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Pharmacokinetics of Three Sulfonamides in the Rabbit

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Abstract □ The blood level–time relationships for sulfamethazine, sulfisomidine, and sulfathiazole were determined in rabbits after intravenous injection. The results were fitted to a two-compartment open model, and the parameters and rate constants for the model were obtained. Differences between drugs, animals, and treatments (water loading) were studied in terms of urinary and metabolic rate constants, intercompartment diffusion constants, and clearance values. Sulfisomidine was the most slowly eliminated of the compounds, mainly because of the rabbit's low capacity to metabolize this compound. Water loading increased the fraction of drug excreted free (f). It reduced the k_m for sulfathiazole but produced no change with the other drugs. The clearances of the compounds from the central compartment were calculated using $V_b \cdot k_u$ as the apparent renal clearance and $V_b \cdot k_m$ as the metabolic clearance. The apparent renal clearance calculated for all drugs was increased by water loading, while the metabolic clearance was unaffected. The metabolic clearance was suggested as a useful basis for quantitative comparison of the metabolism of these drugs.

Keyphrases □ Pharmacokinetics, sulfamethazine, sulfisomidine, sulfathiazole—after intravenous administration, rabbits □ Sulfamethazine—blood level–time relationships after intravenous administration, pharmacokinetic parameters, rate constants, rabbits □ Sulfisomidine—blood level–time relationships after intravenous administration, pharmacokinetic parameters, rate constants, rabbits □ Sulfathiazole—blood level–time relationships after intravenous administration, pharmacokinetic parameters, rate constants, rabbits

One of the most important factors regulating the response of an organism to a drug or toxic agent is the rate at which the compound is eliminated from the body. Williams (1) pointed out that the main difference between man and animals in drug response probably lies in the varying abilities of different species to carry out drug metabolism. Therefore, a knowledge of comparative pharmacokinetics should be useful in both pharmacology and toxicology. In this paper, sulfonamides are used as model compounds to explore the

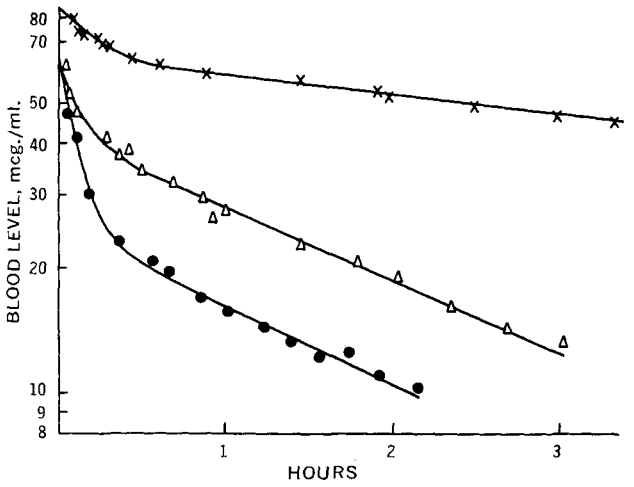


Figure 1—*Blood level curves for the three sulfonamides in the rabbit. Key: ×, sulfisomidine, Rabbit G; Δ, sulfamethazine, Rabbit H; and ●, sulfathiazole, Rabbit P. The solid line in each case is the computer-generated line of best fit.*

pharmacokinetic aspects of drug metabolism in intact animals.

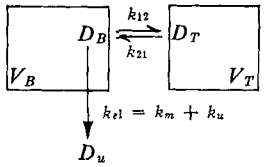
Numerous studies have appeared on the pharmacokinetics of sulfonamides in man (2–4) and in animals (5–7). In these studies, a one-compartment model was found to give an adequate fit to the data. Nelson (8) showed that the blood level curve of sulfaethylthiadiazole did not conform to a one-compartment model but was better described by a two-compartment system. Loo and Riegelman (9) carried out a two-compartment analysis of sulfisoxazole in the rabbit. The application of a two-compartment model to sulfonamide metabolism and excretion in the rabbit is described in this paper.

