

# Effects of Sheep Digoxin-Specific Antibodies and Their Fab Fragments on Digoxin Pharmacokinetics in Dogs

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**ABSTRACT** Intact sheep antidigoxin antibodies and their Fab fragments have both been found to exert profound effects on digoxin pharmacokinetics in [ $^3\text{H}$ ]digoxin-treated dogs. Both classes of molecule remove digoxin from the extravascular space and sequester it in the circulation in protein-bound form, a form in which the digoxin is presumably inactive. These two classes of molecule differ, however, in that the intact antibody molecules interfere with digoxin excretion, thereby promoting the retention of the glycoside; this retained digoxin is eventually released in free, active form when the administered antibody is metabolically degraded. In contrast, urinary excretion of digoxin continues in Fab-treated dogs, with significant quantities of digoxin being excreted promptly in the urine in complex with Fab fragments. These differences in urinary excretion, together with the probable decreased immunogenicity of sheep antidigoxin Fab fragments, suggest that such fragments possess potential advantages over intact antibody molecules for use in the therapy of life-threatening digoxin intoxication in man.

## INTRODUCTION

Experimental animals immunized with synthetic digoxin-protein conjugates form antibodies with speci-

ficity for the unconjugated digitalis glycoside, digoxin (2). In vitro, antibodies to digoxin or to other cardiac glycosides are capable of removing these glycosides from mammalian cells (3, 4) and of preventing or of reversing many of the pharmacologic and toxic effects of the glycosides on such cells (3-10). In vivo, antidigoxin antibodies are capable of preventing or of reversing lethal digoxin intoxication in experimental animals (11-13). These properties of digoxin-specific antibodies have prompted consideration of the possibility that these antisera might be of clinical value in the reversal of severe digoxin intoxication in man (5, 7, 9, 12, 13).

Two major problems, however, might be anticipated if heterologous antidigoxin serum were to be administered in human subjects. One problem is the danger of immunological reactions to foreign serum proteins, as manifested clinically by anaphylaxis or serum sickness. The second problem arises from the fact that the manner in which the administration of antibodies might alter the metabolism and excretion of digoxin has not been established. It is known, however, that digoxin-binding antibodies in the sera of actively immunized rabbits slow the excretion of digoxin and markedly prolong its biologic half-life (14); therefore, if the administration of heterologous antibody had a similar effect, one might anticipate that the eventual immunologic elimination of the foreign protein could be relatively rapid and be accompanied by release of bound glycoside in its free, active, and potentially toxic form (7, 9).

In the hope of minimizing these problems, digoxin-binding Fab fragments of antidigoxin antibodies have been purified. These Fab fragments are smaller (50,000 daltons) than intact IgG antibodies (160,000 daltons). Each Fab fragment contains one digoxin-binding site in contrast to the two binding sites present in intact IgG, but Fab fragments possess biologic activity com-

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parable to that of equivalent concentrations of intact antibodies (10, 15). Since antidigoxin Fab fragments are free of other serum proteins, lack the more immunogenic Fc portion of the heterologous antibody molecule, and have a serum  $t_{1/2}$  of less than 12 h (16–18), these Fab fragments may possess less immunogenic potential than heterologous antidigoxin serum or intact antidigoxin antibody. Furthermore, by virtue of their smaller size, although largely metabolized by the kidney and other tissues (18), Fab fragments are excreted, at least in part, in the urine (17, 18). It has therefore been suggested that, if the antigen-binding sites of such excreted antidigoxin Fab fragments remain intact, these fragments might be capable of binding digoxin *in vivo* and then, in contrast to intact antidigoxin antibody, of permitting or promoting the relatively prompt urinary excretion of digoxin, thereby minimizing the potential hazards connected with late release of bound, retained digoxin from intact heterologous antiglycoside antibody molecules (5, 7, 9, 15).

It is the purpose of this report to present evidence that both sheep antidigoxin antibodies and their Fab fragments exert profound effects on the pharmacokinetics of digoxin in dogs.

## METHODS

**Antisera.** Digoxin was conjugated to bovine serum albumin (BSA)<sup>1</sup> by the periodate oxidation method (19) as described in detail elsewhere (2, 20). The synthetic BSA-digoxin was made up to a final concentration of 1 mg/ml in complete Freund's adjuvant mixture (20) and repetitively injected intramuscularly, 0.5 ml at each of two sites, into adult sheep, usually at 2–4 wk intervals. Control antiserum was obtained from a sheep immunized in a similar manner with a 1-methyladenosine-BSA conjugate also prepared by the periodate oxidation method (19).

Sheep gamma globulin (SGG; fraction II, Miles Laboratories, Inc., Elkhart, Ind.) was purified by chromatography on DEAE-cellulose (standard grade; Schleicher & Schuell, Inc., Keene, N. H.) in 0.02 M phosphate buffer, pH 7.0, as previously described (3). White New Zealand rabbits, weighing 2–3 kg, were immunized by the injection of SGG, 1 mg/ml in complete Freund's adjuvant mixture, according to a schedule described previously (11). Sera obtained from two rabbits after 6–30 wk of immunization contained antibodies to no constituents of sheep serum other than SGG as detected by immunoelectrophoretic analysis (21) against whole sheep serum. For use in studies of canine serum, antibodies which cross-reacted with canine gamma globulin (CGG) were removed by absorption with CGG (fraction II, Miles Laboratories, Inc.) at equivalence, or in later experiments, with an insoluble CGG-agarose immunoadsorbent.

**Immunoadsorbents.** CGG was coupled to agarose by the cyanogen bromide method (22, 23). Bromoacetyl cellulose was prepared according to the method of Robbins et al. (24). Ouabain-ribonuclease was prepared and coupled to bromoacetyl cellulose as previously described (13).

**Preparation of antidigoxin Fab fragments.** A globulin fraction of sheep antidigoxin serum was prepared by an ammonium sulfate precipitation method (25) as follows: to 200 ml antiserum, 100 ml saturated  $(\text{NH}_4)_2\text{SO}_4$  was added with stirring; the suspension was adjusted to pH 7.8 with 5 N NaOH and centrifuged for 30 min at 500 g at room temperature. The globulin precipitate was redissolved in 100 ml 0.15 M NaCl, 0.1 M sodium phosphate, pH 7.5, and dialyzed overnight against 2,000 ml of the same buffer.

The globulin fraction was digested with papain according to the method of Nisonoff (26) in a freshly made reaction mixture containing 0.002 M sodium EDTA and 0.01 M L-cysteine in 0.1 M sodium phosphate buffer, pH 7.5. Papain (twice crystallized, Worthington Biochemical Corp., Freehold, N. J.) was added dropwise with stirring to a protein concentration 1/100<sup>th</sup> that of the globulin in the final reaction mixture. After a 3-h incubation in a stoppered flask with shaking at 37°C, the mixture was placed in an ice water bath, and iodoacetic acid in 0.1 M  $\text{Na}_2\text{HPO}_4$  was added to a final concentration of 15 mM. After 2.5 h at 0°C, the reaction mixture was dialyzed overnight at 4°C against 4 liters of 0.15 M NaCl, 0.04 M sodium phosphate, pH 7.4, and further purified in one of two ways. For studies in three dogs in which large quantities of antidigoxin Fab fragments were required, the mixture was subjected to gel filtration on Sephadex G-150 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.); the first IgG-containing peak eluted from this column was discarded and the second, Fab-containing peak was pooled and administered as "partially purified antidigoxin Fab fragments". For all other studies, the papain-digested globulin mixture was added in eight equal portions to 750-mg aliquots of the bromoacetyl cellulose-ribonuclease-ouabain immunoadsorbent (an immunoadsorbent previously shown to be capable of adsorbing antidigoxin antibodies and their Fab fragments [10, 13]) and stirred for 4 h at 4°C and for 30 min at 37°C. The suspension was centrifuged at 4°C for 10 min at 10,000 g. After decanting and discarding the supernatant solution, the immunoadsorbent was washed three times with chilled 0.15 M NaCl, 0.01 M potassium phosphate, pH 7.4, by centrifuging 10 min at 10,000 g at 4°C. Fab fragments of antibody were eluted as previously described (13) with three successive additions (15, 10, and 10 ml) of 25 mM ouabain (ouabain octahydrate, Sigma Chemical Co., St. Louis, Mo.), stirring 30 min at 37°C and again centrifuging 10 min at 10,000 g at 4°C. The eluates from all portions were pooled, lyophilized, and taken up in distilled water to a 100-ml vol (much of the ouabain did not dissolve, and a substantial removal of free ouabain was achieved at this step). To permit dissociation of ouabain from antibody, the soluble protein was dialyzed overnight at 4°C against 600 ml 7 M guanidine hydrochloride ("ultrapure" grade, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.; or "extreme purity" grade, Heico Inc., Delaware Water Gap, Pa.). To promote relatively gradual denaturation of protein, dialysis bags were allowed to stand for 3 h without stirring, followed by continuous stirring overnight. To remove residual-free ouabain, the protein was then passed over a 6 × 88-cm column of Sephadex G-25 (Pharmacia Fine Chemicals), equilibrated with 6 M guanidine, at a rate of 1 ml/min; fractions were monitored by their absorption of ultraviolet light at 280 nm. The first 280-nm absorbing peak was found to contain antidigoxin Fab fragments, which were pooled and renatured as previously described (13) by dialysis against two changes of 2–3 liters 0.15 M NaCl, 0.04 M sodium phosphate, pH 7.4. The final product was lyophilized or frozen and stored at –20°C until use.

