Table II—Tissue I Concentrations (Micrograms per Gram) in Beagle Dogs that Received 3.8 g/kg intratracheally

Dog Number, Sex, and Weight (kg)	Lung	Fat	Thoracic Lymph	Mesenteric Lymph	Bile	Adrenals	Ovaries	Testes
				1 Week				
1, M, 10.4	0.01	6.5	0.30	0.70	<MQL[a]	0.11	—	<MQL
2, M, 9.2	0.01	0.96	0.26	0.09	<MQL	0.12	—	0.01
3, F, 7.3	0.01	2.5	0.31	0.11	<MQL	0.07	<MQL	—
4, F, 8.6	<MQL	0.86	<MQL	0.14	<MQL	0.05	<MQL	—
				2 Weeks				
5, M, 10.0	<MQL	0.67	<MQL	0.28	<MQL	<MQL	—	<MQL
6, M, 9.3	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—	<MQL
7, F, 8.2	<MQL	0.16	<MQL	0.02	<MQL	<MQL	—[b]	—
8, F, 6.6	<MQL	0.67	0.03	0.05	<MQL	<MQL	<MQL	—
				4 Weeks				
9, M, 10.7	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—	<MQL
10, M, 9.9	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—	<MQL
11, F, 8.2	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	<MQL	—
12, F, 6.9	<MQL	0.06	<MQL	<MQL	<MQL	<MQL	<MQL	—

[a] Mean minimum quantifiable level (MQL) was 0.01 μg/g. [b] No sample.

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Dose-Dependent Protein Binding and Disposition of Prednisolone in Rabbits

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Abstract □ An animal model was sought that would mimic humans with regard to the dose-dependent pharmacokinetics of prednisolone. Four rabbits were each given 0.5 and 10 mg iv of prednisolone, and timed blood samples were obtained. Plasma prednisolone and prednisone concentrations were assayed by high-performance liquid chromatography, and protein binding was assessed using equilibrium dialysis at 37°. Increases in the systemic clearance, volume of distribution at steady state, mean residence time (in three of four rabbits), and variance of residence time occurred as dose was increased. As in humans, prednisolone was partly converted to prednisone in the rabbit. Transcortin and albumin concentrations and their affinity constants for binding prednisolone were also similar to humans.

Keyphrases □ Prednisolone—dose-dependent protein binding and pharmacokinetics, rabbits □ Binding, protein—prednisolone, pharmacokinetics, rabbits □ Pharmacokinetics—prednisolone, protein binding, rabbits

Prednisolone and its pharmacologically inactive prodrug, prednisone, are synthetic glucocorticoids widely used in the treatment of various disease entities including asthma, shock, and nephrotic syndrome. Jusko and Rose (1) demonstrated nonlinear pharmacokinetics of these compounds in humans, characterized by increases in their total plasma clearance with increases in dose. Furthermore, the serum protein binding of prednisolone is nonlinear at therapeutic serum concentrations owing to the existence of two binding proteins (2). This binding appears to explain most, but not all, of the nonlinearity in prednisolone pharmacokinetics in humans (1).

To assess mechanisms contributing to this nonlinear disposition, an animal model is needed that mimics humans with regard to the serum protein binding and disposition of prednisolone. The serum protein binding of prednisolone was previously examined in rat, rabbit, canine, and human serum (3), with the rabbit being most like the human in type and degree of nonlinear binding. This study further evaluated the rabbit as an animal model for dose-dependent disposition of prednisolone.

EXPERIMENTAL

Methods—Four New Zealand White rabbits, 3.0–3.9 kg, received 0.5- and 10-mg iv equivalent doses of prednisolone as the sodium succinate.

