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The Isolation of Digoxin-Specific Antibody and Its Use in Reversing the Effects of Digoxin

(human erythrocytes/^{86}Rb transport/guinea-pig atria/ventricular tachycardia/Fab)

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ABSTRACT **Specific antibodies to digoxin were isolated from antisera of sheep immunized with a digoxin–human serum albumin conjugate. The antibody was purified by adsorption to an immunoadsorbent, synthesized by coupling a ouabain–ribonuclease conjugate to bromoacetyl-cellulose, followed by elution with 25 mM ouabain. Ouabain was dissociated from antibody by denaturation in 6 M guanidine. The renatured antibody bound 1.6 mol of digoxin per mol and had an association constant of 1.6×10^8 M^{-1}. At near-stoichiometric concentrations, either purified antibody to digoxin, or its papain-digested product (Fab–Fc), reversed digoxin-induced: (*a*) inhibition of ^{86}Rb transport in human erythrocytes, (*b*) increase in developed tension in isolated guinea-pig atrial strips, and (*c*) ventricular tachycardia in intact dogs, and also corrected digoxin-induced automaticity in isolated guinea-pig atrial strips.**

Digoxin-specific antisera are capable of reversing the biological effects of the cardiac glycoside digoxin in *in vivo* and *in vitro* preparations* (1, 2). The investigations performed to date with unfractionated antisera do not allow precise quantitation of the interaction of the antibodies with digoxin. Other serum components present in these antisera may obscure the results and their interpretation. Furthermore, the full potential for digoxin-specific antibodies in the therapy of digoxin toxicity, a common clinical problem, will not be realized until they are available in pure form.

In this investigation digoxin-specific antibodies were produced in sheep immunized with a digoxin–human serum albumin conjugate (digoxin-HSA, ref. 3) and subsequently isolated with an immunoadsorbent. The isolated antibodies bound digoxin with an affinity and capacity comparable to that of the antiserum. Both the isolated antibodies and their papain digests (Fab–Fc) were effective in reversing digoxin-induced inhibition of rubidium uptake by human erythrocytes and digoxin-induced inotropic and arrhythmic effects in isolated guinea pig atrial strips. In addition, digoxin-induced ventricular tachycardia in dogs was rapidly abolished by the intravenous administration of Fab–Fc prepared from digoxin-specific antisera.

Abbreviation: Me_2F, dimethylformamide; HSA, human serum albumin; PBS, phosphate-buffered saline, pH 7.4; BAC, bromoacetyl cellulose;

* Schmidt, D. H., and V. P. Butler, Jr., *J. Clin. Invest.*, *48*, Abstr. 74a (1969).

MATERIALS AND METHODS

Antigen

Digoxin (Burroughs Wellcome Co., Inc.) was coupled to human serum albumin (3).

Immunization

A 50-kg sheep was injected intramuscularly each week with 3 mg of digoxin–HSA emulsified in 1 ml of Freund's adjuvant to which 10 mg of killed tubercle bacilli had been added. After 20 weeks, 250-ml aliquots of blood were collected each week. The serum was separated and stored at −20°C.

Preparation of immunoadsorbent

A crossreacting immunoadsorbent was prepared by reacting conjugates consisting of ouabain covalently bound to ribonuclease (ouabain–RNase) with bromoacetyl cellulose (BAC). The ouabain–RNase conjugates were made by modification of the procedure used to make digoxin–HSA (3). In a typical preparation, 1450 mg of ouabain octahydrate (Sigma Chemical Co.) was dissolved in 70 ml of ethanol. Then, 70 ml of 0.1 M sodium metaperiodate was slowly added with stirring, at 22°C. After 45 min, 2.1 ml of 1 M ethylene glycol was added. This mixture was stirred for 5 min, and then added dropwise to 900 mg of bovine pancreatic ribonuclease (RNase) (Sigma Chemical Co.) dissolved in 70 ml of water at pH 9.5 (pH adjusted with 1.0 M NaOH and 5% K_2CO_3). The pH was maintained between 9 and 10 by addition of 5% K_2CO_3. After the pH had stabilized, the mixture was stirred for 1 hr. Then, 1.06 g of sodium borohydride freshly dissolved in 70 ml of H_2O was added slowly. The mixture was stirred for an additional 3 hr. The pH was then lowered to 6.5 by addition of formic acid. The resulting cloudy solution was dialyzed overnight in cold water at 4°C in boiled dialysis bags. The next day the dialysate was lyophilized to dryness. The ratio of ouabain to RNase calculated from a spectrophotometric determination, assuming $E(1\%, 1\text{ cm}) = 80$ at 515 nm in 95% concentrated H_2SO_4 (4), was 6 mol of ouabain per mol of RNase.