All preparations of purified digoxin-specific Fab fragments

<sup>1</sup>Abbreviations used in this paper: BSA, bovine serum albumin; SGG, sheep gamma globulin; CGG, canine gamma globulin.

contained only protein with gamma globulin mobility in cellulose acetate electrophoresis and yielded only a single arc on cellulose acetate immunoelectrophoretic analysis with rabbit antiserum to whole sheep serum (Hyland Lab., Costa Mesa, Calif.; lot 8135H001A1) or with rabbit anti-SGG serum. Analytical ultracentrifugation in a model E ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) employing schlieren optics was carried out on all preparations except that given to dog Fab-1 (S-62<sub>155</sub>) and revealed, in each instance, a single major peak containing 98–100% of the detectable protein and possessing a sedimentation coefficient of approximately 3.5S. However, polyacrylamide disk gel electrophoresis in sodium dodecyl sulfate in the absence of  $\beta$ -mercaptoethanol (27) was performed on some Fab preparations and revealed, in most instances, faint traces of a component with a mobility identical to that of intact sheep IgG.

**Binding of digoxin by antidigoxin antisera and Fab fragments.** The digoxin-binding capacity of antidigoxin sera and of antidigoxin Fab fragments was assessed by equilibrium dialysis of increasing concentrations of [<sup>3</sup>H]digoxin (0.23–62.1 ng/ml) in a 2-ml volume against equal volumes of a constant dilution of antidigoxin serum or against equal volumes of a solution containing a constant concentration of Fab fragments. The dilutions of antidigoxin sera employed were either 1:100,000 or 1:110,000, whereas the concentrations of different Fab preparations studied ranged from 73 to 123 ng/ml. Results were expressed as nanomoles digoxin bound per milliliter undiluted serum or as nanomoles digoxin bound per nanomole Fab fragments (Table I). Average intrinsic association constants were also calculated from the observed data, using methods outlined by Eisen (28).

**Radioiodination of SGG and antidigoxin Fab fragments.** SGG and antidigoxin Fab fragments, prepared as described

above, were radiolabeled with <sup>125</sup>I by the lactoperoxidase method (29, 30). To 100  $\mu$ l of 0.05 M phosphate buffer, pH 7.5, the following reagents were added (with mixing after each addition) in the following order: 5 or 10  $\mu$ l SGG or Fab (1  $\mu$ g/ $\mu$ l in buffer); 5  $\mu$ l bovine lactoperoxidase (B grade, Calbiochem, San Diego, Calif.; 1  $\mu$ g/ $\mu$ l in buffer); 2 mCi <sup>125</sup>I (NaI, in NaOH; carrier-free; Cambridge Nuclear Corp., Cambridge, Mass.); and, 5  $\mu$ l hydrogen peroxide (6 ng/ $\mu$ l in buffer). After a 5-min incubation at room temperature, 0.5 ml buffer was added, and the reaction mixture was passed over a 1.0  $\times$  20-cm column of Sephadex G-50 (Pharmacia Fine Chemicals), over which had been previously passed 1 ml 2% BSA in buffer, followed by 30 ml protein-free buffer. The first <sup>125</sup>I-containing material eluted from this column was frozen and stored at –20°C until use.

**Preparation of digoxin for pharmacokinetic studies.** [<sup>3</sup>H]Digoxin (lot 636-126; generally labeled; 9.0 Ci/mmol; 1 mCi/ml in 90% ethanol, 10% benzene) was purchased from New England Nuclear, Boston, Mass., and shown to contain 91.6% of its radioactivity in a fraction with an *R<sub>f</sub>* identical to that of digoxin on thin-layer chromatography, performed as described previously (3). Nonradioactive digoxin, 0.25 mg/ml in a 40% propylene glycol-10% ethanol solution, was generously provided by the Burroughs Wellcome Co., Research Triangle Park, N. C. (through the courtesy of Dr. Stanley T. Bloomfield). For use in pharmacokinetic studies, 200  $\mu$ l [<sup>3</sup>H]digoxin (17.4  $\mu$ g; 200  $\mu$ Ci) was evaporated to dryness in a 10-ml volumetric flask. 2-ml of nonradioactive digoxin (0.5 mg) was added, followed by 0.85% NaCl to a final volume of 10 ml. The final solution contained 0.052 mg digoxin and 20  $\mu$ Ci <sup>3</sup>H/ml.

**Pharmacokinetic studies.** Female mongrel dogs, 7.3–13.4 kg, were anesthetized with sodium pentobarbital (26 mg/kg administered intravenously). Additional sodium pentobarbital

TABLE I  
Amounts and Properties of Antidigoxin Serum and Fab Fragments Administered to Individual Dogs

Dog	Weight kg	Digoxin dose nmol	Antidigoxin material administered	Immunization details			Digoxin-binding capacity			
				Sheep*	Anti- gen injec- tions	Average intrinsic association constant	nmol digoxin/ ml serum	nmol digoxin/ nmol Fab	Total binding capacity of administered dose	Ratio of nmol bind- ing capacity to nmol digoxin administered
					<i>n</i>				<i>nmol</i>	
WS-1	10.0	256	30 ml serum	S-102 <sub>34</sub>	23	$6.2 \times 10^9$	148		{ 4,449	17.4
WS-2	11.4	292	25 ml serum	S-102 <sub>34</sub>						
WS-3	13.4	343	25 ml serum	S-102 <sub>34</sub>						
WS-5	11.1	284	25 ml serum	S-102 <sub>35</sub>	24	$3.1 \times 10^9$	229		5,720	{ 20.1
WS-6	11.8	302	25 ml serum	S-102 <sub>35</sub>						
WS-7	11.4	292	25 ml serum	S-102 <sub>35</sub>						
WS-8	10.7	274	25 ml serum	S-102 <sub>35</sub>						
Fab-1	10.1	259	43 mg Fab	S-62 <sub>155</sub>	52	$7.5 \times 10^9$		1.09	939	3.3
Fab-2	11.1	284	19 mg Fab	S-62 <sub>156</sub>	52	ND		ND	ND	ND
Fab-3	11.8	302	17 mg Fab	S-62 <sub>156</sub>	52	ND		ND	ND	ND
Fab-8	11.1	284	35 mg Fab	S-102 <sub>28</sub>	21	$5.1 \times 10^9$		0.94	684	2.3
Fab-9	7.3	187	{ 11.2 mg Fab	S-102 <sub>28</sub>	21	$5.1 \times 10^9$		0.94	385	2.0
			{ 9.2 mg Fab	S-61 <sub>64</sub>	27	$7.1 \times 10^9$		>0.90		
Fab-10	11.8	302	33 mg Fab	S-102 <sub>172</sub>	57	$1.5 \times 10^9$		0.77	524	1.7

\* Subscript denotes duration of immunization period in weeks.  
ND, not done.

(60–180 mg) was administered as necessary to keep the animals anesthetized during the initial 6 h of the study, during which time a catheter was kept in place in the urinary bladder and an intravenous infusion of 300 ml 0.9% NaCl or Ringer's lactate solution was administered. After insertion of the catheter and institution of the infusion, [ $^3\text{H}$ ]digoxin in a 0.02-mg/kg body weight dosage, prepared as described above, was administered intravenously over a 10-min period. 2 h later, one group of animals received freshly centrifuged (5,000 g, 15 min, 4°C) whole sheep antidigoxin serum (25 ml, except dog WS-1, which received 30 ml) and a second group of dogs received purified antidigoxin Fab fragments (17–43 mg in 10–60 ml 0.85% NaCl), both administered intravenously over a 10-min period. Details concerning these two groups of dogs and the antiserum or Fab fragments which they received are presented in Table I. A group of eight control animals received no serum or Fab fragments, while two other control dogs received freshly centrifuged sheep anti-1-methyladenosine-BSA serum (25 ml, administered intravenously over a 10-min period). After 6 h, anesthesia was discontinued; the catheters were removed, and the dogs were placed in metabolic cages. Serum and urine specimens were obtained frequently (usually hourly) during the first 6 h of the study and at various intervals thereafter, usually continuing until [ $^3\text{H}$ ]digoxin concentrations were <1% of the peak concentrations observed. Serum was stored at 4° or –20°C, whereas aliquots of urine were stored only in the frozen state until analysis (a few urine specimens were not promptly frozen; these were analyzed for total [ $^3\text{H}$ ]digoxin content and were not analyzed for protein-bound [ $^3\text{H}$ ]digoxin or Fab concentrations).