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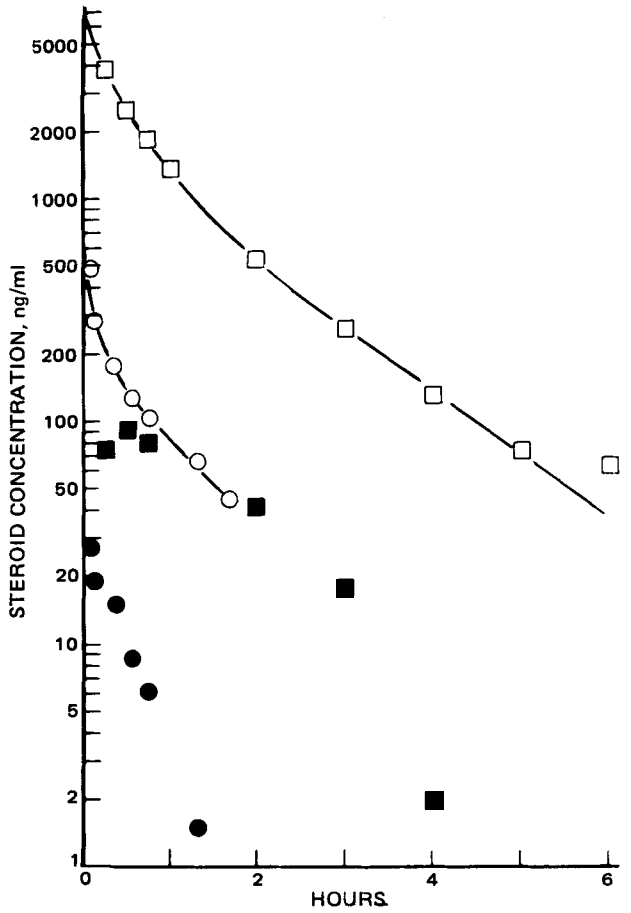

Figure 1—*Serum concentration* versus *time profiles of prednisolone (○,□) and prednisone (●,■) after 0.5 mg of prednisolone (○,●) and 10 mg of prednisolone (□,■) intravenously in Rabbit 2.*

A balanced crossover design was used, with 2 weeks between crossover. Rabbit body weight did not change appreciably over the experimental period. The drug was administered into the lower left marginal ear vein over 15 sec. Blood samples (~3 ml) were obtained from the right lower marginal ear vein just prior to dosing and at 0.08, 0.17, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, and 8 hr following drug administration.

Sample Analysis—Serum samples were analyzed for prednisolone and prednisone using the high-performance liquid chromatographic (HPLC) technique described earlier (4). Serum protein binding of prednisolone was evaluated at 37° by equilibrium dialysis with [^{3}H]-prednisolone. Details of the protein binding technique and data analysis were presented previously (3).

Pharmacokinetics—The area under the curve for prednisone (AUC_P) and prednisolone (AUC_{Pn}), the systemic clearance (Cl_T), mean residence time (MRT), variance of the residence time (VRT), and volume of distribution at steady state (V_D^{ss}) were obtained through use of the LAGRAN computer program, which provides model-independent estimates of these pharmacokinetic parameters based on area/moment analysis[1].

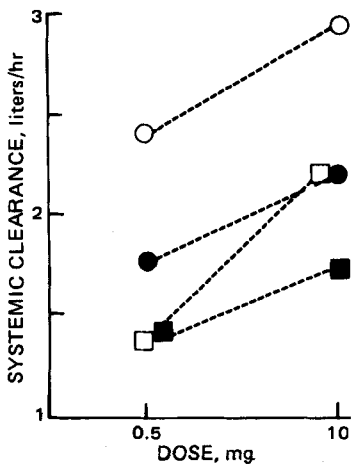

Figure 2—*Systemic clearance (Cl_T) of prednisolone as a function of dose. The stippled lines connect Cl_T values from the same rabbit.*