BAC was synthesized according to Robbins *et al.* (5) and stored as a moist paste at 4°C. About 1 g of lyophilized ouabain–RNase was reacted with 15 g of BAC paste (5). The initial adsorption of the protein conjugate to the BAC was performed in 150 ml of 0.15 M citric acid adjusted to pH 5.5 with 0.15 M Na_2HPO_4. Further adsorption and reaction was performed exactly as described (5). The completed immunoadsorbent (ouabain–RNase–BAC) was then washed with 0.15 M NaCl–

0.01 M K_2HPO_4, pH adjusted to 7.4 with H_3PO_4 (PBS) and stored moist at 4°C. On the basis of nitrogen determination, 49 mg of ouabain–RNase was substituted per gram of dry immunoadsorbent.

Analytic methods

Antibody titers for digoxin-specific antibodies have been defined previously (3). *Antibody affinity and capacity* were determined from saturation curves for small amounts of digoxin-specific antibody. Appropriate dilutions of antiserum, ouabain-eluted antibody after gel filtration, and antibody recovered from guanidine·HCl dialysis were incubated in 0.5 ml of human serum, 0.5 ml PBS, and different concentrations of [^{3}H]digoxin (New England Nuclear Corp.). After incubation at 4° C for 24 hr, free [^{3}H]digoxin was separated from antibody-bound [^{3}H]digoxin by dextran-coated charcoal (6). Bound [^{3}H]digoxin was determined by liquid scintillation counting in Bray's solution (7). The values for antibody-bound digoxin were corrected in each instance by subtraction of a control value of digoxin not bound by charcoal in tubes containing no antibody. The reciprocal of the concentration of free [^{3}H]digoxin at 50% saturation was defined as the average intrinsic association constant of the antibody population under study (Ko) (8). The capacity of the antibody to bind with digoxin was expressed as the number of moles of digoxin bound per mole of antibody at saturation. E_{280} (1%, 1 cm) = 15 and a molecular weight of 150,000 for antibody were assumed in the calculations. *Immunoelectrophoresis* was performed on 2% agar slides according to Scheidegger (9). *Immunodiffusion* for 24 hr was performed in a 3-hole Ouchterlony plate with 2% agar. The same antisera (Hyland rabbit-antisheep gamma-globulin and rabbit antisheep serum) were used in immunodiffusion and in immunoelectorphoresis. *Cellulose acetate electrophoresis* was performed with a Beckman Microzone Cell, model R101. Samples of 0.25 μl were applied to a cellulose-acetate strip saturated with barbital buffer. *Sedimentation velocities* were determined in a Spinco model E analytical ultracentrifuge with a schlieren optical system. Samples were sedimented at 60,000 rpm in PBS and corrected to s_w^{20} values.

ISOLATION, CHARACTERIZATION, AND PAPAIN DIGESTION OF DIGOXIN-SPECIFIC ANTIBODIES

Digoxin-specific antibodies were adsorbed by incubation of sheep antiserum with ouabain-RNase-BAC immunoadsorbent. After buffer washes, which remove unbound serum components, the antidigoxin antibodies were specifically eluted with 25 mM ouabain in PBS. The ouabain that remained associated with the antibodies after gel filtration was removed by denaturation in 6 M guanidine·HCl. The antibodies were recovered after renaturation in PBS.

