packages in excess of that for the control package would indicate sorption by the packaging and/or permeation of the package by nitroglycerin.

The potency–time curves for the control package and the conventional package are not significantly different. Except for a drop in potency shortly after packaging due to absorption (8% at 26°), the potency–time curves for the unit-dose package are indistinguishable from those for the control package. Therefore, permeation of the package by nitroglycerin is not significant.

The mean content uniformity (Table VI) appears to be slightly better for the unit-dose package than for the conventional package. Although the tablets studied were stabilized, a small amount of intertablet migration (~2% increase in standard deviation) apparently occurred as the tablets were aged in conventional containers. Since intertablet migration was prevented in the unit-dose package, the content uniformity remained essentially unchanged upon aging.

In summary, acceptable stability for up to 2 years[6] at 26° was demonstrated for unit-dose nitroglycerin in Package IX. The tablets employed in this research were stabilized tablets; conventional tablets would probably lose excessive nitroglycerin through absorption.

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[6] Since the potency does decrease slightly with time due to decomposition, the exact time period during which the tablets will meet USP standards will depend upon the tablet potency before packaging. The excess over label claim was generally small for the tablets studied. More recent lots of Lilly nitroglycerin tablets are formulated to provide a larger excess over label claim at the time of manufacture.

Disposition of Sulfonamides in Food-Producing Animals V: Disposition of Sulfathiazole in Tissue, Urine, and Plasma of Sheep following Intravenous Administration

RICHARD F. BEVILL *x, GARY D. KORITZ *, LEWIS W. DITTERT ‡, and DAVID W. A. BOURNE ‡

Abstract □ The plasma, urine, and tissue sulfathiazole concentrations were determined at various times following intravenous administration to 12 sheep. The plasma and urine data were consistent with a one-compartment pharmacokinetic model, with an elimination half-life of 1.1 hr and a volume of distribution of 0.39 liter/kg. Sulfathiazole was eliminated by excretion of unchanged drug in urine (67%) and by formation of two metabolites. The data obtained from eight tissue sites were consistent with the one-compartment pharmacokinetic model presented and confirmed that tissue residues of sulfathiazole can be calculated from serum and urine drug concentrations.

Keyphrases □ Sulfonamides—disposition of sulfathiazole in tissue, urine, and plasma of sheep following intravenous administration □ Sulfathiazole—disposition in tissue, urine, and plasma of sheep following intravenous administration, pharmacokinetic model □ Pharmacokinetics—sulfathiazole in sheep following intravenous administration□ Disposition, biological—sulfathiazole in tissue, urine, and plasma of sheep following intravenous administration, pharmacokinetic model □ Antibacterials—sulfathiazole, disposition in tissue, urine, and plasma of sheep following intravenous administration, pharmacokinetic model

When food-producing animals are treated with antibacterial drugs, significant drug concentrations may remain for some time in food tissues. Human consumption of meat containing drug residues may subsequently cause the development of hypersensitivity to drugs used therapeutically or the preferential selection of bacterial strains resistant to those drugs (1).

BACKGROUND

Normally, drug residues in the food tissues of animals are controlled by cessation of treatment at some minimum specified time, *i.e.*, the withdrawal time, before slaughter, allowing the drug to "washout" from the food tissues. However, field surveys reporting the number of carcasses with illegal concentrations of antibacterial drugs suggest that a sufficient withdrawal time often is not allowed (2).

The current method for controlling the appearance of drug-contaminated meat on the market consists of randomly checking carcasses at the slaughterhouse. This method is inefficient because drug assays of tissue are generally expensive and time consuming and the detection of contaminated carcasses may cause the condemnation of complete carcass lots. If a method could be developed to detect animals whose meat contained more than the tolerance limit of a drug before slaughter, it would be possible to delay slaughtering until the drug is below tolerated levels, thereby saving the carcass from needless destruction. Furthermore, if the detection method analyzed blood or urine instead of tissue specimens, it should be possible to reduce the cost and time involved in assay and thereby increase the efficiency of surveillance.