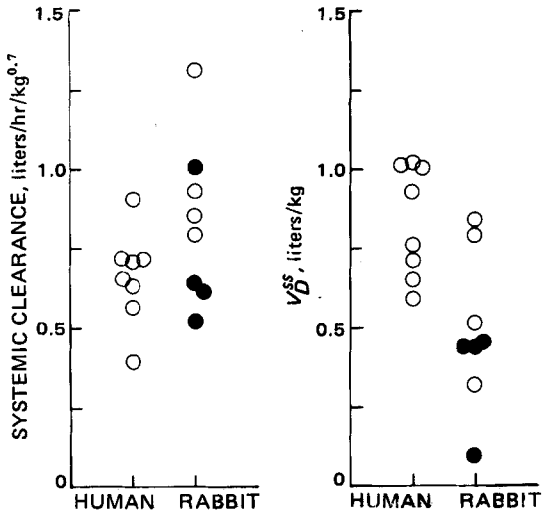

Figure 3—*Rabbit Cl_T values (animal scale-up) and V_D^{ss} values compared to those observed in humans. The solid and open symbols in the rabbit data represent values from the low and high dose studies. Human data are from Rose* et al. *(6).*

The free drug clearance ($Cl_{T,free}$) and the transcortin (CBG) free drug clearance ($Cl_{T,CBG\text{-}free}$) of prednisolone were computed using:

$$Cl_{T,free} = \text{dose}/AUC_{Pn,free} \quad \text{(Eq. 1)}$$

$$Cl_{T,CBG\text{-}free} = \text{dose}/AUC_{Pn,CBG\text{-}free} \quad \text{(Eq. 2)}$$

where $AUC_{Pn,free}$ and $AUC_{Pn,CBG\text{-}free}$ are the areas under the serum concentration *versus* time curves of free and CBG-free prednisolone.

Approximate extraction ratios of prednisolone by kidney (E_K) and liver (E_L) tissues were obtained as the ratio of Cl_T to kidney plasma flow (Q_K) and liver plasma flow (Q_L). The values of Q_K and Q_L used in the computation were 55 and 70 ml/min (5).

RESULTS

Prednisolone Disposition—Typical serum concentration–time profiles for prednisolone and prednisone after low and high doses of prednisolone are presented for Rabbit 2 in Fig. 1. Prednisolone disposition was biphasic in nature, with metabolism of prednisolone to prednisone occurring at each dose. The systemic clearance of prednisolone (Cl_T) at the two doses is shown in Fig. 2. A significant increase ($p < 0.02$) in Cl_T occurred with the increase in dose. The mean ($\pm SD$) Cl_T values after low and high intravenous doses were 1.72 (±0.48) and 2.25 (±0.52) ml/min, respectively. The approximate E_K and E_L values obtained using the mean Cl_T values were 0.036 and 0.028, respectively. There was no dose dependence in prednisolone clearance when corrected for albumin and

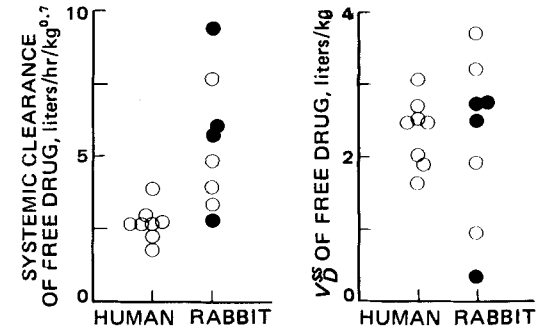

Figure 4—*Rabbit Cl_T and V_D^{ss} values for unbound prednisolone compared to human values (corrected for animal scale-up). The solid and open symbols in the rabbit data represent values from the low and high dose studies. Human data are from Rose* et al. *(6).*

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Table I—Essential Characteristics of Prednisolone Disposition