Adsorption

A suspension of 80 ml of antiserum (32 weeks after initial immunization) and about 600 mg dry weight of ouabain–RNase–BAC was incubated with stirring overnight at 4°C in a 250-ml centrifuge bottle. The next morning, the suspension was stirred at 37°C for 30 min. The immunoadsorbent was then centrifuged at 13,000 × *g* for 10 min. The supernatant was decanted and the immunoadsorbent was resuspended in 200 ml of cold PBS. After 20 min. of stirring at 4°C, the immunoadsorbent was centrifuged again. The supernatant was decanted and discarded. This washing procedure was repeated three additional times. The effectiveness of the washing procedures was established by inclusion of ^{125}I-labeled sheep gamma globulins in the incubation. The supernatant from the third wash contained less than 2% of the counts originally associated with the immunoadsorbent pellet.

Elution

The antidigoxin antibodies were specifically eluted from the washed immunoadsorbent with three washes of 25 mM ouabain in PBS. In repeated elutions, aliquots of 25, 12.5, and 12.5 ml were incubated with stirring with the immunoadsorbent for 30 min at 37°C. The three ouabain elutions yielded 207, 21, and 7 mg of antibody, respectively. The yield of antibody compared favorably with the theoretical capacity of the immunoadsorbent, which was determined to be 0.32 mg of antibody per mg of dry weight.

Gel filtration

The antibody eluate was subjected to gel filtration on a column (100 × 5 cm) of Sephadex G-25m (Pharmacia Fine Chemicals) equilibrated with PBS. Excess ouabain was well separated from the protein peak. Prior equilibration of the antibody solution with [^{3}H]ouabain, however, revealed that 1.8–1.9 mol of ouabain remained associated with each mole of antibody after gel filtration.

Reversible denaturation

Dialysis of 15 ml of the antibody solution recovered from gel filtration against 225 ml of 6 M guanidine·HCl (Schwarz–Mann, Co., Ultra Pure) at 4°C removed 99% of the ouabain associated with the antibodies. The dialysis was performed in $^5/_8$-inch dialysis tubing and the dialysate was changed twice at 12-hr intervals. After 36 hr the protein was renatured at 4°C by three successive 6-hr dialyses in solutions containing the following ratios of 6-M guanidine·HCl to PBS: 2/1, 1/2, and 1/6. The solution was then dialyzed against three changes of PBS over 24 hr. The antibody solution was clear at the end of the guanidine dialysis, but tended to become turbid during the PBS dialysis. The turbidity was removed by centrifugation at 12,000 × *g* for 10 min. The recovery of protein from the denaturation–renaturation procedure varied between 73 and 95%.

Characterization of isolated antibodies

Table 1 lists the binding affinity and effective number of sites per mole of antibody at the various stages of the isolation. It is apparent that the eluted antibody population is characterized by a higher affinity than the antibodies present in the antiserum, whereas some loss of affinity and binding capacity occurred during the guanidine denaturation and subsequent renaturation.

The isolated antibodies were examined by cellulose-acetate electrophoresis, immunoelectrophoresis, and immunodiffusion. Cellulose-acetate electrophoresis of 20 μg of isolated antibodies revealed bands only in the gammaglobulin zone. Immunoelectrophoresis showed that the isolated antibodies migrated identically to known sheep gammaglobulin (Pentex Biochemicals). The immunodiffusion also showed a line of identity with sheep gammaglobulin. A more-rapidly diffusing contaminant was barely perceptible.

The concentration of antibody protein was 3.7% of that of serum proteins in the antiserum. The single-step purification with immunoadsorbent resulted in a product, essentially un-

