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Reversal of the Pharmacological and Toxic Effects of Cardiac Glycosides by Specific Antibodies*

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Introduction

IT has long been known that specific antibodies are capable, under appropriate circumstances, of neutralizing many biologically active macromolecules including bacterial exotoxins, numerous enzymes from various sources, peptide hormones, and transforming deoxyribonucleic acid (DNA). Since antibodies do not ordinarily penetrate the membranes of intact mammalian cells, the neutralizing effects of antibodies specific for these biologically active substances ordinarily have been demonstrated only when the interaction between antigen and antibody has occurred extracellularly (12). It had not been thought that antibodies specific for tetanus toxin or diphtheria toxin, for example, were capable of reversing the effect of the corresponding toxin, once that toxin had interacted with a target cell. In 1966, however, Pastan *et al.* (43) reported that antibodies specific for two polypeptide hormones, insulin and thyrotropin, could reverse established cellular effects of these hormones *in vitro*.

Despite the fact that much useful information has been derived from the interaction of antibodies with macromolecular toxins, enzymes, and peptide hormones, there has been, until recently, relatively little physiological or pharmacological application of antibodies as specific biological antagonists of low molecular weight substances (3, 8). It has been demonstrated that antibodies specific for steroid hormones (11, 21–23, 30, 36, 40, 47), pyridoxal phosphate (62), histamine (6, 14, 16), serotonin (24, 45, 46), chloramphenicol (31), and cardiac glycosides (7, 13, 15, 26, 48, 53, 55, 64) are capable, by virtue of their capacity to bind these substances, of inhibiting certain physiological effects of these compounds on various tissues, either *in vivo* or *in vitro*. Evidence has been obtained recently that antibodies specific for the cardiac glycoside, digoxin (9), not only neutralize this drug but are also capable of removing the drug from certain mammalian cells (64) and of reversing certain of its pharmacological and toxic actions both *in vitro* and *in vivo* (7, 15, 26, 29, 37, 49, 53, 55, 57, (64). It is the purpose of this review to describe the biological properties of digoxin-specific antibodies and the use of these antibodies as specific pharmacological antagonists of the glycoside in studies of the mechanism of digoxin action and in the reversal of digoxin intoxication.

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Digoxin-specific Antibodies: Production and Properties

Digoxin (fig. 1) has a molecular weight of 780.9 and is too small to be antigenic by itself. Therefore, digoxin was chemically coupled as a hapten to antigenic protein carriers (9, 10) by the periodate oxidation method (20) of Erlanger and Beiser (fig. 2, step 1). Rabbits and sheep immunized with digoxin-protein conjugates form antibodies to the protein carrier but, more importantly, they form antibodies to digoxin (fig. 2, step 2). With a variety of immunochemical techniques, including equilibrium dialysis, the dextran coated charcoal method, gel filtration, and the double antibody technique, it has been demonstrated conclusively that these antibodies bind with, but do not precipitate with, tritiated digoxin (7, 9). These antibodies have great specificity for digoxin. For example, digitoxin is almost identical structurally with digoxin; its three glycosidic digitoxose groups are identical and its steroidal digitoxigenin portion differs only in that it lacks a single hydroxyl group at carbon-12 (fig. 1). Yet antidigoxin antibodies react 20 or more times effectively with digoxin than they react with digitoxin. Antidigoxin antibodies possess weak cross reactivity with ouabain, a distantly related cardiac glycoside, and their cross reaction with steroid hormones is barely detectable (9, 56).

Effects on Cellular Digoxin *in Vitro*

To study the effect of antidigoxin antibodies on cellular digoxin uptake (26, 64), two tissues were chosen: the kidney, because the studies of Doherty and Perkins indicated that the highest concentrations of this glycoside were located in this tissue, rather than in the myocardium (18); and the erythrocyte, because this tissue readily lent itself to studies of the effect of antibodies on a pharmacological effect of digoxin, namely inhibition of potassium influx (27, 28, 33, 52, 54).