The three compounds used, sulfamethazine, sulfisomidine, and sulfathiazole, show a preponderence of the *N*-4-acetyl derivative in their metabolism (7, 10–12). Hence, whenever metabolic rates are considered, it is assumed that the rate of biological acetylation is being measured. The one-compartment pharmacokinetics of sulfisomidine and sulfathiazole were studied previously in the rabbit (5, 7), but no data were available on sulfamethazine.

In previous work with orally administered sulfonamides, rabbits were dosed with water to encourage urine flow (7). The possible effects of water loading on sulfonamide pharmacokinetics are examined in this paper by blood level sampling techniques.

EXPERIMENTAL

Sulfathiazole[1], sulfamethazine[2], and sulfisomidine[3] were used without further purification. One milliliter of solution (50 mg./ml. of sulfonamide as the sodium salt) was injected into a marginal ear vein. Adult male New Zealand white rabbits (2–3 kg.) were obtained from a commercial supplier. The animals were fasted overnight before the experiment but were allowed drinking water *ad libitum*. During the experiments the animals were held in restraining cages and were later transferred to metabolism cages for 4–5 days where they were allowed food and water. During this time all urine was collected and retained for assay.



Scheme I—Two-compartment pharmacokinetic model. The central compartment with volume V_B *connects with a peripheral compartment with volume* V_T. *The arrow* $D_B \rightarrow D_u$ *represents the sum of parallel excretory and metabolic pathways (assigned rate constants* k_u *and* k_m).

Experiments were conducted at intervals of 14 days, and the compounds were administered according to a randomized treatment plan in an attempt to avoid time-dependent variations in each rabbit. The animals were starved for the same length of time before each run, and the doses were administered at the same time of day to avoid possible diurnal variations in behavior. With this experimental protocol, it was found that the same rabbit gave consistent experimental behavior over periods of up to 4 months, similar to the results of Yamazaki *et al.* (5).

Collection of Blood Samples—One-milliliter samples of blood were removed at frequent intervals from a marginal ear vein into which a catheter[4] (i.d. 0.017 mm.) had been inserted. Blood samples were taken over a 3–4-hr. period; the number of samples taken from each rabbit averaged 15 (range 14–23).

Water-Loading Procedure—The animals were water loaded by oral administration of 30 ml. of water hourly for 2 hr. before the sulfonamide was administered and then 30 ml. of water each hour for the next 4 hr. (7).

Analysis—The blood samples were added to a centrifuge tube containing sodium oxalate (1 mg.) in distilled water (7 ml.) and were stored in a refrigerator until assay. Urine samples were stored in a similar manner. Free and total sulfonamide in blood and urine were assayed by the Bratton–Marshall (13) technique.

THEORY

When a sulfonamide is injected into the bloodstream, it is distributed to various tissues and undergoes metabolism and excretion. The two-compartment open model is applied to this system (Scheme I) with the same assumptions given by Riegelman *et al.* (14). In addition, all metabolism is assumed to occur in the central compartment. The elimination rate constant (k_{el}) is the sum of the excretion rate constant for free drug (k_u) and the metabolite formation constant (k_m). The pharmacokinetic behavior of the acetyl sulfonamide (M_B) produced will be considered in a later paper. The distribution of sulfonamide into a tissue compartment was assigned distribution rate constants k_{12} and k_{21}; D_B, D_T, and D_u represent the amount of drug in the central, tissue, and urinary compartments, respectively.