**Digoxin determinations.** Buffered human serum albumin, 0.35% in pH 7.4 Tris-buffered saline (11), was used as a diluent for all determinations. The dextran-coated charcoal method (14, 31) was used to estimate protein-bound [ $^3\text{H}$ ]digoxin concentrations in canine serum and urine. Total and protein-bound [ $^3\text{H}$ ]digoxin concentrations were determined simultaneously as follows: to each of four tubes, 0.5 ml of a 1:5 dilution of test serum or of a 1:21 dilution of test urine was added. To two tubes, 0.25 ml of dextran-coated charcoal (2% dextran T-40, 20% charcoal in Tris-buffered saline, pH 7.4) was added, and to two control tubes, was added 0.25 ml buffered albumin. After mixing, the tubes were centrifuged at 900 g for 1 h at 4°C. The supernatant solution was decanted into scintillation vials containing 15 ml of a toluene-Triton X-100 scintillation mixture (11). The vials were heated at 80°C for 30 min, allowed to cool, and then counted in a Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.); internal standards of [ $^3\text{H}$ ]digoxin were added to each sample, and the samples were recounted to correct for quenching. Utilizing the known specific activity of the administered [ $^3\text{H}$ ]digoxin, total digoxin concentrations in test serum and urine specimens were determined from the amounts of digoxin present in control tubes containing buffered albumin without charcoal; protein-bound [ $^3\text{H}$ ]digoxin was then expressed as the fraction of digoxin present in the supernatant of the tubes to which dextran-coated charcoal had been added. Total and protein-bound concentrations were then plotted on a logarithmic scale (ordinate) against time on a linear scale (abscissa).

Since the dextran-coated charcoal method measures [ $^3\text{H}$ ]digoxin bound to other molecules as well as to immunoglobulin molecules, the double-antibody method was used to determine the amount of digoxin bound to immunoglobulins or to Fab fragments in some serum and urine specimens (14, 32). 200  $\mu\text{l}$  of test serum was added to duplicate tubes

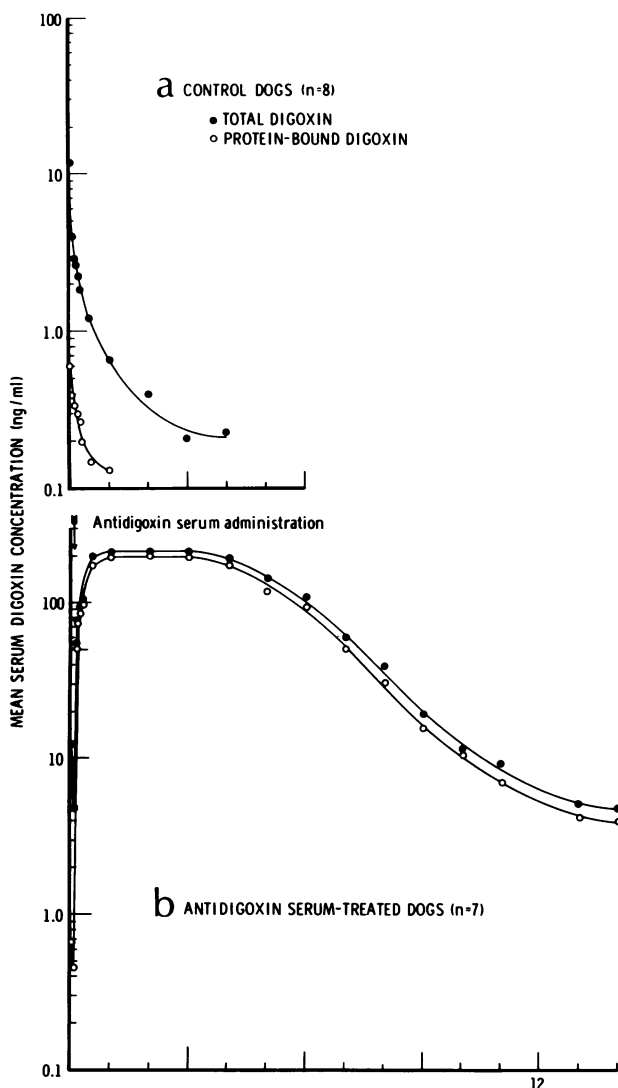
containing 1.0 ml of a 1:10 dilution of CGG-absorbed anti-SGG serum, or 200  $\mu\text{l}$  of a 1:10 dilution of urine was added in duplicate to 1.0 ml of a 1:32 dilution of unabsorbed anti-SGG serum. In duplicate control tubes, 200  $\mu\text{l}$  of test serum or of the 1:10 dilution of test urine was added to 1.0 ml of buffered albumin. After mixing, the tubes were placed in a 37°C water bath for 1 h, refrigerated at 4°C for 5–6 days, and centrifuged for 1 h at 4°C and 1,100 g. The precipitates were washed three times with chilled 0.85% NaCl, digested in 0.5 ml Soluene (Packard Instrument Co. Inc.), transferred to scintillation vials with the aid of two 5-ml portions of Dimilume (Packard Instrument Co. Inc.) and counted in the liquid scintillation spectrometer, using the subsequent addition of [ $^3\text{H}$ ]digoxin internal standards to correct for quenching. In each instance, 1 ml of supernatant solution was counted in 10 ml Dimilume in a similar manner. The remainder of the supernatant solution was assessed for residual antibody-bound or Fab-bound radioactivity by the addition of further anti-SGG antiserum, but in no instance was there precipitation of significant radioactivity. Immunoglobulin-bound or Fab-bound digoxin was calculated as the percent of the recovered serum or urinary radioactivity detected in the original precipitate.

**Radioimmunoassay of SGG and Fab fragments.** A globulin fraction of CGG-absorbed anti-SGG serum was prepared by the sodium sulfate method of Kekwick (33) and Strauss et al. (34) and coupled to bromoacetyl cellulose by the method of Robbins et al. (24). Solid-phase radioimmunoassays were carried out in 0.35% BSA in pH 7.4, Tris-buffered saline (11) as follows: to duplicate tubes to be used in the construction of a standard curve were added 1.0-ml portions of buffer containing 0–1,000 ng unlabeled SGG or Fab and 100  $\mu\text{l}$  of various dilutions (undiluted to 1:1,000) of normal canine serum or urine; corresponding 100- $\mu\text{l}$  volumes of the same dilutions of the serum or urine specimens being analyzed were added in duplicate to 1.0 ml buffer. To all tubes, 50  $\mu\text{l}$  (0.02  $\mu\text{Ci}$ )  $^{125}\text{I}$ -labeled SGG or Fab was added followed, after mixing, by 0.5 ml of a suspension containing sufficient bromoacetyl cellulose-anti-SGG to bind 40% of the added radioactivity in the absence of unlabeled antigen. After a 30-min incubation in a 37°C water bath, the tubes were centrifuged for 10 min at 6,200 g and 4°C; after decantation of the supernatant fluid, both precipitate and supernatant radioactivity were counted in an Auto-Gamma Spectrometer (Packard Instrument Co. Inc.). After construction of a standard curve, plotting precipitate radioactivity (after correction for nonspecific trapping) against the known antigen concentrations (plotted logarithmically), the concentrations of SGG or Fab in unknown specimens were determined from this standard curve by their respective capacities to inhibit binding of radioactivity to the insoluble immunoadsorbent. Using this method, as little as 10 ng SGG or Fab in 100  $\mu\text{l}$  serum or urine could readily be detected.

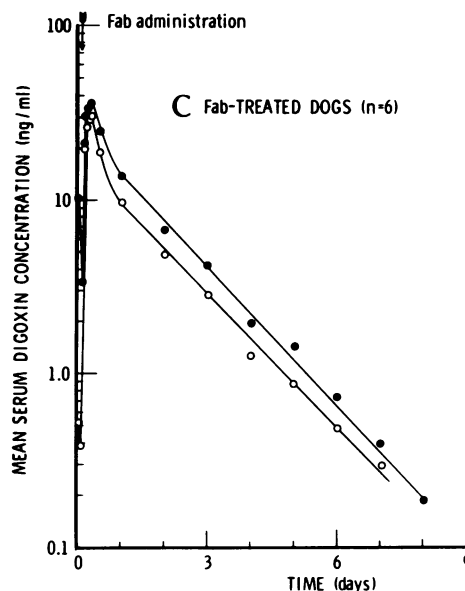
**Serum digoxin-binding capacity.** To determine the total digoxin-binding capacity of sera from [ $^3\text{H}$ ]digoxin-treated dogs receiving sheep antidigoxin serum or Fab fragments, 100- $\mu\text{l}$  aliquots of serum dilutions (1:5–1:1,050) in 0.35% human serum albumin in pH 7.4 Tris-buffered saline were mixed with varying amounts (6.5–52 ng) of [ $^3\text{H}$ ]digoxin (50  $\mu\text{l}$  volume) of the same specific activity (0.39  $\mu\text{Ci}/\mu\text{g}$ ) which had been administered to the dog. After a 10-min incubation at room temperature, 0.25 ml dextran-coated charcoal was added, and the protein-bound digoxin was determined as described above in the section, Digoxin determinations. The maximal amount of digoxin bound under these conditions was considered to represent the total digoxin-binding capacity of the 100- $\mu\text{l}$  serum dilution assayed.