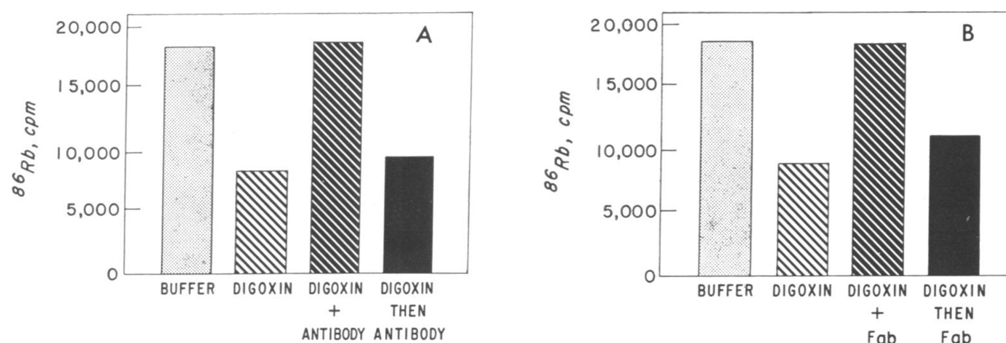

FIG. 1. *A*) Rubidium uptake in erythrocytes under conditions as specified in (*Rubidium uptake in human erythrocytes*). Intact antibody concentration is 2.9×10^{-7} M, digoxin concentration is 5×10^{-8} M. (*B*) Fab–Fc concentration, 6.4×10^{-7} M (assuming a molecular weight 50,000); digoxin concentration, 5×10^{-8}. Identical results were obtained when the digoxin concentration was increased to 1×10^{-7} M.

contaminated with other proteins, representing a 27-fold purification. The inactive material in the final product (20%) probably represents irreversibly denatured antibody.

Papain digestion of ouabain-eluted antibodies

200 mg of the antibody recovered from gel filtration was digested by papain. The conditions used were those previously described for rabbit gammaglobulins (10). After 3 hr of digestion at 37°C, the solution was placed on ice. Sufficient iodoacetic acid freshly dissolved in 0.1 M Na_2HPO_4 was added to the digest to bring the final concentration to 15 mM. The pH was raised to 8.0-8.5 by addition of 1 M NaOH. After 2.5 hr, this solution was dialyzed overnight against PBS at 4°C. Digoxin-binding capacity of the antibody solution decreased less than 10% after the papain digestion and iodoacetic acid alkylation. The digest was submitted to denaturation and renaturation as described for intact digoxin-specific antibodies to remove residual bound ouabain. After renaturation in PBS, the digest did not become turbid and recovery of protein was quantitative. The relative change in affinity of the digested antibodies after the denaturation–renaturation procedure was similar to that observed with intact antibody. Ultracentrifugation revealed that the papain digest sedimented as a single symmetrical peak with an s_w^{20} of 4.3 ± 0.3. This sedimentation value suggests that papain digests intact antibodies into Fab and Fc fragments (Fab–Fc). These results are similar to those of Heimer *et al.* (11).

EFFECTS OF INTACT DIGOXIN-SPECIFIC ANTIBODIES AND FAB ON ESTABLISHED EFFECTS ON DIGOXIN

Rubidium uptake in human erythrocytes

In erythrocytes, active rubidium uptake can be used as a sensitive measure of the activity of the Na^+–K^+ transport mechanism (12), which is inhibited by cardiac glycosides (13). ^{86}Rb-uptake studies were done by a modification of the method of Lowenstein (14). Freshly drawn human erythrocytes were washed four times with a buffer (NaPBS-G) that consisted of 0.15 M NaCl, 1 mg/ml glucose, 0.01 M Na_2HPO_4, and sufficient phosphoric acid to lower the pH to 7.4. 0.4 ml of packed erythrocytes and 0.6 ml of buffer were added to four duplicate sets of test tubes; digoxin was added to sets two through four, and antibody was added to the third set. All the tubes were incubated at 37°C for 1 hr and the erythrocytes were then washed twice by suspension and centrifugation with 4 ml of cold buffer. Antibody was added to the fourth set and all tubes were again incubated for 1 hr at 37°C. The washing procedure was repeated and the volume was reduced to 1 ml. All the tubes were then incubated with 40,000–80,000 cpm of ^{86}Rb; extracellular ^{86}Rb was removed by four successive 4-ml washes with buffer and the cells were counted in a Nuclear Chicago well scintillation counter.