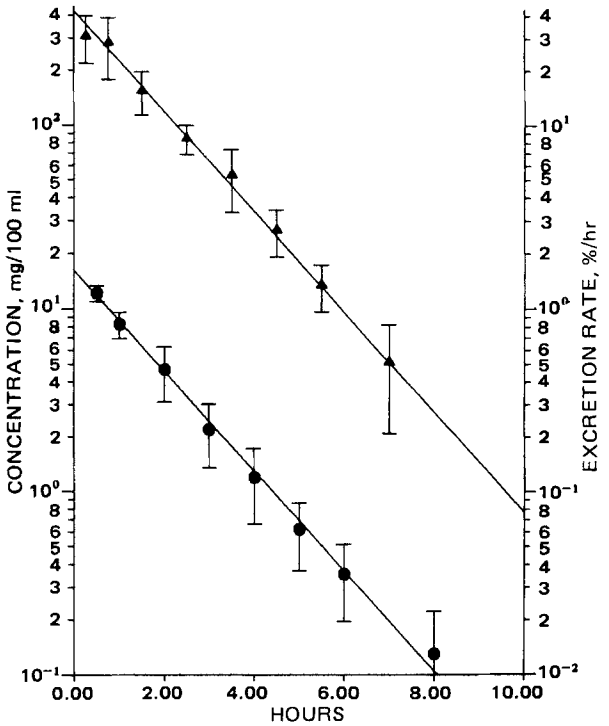


Figure 1—*Semilogarithmic plot of average plasma sulfathiazole concentration (●) and average rate of urinary excretion of unchanged sulfathiazole (▲) versus time following intravenous administration to sheep. The points were experimentally determined (±1 SD), and the lines were calculated using the pharmacokinetic model (Scheme II) and the values of the parameters presented in Table II.*

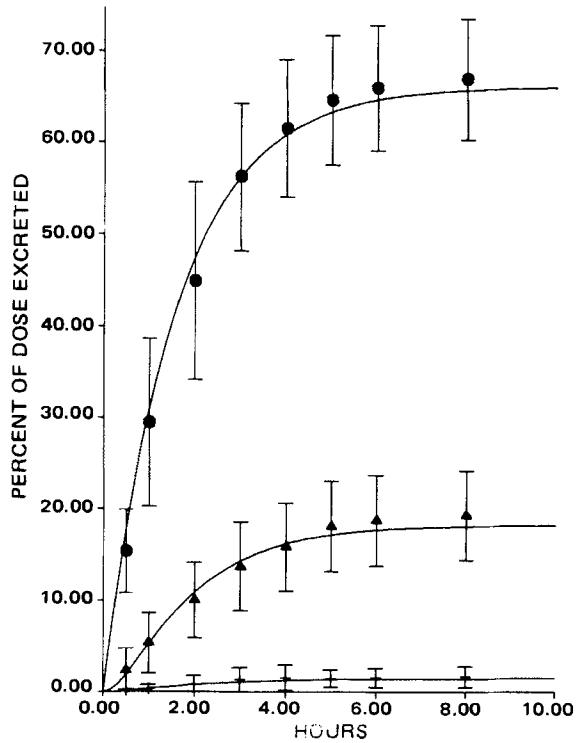


Figure 2—*Plot of cumulative amount of sulfathiazole (●), acetyl metabolite (▲), and polar metabolite (+) excreted in urine versus time following intravenous administration to sheep. The points were experimentally determined (±1 SD), and the lines were calculated using the pharmacokinetic model (Scheme II) and the values of the parameters presented in Table II.*

If a relationship could be established between tissue concentrations and plasma and/or urine concentrations of the drug and if the pharmacokinetics of the drug were known, it would be possible to predict when the tissue concentrations in an animal reach the tolerance limits. Also, by judicious use of pharmacokinetic relationships, the effects of changes in drug products, route of administration, or dosing regimens on withdrawal times could be investigated without the need for expensive slaughter studies.

The preliminary pharmacokinetics of sulfathiazole in sheep following intravenous and oral administrations were presented previously (3). Sulfathiazole disposition was described in terms of a one-compartment pharmacokinetic model (3). In this study, the pharmacokinetic model previously developed was confirmed, and correlations between the drug concentration in various tissues and drug concentrations in plasma and urine following intravenous administration were investigated.

EXPERIMENTAL

Animals—Fifteen mixed-breed female lambs[1] were randomly assigned to groups of three and placed in individual metabolism cages 72 hr prior to drug administration. Twenty-four hours prior to dosing, each animal was weighed and fitted with a Foley retention catheter[2], and the area over each jugular vein was clipped. Hay and water were provided *ad libitum*, but grain was limit fed throughout the acclimation and treatment periods.

Drug Administration and Specimen Collection—A 12.5% solution of sulfathiazole sodium[3] (72 mg/kg) was injected rapidly into the right jugular vein of each animal. Plasma specimens were collected *via* the left jugular vein at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, and 24 hr after administration. The total voided urine volume was measured, and urine specimens were collected at 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, and 24 hr following administration.

Groups of three animals were sacrificed at 2, 4, 8, 16, and 24 hr after drug administration. Three untreated control animals[1] were slaughtered after the other treatments were completed. Portions of liver, heart, kidney, shoulder muscle, loin muscle, hindlimb muscle, body fat, and omental fat tissues were obtained from each slaughtered animal.