Renal cortical slices of rats accumulate digoxin against a concentration gradient. At a concentration of 10^{-5} M, digoxin rapidly accumulated in slices and reached a steady-state distribution ratio (of intracellular to extracellular digoxin concentration) in the 1.8 range by 60 min. In the presence of digoxin-specific antisera, this uptake of

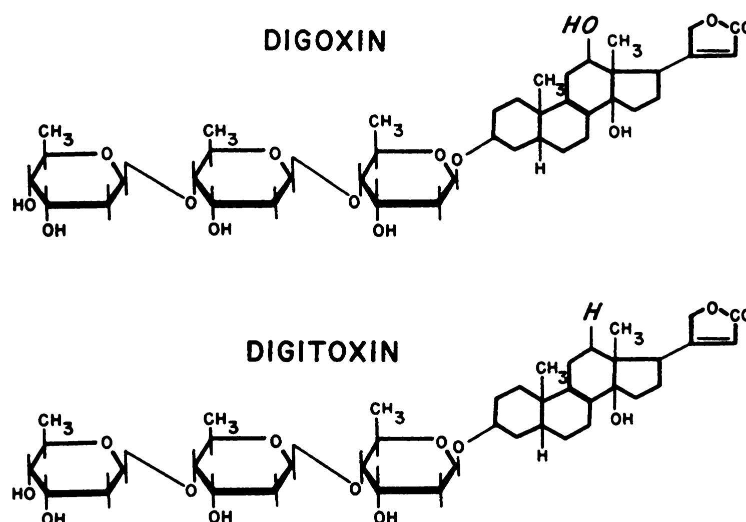


FIG. 1. Structural formulas of digoxin and digitoxin. The difference at carbon-12 of the steroidal portion is shown in boldface. (Reprinted by permission from V. P. Butler, Jr.: Digoxin: immunologic approaches to measurement and reversal of toxicity. N. Engl. J. Med. 283: 1150–1156, 1970.)

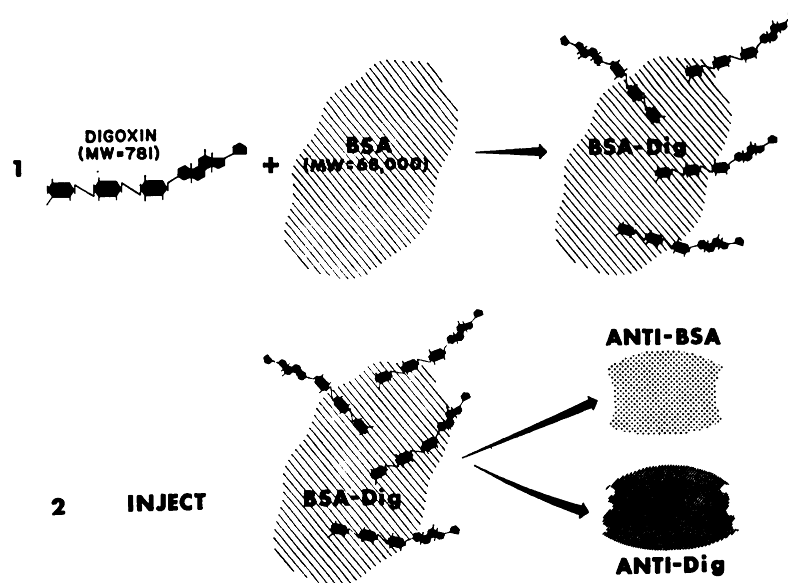


FIG. 2. Schematic representation of experimental production of digoxin-specific antibodies: 1) Digoxin (Dig) was chemically conjugated to bovine serum albumin (BSA) by the periodate oxidation method. 2) The BSA-digoxin conjugate was suspended in complete Freund's adjuvant mixture and injected into animals. These animals formed antibodies to BSA, and antibodies to digoxin, as indicated by the bivalent structure at the lower right with two combining sites, each capable of binding one molecule of digoxin. (Reprinted by permission from V. P. Butler, Jr.: Digoxin: immunologic approaches to measurement and reversal of toxicity. N. Engl. J. Med. 283: 1150–1156, 1970.)

