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The Plasma Disposition and Renal Elimination of Digoxin-specific Fab Fragments and Digoxin in the Rabbit

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Abstract—Administering digoxin-specific antibody fragments (DSFab, 1·9 mg kg^{-1}, i.v.) to rabbits 1 h after digoxin (15 μg kg^{-1} or 12·5 μCi kg^{-1}, i.v.) produced a redistribution of digoxin associated with a 5-fold elevation in total plasma concentration and 36–86% reductions in elimination half-life, apparent volume of distribution at steady-state and total body clearance (CL_T). Renal clearance (CL_R) was also reduced (54%), but urinary digoxin excretion was increased by one-third (35% vs 25%). This apparent anomaly is due to the large rise in total plasma digoxin concentration with a consequent increase in the area under the plasma concentration curve (AUC). The AUC, which is the denominator term in calculating CL_R (and CL_T), was increased to a greater extent than urinary digoxin excretion (numerator term in calculating CL_R) so that an overall reduction in CL_R occurred. The initial presence of digoxin appeared to alter the distribution of DSFab, since their plasma concentrations were markedly higher when the antibody was given after the hapten. The digoxin also reduced (from 3 to 1%) the amount of detectable DSFab in the urine.

The use of digoxin-specific Fab fragments (DSFab), derived from corresponding sheep immunoglobulin G, is well established in the treatment of severe cardiac glycoside intoxication (Antman et al 1990). It appears that intravenously injected DSFab, penetrate to the exctracellular space, where they bind the free drug, rendering it inactive. In addition, since the drug has a higher affinity for DSFab than for the ATPase receptor, when tissue-bound cardiac glycoside dissociates it becomes preferentially bound to the excess of antibody present, resulting in a decrease in pharmacological effect (Capps et al 1985). However, although rapid DSFab-induced digoxin redistribution is a crucial feature of toxicity reversal, the subsequent removal of the drug from the body is of great importance, particularly as the influence of the antibody fragments on elimination is unclear. For instance, we found in studies on plasma kinetics in rabbits (Timsina & Hewick 1991), that DSFab markedly reduced the total body clearance of digoxin, apparently contrasting with earlier reports indicating that urinary hapten excretion is increased in rats (Johnston et al 1987) or unchanged in dogs (Butler et al 1977). To investigate this anomaly, urinary excretion as well as plasma concentration data are needed to characterize the disposition of DSFab and digoxin.

In the present study we used a rabbit model, in which both plasma and urinary elimination of DSFab and the hapten digoxin are monitored. Furthermore, in addition to studying the effect of DSFab on digoxin disposition, the influence of the presence of the hapten on the disposition of DSFab will be investigated.

Materials and Methods

Materials

Digoxin-specific Fab fragments (lyophilized powder, Digibind) derived from anti-digoxin immunoglobulin G raised in sheep, were received as a gift from the Wellcome Foundation Ltd, Beckenham, UK.

Unlabelled digoxin (Lanoxin) was obtained from Wellcome Foundation Ltd, Beckenham, UK, while 12α-[^{3}H]digoxin, (sp. act. 10·0 Ci $mmol^{-1}$) was obtained from Du Pont, NEN, Southampton, UK. The purity of the labelled digoxin was checked by thin-layer chromatography using commercial silica gel sheets (Eastman Chromatogram Sheet, Kodak 100 μm gel thickness) with chloroform: methanol (9:1) as the solvent system. The plate was scanned for radioactivity using a radiochromatogram thin-layer scanner (Panax system E 0111/p7900A) and a single sharp symmetrical peak obtained corresponding to unlabelled digoxin. The quenched tritium standards were obtained from Amersham International, Amersham, UK. The liquid scintillation fluid (NE260) was supplied by New England Nuclear, Edinburgh, UK. Standard enzyme-linked immunosorbent assay (ELISA) reagents for quantitating sheep polyclonal DSFab, exploiting the sheep-specificity, were obtained as described previously (Timsina & Hewick 1990). All other reagents were obtained from British Drug Houses, Dorset, UK, and were of analytical grade, unless otherwise stated.