Plasma and urine specimens were stored at −5° until assayed. Tissue specimens were frozen in liquid nitrogen, blended to a powder, and stored at −10° until assayed (4).

Assay Methods—Plasma was analyzed for sulfathiazole by the modified Bratton–Marshall method of Annino (5).

Urine was analyzed for unchanged sulfathiazole and its acetyl and polar metabolites by the method of Bevill *et al.* (4), except that ethyl acetate was used to develop the TLC plates instead of chloroform–acetone as used previously.

Tissues were analyzed for sulfathiazole by the method of Bevill *et al.* (4), except that three 25-ml volumes of hexane were used to remove the eluates from the round-bottom flasks after the flash evaporation step, and the flasks were washed with 5 ml of acetone after each hexane wash. Standard curves were prepared as described previously (4). Tissue samples containing 0.1–20 ppm of sulfathiazole were employed.

RESULTS AND DISCUSSION

Analytical Results—The average plasma sulfathiazole concentrations at various times following intravenous administration are shown in Fig. 1. The plasma concentrations decreased rapidly with time, reaching 0.1 mg % (approximate limit of detection) in about 10 hr. The average plasma concentration was below 5 mg % (a therapeutically effective concentration) within 2 hr.

The results of analysis of urine collected at various times following intravenous administration are presented in Fig. 2 as the average cumulative percent of dose excreted as unchanged sulfathiazole and as the acetyl and polar metabolites. Approximately 67% of the dose was excreted as unchanged sulfathiazole, 19% as the acetyl metabolite, and less than 2% as the polar metabolite.

The results of residue analysis of various tissues of lambs slaughtered at various times following drug administration are shown in Table I.

Selection of a Pharmacokinetic Model—Semilogarithmic plots of the average plasma concentration *versus* time and the average rate of excretion of unchanged sulfathiazole in urine *versus* time are presented in Fig. 1. Both these plots were linear with constant and similar slopes,

[1] From the University of Illinois Agricultural Experiment Station, Dixon Springs, Ill.

[2] Bardex, 16 Fr, C. R. Bard Inc., Murray Hill, N.J.

[3] Holmes Serum Co., Springfield, Ill.

Table I—Average Sulfathiazole Concentrations in Tissues of Lambs at Various Times after Administration of Sulfathiazole, 72 mg/kg iv

Hours after Dosing	Average Tissue Concentration, ppm ± *SD*							
	Kidney	Heart	Leg Muscle	Shoulder Muscle	Loin Muscle	Liver	Body Fat	Omental Fat
2.0	308 ±145	34 ±11	22 ±7.5	23 ±8.1	22 ±5.7	40 ±13	11 ±5.2	6.7 ±2.9
4.0	55 ±24	9.3 ±5.9	5.0 ±2.6	4.7 ±2.5	4.9 ±2.8	9.4 ±4.9	3.5 ±1.9	1.4 ±0.54
8.0	2.3 ±1.3	0.32 ±0.12	0.26 ±0.11	0.27 ±0.12	0.23 ±0.11	0.70 ±0.29	0.26 ±0.13	0.12 ±0.04
16.0	0.36 ±0.13	0.08 ±0.03	n.s.[a]	n.s.	0.05 ±0.02	0.30 ±0.14	n.s.	n.s.
24.0	0.11 ±0.06	0.07 ±0.03	n.s.	n.s.	n.s.	0.12 ±0.05	n.s.	n.s.

[a] n.s. = not significant ($p < 0.01$).

Table II—Values of the Parameters of the Pharmacokinetic Model Describing Sulfathiazole Disposition in Lambs (Scheme II)

	V_d[a]	k_{el}[b]	$k_{sulfathiazole}$[b]	$k_{miscellaneous}$[b]	k_{AB}[b]	k_{PB}[b]	k_{AU}[b]	k_{PU}[b]
$\bar{x}$[c]	0.389	0.625	0.424	0.071	0.116	0.013	2.53	1.88
SD[c]	0.044	0.144	0.120	0.046	0.047	0.015	2.4	2.70
Averaged[d]	0.373	0.629	0.417	0.088	0.115	0.009	2.03	1.32

[a] Volume of distribution, liter per kilogram. [b] Rate constants, hour^{-1}. [c] Average and standard deviation of values from individual animals 4–15. [d] Results from fitting Scheme II to averaged plasma and urine data.

suggesting that the overall elimination of sulfathiazole in lambs follows first-order kinetics:

$$\text{rate of elimination} = k_{el}C_p \qquad \text{(Eq. 1)}$$

where C_p is the concentration of unchanged sulfathiazole in plasma.