The differential equations describing this model may be solved to give Eq. 1 for the blood level of drug (C_b) (15):

$$C_b = Ae^{-r_1 t} + Be^{-r_2 t} \qquad \text{(Eq. 1)}$$

A, B, r_1, and r_2 are hybrid parameters containing the rate constants of the model and have the following values (Eqs. 2–5):

$$A = \frac{C_b^0(k_{21} - r_1)}{r_2 - r_1} \qquad \text{(Eq. 2)}$$

$$B = \frac{C_b^0(k_{21} - r_2)}{r_1 - r_2} \qquad \text{(Eq. 3)}$$

where C_b^0 is the initial concentration of drug in the central compartment, and:

$$r_1 = {}^1/_2(k_{el} + k_{12} + k_{21} + \sqrt{(k_{el} + k_{12} + k_{21})^2 - 4k_{el}k_{21}}) \qquad \text{(Eq. 4)}$$

[1] Shawinigin, McArthur Chemical Co., Ltd., Montreal, Canada.
[2] British Drug Houses, Ltd.
[3] Nutritional Biochemicals Corp.

[4] Bardic 1619-R.

$$r_2 = {}^1/_2(k_{el} + k_{12} + k_{21} - \sqrt{(k_{el} + k_{12} + k_{21})^2 - 4k_{el}k_{21}}) \quad \text{(Eq. 5)}$$

The values of the rate constants of the model may be obtained from these equations or by use of the relationships given by Riegelman *et al.* (14). Estimates of the values of A, B, r_1, and r_2 were obtained by graphical analysis of semilog plots of blood levels *versus* time (16). Such values are biased and give no statistical information. Most workers in the field have fitted the data by an iterative digital computer program, using the values of the hybrid constants as input with the blood level data (9, 17). This method gives a line of best fit for the blood level plot and statistical information on the hybrid parameters. It gives no direct statistics on the rate constants of the model, and Nogami *et al.* (18) rightly pointed out the shortcomings of this method.

To overcome this defect, the graphical estimates of the hybrid parameters were refined by the use of the NONLIN program for iterative nonlinear curve fitting. The model parameters (k_{el}, k_{12}, and k_{21}) were calculated from the refined hybrid parameters and then fed back into the computer. Two separate subroutines were written to fit the estimated rate constants to the blood level data; one utilized the algebraic expressions shown in Eqs. 2–5, and the other used the differential equations of the model which were solved in the computer by a Runge-Kutta procedure. Both methods gave similar results with slightly better fit by the differential equation subroutine, but the latter was much less economical in computer time.

RESULTS

Blood Level Curves—Blood level curves were constructed for each sulfonamide in each rabbit; typical examples are shown in Fig. 1 and the values of the hybrid parameters are shown in Table I. In all cases, sulfisomidine gave a higher blood level than the other compounds and the level declined more slowly. Sulfathiazole generally gave the lowest blood levels of the three compounds. The water-loaded animals did not appear to differ greatly from the untreated series with sulfamethazine and sulfisomidine, but water loading caused a marked reduction in the values of A and B for sulfathiazole in water-loaded animals. This finding could reflect a considerable change in the apparent volume of distribution of this drug in such animals since $A + B = C_b^0$, the initial blood level of the drug (C_b^0) (14), and the same dose was given throughout.

Rate Constants of the Model—The rate constants of the model were computed and the values are shown in Table II. The errors (standard deviation) in the estimation of the overall elimination rate constant are generally low but tend to be higher for distribution rate constants k_{12} and k_{21}.

The elimination rate constant (k_{el}) is the sum of the rate constants for parallel excretory and metabolic pathways followed in the model (Scheme I). By the same method of derivation used for the one-compartment model (2), it may be shown that in the model used here the following relationship holds (Eq. 6):

$$f = \frac{D_u^\infty}{D_u^\infty + M_u^\infty} = \frac{k_u}{k_{el}} = \frac{k_u}{k_m + k_u} \quad \text{(Eq. 6)}$$

where f is the fraction of drug excreted free by the time at which excretion is essentially complete; and D_u^∞ and M_u^∞ are the total cumulative free drug and metabolite excreted, respectively. The values for f, k_m, and k_u are shown in Table III.