Methods

Preparations of [^{3}H]digoxin for injection. An appropriate volume (1000 μCi mL^{-1}, 78 μg mL^{-1} ethanol) was evaporated to dryness under nitrogen. Digoxin (250 μg mL^{-1}) was added and the solution diluted with 0·9% NaCl (saline) such that the total amount of digoxin in the injection solution was 15 μg mL^{-1} (12·5 μCi mL^{-1}). The [^{3}H]digoxin solution was stored at -20°C until required.

Procedures in conscious rabbits. Five female New Zealand-White rabbits (3·2–5·1 kg) were used throughout. During dosing with DSFab and digoxin they were housed in metabolic cages for 7 days with free access to food and water.

Each rabbit was dosed (i.v., left ear marginal vein) with

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DSFab (1·9 mg kg^{-1}), or [^{3}H]digoxin (12·5 μCi kg^{-1}, 15 μg kg^{-1}), or [^{3}H]digoxin (12·5 μCi kg^{-1}, 15 μg kg^{-1}) followed 1 h later by DSFab (1·9 mg kg^{-1}). This dose of DSFab was estimated to be twice-molar to the total amount of digoxin in the body just before DSFab administration (Timsina & Hewick 1991). There was a four-week interval between each treatment, with the order of treatment being randomized.

Blood was collected from the right ear marginal vein at 5, 10, 15, 30 and 60 min and then every hour until 9 h (when DSFab was given alone) or at 4, 8, 12 h and then at 24, 28, 32, 48, 52, 56, 72, 76, 80, 96, 100 and 104 h (when digoxin or digoxin followed by DSFab was given). Plasma samples were obtained by centrifuging blood (3000 *g*, room temperature (21°C), 15 min) and aliquots (0·2 mL) were stored at −20°C until the time of assay. Urine voided during the intervals 0–6, 6–12, 12–24, 24–30, 30–36, 36–48, 48–72, 72–96, 92–120, 120–144, 144–168 and 168–192 h after the intitial drug injection was collected, centrifuged and stored as for plasma samples.

Determination of total [^{3}H]digoxin. For total digoxin, plasma or urine samples (0·05 mL) were mixed with 5 mL liquid scintillant and counted using a Packard Tricarb 300 liquid scintillation counter. All determinations were carried out in triplicate and expressed as equivalents of digoxin.

Determination of DSFab concentrations. Plasma DSFab concentrations were determined by an ELISA based on anti-sheep reagents as described previously (Timsina & Hewick 1990). Urine was assayed in an analogous manner. Plasma samples were diluted 1 in 20 (samples from 5 min to 2 h blood collection period) or 1 in 10 (from 3 to 11 h blood collection period). Urine samples were diluted 1 in 20 (samples from 0 to 12 h collection intervals) or 1 in 10 (samples from 24 to 36 h collection intervals) or 1 in 5 (sample from 48 h collection interval). All determinations were carried out in triplicate.

Determination of extracellular fluid volume. The thiocyanate space, used as an index of an extracellular fluid volume, was determined as described previously (Timsina & Hewick 1990).

Pharmacokinetic and statistical analysis. In control experiments, the DSFab plasma concentration vs time data were analysed as follows. The elimination rate constant (k_{el}) and elimination half-life ($t\frac{1}{2}_\beta$) were obtained by a model-independent method from the terminal part (3-9 h) of the log concentration time plot using linear least-squares regression analysis. The total area under the concentration vs time curve (AUC) was obtained from 0 to 9 h using the linear trapezoidal rule and from 9 h to infinity using k_{el} to determine the extrapolated area. The total digoxin plasma concentration vs time data either for the control experiment or after DSFab administration were analysed as follows. The k_{el} and $t\frac{1}{2}_\beta$ were obtained by a model-independent method from the terminal part (24–104 h) of the log concentration vs time plot using linear least-squares regression analysis. The AUC was obtained from 0 to 104 h using the linear trapezoidal rule and from 104 h to infinity by extrapolation using k_{el}. Using the parameters obtained, the apparent volume of distribution at steady-state (Vd_{ss}) was calculated by dividing the product of dose and area under the moment curve (AUMC) by AUC squared (Gibaldi & Perrier 1982). The AUMC (the area under the curve of the product of time and plasma concentration over the time-span zero to infinity) was calculated in the same way as AUC (see above). Total body clearance CL_T was calculated by dividing the dose by AUC. Renal clearance (CL_R) was calculated by dividing total amount excreted to infinity by plasma $AUC_{0-\infty}$ and was adjusted for body weight.