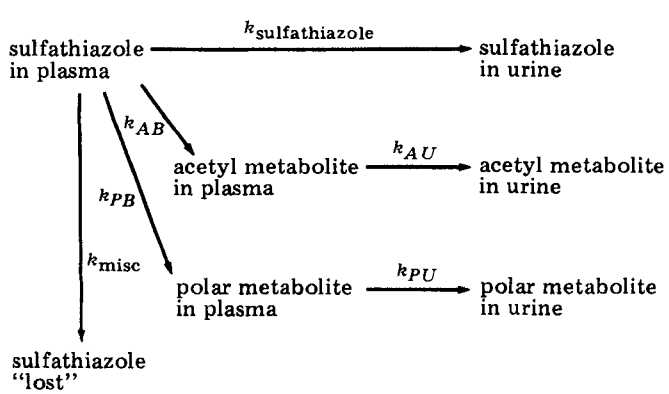
The biological half-life of sulfathiazole in lambs (Fig. 1) is approximately 1.1 hr; therefore, k_{el}, the overall elimination rate constant, is approximately 0.63 hr^{-1}. Since there was no apparent distribution phase in Fig. 1, it was concluded that sulfathiazole pharmacokinetics in lambs could be described by a one-compartment model such as that shown in Scheme I.

sulfathiazole in plasma $\xrightarrow[0.63\ hr^{-1}]{k_{el}=}$ sulfathiazole eliminated from plasma

Scheme I

Urinary excretion data also were collected for unchanged sulfathiazole and its acetyl and polar metabolites, so the pharmacokinetic model could be expanded to include these features. Moreover, since the entire dose was not recovered in the urine, the amount not recovered was represented by a "miscellaneous" process. It was assumed that unchanged sulfathiazole entered the urine directly from the plasma compartment whereas the metabolites were first formed in the plasma (or body) compartment and then cleared into the urine. Based on these assumptions, the model in Scheme II was selected.

Fitting Model to Data—Scheme II was fitted to the data for unchanged sulfathiazole in plasma and urine and for the metabolites of sulfathiazole in urine by obtaining values for each rate constant. First, initial estimates of the rate constants were obtained by the method described previously (6). Then the averaged plasma concentrations of sulfathiazole and averaged urine outputs of sulfathiazole and its metabolites *versus* time were fitted to the model in Scheme II by iterative least-squares computation, using the SAAM 23 program (7) and a digital computer[4].



Scheme II

The calculated values of the parameters of Scheme II for averaged plasma and urine data are presented in Table II. The overall elimination rate constant, k_{el}, was 0.63 hr^{-1} (biological half-life of 1.1 hr), and the volume of distribution was 0.37 liter/kg.

The close agreement between the model and the averaged experimental data is illustrated in Figs. 1 and 2. The lines in these figures were generated by the pharmacokinetic model shown in Scheme II with the "best

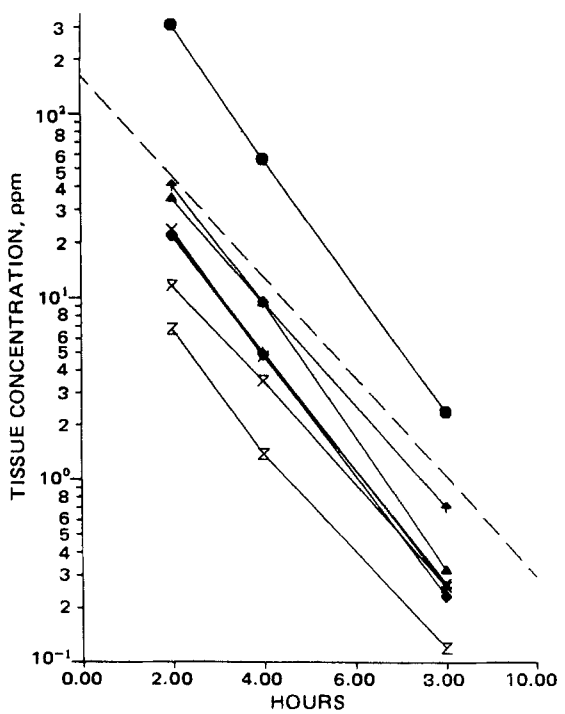


Figure 3—*Semilogarithmic plot of sulfathiazole concentration in various tissues* versus *time following intravenous administration to sheep. The points (with solid connecting lines) were experimentally determined in kidney (●), heart (▲), liver (↑), loin muscle (♦), leg muscle (+), shoulder muscle (×), body fat (⊠), and omental fat (Z) tissue. The dashed line represents the plasma concentration calculated using the pharmacokinetic model (Scheme II) and the values of the parameters presented in Table II.*

[4] IBM 370/165.