digoxin by kidney slices was inhibited almost completely whereas, in the presence of normal rabbit serum or of antisera to other antigens, no effect on digoxin uptake was observed. The capacity of renal cortical slices to accumulate digoxin against a concentration gradient provided a useful system in which the ability of antidigoxin antibodies to remove tissue-bound glycoside could be studied. Accordingly, after 60 min of incubation of slices with digoxin-^{3}H, at which time the distribution ratio was in the 1.8 range, 0.5 ml of various sera were added to individual slices and incubation was continued for another hour. When serum from a non-immunized control rabbit was added to slices, a slight fall in distribution ratio occurred due to dilution. In contrast, when antidigoxin serum was added, rapid removal of digoxin-^{3}H occurred (fig. 3); 50% of the glycoside was removed within 3 min, and more than 90% was removed in the first 30 min (64).

Antidigoxin antibodies prevent the uptake of digoxin by human erythrocytes. Antibodies to the glycoside are capable of removing digoxin rapidly from these cells. It was originally thought that the rapid removal of digoxin was almost complete (64), but evidence has been obtained recently that this rapid removal is restricted to that fraction of the erythrocyte digoxin which has been taken across the membrane into the cell (26). Membrane-bound digoxin is also removed from red cells by antibody, but at a much slower rate (table 1). Data obtained in these erythrocyte studies suggest that antibodies bind this latter fraction of digoxin in the extracellular space after dissociation from membrane binding sites and that these antibodies lower membrane digoxin concentrations by preventing reassociation rather than by "prying" digoxin from binding sites (26).

These studies with renal cortical slices and with erythrocytes have broad significance because they provide direct evidence that antibodies are capable, under certain circumstances, of removing biologically active substances from mammalian cells.

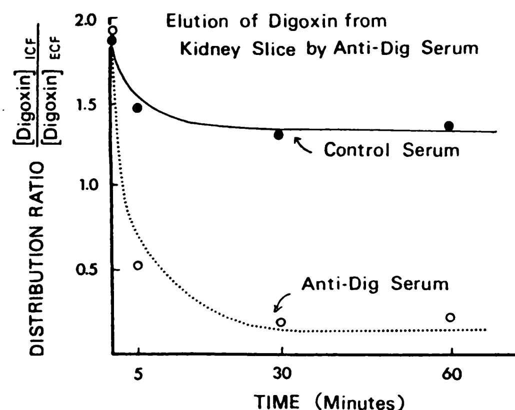


FIG. 3. Removal of digoxin from rat renal slices by rabbit antidigoxin serum (open circles), as compared with control rabbit serum (closed circles). Slices have been allowed to incubate 1 hr with 2 ml 10^{-8} M digoxin to achieve steady-state distribution ratio before adding 0.5 ml serum at 0 min. Distribution ratio (ratio of intracellular to extracellular digoxin concentration) is plotted as a function of time. (Reprinted by permission from J. F. Watson and V. P. Butler, Jr.: Biologic activity of digoxin-specific antisera. J. Clin. Invest. 51: 638–648, 1972.)

Immunological Reversal of Cellular Effects of Digoxin

To determine whether the removal of cellular digoxin by specific antibody was associated with reversal of a pharmacological action of the glycoside, the effect of the antibodies on digoxin-mediated inhibition of cation flux was studied in human erythrocytes. Digoxin inhibits the influx of potassium into these cells and, when antidigoxin antibody is added to digoxin-treated cells, this inhibitory effect of the glycoside is reversed (64). This reversal is relatively slow (15, 26, 64) and appears to be correlated temporally with the removal of membrane-bound digoxin (table 1). These immunological observations lend strong support to other pharmacological data indicating that there are at least two fractions of red cell digoxin, only one of which, *viz.* the membrane-bound fraction, is responsible for the effect of the glycoside on cation transport (26).