The F-test ($P<0·05$) was used to determine which compartmental model best fitted the plasma concentration vs time profile. For control DSFab plasma concentration vs time data, a biexponential solution was found to be appropriate and this was also the case for control digoxin in these experiments.

To obtain DSFab, $t\frac{1}{2}_\alpha$, an exponential stripping programme (Brown & Manno 1978) was used.

To test for significant differences ($P<0·05$) Student's paired *t*-test was used.

Results

The well-established effect of DSFab in elevating plasma digoxin concentrations is shown in Fig. 1. Following an initial 5-fold elevation, a significant increase was maintained for at least 32 h. DSFab administration produced significant reductions in plasma kinetic parameters of digoxin (Table 1).

DSFab induced faster urinary digoxin excretion initially (Fig. 2) and increased the total amount excreted by this route by about one-third (35% vs 25%). By 144 h, essentially no

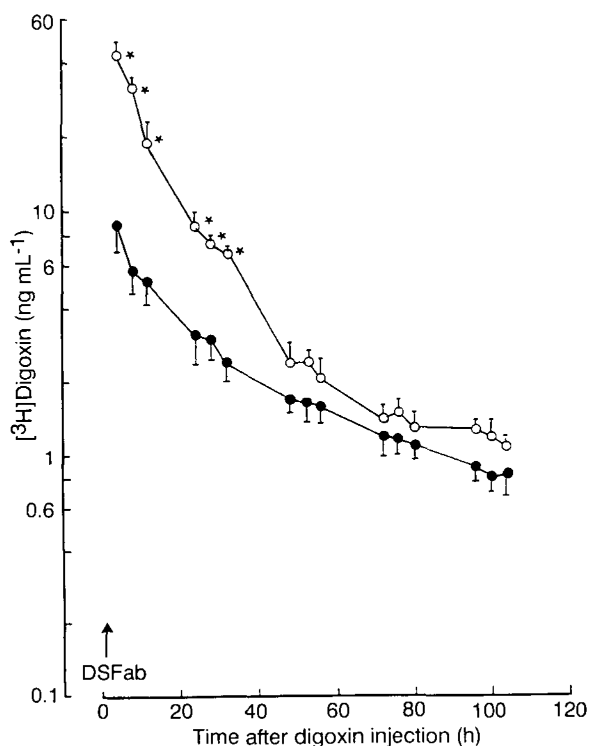

FIG. 1. Plasma digoxin concentrations with (○) and without (●) DSFab administration in rabbits. The i.v. doses of [^{3}H]digoxin and DSFab were 15 μg kg^{-1} (12·5 μCi kg^{-1}) and 1·9 mg kg^{-1}, respectively. Means ± s.e.m. are given (n = 5). *$P<0·05$.

Table 1. The effect of digoxin-specific Fab fragments (DSFab) on digoxin pharmacokinetics in rabbits.

Parameter	Control	Digoxin/DSFab	% reduction by DSFab
$t_{\frac{1}{2}\beta}$ (h)	43·5±4·3	27·8±1·0*	36
Vd_{ss} (L kg^{-1})	3·4±1·0	0·48±0·04*	86
CL_T (mL kg^{-1} h^{-1})	55·3±10·4	18·4±1·6*	67
CL_R (mL kg^{-1} h^{-1})	13·0±1·9	6·0±0·3*	54

The i.v. doses of [^{3}H]digoxin and DSFab were 15 μg kg^{-1} (19 μmol kg^{-1}) and 1·9 mg kg^{-1} (38 μmol kg^{-1}), respectively. Means ± s.e.m. are given (n = 5). *$P < 0·05$ compared with control value.

further [^{3}H]digoxin-derived radioactivity was being excreted. DSFab reduced digoxin renal clearance significantly (Table 1).

