoretic disadvantage, but in practice, serum immunoreactive digoxin and digitoxin concentrations have correlated well with values obtained by other methods and with the clinical state of the patients studied. In the case of digoxin, this correlation may reflect the findings that very little of this glycoside is metabolized in man (Doherty, 1968) and that most of its known metabolites (digoxigenin and its mono- and bisdigitoxosides) are both cardioactive and immunoreactive (Butler, 1972). In the case of digitoxin, metabolic degradation is more extensive (Doherty, 1968), and the contribution of inactive metabolites to the serum concentration of immunoreactive digitoxin is not yet clear (Oliver et al., 1968). Although this has not been a major limitation in a practical sense, the precise role of metabolites in radioimmunoassay procedures has not yet been clearly delineated.

Although the antibodies in most antidigitalis sera are directed largely toward the aglycone portion of the digitalis molecule, all antisera to cardiac glycosides that have been characterized cross-react to some extent with other glycosides and