Clearance and Volume of Distribution—In blood level studies, the blood level measured is a concentration parameter of drug in the central compartment. The apparent volume of distribution of drug in the central compartment (V_b) may be calculated from Eq. 7 (19):

$$V_b = \frac{\text{dose (mcg.)}}{C_b^0\ \text{(mcg./ml.)}} \quad \text{(Eq. 7)}$$

In these experiments, dose was taken to equal total sulfonamide recovered in the urine at infinite time. The values of V_b are shown in Table IV. The clearance (C_f) of material from the central compartment may be defined (16) as:

$$C_f = V_b k_{el} \quad \text{(Eq. 8)}$$

Table I—Values of Parameters for $C_b^0 = Ae^{-r_1 t} + Be^{-r_2 t}$ for Sulfonamides in Three Rabbits[a]

Rabbit	A, mcg./ml.	r_1, hr.$^{-1}$	B, mcg./ml.	r_2, hr.$^{-1}$
		Sulfamethazine		
P	23.42 ± 1.03	2.86 ± 0.10	12.87 ± 0.55	0.342 ± 0.024
G	41.46 ± 0.87	2.71 ± 0.09	15.39 ± 0.47	0.362 ± 0.018
H	22.08 ± 1.75	7.13 ± 0.62	41.04 ± 0.97	0.400 ± 0.019
S	29.80 ± 2.21	4.16 ± 0.41	8.41 ± 0.81	0.256 ± 0.043
		Water Loaded		
P	26.41 ± 2.60	2.97 ± 0.33	13.32 ± 0.98	0.333 ± 0.065
G	34.68 ± 1.91	4.42 ± 0.28	12.18 ± 0.59	0.337 ± 0.046
H	26.03 ± 1.52	5.79 ± 0.37	37.80 ± 0.97	0.384 ± 0.025
S	31.65 ± 1.86	4.23 ± 0.31	9.12 ± 0.87	0.286 ± 0.032
		Sulfisomidine		
P	25.40 ± 3.83	5.00 ± 0.78	60.03 ± 1.66	0.091 ± 0.014
G	22.47 ± 2.45	4.57 ± 0.34	66.43 ± 0.94	0.107 ± 0.008
H	13.80 ± 3.52	4.45 ± 1.08	67.66 ± 2.16	0.071 ± 0.012
S	23.40 ± 2.68	4.72 ± 0.54	63.25 ± 0.87	0.104 ± 0.009
		Water Loaded		
P	23.05 ± 3.11	5.07 ± 0.58	54.73 ± 1.87	0.138 ± 0.020
G	21.57 ± 1.72	4.63 ± 0.27	64.20 ± 0.83	0.106 ± 0.006
H	9.89 ± 2.47	6.07 ± 0.96	60.48 ± 0.95	0.070 ± 0.008
S	21.07 ± 2.04	5.02 ± 0.38	60.20 ± 0.82	0.105 ± 0.008
		Sulfathiazole		
P	43.94 ± 3.94	8.90 ± 0.68	25.11 ± 0.93	0.438 ± 0.034
G	43.41 ± 3.72	6.79 ± 0.58	28.60 ± 1.61	0.530 ± 0.041
H	30.74 ± 2.08	5.38 ± 0.30	32.10 ± 0.99	0.406 ± 0.025
S	44.24 ± 2.86	7.24 ± 0.64	28.84 ± 1.24	0.520 ± 0.031
		Water Loaded		
P	26.69 ± 2.51	4.62 ± 0.39	17.68 ± 1.12	0.525 ± 0.063
G	23.47 ± 1.46	5.31 ± 0.26	20.70 ± 0.68	0.407 ± 0.022
H	17.96 ± 2.30	4.08 ± 0.34	30.05 ± 1.35	0.363 ± 0.028
S	24.14 ± 1.86	5.21 ± 0.41	18.64 ± 1.12	0.490 ± 0.027

[a] Results are quoted ± SD of the parameter.