TABLE 1

Effect of digoxin-specific antiserum on loss of cellular digoxin and restoration of potassium influx

Erythrocytes were incubated for 3 hr at 37°C with ^{3}H-digoxin (1.53 × 10^{-7} M). Aliquots were then taken for determination of total cellular digoxin accumulation, the amount of digoxin bound to the cell membrane and potassium influx just before and at various times after addition of digoxin-specific antiserum or control antiserum. Intracellular digoxin was calculated as total cellular digoxin minus membrane bound digoxin. Potassium influx was determined over a 20-min period with ^{42}K and a final potassium concentration of 15.3 mM. Potassium influx in control erythrocytes not treated with digoxin was 2.41 ± 0.18 mmoles/L cells per hr. Each value represents the mean of 6 experiments ± 1 S.D.

Time	Intracellular Digoxin	Membrane Bound Digoxin	Potassium Influx
min	*pmoles/ml cells*	*pmoles/ml cells*	*mmoles/L cells/hr*
0	23.2 ± 2.1*	20.8 ± 1.7*	0.93 ± 0.07*
2	9.3 ± 1.8	20.5 ± 1.6	
4	3.6 ± 1.6	21.0 ± 2.1	
6	0.2 ± 2.0	20.7 ± 1.4	
8	0.1 ± 2.2	20.9 ± 1.3	
10	−0.3 ± 1.7	20.8 ± 1.8	
60	0.2 ± 1.9	19.6 ± 1.1	1.08 ± 0.04
120	0.4 ± 1.8	18.3 ± 1.2	1.21 ± 0.06
180	1.1 ± 2.3	17.3 ± 1.4	1.32 ± 0.09
240	−0.4 ± 1.6	16.0 ± 1.2	1.43 ± 0.09
300	0.6 ± 1.7	14.8 ± 0.9	1.54 ± 0.08

* These values did not change significantly after the addition of control serum containing antibodies to an antigen structurally unrelated to digoxin.

Digoxin-specific antibodies are also capable of reversing the effect of digoxin on myocardial cells *in vitro* (15, 29, 37, 53, 57). For example, 5 × 10^{-7} M digoxin produces an increase in the active tension developed by electrically stimulated, isometrically contracting, isolated cat papillary muscle preparations. Replacement of the bathing solution with a digoxin-free solution produces a slow decrease in tension, but the addition of antidigoxin serum without removal of digoxin causes a rapid and marked decrease in the positive inotropic effect of the glycoside. Moreover, the anti-inotropic effect of antidigoxin serum can be overcome at any time by the addition of sufficient digoxin. This latter observation suggests that the inhibition of digoxin action does not reflect the presence of a digoxin-antibody complex at

digoxin receptor sites on the myocardial cell membrane. This observation further suggests that the inadvertent reversal of positive intropism in the clinical reversal of digoxin toxicity by antibody (see below) can be overcome by the judicious administration of additional digitalis (53). In other myocardial studies, it has been demonstrated that antidigoxin antibodies can reverse the toxic electrophysiological effects of the glycoside on isolated perfused canine Purkinje fibers and rabbit atrioventricular node preparations (37).

Immunological Protection against Digoxin Intoxication

Antidigoxin antibodies protect rabbits from the adverse electrocardiographic effects of an otherwise lethal dose of digoxin. Shown in figure 4 are the heart rates of normal control rabbits and of digoxin-immunized rabbits after the slow intravenous administration of the glycoside. Non-immunized rabbits, given 0.5 mg of digoxin per kg of body weight, showed rhythm disturbances accompanied usually by bradycardia and followed by death within 1 to 2 hr. In contrast, rabbits immunized with digoxin-albumin conjugates and whose sera contained antidigoxin antibodies showed no significant abnormalities during 2 hr of continuous electrocardiographic monitoring after 0.6 mg of digoxin per kg of body weight (48).