Results were expressed in nanograms digoxin per milliliter canine serum.

**Density gradient ultracentrifugation.** 1 ml of serum or urine, diluted 1–3 in 10% sucrose, was layered onto an 11.8 or 12.4 ml linear gradient of 10–40% sucrose and centrifuged for 18 h at 199,000  $g_{av}$  at 20°C in a model L2-65B ultracentrifuge (Beckman Instruments Inc.), employing a model SW 40Ti rotor. After removal from the centrifuge, the tubes were punctured at their bottoms and 0.5-ml fractions collected. The 19S (IgM)- and 7S (IgG)-containing fractions were identified by their capacity to form precipitates in agar diffusion against rabbit anti-CCG serum (Miles Laboratories Inc.); the 4S albumin-containing fractions were identified by their capacity to bind bromphenol blue. The presence of [ $^3$ H]digoxin in these fractions was determined by assay of 250- $\mu$ l aliquots in a toluene-Triton X-100



**FIGURE 1** Mean serum [ $^3$ H]digoxin concentrations at various time intervals after [ $^3$ H]digoxin administration in three groups of dogs: (a) control dogs which received no sheep serum or Fab fragments; (b) dogs which received sheep antidigoxin serum 2 h after [ $^3$ H]digoxin administration.



**FIGURE 1c** Dogs which received purified sheep antidigoxin Fab fragments 2 h after [ $^3$ H]digoxin administration.

liquid scintillation mixture (11), using the liquid scintillation spectrometer.

**Detection of antibodies to SGG or sheep Fab fragments in dog sera.** Canine sera were assessed for the presence of antibodies to SGG or to sheep Fab fragments by measuring the ability of these sera to agglutinate human group O erythrocytes, coated with SGG or with sheep Fab fragments by the bisdiazotized benzidine passive hemagglutination method, performed as previously described (35).

## RESULTS

In Fig. 1a are shown mean serum [ $^3$ H]digoxin concentrations at various time intervals after the intravenous injection of a single 0.02-mg/kg dose of [ $^3$ H]digoxin to a group of eight control dogs, which received no serum or Fab fragments. The mean  $t_1$  of serum digoxin was  $30.9 \pm 12.4$  h (SD), a value comparable to that of 23–26.9 h, reported by earlier workers (36–38). Serial determinations of protein-bound [ $^3$ H]digoxin as determined by the dextran-coated charcoal method are also depicted in Fig. 1a. A mean of 11.3% of the [ $^3$ H]digoxin was protein-bound.

In Fig. 2a, the mean urinary [ $^3$ H]digoxin excretion rate during various time intervals after [ $^3$ H]digoxin administration is shown. As anticipated, the urinary digoxin excretion rate decreased as the serum digoxin concentration fell; a mean of 6.7% of the urinary [ $^3$ H]digoxin was bound to proteins or other macromolecules, as determined by the dextran-coated charcoal method. In two additional control dogs given 25 ml of sheep anti-1-methyladenosine-BSA serum 2 h after receiving [ $^3$ H]digoxin, comparable serum digoxin

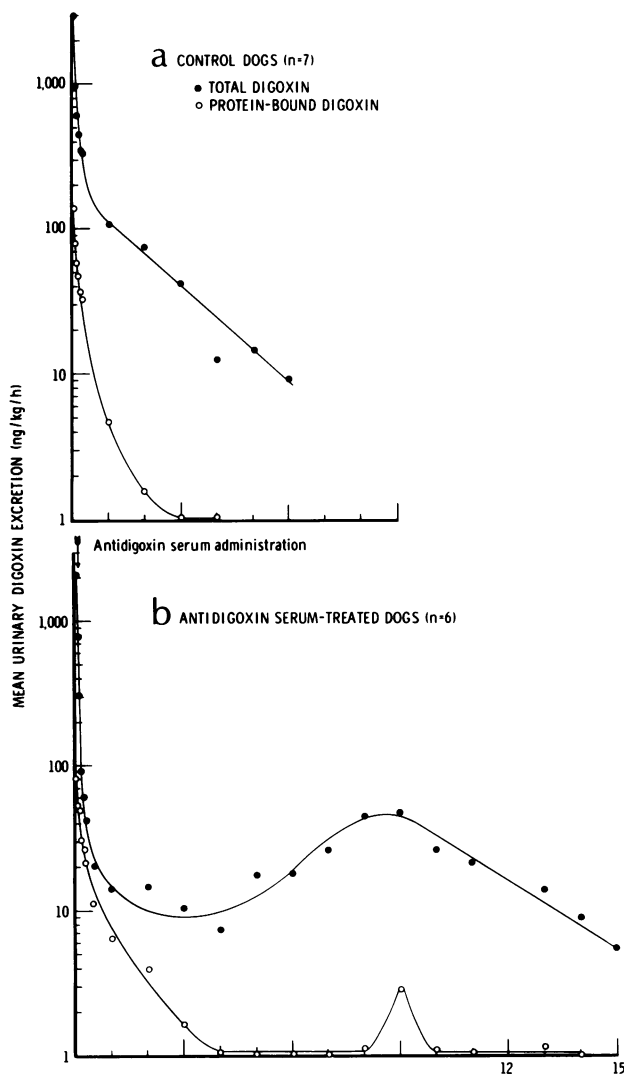


FIGURE 2 Mean urinary [ $^3\text{H}$ ]digoxin excretion rates at various time intervals after [ $^3\text{H}$ ]digoxin administration in three groups of dogs: (a) control dogs which received no sheep serum or Fab fragments; (b) dogs which received sheep antidigoxin serum 2 h after [ $^3\text{H}$ ]digoxin administration.

concentrations and urinary excretion rates were observed.

Fig. 1b depicts mean serum digoxin concentrations after various time intervals in seven dogs, each of which received 25–30 ml of sheep antidigoxin serum 2 h after receiving a single dose of [ $^3\text{H}$ ]digoxin. Fig. 3a shows a representative serum [ $^3\text{H}$ ]digoxin disappearance curve observed in dog WS-8. After administration of the antidigoxin serum, containing intact antidigoxin antibodies, a 13- to 31-fold increase in serum digoxin, most of it protein-bound, occurred over the next 4 h. Serum digoxin concentrations continued to rise, but more slowly, during the subse-

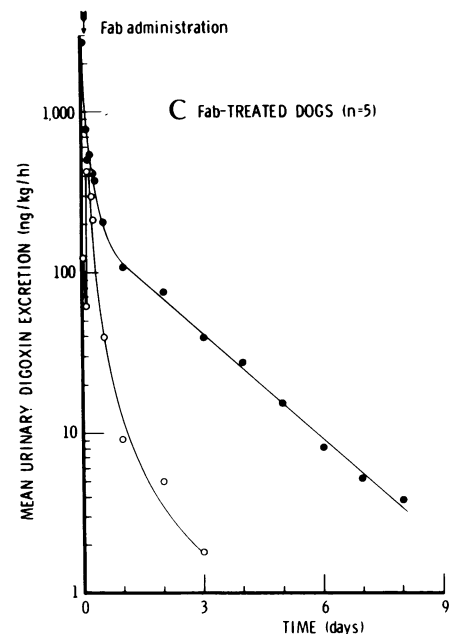


FIGURE 2c Dogs which received purified sheep antidigoxin Fab fragments 2 h after [ $^3\text{H}$ ]digoxin administration.

quent 18 h so that at the end of the 1st day of the study, the increases in serum digoxin levels produced by intact antidigoxin antibodies ranged from 33-fold in dog WS-3 to 57-fold in dog WS-5. Evidence that the protein-bound [ $^3\text{H}$ ]digoxin in these dog sera was largely bound to sheep antibody was obtained in two ways. (a) In sucrose density gradient ultracentrifugation experiments with serum from dog WS-3, [ $^3\text{H}$ ]digoxin, which had remained near the top of the gradient in serum obtained from this animal before antibody administration, sedimented with the 7S immunoglobulin-containing fraction in serum specimens obtained 1 and 11 days after antibody administration; a similar result was observed with serum obtained from dog WS-8 4 h after the antibody was given. (b) A more direct demonstration that most of the [ $^3\text{H}$ ]digoxin, which was protein-bound as assessed by the coated charcoal method, was indeed bound to SGG, was obtained by demonstrating that the labeled glycoside coprecipitated with SGG when CGG-absorbed anti-SGG serum was added to representative canine serum specimens (Table II).