The pharmacokinetic parameters of DSFab (in the absence of digoxin) derived from the data shown in Fig. 3 were $t_{\frac{1}{2}\alpha}$, 0·21±0·01 h; $t_{\frac{1}{2}\beta}$, 2·3±0·2 h; Vd_{ss}, 189·1±13·4 (mL kg^{-1}); CL_T 86·5±10·9 (mL kg^{-1} h^{-1}) and CL_R, 2·8±0·8 (mL kg^{-1} h^{-1}). The initial presence of digoxin appeared to modify the distribution of DSFab, since their plasma concentrations were markedly higher (Fig. 3) when the antibody fragments were given after the hapten.

The presence of hapten also affected urinary excretion of DSFab, reducing it by two-thirds (Fig. 4). It was also noted that even without the effect of digoxin, only about 3% of the DSFab dose was excreted in the urine in a form that can be detected by the ELISA. DSFab were not detected in the plasma and urine when tested at 24 and 48 h, respectively.

The extracellular fluid volume, (determined by thiocyanate space), was found to be 209·8±4·3 mL kg^{-1} (n = 5).

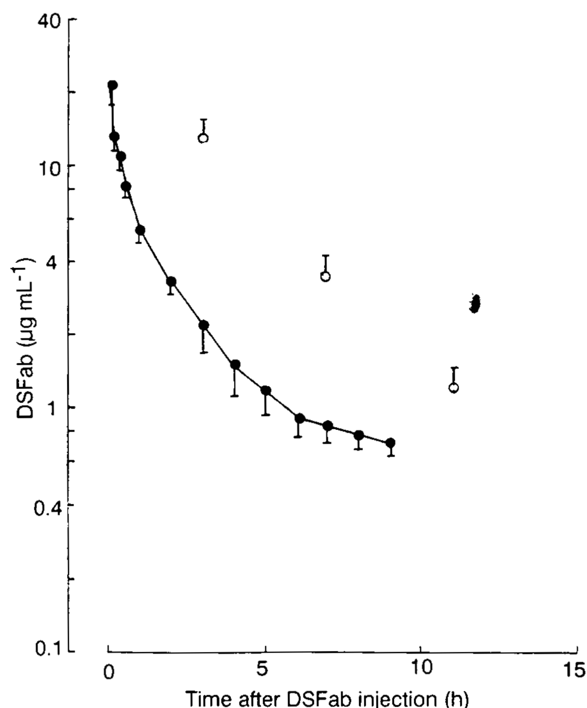

FIG. 3. Plasma DSFab concentrations in rabbits with (○) and without (●) digoxin being administered 1 h previously. The i.v. doses of [^{3}H]digoxin and DSFab were 15 μg kg^{-1} (12·5 μCi kg^{-1}) and 1·9 mg kg^{-1}, respectively. Means ± s.e.m. are given (n = 5).

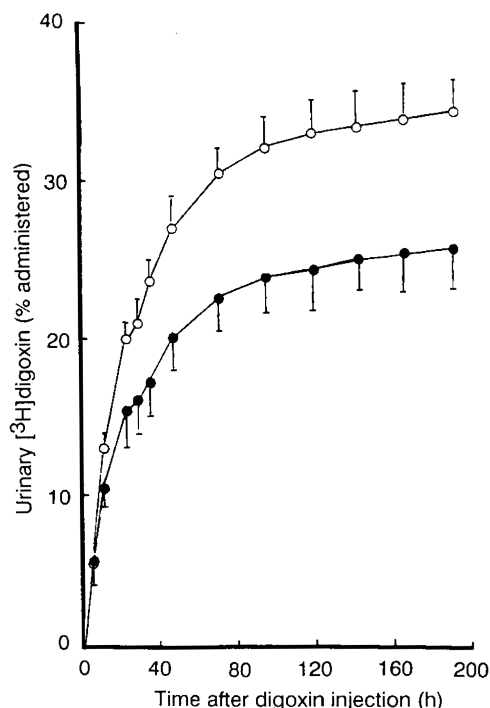

FIG. 2. The urinary elimination of digoxin with (○) and without (●) DSFab administration in rabbits. The i.v. doses of [^{3}H]digoxin and DSFab were 15 μg kg^{-1} (12·5 μCi kg^{-1}) and 1·9 mg kg^{-1}, respectively. Each point represents mean ± s.e.m. (n = 5). Data at each collection interval after 6 h in digoxin/DSFab experiments are significantly different ($P < 0·05$) from those in controls.