Antidigoxin antibodes markedly alter the metabolic turnover of the glycoside in the rabbit. Although the binding of digoxin by antibodies protects the myocardium, the presence of antibodies results in approximately 100-fold greater serum concentrations, decreased urinary excretion, and a 27-fold pro-

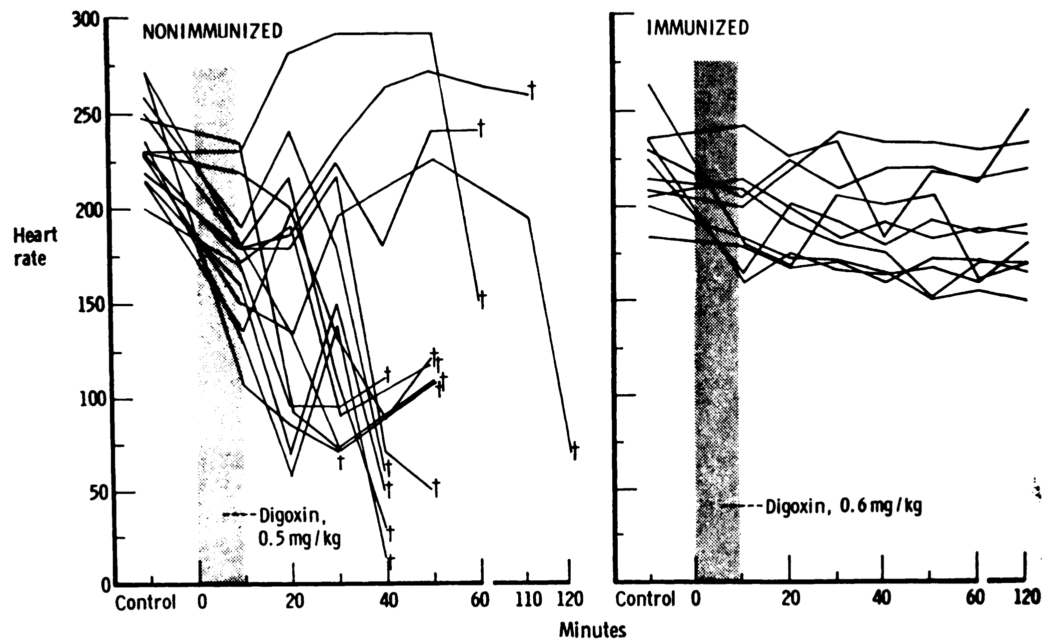


FIG. 4. The effect of digoxin on the heart rate of 15 non-immunized rabbits given a lethal dose of digoxin (0.5 mg/kg of body weight) and on the heart rate of 10 rabbits immunized with digoxin-protein conjugates and given 0.6 mg digoxin per kg of body weight; the sera of the latter group of animals contained antidigoxin antibodies in high titer. Heart rates were determined during a 30-min control period prior to the administration of digoxin, during the 10-min intravenous infusion of digoxin (the shaded area), and for up to 2 hr thereafter. A cross indicates the time of death. (Reprinted by permission from D. H. Schmidt and V. P. Butler, Jr.: Immunological protection against digoxin toxicity. J. Clin. Invest. 50: 866–871, 1971.)