Serum digoxin concentrations remained elevated for several days after antibody administration but then, in most animals, began to decrease sharply after about 6 or 7 days (Figs. 1b, 3a). For example, in dog WS-8, a 100-fold decrease in serum digoxin from 112 to 1.1 ng/ml, occurred between days 6 and 9 (Fig. 3a); similarly, a 50-fold decrease from 70 to 1.4 ng/ml occurred between days 6 and 8 in dog WS-5, and a 70-fold decrease from 77 to 1.1 ng/ml occurred in dog

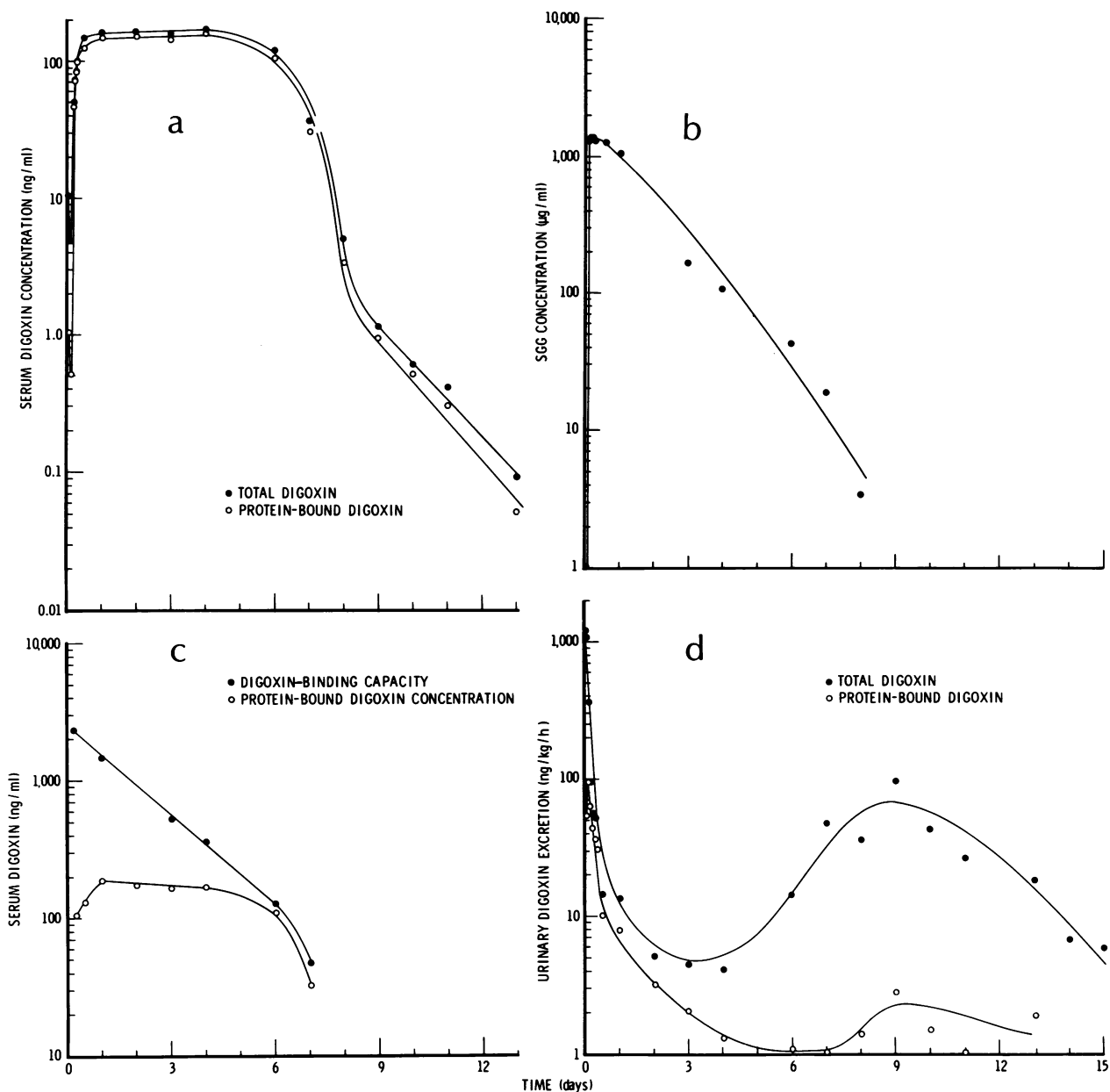


FIGURE 3 Serial determinations of various parameters in dog WS-8, which received 25 ml sheep antidigoxin serum 2 h after administration of [ $^3\text{H}$ ]digoxin at 0 h: (a) total and protein-bound serum [ $^3\text{H}$ ]digoxin concentrations; (b) serum SGG concentration; (c) serum digoxin-binding capacity and serum protein-bound [ $^3\text{H}$ ]digoxin concentration; and, (d) urinary excretion rate of total and protein-bound [ $^3\text{H}$ ]digoxin.

WS-7 between days 7 and 9. In one dog, WS-3, a sharp decrease in serum digoxin concentration was not observed, and the disappearance of serum digoxin occurred more gradually over a 4-wk period.

To determine whether the observed decreases in serum digoxin concentrations could be correlated with the disappearance of SGG from canine serum, SGG concentrations were measured serially in

serum specimens obtained from five dogs which had received sheep antidigoxin serum (Figs. 3b, 4a); in specimens obtained from two of these dogs, the total digoxin-binding capacity of the dog serum was also determined at various time intervals (Fig. 3c). The serum concentrations of SGG began to decrease immediately after the 1st day (Figs. 3b, 4a) but, as can be seen in Fig. 3c, the amount of anti-

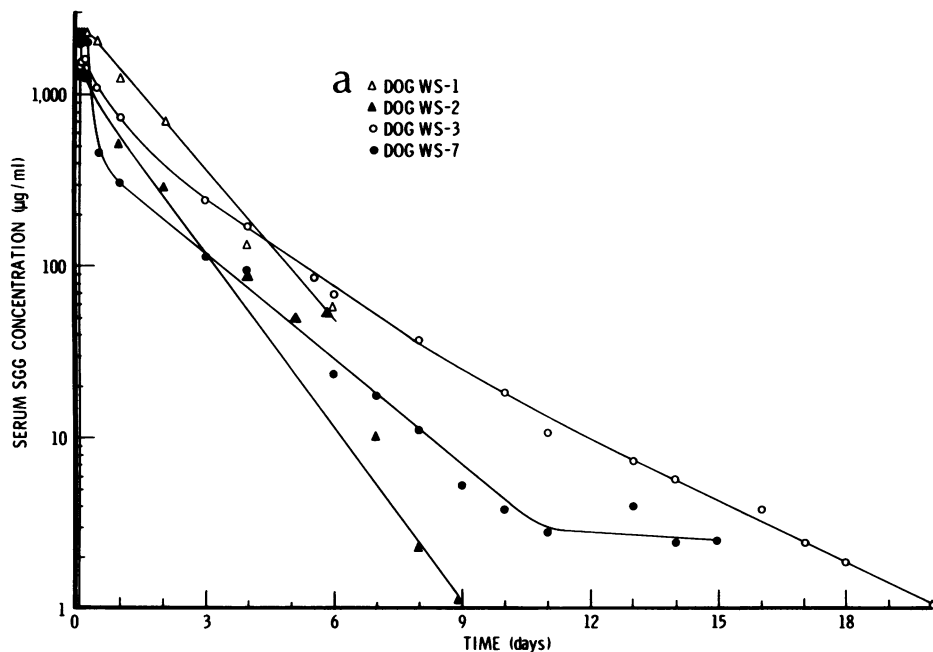


FIGURE 4 Serial serum concentrations of SGG or Fab fragments at various time intervals after [ $^3\text{H}$ ]digoxin administration: (a) serum SGG concentrations in four dogs which received sheep antidigoxin serum 2 h after [ $^3\text{H}$ ]digoxin administration.

body administered was initially capable of binding considerably more digoxin than was present in the canine serum specimens being analyzed. Presumably because of this considerable residual digoxin-binding capacity, serum protein-bound digoxin concentrations

remained relatively stable for several days, despite the decreasing SGG concentration. Then, as the serum digoxin-binding capacity more nearly approached the serum digoxin concentration, a decrease in the serum protein-bound digoxin concentration occurred which appeared to be correlated with the decreasing SGG concentration (Figs. 3, 4a). In dog WS-8 (Fig. 3) and in two of the four dogs depicted in Fig. 4a, it was noteworthy that sharp decreases in SGG concentration (and, in the case of dog WS-8, also a decrease in total digoxin-binding capacity) occurred at about the times at which sharp decreases in serum digoxin concentrations had been observed. This observation was consistent with the hypothesis that immunologically mediated clearance or degradation of SGG may have accelerated the release of antibody-bound [ $^3\text{H}$ ]digoxin, with the associated decreases in serum digoxin concentrations being caused by uptake of newly released glycoside in tissue-binding sites and by excretion of newly released glycoside in the urine.