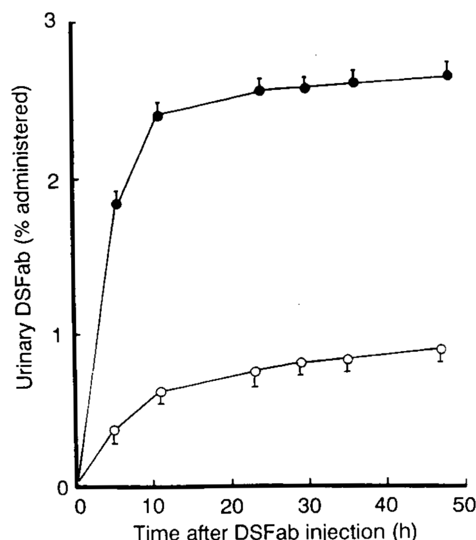

FIG. 4. The urinary elimination of DSFab in rabbits with (○) and without (●) 1 h prior administration of digoxin. The i.v. doses of [^{3}H]digoxin and DSFab were 15 μg kg^{-1} (12·5 μCi kg^{-1}) and 1·9 mg kg^{-1}, respectively. Each point is the mean ± s.e.m. (n = 5). Data at each time point in digoxin/DSFab experiments are significantly different ($P < 0·05$) from those in controls.

Discussion

The DSFab-induced increase in plasma digoxin is still apparent at 32 h even though we could not detect DSFab in plasma by 24 h. This 'residual' plasma digoxin elevation in the absence of antibody fragments was also noted in previous work in rabbits (Timsina & Hewick 1991) although it persisted for as long as 100 h. In the present study, however, the prolonged difference between control and experimental digoxin concentrations was less marked and was not significant by 48 h. Plasma pharmacokinetic parameters of digoxin in control, and DSFab/digoxin experiments in the present study are comparable with those previously reported (Timsina & Hewick 1991). The fact that the renal clearance of digoxin in control experiments was only 25% of the total body clearance indicates that there is considerable clearance by other routes, probably via the bile and with some direct secretion into the intestine (Schafer & Fichtl 1984). The importance of elimination by non-renal routes in the rabbit has been demonstrated by Ochs et al (1978) who showed that after administration of [^{3}H]digoxin, recovery of radioactivity was 15% in urine and 75% in the faeces over a 96 h period.

After the administration of DSFab, digoxin is avidly bound by the antibody distributed in the extracellular space, resulting in a dramatic reduction in Vd_{ss}. This is reflected by the marked rise in plasma drug concentration with a consequent increase in plasma AUC. It is the large value of this, which is the denominator term in calculating both CL_T and CL_R, that is responsible for the DSFab-induced decrease in these two parameters. The reduction in clearance values after administration of drug-specific Fab fragments, as well as being previously reported for digoxin (Timsina & Hewick 1991) has been observed for phencyclidine (Owens & Mayersohn 1986). The large effect of increased protein binding on digoxin clearance is as expected (Guentert & Oie 1980), since digoxin has low hepatic and renal extraction ratios (organ clearance divided by organ blood flow). Using reported values for rabbit hepatic and renal blood flows (Kozma et al 1974; Guentert & Oie 1980), and assuming that non-renal clearance determined in the present study approximates to the hepatic clearance, gives extraction ratios for both organs of about 0·01.

Despite halving renal digoxin clearance, DSFab significantly increased the rate of urinary digoxin elimination and the amount of drug excreted by this route. However, in the calculation of renal clearance, the increase in urinary excretion (numerator) was less marked than the increase in the plasma AUC (denominator), so that an overall reduction in the clearance term resulted.