Table II—Two-Compartment Rate Constants for Two Treatments[a]

Rabbit	k_{el}, hr.$^{-1}$	k_{12}, hr.$^{-1}$	k_{21}, hr.$^{-1}$
	Sulfamethazine		
No water			
P	0.799 ± 0.031	1.147 ± 0.072	1.295 ± 0.068
G	0.980 ± 0.046	1.092 ± 0.075	0.992 ± 0.077
H	0.608 ± 0.019	2.473 ± 0.170	5.201 ± 0.345
S	0.955 ± 0.036	2.346 ± 0.090	1.115 ± 0.082
Average	0.834[b]	1.765[b]	2.151[b]
Water loaded			
P	0.791 ± 0.133	1.291 ± 0.304	1.160 ± 0.256
G	1.131 ± 0.102	2.310 ± 0.277	1.525 ± 0.132
H	0.623 ± 0.020	1.968 ± 0.106	3.644 ± 0.184
S	0.952 ± 0.096	2.291 ± 0.286	1.147 ± 0.141
Average	0.874[b]	1.965[b]	1.869[b]
	Sulfisomidine		
No water			
P	0.124 ± 0.017	1.396 ± 0.290	3.393 ± 0.475
G	0.142 ± 0.012	1.094 ± 0.091	3.441 ± 0.276
H	0.084 ± 0.020	0.713 ± 0.219	3.601 ± 0.453
S	0.140 ± 0.014	1.119 ± 0.104	3.474 ± 0.325
Average	0.123[b]	1.081[b]	3.477[b]
Water loaded			
P	0.189 ± 0.019	1.395 ± 0.153	3.583 ± 0.400
G	0.140 ± 0.006	1.113 ± 0.085	3.520 ± 0.153
H	0.080 ± 0.009	0.664 ± 0.158	4.653 ± 0.717
S	0.141 ± 0.008	1.227 ± 0.135	3.735 ± 0.212
Average	0.138[b]	1.100[b]	3.873[b]
	Sulfathiazole		
No water			
P	1.112 ± 0.080	4.816 ± 0.276	3.595 ± 0.351
G	1.190 ± 0.061	3.020 ± 0.312	3.110 ± 0.266
H	0.775 ± 0.029	2.280 ± 0.131	3.196 ± 0.187
S	1.166 ± 0.076	3.402 ± 0.243	3.145 ± 0.241
Average	1.061[b]	3.380[c]	3.262[c]
Water loaded			
P	1.173 ± 0.088	1.966 ± 0.273	2.460 ± 0.326
G	0.797 ± 0.027	2.235 ± 0.118	2.750 ± 0.155
H	0.557 ± 0.023	1.275 ± 0.102	2.792 ± 0.266
S	0.998 ± 0.032	2.160 ± 0.112	2.536 ± 0.212
Average	0.881[b]	1.909[c]	2.635[c]

[a] Results are quoted ± SD of the parameter. [b] Mean values of constants for rabbits not given water and water loaded are not significantly different, $p < 0.05$, one-tail t test. [c] Mean values for untreated and water-loaded rabbits are significantly different, $p < 0.05$, one-tail t test.

The sulfonamides are eliminated from the central compartment by urinary excretion of the free drug and metabolic conversion to the acetyl derivative. Therefore, two clearance parameters may be defined for each sulfonamide, a renal and a metabolic clearance (C_u and C_m, respectively) (20):

$$C_u = V_b k_u \quad \text{(Eq. 9)}$$

$$C_m = V_b k_m \quad \text{(Eq. 10)}$$

The calculated values of the renal and metabolic clearances are shown in Table IV. It should be remembered that these clearances are based on removal of drug from a model compartment and may not be comparable to a physiological renal clearance.