Attempts were made to detect circulating SGG-anti-SGG complexes in the sera of dogs receiving antidigoxin serum by examining the sedimentation characteristics of protein-bound [ $^3\text{H}$ ]digoxin as studied by sucrose density gradient ultracentrifugation. No evidence for [ $^3\text{H}$ ]digoxin sedimenting with a fraction with a sedimentation coefficient greater than that of IgG was detected at 25 and 264 h in the case of

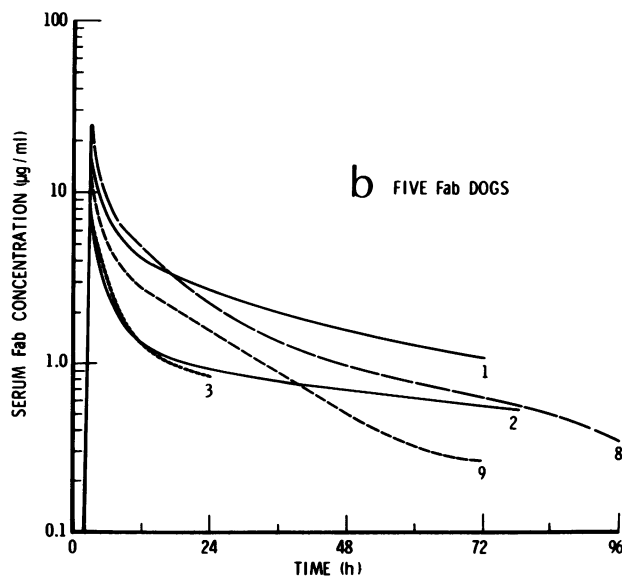


FIGURE 4b Serum immunoreactive Fab concentrations in five dogs which received purified sheep antidigoxin Fab fragments 2 h after digoxin administration.



dog WS-3. At 408 h, a small amount of [ $^3\text{H}$ ]digoxin was detected in fractions 4–6 ml from the bottom of the gradient, consistent with the presence of digoxin-binding antibodies in the >7S “intermediate complex” region; however, the low levels of [ $^3\text{H}$ ]digoxin in this late serum specimen and its ultracentrifugal fractions made it difficult to be certain of the presence of circulating immune complexes.

The urinary excretion of [ $^3\text{H}$ ]digoxin was studied in most of the dogs which received sheep antidigoxin serum (Figs. 2*b*, 3*d*). In these animals, presumably as a result of the binding of digoxin by antibody, urinary digoxin excretion rates decreased to negligible values immediately following antibody administration. In most animals, there was a slight increase in the percentage of urinary digoxin which appeared to be

TABLE III  
*Immunoglobulin-Bound or Fab-Bound [ $^3\text{H}$ ]Digoxin in Urine of Dogs Receiving Antidigoxin Serum or Fab Fragments*

Dog	Time	[ $^3\text{H}$ ]Digoxin concentration	[ $^3\text{H}$ ]Digoxin-bound, %	
			Dextran-coated charcoal method	Double-antibody method
	<i>h</i>	<i>ng/ml</i>		
WS-5	2	737	10	<1
	3	371	15	<1
	5	58	51	<1
WS-6	2	1,327	7	<1
	3	310	19	<1
	5	64	55	<1
	6	112	64	<1
WS-7	0.5	2,325	5	<1
	5	47	25	3
Fab-8	2	655	5	<1
	3	81	77	41
	4	28	70	21
	5	30	64	4
	12	95	22	3
	24	54	8	<1
	48	40	4	<1
Fab-9	2	1,450	10	<1
	3	88	84	66
	5	576	80	44
	24	209	13	<1
Fab-10	48	99	3	<1
	2	835	7	<1
	3	518	78	52
	5	47	59	40

TABLE II

*Immunoglobulin-Bound or Fab-Bound [ $^3\text{H}$ ]Digoxin in Serum of Dogs Receiving Antidigoxin Serum or Fab Fragments*

Dog	Time	[ $^3\text{H}$ ]Digoxin concentration	[ $^3\text{H}$ ]Digoxin-bound, %	
			Dextran-coated charcoal method	Double-antibody method
	<i>h</i>	<i>ng/ml</i>		
WS-2	0.5	7.3	4	<1
	48	106	90	>99
	193	3.5	83	85
WS-3	336	11.1	84	90
	408	3.0	82	84
WS-7	0.5	10.9	8	<1
	48	260	97	>99
	96	177	>99	>99
WS-8	0.5	9.3	8	<1
	48	152	>99	>99
	192	3.5	66	84
Fab-2	2	4.1	16	<1
	7	29.8	76	81
	24	14.0	68	71
Fab-3	2	3.4	7	<1
	6	20.4	81	86
	24	8.2	58	26
Fab-8	0.5	12.0	5	<1
	6	51.3	79	95
	12	37.5	79	95
	24	17.4	69	84
	48	9.2	66	93
Fab-9	5	41.8	80	85
	12	17.5	75	82
Fab-10	0.5	10.1	6	<1
	6	36.2	93	93

bound to proteins or other macromolecules during the 1st day after antiserum administration (Figs. 2*b*, 3*d*); however, this fraction of the urinary digoxin did not appear to be bound to sheep immunoglobulin-derived molecules, as assessed by the double-antibody method (Table III). The urinary digoxin excretion rate remained low for several days and then, at about the time at which the serum concentration began to decrease rapidly, an increase in urinary digoxin excretion, mainly in free nonprotein-bound form, was observed in most instances (Figs. 2*b*, 3*d*).

Six dogs were studied extensively after having been given purified sheep antidigoxin Fab fragments (1.4–4.3 mg/kg) intravenously 2 h after receiving a single dose of [ $^3\text{H}$ ]digoxin. During the 4 h after administration of Fab fragments, an 8- to 16-fold increase in serum [ $^3\text{H}$ ]digoxin from the 3.1–3.7 ng/ml range to the 23–57 ng/ml range, most of the [ $^3\text{H}$ ]digoxin being protein-bound, occurred (Figs. 1*c*, 5*a*). Most of the

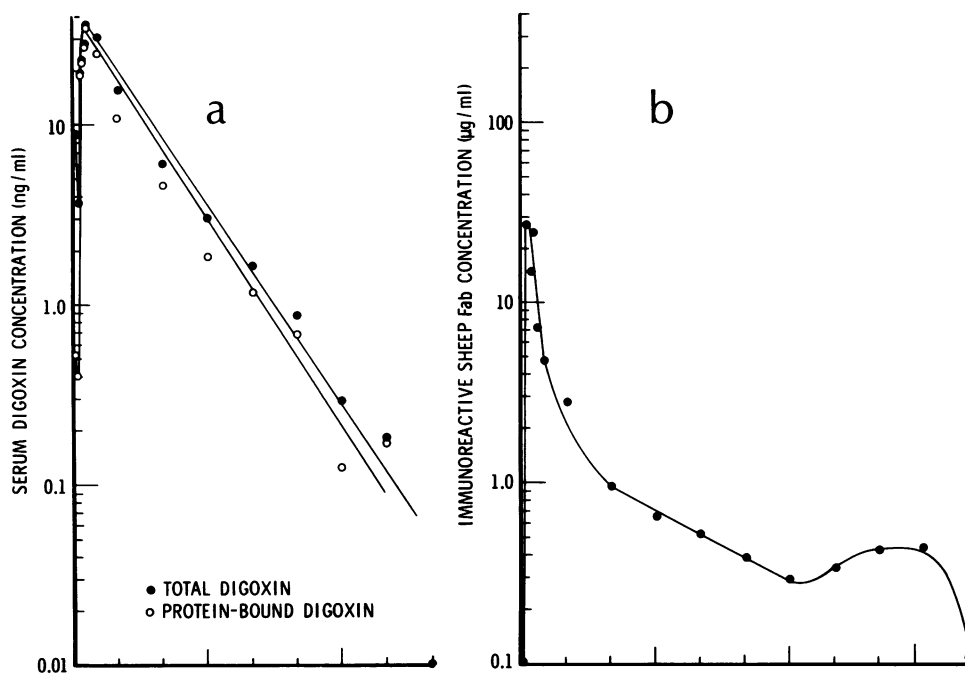


FIGURE 5a, b Serial determinations of various parameters in dog Fab-10, which received 33 mg purified sheep antidigoxin Fab fragments 2 h after administration of [ $^3$ H]digoxin at 0 h: (a) Total and protein-bound serum [ $^3$ H]digoxin concentrations; (b) serum immunoreactive Fab concentration.

protein-bound [ $^3$ H]digoxin was Fab-bound, as shown by its coprecipitation with Fab fragments when CCG-absorbed anti-SGG serum was added to representative canine serum specimens (Table II) and by its sedimentation in the 3.5S fraction in sucrose density gradient ultracentrifugation. Unlike dogs receiving intact antidigoxin antibodies, in whose sera digoxin concentrations initially decreased slowly, serum digoxin concentrations fell rather rapidly over the next 18 h.