Table III—Linear Regression Analysis of Average Plasma Sulfathiazole Concentration and Excretion Rate of Unchanged Sulfathiazole *versus* the Sulfathiazole Concentration in Various Tissues following Administration of Sulfathiazole, 72 mg/kg iv

Hours	Average Plasma Concentration, mg/100 ml	Average Excretion Rate, % dose/hr	Concentration in Tissues, ppm					
			Kidney	Liver	Heart	Muscle	Body Fat	Omental Fat
2.0	4.7	13.4	308	40	34	22	11	6.7
4.0	1.2	4.3	55	9.4	9.3	4.9	3.5	1.4
8.0	0.1	0.7	2.3	0.7	0.3	0.3	0.3	0.1
			r (between plasma and tissue concentrations)					
			1.000	1.000	1.000	0.999	0.998	0.998
			r (between excretion rate and tissue concentration)					
			0.990	0.996	0.998	0.995	1.000	0.992

fit" constants for averaged data shown in Table II. In each figure, the points are the averaged values from the remaining lambs at each sampling time, with 1 *SD* indicated by error bars.

Variations in the values of the rate constants due to variations within the lamb populations were estimated by fitting the data from each animal individually to the model in Scheme II. The results obtained from the averaged data were used as initial estimates. The values obtained for the parameters of Scheme II following iterative least-squares fitting to the individual animal data are presented in Table II. The average overall elimination rate constant was 0.62 ± 0.14 hr^{-1} (biological half-life of 1.1 ± 0.2 hr) and the average volume of distribution was 0.39 ± 0.04 liter/kg. These figures compare favorably with the results obtained by fitting averaged plasma and urine data and with the results obtained previously in sheep (3).

The observed one-compartment pharmacokinetics in lambs imply that the extravascular tissues into which sulfathiazole penetrates are in rapid equilibrium with plasma throughout the entire time that the drug is in the body. In the present study, the one-compartment nature of sulfathiazole pharmacokinetics beyond 2 hr can be observed in the experimentally determined drug concentrations in representative tissue specimens. This result is illustrated in Fig. 3, which shows plasma concentrations of unchanged sulfathiazole calculated using the pharmacokinetic model and concentrations of unchanged sulfathiazole in eight tissues obtained from the animals at slaughter.

According to the proposed model, the drug concentration in each tissue should be directly proportional to the plasma concentration and to the urinary excretion rate of unchanged sulfathiazole determined at the same time. The correlation coefficients obtained by linear regression of the plasma and urine data with the residual concentrations in various tissues are shown in Table III. These excellent correlations demonstrate that the plasma concentrations and the urine outputs of sulfathiazole accurately reflect tissue residues of sulfathiazole in the lamb. Thus, the time required for sulfathiazole to reach negligible levels in various tissues that might be used as food can be determined by plasma and/or urine analysis without slaughtering the animals.

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Cascarosides A and B

J. W. FAIRBAIRN ˣ, F. J. EVANS, and J. D. PHILLIPSON

Abstract □ Electron-impact and field desorption mass spectrometry, together with NMR and circular dichroism spectroscopy, were used to confirm that cascarosides A and B are C-10 isomers of 8-*O*-(β-D-glucopyranosyl)barbaloin. Several batches of cascarosides A and B were prepared and oxidatively hydrolyzed to aloe-emodin. The results are discussed in relation to the assay for cascara given in the European Pharmacopoeia, 1971.

Keyphrases □ Cascarosides A and B—electron-impact and field desorption mass spectrometric and NMR and circular dichroism spectroscopic structural identification □ Barbaloin derivatives—cascarosides A and B, electron-impact and field desorption mass spectrometric and NMR and circular dichroism spectroscopic structural identification □ Mass spectrometry, electron impact and field desorption—structural identification of cascarosides A and B □ NMR spectroscopy—structural identification of cascarosides A and B □ Circular dichroism spectroscopy—structural identification of cascarosides A and B

Cascarosides A and B previously were isolated from *Rhamnus purshiana* DC. bark and shown to contain glucose and barbaloin (1). Later work proved that cascarosides A and B were glucosides of (+)- and (−)-barbaloin, respectively, although it was suggested that the molecules contained additional carbon fragments (2). This suggestion was partly based on the fact that the yield of barbaloin and aloe-emodin after hydrolysis was significantly less than theory for a glucoside of barbaloin. Molecular weight determinations, mass spectrometry, NMR spectroscopy, and