DISCUSSION

The plot of log blood level of drug *versus* time is a curve and, therefore, the data will not fit a simple one-compartment model (8, 14). The data were fitted satisfactorily to a two-compartment open model (Table I and Scheme I). Riegelman *et al.* (14) presented evidence that the two-compartment open model will fit blood level data for a wide variety of drugs. Krüger-Thiemer (21–23) discussed more complex models for the pharmacokinetic behavior of sulfonamides, with particular reference to protein binding effects. Other complex models which may fit the data were proposed by Rowland and his coworkers (24, 25), but here it is proposed to show that the parameters of a simple two-compartment model may be related to the biological behavior of the drugs in response to water loading. In later publications, more complex models involving metabolite blood levels and quantitative protein binding effects will be considered.

Care was taken in the experimental design to exclude possible random sources of variation in pharmacokinetic behavior. It is hoped that the data obtained allow valid conclusions to be drawn about the difference in pharmacokinetic parameters between drugs, between the two treatments (water loading or not), and between individual rabbits. The sample was small and conclusions, therefore, are tentative, but the evaluation of errors should give guidance as to the reliability of the parameters.

The three compounds exhibit differences in pharmacokinetic behavior. Sulfisomidine is always eliminated more slowly than the other two compounds (k_{el}, Table II). The slower elimination of sulfisomidine would appear to be related to a limited ability of the rabbit to metabolize the compound (low k_m, Table III). Since the major pathway of metabolism is *via* acetylation, it is difficult to understand how the minor structural differences between sulfamethazine (the 4,6-dimethylaminopyrimidine sulfonamide) and sulfisomidine (the 2,6-dimethylaminopyrimidine analog) might cause a large difference in k_m (k_m sulfamethazine is approximately 20 × k_m sulfisomidine. Other workers (11, 12) observed that sulfisomidine is acetylated to a much smaller extent than sulfamethazine in the rabbit and man.

Several studies demonstrated that increased protein binding decreases sulfonamide acetylation in liver perfusion systems (26) and in liver preparations (27). Sulfisomidine is known to be strongly bound to rabbit plasma proteins, while sulfathiazole and sulfa-

Table III—Values of Metabolite Formation Constant (k_m) and Free Drug Urinary Excretion Constant (k_u) for Sulfonamides in Rabbits

Rabbit	f — No Water	f — Water Loaded	k_m, hr.$^{-1}$ — No Water	k_m, hr.$^{-1}$ — Water Loaded	k_u, hr.$^{-1}$ — No Water	k_u, hr.$^{-1}$ — Water Loaded
Sulfamethazine						
P	0.103	0.109	0.717	0.705	0.082	0.086
G	0.189	0.240	0.795	0.860	0.185	0.274
H	0.284	0.430	0.435	0.355	0.173	0.268
S	0.175	0.251	0.788	0.713	0.167	0.239
Average	0.188[a]	0.258[a]	0.684[b]	0.658[b]	0.152[a]	0.217[a]
Sulfisomidine						
P	0.712	0.765	0.035	0.044	0.088	0.145
G	0.697	0.736	0.043	0.037	0.099	0.103
H	0.789	0.830	0.017	0.014	0.064	0.070
S	0.703	0.771	0.039	0.032	0.102	0.109
Average	0.725[a]	0.776[a]	0.034[b]	0.032[b]	0.088[b]	0.107[b]
Sulfathiazole						
P	0.357	0.482	0.715	0.608	0.397	0.565
G	0.276	0.496	0.862	0.401	0.328	0.395
H	0.504	0.597	0.384	0.235	0.391	0.323
S	0.340	0.502	0.770	0.497	0.396	0.501
Average	0.369[a]	0.519[a]	0.683[a]	0.435[a]	0.378[b]	0.446[b]

[a] Mean values of constants between treatments are significantly different, $p < 0.05$, one-tail t test. [b] Mean values of constants between treatments (with and without water) are not significantly different, $p < 0.05$, one-tail t test.