Rabbit	Body Weight, kg	Dose, mg	Total Plasma Clearance, Cl_T, liters/hr/kg	Free Drug Clearance, $Cl_{T,free}$, liters/hr/kg	CBG-Free Drug Clearance, Cl_T, CBG_{free}, liters/hr/kg	AUC_P/AUC_{Pn}	Volume of Distribution at Steady State, V_D^{ss}, liters/kg	Mean Residence Time, MRT, hr	Variance of Residence Time, VRT, hr
1	3.2	0.5	0.75	6.59	1.27	0.040	0.44	0.59	0.39
	3.2	10.0	0.92	5.31	1.03	0.028	0.84	0.92	1.38
2	3.4	0.5	0.51	3.94	0.87	0.060	0.46	0.89	1.16
	3.4	10.0	0.64	3.32	0.74	0.052	0.79	1.23	2.18
3	3.9	0.5	0.35	1.85	0.54	—	0.10	0.29	0.11
	3.9	10.0	0.56	2.20	0.65	0.044	0.32	0.57	0.68
4	3.0	0.5	0.46	4.3	0.95	—	0.44	0.95	1.14
	3.0	10.0	0.56	2.8	0.63	0.076	0.51	0.91	1.51
Significance[a] (between doses)		—	$p < 0.02$	NS[a]	NS	—	$p < 0.05$	NS	$p < 0.02$

[a] NS = not significant.

transcortin binding ($Cl_{T,free}$) nor in the clearance corrected for only transcortin binding ($Cl_{T,CBG\text{-}free}$) (Table I).

Significant increases in the volumes of distribution at steady state (V_D^{ss}) and the variance of residence time (VRT) were observed at the higher prednisolone dose. In contrast, the mean residence time (MRT) increased with dose in only three of four rabbits. This total change in MRT was not statistically significant (Table I).

Figure 3 illustrates V_D^{ss} and Cl_T using animal scale-up corrections (5) for the rabbit compared to the human (6). There was no significant difference in the Cl_T values between species. In contrast, V_D^{ss} values were significantly higher in the human than in the rabbit ($p < 0.01$). Comparison of Cl_T and V_D^{ss} for unbound prednisolone between humans and rabbits using animal scale-up corrections demonstrated no significant species difference in V_D^{ss}, while the Cl_T values for free prednisolone were significantly higher in the rabbit ($p < 0.05$) (Fig. 4).

Protein Binding—A plot of percent bound *versus* postequilibrium dialysis serum prednisolone concentrations for a rabbit is presented in Fig. 5. The mean ($\pm SD$) serum albumin concentration and the binding affinity constant obtained from computer fitting of the binding data (2) were 5.51 ($\pm$0.46) $\times 10^{-4}$ M and 6.24 ($\pm$1.5) $\times 10^{3}$ M^{-1}, respectively. The mean ($\pm SD$) transcortin concentration and binding affinity constant, also obtained through computer fitting, were 4.70 ($\pm$2.4) $\times 10^{-7}$ M and 2.84 ($\pm$2.16) $\times 10^{7}$ M^{-1}, respectively. The binding parameters obtained in each rabbit are compared in Figs. 6 and 7 with values obtained from a study (6) performed in normal healthy volunteers.

DISCUSSION

The pharmacokinetic parameters derived from these disposition studies are dose and time average values since the disposition of prednisolone is nonlinear, as indicated by the increase in Cl_T, V_D^{ss}, MRT, and VRT with dose. Interconversion between these corticosteroids occurs as well, providing an additional confounding influence on the pharmacokinetic parameters obtained in the present investigation. Such interconversion of prednisone and prednisolone was demonstrated in humans (1, 6) and in the isolated perfused rat kidney (7). The increases in Cl_T and V_D^{ss}, as well as the formation of prednisone after intravenous doses of prednisolone, are all characteristic of the disposition of this steroid in humans (1). Corrections of Cl_T for total protein binding and transcortin binding partially eliminates the differences in Cl_T between low and high doses of prednisolone. This result also was observed in the human and suggests that nonlinear protein binding largely produces dose-dependent changes in Cl_T (6).

The dose-dependent increase in V_D^{ss} is consistent with the changes in protein binding that occur as a function of serum prednisolone concentration (Fig. 4). High prednisolone doses produce higher initial serum concentrations. The reduced binding of prednisolone at these high concentrations (Fig. 4) results in increased distribution of prednisolone to tissues, as reflected by an increase in V_D^{ss}.

