Advanced digitalis toxicity unresponsive to conventional therapy continues to be an important clinical problem (Smith and Willerson, 1971; Bismuth et al., 1973; Lely and van Enter, 1970). The cardiac glycoside digitoxin is used in about 16%-20% of digitalis-treated patients in the United States (National Prescription Audit) and is in more common use in some European countries such as France, where 96% of a series of 115 patients treated for acute digitalis poisoning had taken digitoxin with a resulting morality of 22% (Bismuth et al., 1973). Cardiac glycoside-specific antibodies or their Fab fragments have been shown to be capable of reversing a number of pharmacologic and toxic effects of digoxin and ouabain (Butler et al., 1973; Smith et al., 1977). Purified Fab fragments of digoxin-specific antibodies have recently been used clinically to reverse intractable hyperkalemia and advanced atrioventricular block following massive suicidal digoxin ingestion (Smith et al., 1976). Due to the high mortality rate associated with overwhelming digitoxin toxicity and the need for more effective therapy, we have undertaken studies with high affinity digitoxin-specific antibodies to determine their effects in an experimental model of lethal digitoxin toxicity. We have also extended earlier studies of Butler et al. (Butler et al., 1977) with digoxin-specific antibodies and Fab fragments to determine how intact antibodies and Fab fragments with high affinity for digitoxin would influence the pharmacokinetics of digitoxin in an animal model. The latter pharmacokinetic studies required the use of a primate species, the rhesus monkey, to provide an adequate model for digitoxin pharmacokinetics in man.

Antibody Production

To obtain antibodies with high affinity for digitoxin, digoxin was coupled to bovine serum albumin (BSA) by periodate oxidation and Schiff's base formation and reduction (Butler and Chen, 1967; Erlanger and Beiser, 1967). Sheep were immunized with BSA-digoxin in complete Freund's adjuvant and serially boosted and bled (Smith et al., 1970). Initial studies identified an animal that responded to immunization with a high titer of antibodies having high affinity

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for digitoxin. Pooled antiserum from consecutive bleedings of this animal was then used in subsequent experiments. The IgG fraction was obtained by ammonium sulfate precipitation (Campbell et al., 1963), and Fab fragments were prepared by papain digestion (Nisonoff, 1964). Undigested IgG was removed by gel filtration chromatography on Sephadex G-150. Digitoxin binding capacities of Fab fragments and IgG preparations were determined by a dextran-coated charcoal method (Herbert et al., 1965). Control (nonspecific) IgG and Fab fractions were prepared in identical fashion from sera of sheep not previously immunized with cardiac glycoside conjugates. The average intrinsic association constant ($K_\text{d}$) of the antibody population for digitoxin was determined by equilibrium dialysis (Smith et al., 1970). The $K_\text{d}$ for digitoxin of the pooled antiserum used in experiments discussed in this paper, as determined by equilibrium dialysis and Scatchard analysis, was $1.4 \times 10^{10} \text{ M}^{-1}$, confirming the high affinity for digitoxin of this sheep antibody population. Studies of rate constants for formation and dissociation of this antibody-digitoxin complex further document an affinity constant in the $10^{10} \text{ M}^{-1}$ range, with rapid association and slow dissociation kinetics (Smith and Skubitz, 1975; Skubitz and Smith, 1975).

**Toxicity Reversal Experiments**

Experiments to determine the ability of Fab fragments of specific antibodies to reverse established, potentially lethal digitoxin intoxication were carried out in 16 mongrel dogs. Animals were anesthetized with intravenous pentobarbital (30 mg/kg) and ventilated with a Harvard respirator at 12 cycles per min with a tidal volume adjusted to the weight of the animal. Digitoxin, 0.5 mg/kg, was injected intravenously over 10 min. Ventricular tachycardia occurred in all animals; 5 min after its onset, eight control dogs were then given control Fab fragments intravenously. The remaining eight dogs received an amount of specific Fab fragments equal in molar terms to the digitoxin dose over 3 min, followed by a 30-min infusion of an additional one-third of the initial dose. Blood samples for determination of serum digitoxin concentrations were drawn at the onset of ventricular tachycardia and at hourly intervals after administration of Fab fragments. After 3 h in stable sinus rhythm, surviving dogs were allowed to breathe spontaneously and to awaken; electrocardiograms were again recorded 24 h later.

The usual pattern of digitoxin toxicity was an initial sinus bradycardia with varying degrees of atrioventricular block shortly after glycoside administration, sometimes followed by supraventricular tachycardia. Ventricular tachycardia ensued shortly after the appearance of the first ventricular premature beats, at an average time of $23.4 \pm 3.8$ (SEM) min after digitoxin injection in the eight control dogs. All of these animals died, with ventricular fibrillation occurring terminally in six animals and ventricular standstill in the other two. Average time of death in the eight control dogs was $101.4 \pm 36.1$ min after digitoxin injection.