Table IV—Apparent Volume of Distribution and Renal and Metabolic Clearances for Sulfonamides from the Central Compartment

Rabbit	V_b, ml. — No Water	V_b, ml. — Water Loaded	Renal Clearance, ml./min.[a] — No Water	Renal Clearance, ml./min.[a] — Water Loaded	Metabolic Clearance, ml./min.[a] — No Water	Metabolic Clearance, ml./min.[a] — Water Loaded
Sulfamethazine						
P	1377	1259	1.88	1.81	16.46	14.79
G	880	1067	2.71	4.87	11.66	15.29
H	792	783	2.28	3.50	5.74	4.63
S	1371	1226	3.76	4.88	17.74	14.57
Average	1100[b]	1084[b]	2.66[c]	3.77[c]	12.90[b]	12.32[b]
Sulfisomidine						
P	585	643	0.85	1.55	0.35	0.47
G	563	583	0.93	1.01	0.40	0.36
H	614	711	0.66	0.83	0.17	0.17
S	577	615	0.85	1.10	0.33	0.33
Average	585[c]	638[c]	0.82[b]	1.12[b]	0.31[b]	0.33[b]
Sulfathiazole						
P	724	1127	4.79	10.61	8.63	11.42
G	694	1132	3.79	7.42	9.97	7.53
H	796	1041	5.19	6.07	5.09	4.41
S	685	1169	4.79	9.76	8.79	9.33
Average	725[c]	1117[c]	4.64[c]	8.47[c]	8.12[b]	8.17[b]

[a] Clearances are uncorrected for protein binding. [b] Mean values between treatments (with and without water) are not significantly different, $p < 0.05$, one-tail t test. [c] Mean values between treatments (with and without water) are significantly different, $p < 0.05$, one-tail t test.

methazine are less so[5] (28). It is well known that protein binding affects the urinary clearance of compounds including sulfonamides (27). The renal clearance values for the three compounds differ (Table IV), but the range of difference is much smaller than the range of values for metabolic clearance and is in a different order, and it may be concluded that the differences in metabolic conversion of the drugs involve other factors besides protein binding. Sulfisomidine, the compound most protein bound, exhibits the lowest values of the three compounds for both metabolic and renal clearances.

The urinary excretion rate constant (k_u, Table III) varies between drugs but to a lesser degree than k_m. Yamazaki *et al.* (5, 6) found a correlation between the one-compartment excretion rate constants for various sulfonamides and their lipophilic character (measured as CH_3Cl-buffer partition coefficient). When the renal clearances of the three drugs are calculated (Table IV), the values are in the same range as those previously obtained for other sulfonamides in the rabbit by standard clearance methods (29).

The distribution rate constants (k_{12} and k_{21}, Table II) are defined in terms of a simple diffusion process between two model compartments. Any interpretation of these constants in relation to differences in tissue distribution of drug must be extremely cautious. The rate constants for diffusion from the central compartment (k_{12}) are similar for sulfamethazine and sulfisomidine but higher for sulfathiazole; for k_{21} the values for sulfisomidine and sulfathiazole are similar, while sulfamethazine tends to have lower values. Interpretation of the apparent volume of distribution (V_b) must also be cautious (30, 31). It appears that V_b is consistently lowest for sulfisomidine, and this may reflect a tendency by a protein bound material to remain in a more restricted central compartment.