Further support for the importance of protein binding in prednisolone disposition is provided by the low extraction ratios of prednisolone by the kidney and liver, suggesting that only drug not bound to serum proteins is available to these tissues (*i.e.*, restrictive clearance). The extraction ratios calculated in the present investigation are overestimates of the true extraction ratios since total and not individual organ clearances were used. The E_K value, however, agrees very well with those (mean = 0.044) obtained during prednisolone perfusion in isolated rat kidneys (7).

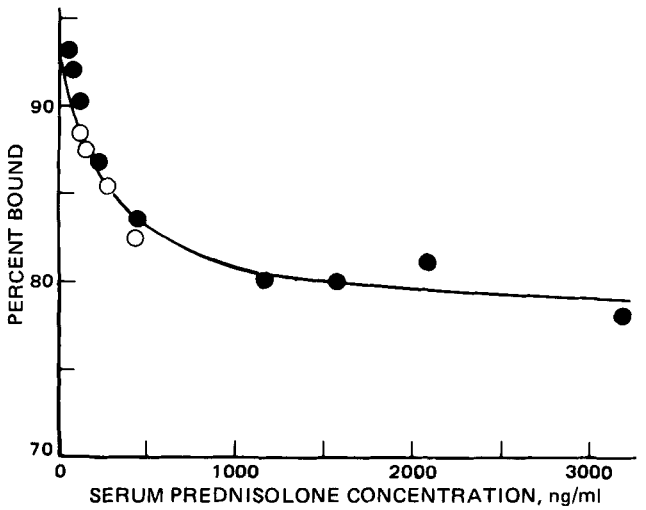

Figure 5—*Percent of prednisolone bound* versus *postdialysis serum prednisolone concentration after low (○) and high (●) intravenous prednisolone doses. The solid line represents the nonlinear least-squares regression line for the protein binding data using a modified Rosenthal relationship for two classes of binding sites (see Ref. 2).*

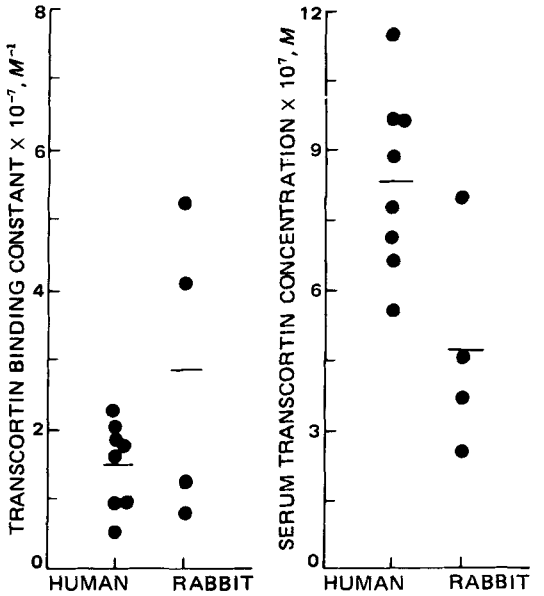

Figure 6—*Serum transcortin concentrations and binding affinity constants for prednisolone in the rabbit and in normal healthy human volunteers (from Ref. 6).*

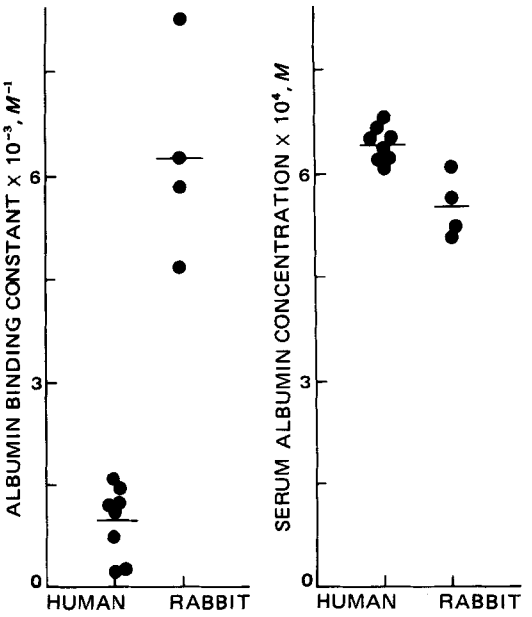

Figure 7—*Serum albumin concentrations and binding affinity constants for prednisolone in the rabbit and in normal healthy volunteers (from Ref. 6).*