The results of erythrocyte rubidium-uptake studies under the four conditions shown in the protocol are summarized in Fig. 1*A* and *B*. It is apparent that isolated intact antibodies or Fab–Fc are capable of preventing the establishment of digoxin inhibition of ^{86}Rb uptake when they are present in near stoichiometric amounts during the first incubation.

Exposure of erythrocytes to 5×10^{-8} M digoxin during the first incubation before the addition of antibody results in inhibition of ^{86}Rb uptake, which is not substantially reversed by subsequent exposure to antibody for 1 hr. However, when exposure to antibody was extended to 12 hr, reversal of inhibition could be demonstrated. Control uptake of ^{86}Rb was 8472 ± 186 (SE) cpm; after incubation with 5×10^{-8} M digoxin, this fell to 7300 ± 129 cpm; after an identical incubation with digoxin, followed by 12 hr incubation with 2.9×10^{-7} M antibody, ^{86}Rb uptake was 8410 ± 131 cpm. The difference in the mean uptakes of ^{86}Rb in nine experiments between the digoxin-exposed cells and those exposed to antibody was significant, with $P < 0.001$. There was no significant difference between the buffer control and the antibody-exposed cells. The relatively small digoxin effect noted in these experiments

TABLE 1. *Studies on the binding of antisera and purified antibody**

Sample	Ko† (M^{-1})	Binding sites (per mole)	Mol of ouabain/mol of antibody
Antiserum	1.8×10^8	(2)‡	—
Antibody after elution from adsorbent with ouabain	3.4×10^8	1.8	1.8
Antibody after removal of ouabain by guanidine	1.6×10^8	1.6	<0.1

* A 27-fold purification of antibody with respect to serum proteins is achieved.

† Defined as the reciprocal of the concentration of unbound digoxin at half-saturation of antibody sites.

‡ Assumed.

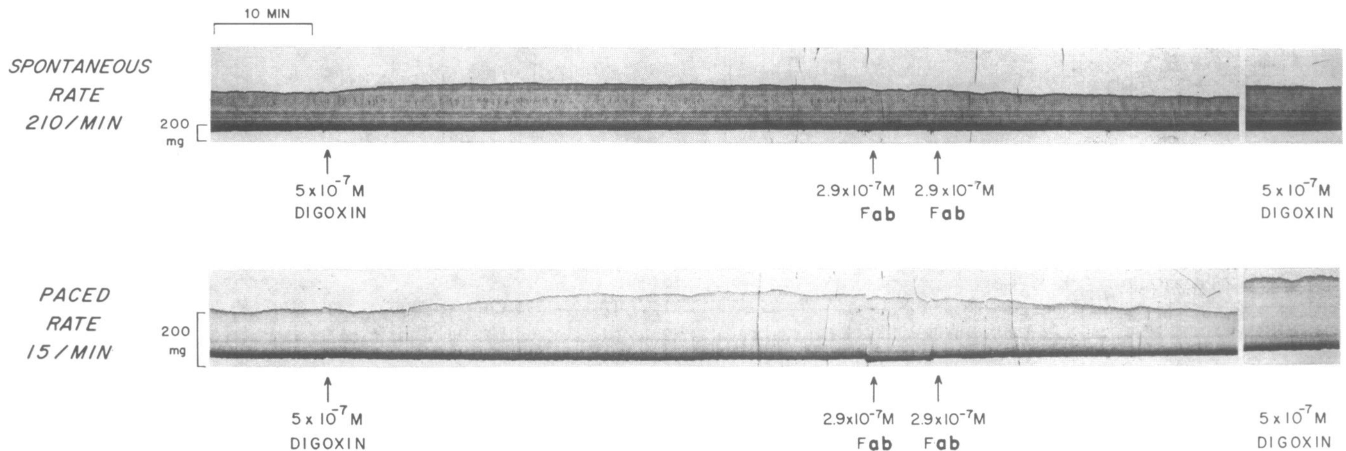

FIG. 2. Tension recordings from isolated guinea-pig atrial strips. Conditions are explained in the text.

is related to the long incubation. Thus, reversal of an established digoxin effect on the Na^+–K^+ transport mechanism of erythrocytes is relatively slow but complete. No effect on ^{86}Rb uptake could be demonstrated when nonspecific gammaglobulin was used.