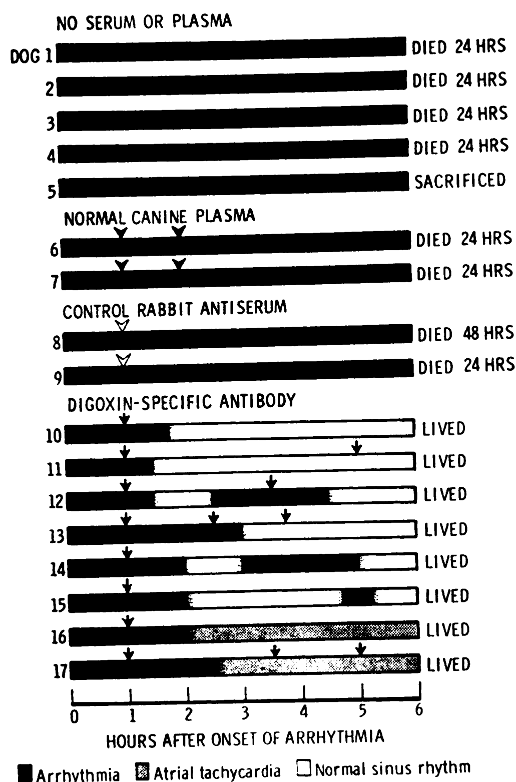


FIG. 5. The course of digoxin-induced arrhythmias in 17 non-immunized dogs which had received 0.09 mg digoxin per kg of body weight, intramuscularly, once daily for 3 days. Black bars indicate the presence of a digoxin-toxic arrhythmia (atrioventricular dissociation with ectopic ventricular beats; atrioventricular dissociation with a junctional rhythm; various forms of atrioventricular block; slow idioventricular rhythm with no evidence of atrial activity; and ventricular tachycardia). Shaded bars denote atrial tachycardia with variable atrioventricular block and a ventricular rate of 80 to 100. White bars indicate normal sinus rhythm. Normal canine plasma, control rabbit antisera (containing antibodies unrelated to digoxin), and antidigoxin sera were infused at the times indicated by the symbols. The horizontal axis represents the duration of the study period in hours after the onset of the arrhythmia. (Reprinted by permission from D. H. Schmidt and V. P. Butler, Jr.: Reversal of digoxin toxicity with specific antibodies. J. Clin. Invest. 50: 1738–1744, 1971.)

longation of the biological half-life of digoxin. Significant concentrations of digoxin are present in the serum 1 year after a *single* intravenous injection of the glycoside (50).

Immunological Reversal of Digoxin Intoxication

To study the effect of digoxin-binding antibodies on established digoxin intoxication in non-immunized animals, 17 dogs were given 0.09 mg of digoxin per kg of body weight, intramuscularly, once daily for 3 days. All animals developed nausea, vomiting, weakness, lethargy, and within 1 to 3 hr after the last dose, a digoxin-toxic arrhythmia (fig. 5). Dogs 1 through 5 received only digoxin, while dogs 6 through 9 also received control serum or plasma which did not contain antidigoxin antibodies. These nine animals exhibited arrhythmias which persisted throughout a 6-hr period; normal sinus rhythm was not observed at any time. Seven dogs were dead within 24 hr, and one moribund animal was sacrificed at this time; the last dog died within 48 hr. In contrast, in dogs 10 through 15, antidigoxin antibodies reversed the toxic arrhythmia and restored normal sinus rhythm during the 6-hr study period. In the two remaining dogs given antidigoxin antibodies, serious ventricular arrhythmias were replaced by atrial tachycardia with variable atrioventricular block and a relatively slow ventricular rate; both animals later reverted to normal sinus rhythm. All eight animals treated with antibodies were also relieved of their gastrointestinal and other clinical manifestations of digoxin intoxication. It was concluded that digoxin-specific antibodies are capable of reversing the toxic cellular effects of excessive myocardial digoxin in the non-immunized dog (49).

It seems probable that reversal of digoxin-toxic arrhythmias is due to the removal of digoxin from myocardial cells by antibody. Evidence of decreased myocardial concentrations of digoxin after antibody administration is not definitive, but 14-fold rises in serum digoxin concentration after antibody administration are certainly suggestive of this mechanism (51). It is of importance to note that, in the passively immunized dog as in the actively immunized rabbit, not only are serum digoxin concentrations markedly elevated, but urinary excretion is also di-

minished and the biological half-life of digoxin is markedly prolonged.[1]