Adolph (8) demonstrated that many metabolic and physiological properties among mammals are proportional to some exponential power of their body weight, and Dedrick (9) indicated that the exponent is usually in the 0.7–0.8 range. Excellent agreement between humans and rabbits was observed in the Cl_T values normalized for weight raised to the 0.7 power (Fig. 3). In addition, V_D^{ss} values, although significantly different between species, are in the same general range. The absence of significant interspecies differences in V_D^{ss} for unbound prednisone (corrected for animal scale-up) (Fig. 4) suggests that differences in binding between species accounts for the differences in V_D^{ss} observed for total drug. The significantly higher free Cl_T in the rabbit (Fig. 4) should not be a major complication in its use as a model since the interspecies difference in this parameter is not large. While the doses used for these comparisons are not equivalent, the dose range examined in the rabbit (0.15–2.98 mg/kg) encompasses the dose (0.52 mg/kg) employed in the human studies (6). This comparison establishes that the normalized Cl_T and V_D^{ss} values in the rabbit are reasonably similar to those observed in humans and further demonstrates the applicability of the rabbit in mimicking prednisolone disposition in humans.

The relative changes in Cl_T and V_D^{ss} with prednisolone dose are greater in the human than in the rabbit. Rose *et al.* (6) observed that both Cl_T and V_D^{ss} approximately doubled over an eightfold dose range. The relative increases in Cl_T and V_D^{ss} with dose in the present study averaged 33 and 102%, respectively. These increases were observed over a 20-fold dose range. The lesser magnitude of the changes in Cl_T and V_D^{ss} in the rabbit may be due to less pronounced nonlinearity of prednisolone plasma binding (2).

The apparent increase in *MRT* (in three of the four rabbits studied) at the higher dose directly reflects an increase in the average persistence time of prednisolone in the body. The fractional increase in V_D^{ss} at the higher dose exceeds the relative increase in Cl_T that occurs as the dose is increased. Since $MRT = V_D^{ss}/Cl_T$, the net effect of these changes is an increase in *MRT*. The increase in *VRT* with dose indicates a wider spread of residence time of the mass of drug molecules at the higher dose. This finding appears to be congruous with the enhanced Cl_T and the larger V_D^{ss} that occur at the higher dose. Increases in Cl_T serve to diminish the residence time of prednisolone, while more extensive tissue distribution of prednisolone prolongs the residence time. Concomitant increases in each of these processes would increase the variance (spread) of the residence times of the drug molecules.

The transcortin and albumin serum concentrations and affinity constants for prednisolone binding in rabbits are in the same general range as those determined in normal healthy volunteers after the 40-mg iv dose of prednisolone phosphate (Figs. 6 and 7). This finding confirms the similarity in prednisolone binding between human and rabbit reported previously (3). The apparent superposition of the binding data after low and high prednisolone doses suggests that prednisolone does not compete with prednisone or other metabolites for protein binding sites on transcortin and albumin. The concentrations of these potential displacers is higher after larger prednisolone doses. Competitive binding studies are required, however, to definitively ascertain whether such competition actually occurs.

In conclusion, the rabbit appears to be a suitable model for prednisolone disposition in humans. This species is also desirable from a technical standpoint in that blood samples are easily obtainable and may be taken in sufficient volume to permit examination of serum prednisolone protein binding in addition to HPLC assay for total serum prednisolone and prednisone concentrations.

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