Developed tension in isolated guinea pig atrial strips

The isolated digoxin-specific antibody and fragments rapidly reversed the effects of digoxin on developed tension in isolated guinea pig atrial strips. The atria of guinea pigs (350–450 g) were dissected and strips were mounted in an isolated muscle-bath apparatus described by Blinks (15). The bath contained 50 ml of a physiological buffer solution containing 6.6 g of NaCl, 0.35 g of KCl, 0.277 g of $CaCl_2$, 0.162 g of KH_2PO_4, 0.147 g of $MgSO_4$, 2.10 g of $NaHCO_3$, and 2.08 g of glucose per liter (138 mM Na, 5.9 mM K) at 35 ± 1°C. 95% O_2–5% CO_2 was bubbled continuously through the bath to maintain the pH and oxygenation of the muscle. The right-atrial strips were allowed to beat spontaneously, while left-atrial strips were driven at 15 beats per min with square-wave pulses of 1 msec duration and voltage 10% above threshold. Tension was recorded on a Hewlett-Packard recorder. The resting tension was adjusted to one-half of that which resulted in a maximal developed tension. The preparations were allowed to equilibrate under these conditions for 2–8 hr. After equilibration, digoxin was added to the bath to produce final concentrations of 5×10^{-7} M. This induced an increase in the developed tension in both the spontaneously beating and the paced atria that reached a plateau in 15–30 min, as is shown in the representative tracing (Fig. 2). After the digoxin-induced increment in developed tension had reached a plateau, either intact digoxin-specific antibody or Fab–Fc was added to the bath. Within 25 min, the developed tension returned to the same baseline levels observed before the addition of digoxin. In order to demonstrate that the digoxin-specific antibody and fragments had not permanently affected the atrial strips, the bath was emptied to remove digoxin and antibody or Fab–Fc. After 10–20 min of equilibration in fresh physiological buffer, an inotropic response to the addition of 5×10^{-7} M digoxin was again observed. The addition of sheep gammaglobulins or digoxin-specific antibodies to isolated atria that had not been exposed to digoxin did not result in a significant decrease in developed tension, nor did sheep gammaglobulin alter the developed tension in atria exposed to digoxin. The data from eight experiments performed on five isolated atrial strips are shown in Table 2. The intropic effects of digoxin on the isolated muscle preparations are fully reversed, in 10–15 min, by either intact antibody or Fab–Fc in a molar concentration of antibody or Fab–Fc combining sites that are less than twice the amount of active digoxin added to the bath.

TABLE 2. *Change in developed tension in guinea-pig atrial strips**

	Developed tension after digoxin (% of baseline)	Developed tension after digoxin followed by antibody (% of baseline)	
		Whole antibody	Fab–Fc
Spontaneous beating	119	93	—
	119	96	—
	162	—	94
	127	—	105
Paced (15/min.)	118	79	—
	118	80	—
	176	—	107
	139	—	93

* The difference between the means of developed tensions in digoxin-treated muscles before and after the addition of antibody or Fab–Fc was significant, with $P < 0.001$.

In one of the paced preparations the addition of digoxin resulted in some increase in developed tension, but automaticity emerged before the digoxin exerted its full effect on developed tension (Fig. 3). The irregularity shown in the tracing was due to spontaneous contractions arising from an irritable focus in the atrium. Changes in the frequency of contraction resulted in gross irregularity of developed tension. This irregularity persisted for about 30 min before digoxin-specific antibody was added to the bath. The addition of antibody resulted