The DSFab-increased urinary excretion of digoxin that we have reported also agrees with the findings of Johnston et al (1987) for rats. In the dog, DSFab appear to produce either a small increase (Butler et al 1973) or no effect (Butler et al 1977) on urinary digoxin excretion. Presumably whether or not elimination of digoxin is enhanced by DSFab administration in the particular animal species studied depends on the relative DSFab/digoxin elimination rates and the relative importance of renal and non-renal routes of digoxin excretion.

With the present experimental design in which each rabbit is used as its own control, there is a possiblity that the second injection of DSFab could induce the production of anti-DSFab antibodies. Smith et al (1979) found that when rabbits were given 1 mg kg^{-1} DSFab i.v., two out of eight animals showed a slight immunogenic response after three weeks. If a second DSFab dose was given at this time, two to four rabbits responded during the subsequent two weeks. In our experiments in which DSFab, digoxin/DSFab and digoxin treatments were given in a random order, we had no evidence of an immunogenic effect of DSFab. Examination of data in individual rabbits with respect to the relative disposition of DSFab or digoxin, indicated that the order in which the various treatments were given had no effect on the pattern of changes observed. The presence of any anti-Fab antibodies (by neutralizing the action of DSFab on its hapten digoxin) would tend to reduce the influence of DSFab on digoxin pharmacokinetics, but would not alter the general picture. Although we had no evidence of the existence of anti-DSFab antibodies, their presence could cloud interpretation of any possible influence the hapten may have on the disposition of the antibody fragments, because endogenously formed antibodies could themselves affect DSFab disposition.

With this proviso in mind, it does seem that the prior presence of digoxin alters the distribution of DSFab, in that elevated plasma DSFab concentrations were detected. This implies that the drug reduces the apparent volume of distribution. Reference to the data on thiocyanate disposition suggests that, in the absence of digoxin, DSFab distributes into the extracellular fluid. The mechanism by which digoxin could alter this situation is not known; further plasma DSFab concentration data would be required to confirm and assess the apparent influence of digoxin on antibody disposition.

The presence of the hapten digoxin also appears to have an effect on the urinary excretion of DSFab. In the absence of drug, we found that less than 3% of the injected dose of DSFab was detected in urine, but with the hapten present the urinary excretion was reduced by two-thirds. The mechanism of this reduction is not known. The small percentage of the DSFab dose detected in the urine supports other reports (Spiegelberg & Weigle 1965; Janeway et al 1968; Arend & Silverblatt 1975; Keyler et al 1991) that both homologous and heterologous Fab fragments are rapidly and extensively reabsorbed and catabolized by proximal tubule cells after glomerular filtration in small animals (mouse, rat, guinea-pig, rabbit and dog) and in man. However, the extent of Fab metabolism does appear to vary amongst species. For instance, in baboons, over the first 24 h after administration, 30–45% of administered sheep DSFab was recovered in active form in urine (Smith et al 1979), while for man Schaumann et al (1986) reported that the amount of DSFab excreted in urine was only 0·2% of the administered dose. In the present study the low urinary DSFab recoveries cannot be ascribed to problems with urinary collection and bacterial contamination as encountered by Butler et al (1977) and Sinclair et al (1989).

It is clear that the renal handling of drug-specific antibody fragments along with the associated hapten needs to be examined further.

References

Antman, E. M., Wenger, T. L., Butler, V. P., Haber, E., Smith, T. W. (1990) Treatment of 150 cases of life-threatening digitalis intoxication with digoxin-specific Fab antibody fragments: final report of a multicentre study. Circulation 81: 1744–1752

Arend, W. P., Silverblatt, J. F. (1975) Serum disappearance and catabolism of homologous immunoglobulin fragments in rats. Clin. Exp. Immunol. 22: 502–513

Brown, R. D., Manno, J. E. (1978) ESTRIP, a basic computer programme for obtaining initial polyexponential parameter estimates. J. Pharm. Sci. 67: 1687–1691

Butler, V. P., Schmidt, D. H., Smith, T. W., Haber, E., Raynor, B. D., Demartini, P. (1973) Effect of Fab fragments of sheep antidigoxin antibody on serum concentrations and urinary excretion of digoxin. Circulation IV: 130