Possible Clinical Applications

In discussing the problem of digitalis toxicity, Withering (65) said in 1785: "If inadvertently the doses of the Foxglove should be prescribed too largely, exhibited too rapidly, or urged to too great a length, the knowledge of a remedy to counteract its effects would be a desirable thing. Such a remedy may perhaps in time be discovered." Digoxin-specific antibodies may constitute such a remedy but, several potential difficulties remain to be overcome or minimized before the use of rabbit or sheep antidigoxin antibodies in the therapy of digoxin intoxication in man can be considered (table 2). Firstly, there is the possibility of a hypersensitivity reaction to the intravenous injection of foreign proteins; such a reaction might be immediate, in the form of an acute anaphylactic reaction, or it could be a delayed reaction of the serum sickness type. The incidence and severity of acute hypersensitivity reactions to foreign serum can be minimized by appropriate skin-testing and by "desensitizing," when indicated (42). Nevertheless, the possible occurrence of such a hypersensitivity reaction must always be kept in mind when foreign serum proteins are administered. Another possible hazard connected with the clinical use of antidigoxin antibodies is that, if an excessive amount of antibody is administered to a digoxin-intoxicated patient with severe organic heart disease with reversal of toxic manifestations, the therapeutic effects of the drug on cardiac rhythm or contractility may also be reversed (29, 37, 53), with serious clinical consequences. Theoretically, this undesirable effect could be overcome by the administration of additional cardiac glycoside (53) but it would be difficult to predict the dosage of glycoside which might be required. Finally (table 2), inactivated, antibody-bound digoxin should be released eventually in the form of active glycoside as the body metabolizes or immunologically degrades the foreign antibody molecules. Such degradation, particularly if it occurs rapidly, might result in the sudden presentation of relatively large amounts of digoxin to binding sites in the myocardium and other tissues. Such release of digoxin from antibody could be particularly hazardous in cardiac patients who require additional digitalis administration after the therapeutic use of antibody and whose total body stores of glycoside are therefore quite large. The removal of a large percentage of antibody-bound digoxin could probably be achieved by the process of plasmapheresis (1, 60), but this latter procedure is not without hazard in hemodynamically unstable, hypotensive patients.

TABLE 2

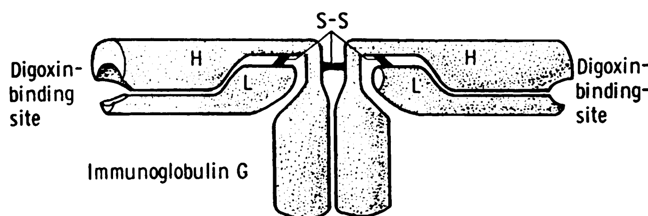
Possible problems associated with the clinical use of antidigoxin antibodies

1. Hypersensitivity reaction to foreign protein
   - Acute (anaphylaxis)
   - Delayed (serum sickness)
2. Excess removal of myocardial digoxin
   - Reversal of inotropic and beneficial electrophysiological effects
3. Late release of inactivated, antibody-bound digoxin in free, active form
   - Especially during rapid immune degradation of foreign γ-globulin

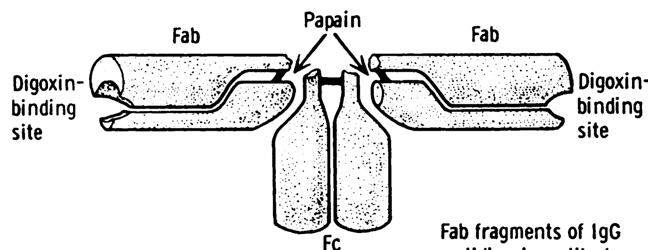
Attempts are now being made to minimize the above hazards connected with the possible clinical use of digoxin-specific antibodies. Curd *et al.* (14) have developed an immunoadsorbent method to purify antidigoxin antibodies. This method removes all foreign proteins except for a single class, γ-globulin, and thus restricts the antigenic challenge to the recipient of the antibody (15). More recently Smith *et al.* (57) with the method of Porter (44), have prepared enzymatic digests of antidigoxin antibody (m.w. = 160,000) and have obtained two relatively small (m.w. = 50,000) antigen-binding (Fab) fragments from each molecule of intact antibody (fig. 6). These Fab fragments possess full biological activity in terms of digoxin inactivation (57). Theoretically, Fab fragments

[1] V. P. Butler, Jr., D. H. Schmidt, B. D. Raynor, P. Demartini and E. Pilliner: Unpublished observations.