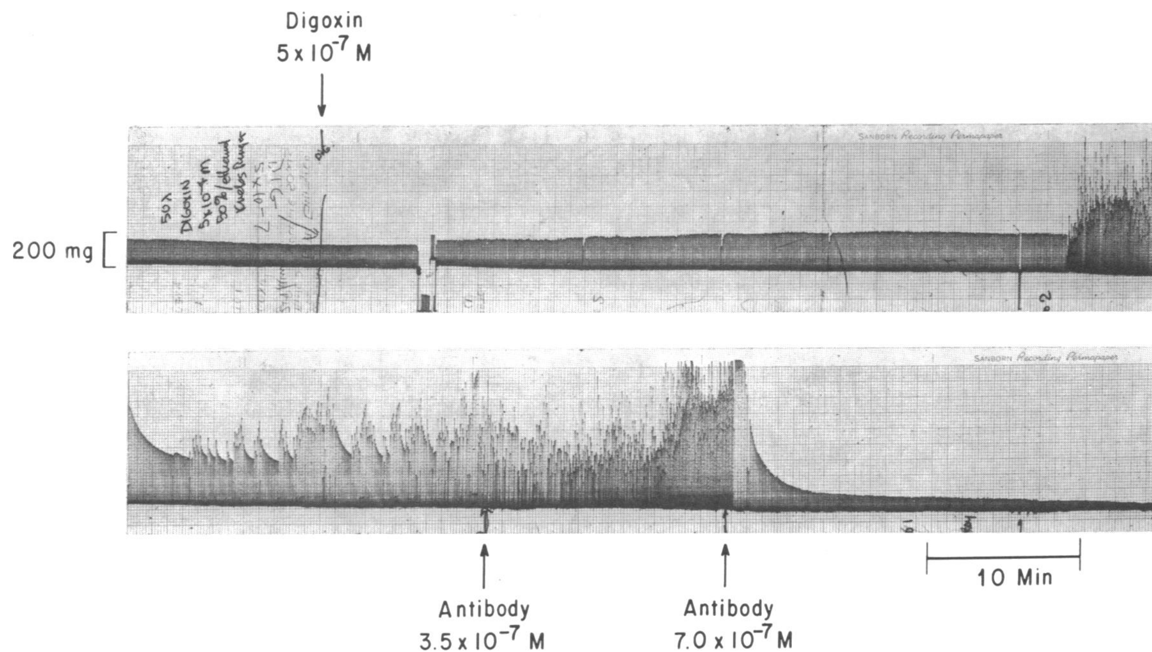

FIG. 3. Tension recording from an atrial strip (paced at 15 beats/min), that developed spontaneous contractions after digoxin was added. Final antibody concentration in the bath was 7×10^{-7} M.

in the cessation of automaticity and the recurrence of a regular-paced rhythm associated with a developed tension less than the baseline value. Antibody and digoxin were then removed by replacing the contents of the bath with fresh physiological buffer solution. After equilibration, the addition of digoxin again resulted in automaticity of the preparation. These spontaneous contractions are probably the result of a toxic effect of digoxin, which was reversed by digoxin-specific antibody.

Reversal of digoxin-induced ventricular tachycardia in the dog

Intravenous injection of 0.1 mg of digoxin/kg of body weight resulted in sustained ventricular tachycardia in 9 of 12 dogs. Mean duration of the arrhythmia was 157 ± 26 (SE) min. (I. Barr, T.W. Smith, M.D. Klein, and B. Lown, unpublished observations.) In marked contrast, three dogs given stoichiometric amounts of Fab–Fc after onset of ventricular tachycardia in response to 0.1–0.2 mg of digoxin/kg of body weight were out of this arrhythmia in 8 ± 3 min and in stable sinus rhythm after 17 ± 3 min ($P < 0.01$).

DISCUSSION

Purified digoxin-specific antibodies and antibody fragments of high affinity and capacity were obtained by immunoadsorbent-isolation and papain digestion. The purified antibodies bind 1.6 mol digoxin per mol, are of the IgG class, and are essentially free of contaminating proteins. These antibodies are effective in reversing established effects of digoxin on erythrocytes and isolated cardiac muscle, and rapidly abolish digoxin-induced ventricular tachycardia in dogs.