The effect of water loading on the model parameters is of interest. Water loading should increase the urinary output of the animals. An increase in k_u and not in k_m would correlate the rate constants of the model with kidney function in the animal and thus help confirm the biological validity of the model. An increase in the one-compartment excretion rate constant for sulfafurazole in man has been demonstrated under urinary alkalosis conditions (32). Water loading does not significantly alter k_{el} in these rabbits (Table II). With k_{12} and k_{21}, a reduction in both constants occurs only with sulfathiazole. Blood sampling may have an inhibitory effect on urinary secretion due to emotional stress in the animals (33). The long-term measurement of f (over 48–72 hr.) may unmask the effect of this inhibition. Water loading causes a significant increase in the fraction excreted as free drug (f) with all compounds (Table III). When k_{el} is partitioned into k_m and k_u with f, the effect of water loading is significant only in causing a decrease in k_m for sulfathiazole. This observation appears to indicate an inhibitory action of water loading on the metabolism of sulfathiazole. When the metabolic clearances are calculated (Eq. 10, Table IV), water loading is found to have no effect on C_m for any of the drugs. Thus, as Riggs (16) pointed out, "the magnitude of the rate constant depends as much upon the volume of the compartment as it does on the effectiveness of the process of removal." The clearance is a measure of overall effectiveness of removal. Metabolic clearance may provide a useful basis on which to compare the rates of metabolism of related drugs in the same species. As expected, water loading induces an increase in renal clearance (C_u) with each drug.

The water-loading technique appears to cause significant change in V_b only with sulfathiazole (Table IV). It is unlikely that this apparent volume change represents a real change in some body compartment volume, and further work will attempt to correlate these effects with the renal excretion mechanism of the drugs.

The experimental design of this study permits comparisons to be made between individual rabbits in regard to their response to drugs and treatments. While the rabbits tended to have similar values with the same drug for renal clearance, k_u and V_b, Rabbit H showed consistently lower values than the other rabbits for the metabolic rate constant (k_m, Table III) and metabolic clearance (C_m, Table IV). No obvious explanation for this difference is available; the rabbit was similar to the others in age and weight, and the difference was consistent over several months of use during which the animal appeared to be in good health. Since this behavior appeared with all three drugs, it supports the view that they are metabolized by a similar mechanism. Since rabbits are known to display pharmacogenetic dimorphism with respect to sulfadiazine acetylation, it could be that Rabbit H was a slow acetylator (34).

Pharmacokinetic studies may provide a basis for comparative work on drug metabolism. In this study, three parameters (k_m, C_m, and f) were obtained which could be used to determine the ability of the rabbit to metabolize the three drugs. The value f is analogous to the "% excreted as metabolite" term commonly used in drug metabolism work. On the basis of f, metabolic ability is in the order: sulfamethazine > sulfathiazole > sulfisomidine. It should be noted (from Eq. 6) that f has an excretory as well as a metabolic component, and this could complicate its use for metabolic com-

[5] Unpublished data from this laboratory.

parisons. The metabolic rate constants (k_m, Table III) are in the order: sulfathiazole ≡ sulfamethazine > sulfisomidine; metabolic clearance is in the order: sulfamethazine > sulfathiazole ≫ sulfisomidine. Further work will explore the utility of these parameters (k_m and C_m) in the metabolic comparison of drugs and species.

The slow distribution rate constant (r_2, Table I) is equivalent to the elimination rate constant of the one-compartment model (14). The biological half-life ($t_{1/2}$) of the compounds is calculated from the relationship $t_{1/2} = 0.693/r_2$. The average values obtained are 2.04, 7.43, and 1.46 hr. for sulfamethazine, sulfisomidine, and sulfathiazole, respectively, while in water-loaded animals they are 2.07, 6.62, and 1.55 hr., respectively. Water loading has little obvious effect on the biological half-lives of the compounds. From various sources, Krüger-Thiemer and Bünger (4) quoted the $t_{1/2}$ values for sulfamethazine, sulfisomidine, and sulfathiazole in man as 7, 7.4, and 3.5 hr., respectively, and it is interesting to compare these values with the rabbit values. Sulfamethazine and sulfisomidine, while very similar in structure, have similar half-lives in man but the half-lives differ widely in the rabbit, as do the other pharmacokinetic parameters discussed earlier. Later publications will explore the possible origin of these pharmacokinetic differences.

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