Serum immunoreactive sheep Fab fragment concentrations were measured serially in six Fab-treated dogs (Figs. 4b, 5b). Unlike serum SGG concentrations which initially decreased slowly in dogs receiving intact antibody (Figs. 3b, 4a), serum immunoreactive Fab concentrations fell rather rapidly over the first 24 h of study (Figs. 4b, 5b) with a mean dominant  $t_{1/2}$  of less than 5 h, a value similar to the  $t_{1/2}$  of 3.6 h reported by Wochner et al. (18) for rabbit Fab fragments in mouse serum. After the first 24 h, the serum disappearance rate of immunoreactive Fab, like that of [ $^3$ H]digoxin, decreased considerably. The fact that most of the serum [ $^3$ H]digoxin was protein-bound after the first 24 h (Figs. 1c, 5a), together with the slower disappearance rate of immunoreactive Fab after the 1st day, suggested that intact antibody molecules or fragments of sizes larger than Fab fragments might have been present in the circulation of

these Fab-treated dogs. In the case of dog Fab-10, sucrose density gradient ultracentrifugation of serum obtained as soon as 4 h after Fab administration revealed that, in contrast to a specimen obtained 1 h after Fab administration, much of the [ $^3$ H]digoxin was in the 7S IgG portion of the gradient. Polyacrylamide disk gel electrophoresis in sodium dodecyl sulfate was then performed retrospectively on some of the Fab preparations used in this study and revealed, in most instances, faint traces of a component with a mobility identical to that of intact sheep IgG. Efforts are now being made to isolate Fab preparations which are free of intact immunoglobulin molecules.

In the serum of dog Fab-10, the disappearance rate of the digoxin-binding capacity was studied and found to parallel closely the serum disappearance rate of immunoreactive Fab (Figs. 5b, c), suggesting that most of the circulating immunoreactive Fab fragments had retained their digoxin-binding capacity and had not been inactivated by canine tissues or plasma. During the first 4 h after Fab administration, the Fab fragments present in the serum were capable of binding considerably more digoxin than was present in the dog's serum at that time. In later specimens, as serum Fab concentrations fell, most of the digoxin-binding sites appeared to have been occupied *in vivo* (Fig. 5c).

In marked contrast with the results observed in

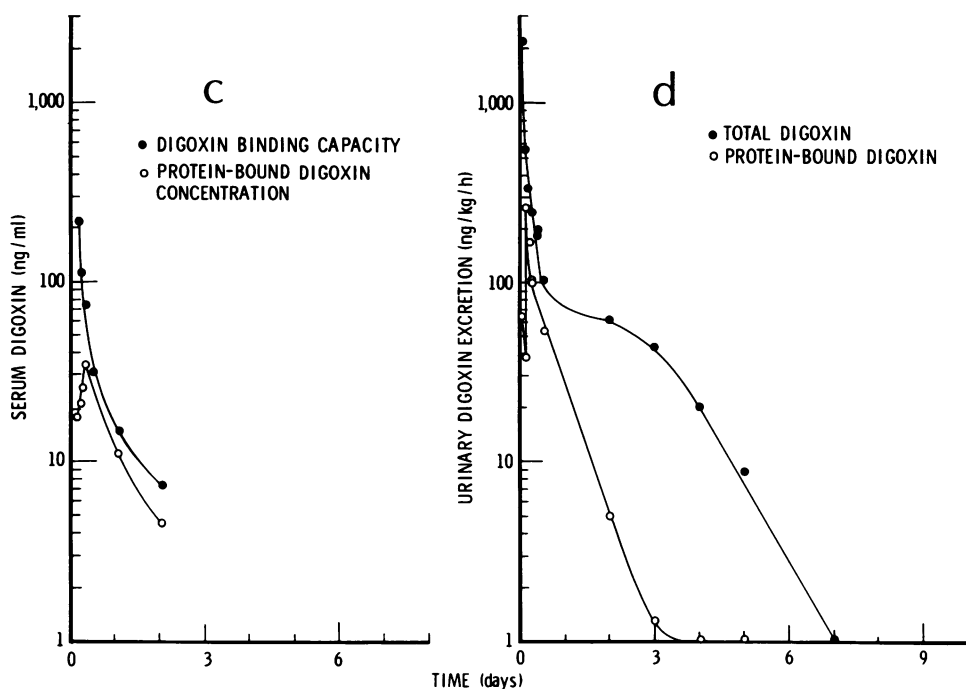


FIGURE 5c, d (c) Serum digoxin-binding capacity and serum protein-bound [ $^3\text{H}$ ]digoxin concentration; and (d) urinary excretion rate of total and protein-bound [ $^3\text{H}$ ]digoxin.

dogs given whole antidigoxin serum, the early rise in serum [ $^3\text{H}$ ]digoxin concentration was not accompanied by a sharp decrease in the urinary excretion rate (Figs. 2c, 5d). Not only was digoxin excreted, but Fab fragments were also excreted in the urine (Fig. 6). As a result of the urinary excretion of Fab fragments, 74.9% of the urinary digoxin excreted in the first 4 h after Fab administration was protein-bound (Figs. 2c, 5d; Table IV); in sucrose density gradient ultracentrifuge analysis of urine obtained 1 h after Fab administration to dog Fab-10, most of the protein-bound [ $^3\text{H}$ ]digoxin sedimented in the 3.5S range with a pattern suggesting that it was Fab-bound. Further evidence that much of the urinary [ $^3\text{H}$ ]digoxin was bound to excreted Fab fragments was obtained in double-antibody studies, in which it was demonstrated that significant amounts of urinary [ $^3\text{H}$ ]digoxin coprecipitated with urinary sheep Fab fragments after the addition of rabbit anti-SGG serum to the urine of Fab-treated dogs (Table III).

Despite the fact that a significant amount of the urinary digoxin was excreted in complex with Fab fragments, the urinary excretion of digoxin during the 22-h period after Fab administration (Table IV) was not significantly greater (41.3% of the recovered urinary digoxin) than in control dogs (38.4%). Furthermore, in four additional dogs in which, after an initial 1-mg dose of purified antidigoxin Fab fragments per kg body weight, additional Fab fragments were infused at a rate

of 0.25 mg/kg per h for 4 h, urinary digoxin excretion during the same 22-h period (49.0%) was not significantly greater than in control animals. Similarly, urinary digoxin excretion during this period was not

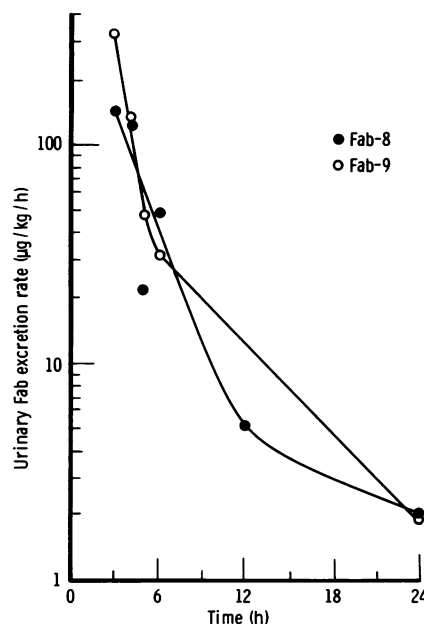


FIGURE 6 Urinary excretion rates of sheep Fab fragments in two dogs given purified Fab fragments intravenously 2 h after [ $^3\text{H}$ ]digoxin administration.