The isolation procedure is relatively simple. The adsorption and elution is dependent upon the crossreactivity of digoxin-specific antibody with ouabain on the ouabain–RNase–BAC immunoadsorbent. Digoxin-specific antibodies have a high affinity for digoxin, but bind to ouabain with an affinity about 100–1000 times less than that for digoxin. There is sufficient affinity and specificity of interaction to allow selective adsorption. Lower affinity for ouabain and relatively high aqueous solubility, in contrast to that of digoxin, facilitated their desorption from the immunoadsorbent. The lower affinity also facilitated dissociation of the ligand from the eluted antibody.

Even with ouabain as the eluting hapten, dissociation was difficult. Repeated prolonged dialysis would not remove the ouabain. Gel filtration in PBS, 0.1 N acetic acid, and 8 M urea were also ineffective. Dialysis in 10 M urea, 15% (v/v) dimethylformamide (Me_2F), 25% Me_2F, 40% Me_2F, or 17% (v/v) and 35% dimethylsulfoxide were either ineffective or resulted in loss of antibody sites or affinity. The renaturation procedure after 6 M guanidine·HCl treatment results in 5–27% loss of intact antibody by precipitation. This may represent aggregation or irreversible denaturation. Precipitation does not occur when the intact antibodies are digested with papain before denaturation and renaturation. The present isolation procedure can be readily increased in scale. The sheep studied here produced about 1 g of digoxin-specific antibody each week over a year's period.

Two types of effects of specific antibody and fragments on digoxin inhibition of ^{86}Rb uptake by erythrocytes were noted. The prior presence of antibody presumably prevented the binding of digoxin to red-cell receptors. Established digoxin binding was apparently reversed when antibody was added to erythrocytes that had been previously treated with digoxin. No measurable inhibition of ^{86}Rb transport occurs when a 2-fold molar excess of antibody sites is added before the addition of digoxin. On the other hand, reversal of established inhibition of ^{86}Rb uptake effected by prior incubation of erythrocytes with digoxin requires considerable time. After washing of digoxin-treated erythrocytes, little reversal can be demonstrated by a 10^{-7} M concentration of antibody at the end of 1 hr. At the end of 12 hr of incubation with antibody, however, 95% reversal was observed. These observations are consonant with those of Dunham and Hoffman (16), who showed that washing of erythrocytes to which [^3H]ouabain had been bound,

removed the glycoside very slowly. The slow reversal observed with antibody may be the result of very high affinity of the erythrocyte receptor, a small rate constant for dissociation, or a binding site in a sterically hindered area. Slow reversal of digoxin inhibition of potassium flux across the erythrocyte membrane by digoxin specific antisera has been noted by Watson and Butler (personal communication).

Reversal of digoxin effects in the atrial-strip preparation isolated from guinea-pigs was more rapid than in erythrocytes. Minutes were required for developed tension to return to baseline values, in contrast to the hours necessary to demonstrate an effect in erythrocytes. A fall to contractile levels somewhat below baseline values has been observed after the removal of acetylstrophanthidin from guinea-pig atrial strips by washing (17). The average time for full reversal of inotropic effects by antibody was 25 min. Half-times for removal of [^{3}H]digoxin and for reversal of inotropic effects by washing were longer at 48 and 45 min, respectively, under the similar conditions used by Roth-Schechter *et al.* (18). The explanation for the difference between erythrocytes and heart muscle may relate to differences in affinity constants or in effective dissociation rate constants.

Rapid reversal of cardiac arrhythmogenic effects of digoxin was observed in dogs. Prompt correction of these rhythm disturbances *in vivo* by intravenous administration of Fab–Fc suggests substantial human therapeutic implications. The digitalis glycosides have a narrow therapeutic range and a low toxic to therapeutic ratio. Intoxication in the course of clinical therapy is frequent (19).

Conventional therapeutic measures are sometimes inadequate for the correction of severe cardiac rhythm disturbances. Purified antibody may provide a means of specific reversal of intoxication in the gravely ill patient in whom conventional therapy has failed. There is a substantial precedent for the use of foreign antisera in therapy (20). Neutralization of a body burden of 2 mg of digoxin by only 130 mg of Fab–Fc, which has a probable half-life of about 5 hr (21), may minimize the problems of antigenicity of a foreign protein.

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