PAPAIN DIGESTION OF IgG ANTIDIGOXIN ANTIBODY



	IgG antidigoxin antibody
Digoxin-binding sites	two per molecule
Molecular weight	160,000
Urinary excretion	no
Serum half-time	23 days*



	Fab fragments of IgG antidigoxin antibody (2 Fab fragments / IgG molecule)
Digoxin-binding sites	one per fragment
Molecular weight	50,000
Urinary excretion	yes
Serum half-time	4.3 hours**

*$t_{1/2}$ of human IgG in man **$t_{1/2}$ of rabbit Fab in rabbit

FIG. 6. Schematic representation of formation of Fab fragments from antidigoxin antibody, based on model of Nisonoff (41). When IgG antidigoxin antibody is treated with papain, it is cleaved into three parts: one Fc fragment and two Fab fragments. Each Fab fragment (ab = "antigen-binding") contains one digoxin-binding site.

possess several advantages over intact antidigoxin antibody molecules for clinical use (table 3). Firstly, they lack the non-antigen-binding Fc fragment of γ-globulin. Since this fragment contains many of the antigenic determinant sites of the immunoglobulin molecule, its removal should result in a significant decrease in immunogenicity in man. Secondly, their smaller molecular size may permit Fab fragments to diffuse more rapidly than intact antibody molecules to digoxin-binding sites in the myocardium and other tissues. Finally and perhaps most importantly, Fab fragments are excreted in the urine, with the result that their biological half-life is usually less than 5 hr (61, 63). Preliminary results obtained in our laboratory in close collaboration with Smith and Haber indicate that Fab fragments of antidigoxin antibody rapidly produce a dramatic rise in serum digoxin-^{3}H in digoxin-treated dogs analogous in the rise observed when intact antidigoxin antibody is administered. In contrast with dogs treated with intact an-

TABLE 3

Theoretical advantages of Fab fragments over intact antidigoxin antibodies

1. Lack species-specific antigenic determinants of Fc fragment
   Less immunogenic
2. Smaller molecular size (50,000 *versus* 160,000)
   May permit more rapid diffusion to digoxin-binding sites in tissue
3. Rapid urinary excretion ($T_{1/2}$ = 4.3 hr)
   May decrease body stores of digoxin rapidly

tibodies, however, the urinary excretion of digoxin in Fab-treated dogs appears to be comparable to that observed in control animals and hence the serum concentrations of digoxin fall more rapidly in Fab-treated dogs than in animals receving intact antibodies. These recent results suggest that Fab fragments share with intact antidigoxin antibody molecules the capacity to remove digoxin from tissue and that, in addition, Fab fragments are capable of promoting the relatively prompt urinary excretion of bound digoxin, a capacity not present in the larger intact antibody molecule.[1] Further studies will be required, however, first to confirm these preliminary observations and then to determine the amounts of Fab fragments required to reverse the toxic effects of digoxin without also reversing its pharmacological effects in patients with advanced heart disease who require digitalis therapy.

Until some of the problems discussed above have been better resolved, the clinical use of antidigoxin antibodies can be considered only in the most serious, potentially lethal instances of digoxin intoxication: for example, deliberate suicidal overdosage (2, 5, 32, 58), inadvertent ingestion of large doses (25, 35, 39, 58), or major overdosage in patients who cannot excrete digoxin effectively due to renal failure (4, 17, 19, 38). At this time, the safest course in most digoxin-intoxicated patients is to withhold the drug and to treat toxic manifestations, including rhythm disturbances, symptomatically (34, 59).

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