Butler, V. P., Schmidt, D. H., Smith, T. W., Haber, E., Raynor, B. D., Demartini, P. (1977) Effect of sheep digoxin-specific antibodies and their Fab fragments on digoxin pharmacokinetics in dogs. J. Clin. Invest. 59:345–359

Capps, N. E., Nigdikar, S., Burns, J. H., Walker, E. J., Dow, J. W. (1985) Characterisation of digoxin binding and daunorubicin uptake by isolated mature rat cardiac myocytes. Biochem. Pharmacol. 34: 1957–1961

Gibaldi, M., Perrier, D. (1982) In: Gibaldi, M., Perrier, D. (eds) Pharmacokinetics. Marcel Dekker, New York, pp 199–219, 410

Guentert, T. W., Oie, S. (1980) Effect of plasma protein binding on quinidine kinetics in the rabbit. J. Pharmacol. Exp. Ther. 215: 165–171

Janeway, C. A., Merler, E., Rosen, F. S., Salmon, S., Crain, J. D. (1968) Intravenous gamma globulin: metabolism of gamma globulin fragments in normal and agammaglobulinaemic persons. New Eng. J. Med. 278: 919–923

Johnston, P. C., Stevenson, I. H., Hewick, D. S. (1987) The influence of digoxin-specific antibody fragments on digoxin disposition in the rat. Biochem. Pharmacol. 36: 2215–2220

Keyler, D. E., Dalerno, D. M., Murakami, M. M., Ruth, G., Pentel, P. R. (1991) Rapid administration of high-dose human antibody Fab fragments to dogs: pharmacokinetics and toxicity. Fundam. Appl. Toxicol. 17: 83–91

Kozma, C., Macklin, W., Cummins, L. M., Flatt, R. E., Mauer, R. (1974) Anatomy, physiology and biochemistry of the rabbit. In: Weisbroth, S. H., Flatt, R. E., Kraus, A. L. (eds) The Biology of the Laboratory Rabbit. Academic Press, London, pp 50–72

Ochs, H. R., Bodem, G., Bales, G., Greenblatt, D. G., Smith, T. W. (1978) Increased clearance of digoxin in rabbits during repeated administration. J. Pharmacol. Exp. Ther. 204: 262–270

Owens, S. M., Mayersohn, M. (1986) Phencyclidine-specific Fab fragments alter phencyclidine disposition in dogs. Drug Metab. Dispos. 14: 52–58

Schafer, S. G., Fichtl, B. (1984) Effect of quinidine on the intestinal secretion of digoxin on guinea-pigs. Biopharm. Drug Dispos. 5: 163–168

Schaumann, W., Kaufmann, B., Neubert, P., Smolarz, A. (1986) Kinetics of the Fab fragments of digoxin antibodies and of bound digoxin in patients with severe digoxin intoxication. Eur. J. Clin. Pharmacol. 30: 527–533

Sinclair, A. J., Hewick, D. S., Johnston, P. C., Stevenson, I. H., Lemon, M. (1989) Kinetics of digoxin antibody fragments during treatment of digoxin toxicity. Br. J. Clin. Pharmacol. 28: 352–356

Smith, T. W., Lloyd, B. L., Spicer, N., Haber, E. (1979) Immunogenicity and kinetics of distribution and elimination of sheep digoxin-specific I_gG and Fab fragments in the rabbit and baboons. Clin. Exp. Immunol. 36: 384–396

Spiegelberg, H. L., Weigle, W. O. (1965) The catabolism of homologous and heterologous 7S gamma globulin fragments. J. Exp. Med. 121: 323–338

Timsina, M. P., Hewick, D. S. (1990) The use of enzyme-linked immunosorbent assays to study the plasma disposition of sheep polyclonal and rat monoclonal digoxin-specific Fab fragments in the rabbit. J. Pharm. Pharmacol. 42: 572–576

Timsina, M. P., Hewick, D. S. (1991) The plasma kinetics of digoxin-specific Fab fragments and digoxin in the rabbit. Ibid. 43: 807–810