**TABLE IV**  
*Percent Recovered [<sup>3</sup>H]Digoxin Excreted during Various Time Intervals after [<sup>3</sup>H]Digoxin Administration*

Dog group	n	Recovery of administered [ <sup>3</sup> H]digoxin, %		Recovered [ <sup>3</sup> H]digoxin excreted during various time intervals, * %					
				Before treatment	After treatment				
				0-2 h	2-6 h	6-24 h	24-48 h	48-96 h	96+ h
Control	7	47.5	Total (bound)‡	32.5 (1.9)	18.6 (1.8)	19.8 (1.0)	14.2 (0.3)	9.1 (0.2)	6.5 (0.1)
Antidigoxin serum-treated	7	42.6	Total (bound)	25.1 (1.5)	4.4 (1.2)	2.5 (1.5)	4.0 (1.3)	5.6 (0.6)	58.2 (2.1)
Fab-treated (1.4-4.3 mg/kg)	5	52.3	Total (bound)	23.5 (1.4)	18.3 (13.7)	23.0 (3.6)	17.9 (1.0)	13.9 (0.6)	7.4 (0.4)
Fab-infusion§	4	59.9	Total (bound)	23.1 (2.4)	14.7 (10.6)	34.3 (15.1)	10.9† (1.2)	18.0 (2.4)	4.4 (0.3)
Fab-treated (22.1-22.6 mg/kg)	3	85.4	Total (bound)	16.8 (1.3)	9.5 (8.3)	42.9 (33.8)	21.2 (9.8)	7.3 (1.5)	2.3 (0.6)

\* [<sup>3</sup>H]Digoxin given at time zero and antibodies or Fab infusions begun at 2 h; see text for details.

† Expressed as percentage of total recovered urinary digoxin excreted in protein-bound form during each time interval.

§ Dogs given initial 1-mg/kg Fab dose followed by infusion of 0.25 mg/kg per h for 4 h; see text for details.

† Specimens from two dogs during this interval not available for analysis.

significantly greater (52.4%) in three dogs which were given single large doses of partially purified antidigoxin Fab fragments of 22.1-22.6 mg/kg (Table IV), doses comparable in digoxin-binding capacity with the doses of intact antibodies administered to the antibody-treated group (Table I). It may be noted, however, that 42-80% of the digoxin excreted during hours 2 through 24 by the three Fab-treated groups was in protein-bound form (Table IV).

Using the bisdiazotized benzidine hemagglutination technique, no antibodies to SGG or to sheep Fab fragments could be detected in the last serum specimens obtained from five dogs receiving antidigoxin serum and from five dogs receiving purified antidigoxin Fab fragments.

## DISCUSSION

Both intact antidigoxin antibodies and their Fab fragments produced prompt and striking increases in the serum concentrations of digoxin and of its protein-bound fraction. Since it has been previously demonstrated that antidigoxin antibodies and their Fab fragments are capable of reversing the pharmacologic and toxic effects of digoxin (3-7, 9, 10, 12, 13, 15, 39), it is presumed that the circulating antibody-bound and Fab-bound digoxin is pharmacologically inactive. In the course of these prior studies of the pharmacologic effects of antidigoxin antibodies, it has been

shown that these antibodies are capable of removing digoxin from mammalian cells (3, 4). Thus, the striking rises in serum digoxin concentrations in both the antibody-treated and the Fab-treated dogs are most consistent with the removal of digoxin from an extravascular compartment or compartments. Since canine tissues were not analyzed in the current study, the extravascular sources of digoxin cannot be identified. It is of interest, however, that tissue digoxin appears to consist of intracellular and cell membrane-bound components (4, 40) and that antidigoxin antibodies remove intracellular glycoside from human erythrocytes more rapidly than they remove the glycoside from cell membrane-binding sites (4). In this connection, it is possible that the prolonged phase of removal of digoxin from an extravascular compartment during hours 6-24 in the antibody-treated dogs is contributed to by slow dissociation of digoxin from specific binding sites on cell membranes, analogous to the slow dissociation of digoxin from such sites on human erythrocyte membranes with consequent slow sequestration by antidigoxin antibodies (4).

Serum digoxin concentrations remained elevated for 5-7 days in most antibody-treated dogs (Figs. 1, 3); then, as the heterologous sheep antibodies were eliminated, 50- to 100-fold decreases in serum digoxin occurred within 48-72 h, presumably reflecting both tissue uptake of released digoxin and its urinary excretion in unbound form (Figs. 2, 3; Table

IV). In contrast with the protracted elevations in the antibody-treated group, serum digoxin concentrations began to fall within 4–10 h after Fab administration; this decrease presumably reflects urinary excretion of digoxin and catabolic degradation of Fab fragments.

The mean maximal rise in serum digoxin in 13 dogs treated with various doses of Fab fragments was 8.2-fold in contrast with the 50.5-fold rise caused by intact antibodies. The basis for this difference has not been established in the current study. Since the maximal rise was only 6.1-fold in the three dogs given doses of Fab fragments comparable with antibody doses, it does not appear that dosage differences were responsible. Continued urinary digoxin excretion and rapid Fab catabolism may have been factors in the Fab-treated dogs. Another possible contributory factor may have been a greater volume of distribution for Fab fragments than for the larger intact antibody molecules but, at this time, there is no direct evidence to support this possibility.

Although urinary recovery of administered [ $^3\text{H}$ ]digoxin was not complete, it can be seen that urinary excretion of digoxin was markedly delayed in antibody-treated dogs, while continuing in the Fab-treated animals (Table IV). During the 22-h period after antibody administration (hours 2–24 of study), only 6.9% of the recovered digoxin was found in the urine of antibody-treated dogs, in contrast with 38.4% in the control group and 41.3–52.4% in the three Fab-treated groups. During the period after more than 96 h had elapsed, 58.2% of the recovered digoxin was found in the urine of the antibody-treated animals in contrast with 2.3–7.4% in the control and Fab-treated groups.

It seems likely that the protracted sequestration of digoxin in antibody-bound form in plasma retards its urinary excretion by the antibody-treated dogs. Since body stores of digoxin are initially maintained at the level present before antibody administration, the clinical use of intact antibody to reverse digoxin intoxication could conceivably be accompanied by the retention of potentially toxic digoxin stores within the body. If large stores of the glycoside were to be abruptly released from antibody during the rapid immunologic degradation of heterologous sheep immunoglobulin, toxic complications caused by the released digoxin could conceivably ensue several days after antibody administration. Inasmuch as excretion of digoxin by Fab-treated dogs continued, it was concluded that the use of Fab fragments would not only reverse digoxin intoxication, but would also permit the prompt excretion of toxic body stores of the drug, much of it in protein-bound, and presumably inactive, form.

In dogs receiving the smaller doses of antidigoxin

Fab fragments, much of the urinary digoxin was not protein-bound; this observation was not entirely unexpected inasmuch as Wochner et al. (18) have presented evidence that, although rabbit Fab fragments are excreted in the urine, the major factor in their overall metabolism is endogenous catabolism, some of which occurs in the kidney. An additional factor which may have artifactually decreased the percentage of protein-bound digoxin in some urine specimens could have been the presence of proteolytic bacterial enzymes, particularly in samples obtained from metabolic cages.

One of the initial hypotheses prompting the performance of the current study was the theory that antidigoxin Fab fragments might actually increase the rate of digoxin excretion. Although somewhat more digoxin was excreted by Fab-treated dogs during hours 2–24 after Fab administration than by control dogs during the same interval, the observed difference was not statistically significant, and hence further studies with large doses of Fab fragments will be required to investigate this hypothesis.

No dog in this study developed overt serum sickness, but the presence of serum sickness in this species could have escaped our attention. Furthermore, we did not detect anti-SGG or anti-Fab antibodies in the sera of these dogs. Thus, no conclusions can be drawn from this study concerning the relative immunogenicity of sheep serum (containing intact antibodies) and of purified Fab fragments. However, in our experience<sup>2</sup> and that of others,<sup>3</sup> Fab fragments are less immunogenic than intact immunoglobulin molecules in eliciting antibody formation in experimental animals. Certainly, in man, the administration of substantial quantities of heterologous serum would be accompanied by an appreciable incidence of serum sickness (41), whereas the use of proteolytic fragments of purified immunoglobulins reduces the risk of serum sickness (42).

The fact that digoxin is excreted promptly, much of it in protein-bound form, in Fab-treated dogs together with the probable lesser immunogenicity of Fab fragments suggest that antidigoxin Fab fragments have theoretical advantages over intact antibody molecules in the immunologic treatment of severe, potentially lethal, digoxin intoxication in man (43–46) which does not respond to currently available forms of antiarrhythmic, symptomatic, and supportive therapy (45). One patient with advanced digoxin intoxication has recently been successfully treated with antidigoxin Fab fragments; digoxin pharmacokinetics in this patient were similar to digoxin pharmacokinetics in dogs given Fab fragments in the current study (47).

<sup>2</sup> Smith, T. W., and E. Haber. Unpublished experiments.

<sup>3</sup> Spiegelberg, H. L. Personal communication.

Finally, we suggest that Fab fragments of antibodies to other small molecules, notably hormones, toxins, and drugs, might be more useful than intact antibodies in instances in which reversal of the effects of toxic concentrations of such a compound are clinically indicated and in which prolonged presence of the inactivating antibody is not desired.

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