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Pharmacokinetics of Sulfisoxazole Compared in Humans and Two Monogastric Animal Species

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Abstract □ The pharmacokinetic profile of sulfisoxazole was studied and compared in dogs, swine, and humans. The trial was conducted over a 72-hr period after intravenous administration and a 96-hr period after oral administration in dogs and swine. In humans, the trial was conducted over an 8-hr period after oral administration. A two-compartment model system was used to define the pharmacokinetic profile. The mean half-lives for the distribution phase were 4.08, 1.30, and 0.56 hr in dogs, swine, and humans, respectively. For the elimination phase, the mean half-lives were 33.74, 46.39, and 7.40 hr in dogs, swine, and humans, respectively. The mean volume of the central compartment was approximately the same in dogs and swine, 10.6 and 10.5 liters, respectively. Humans had a smaller volume of distribution, 7.7 liters. The steady-state volumes of distribution were 17.2, 30.3, and 16.2 liters in dogs, swine, and humans, respectively. Dogs and swine excreted 42.2 and 30.7%, respectively, of the intravenous dose and 29.4 and 18.3%, respectively, of the oral dose. The bioavailability was 69.8% in dogs and 100.0% in swine. The fraction of drug bound ranged from 30 to 50% in dogs, 40 to 60% in swine, and 25 to 40% in humans.

Keyphrases □ Sulfisoxazole—pharmacokinetics in humans, dogs, and swine □ Pharmacokinetics—sulfisoxazole, comparison in humans, dogs, and swine □ Antibacterials—sulfisoxazole, pharmacokinetics in humans, dogs, and swine

Sulfisoxazole is an effective antibacterial agent often used in the treatment of urinary tract infections. Previous studies in dogs, swine, and cattle only measured blood levels of sulfisoxazole and sulfisoxazole acetyl following intravenous (1), subcutaneous (1), oral (2), and peritoneal (1) administrations. No detailed pharmacokinetic analyses were undertaken to characterize the absorption, distribution, metabolism, and excretion of sulfisoxazole in these animals. Likewise, human studies (3–5) of sulfisoxazole after single oral ingestion were limited to the measurement of blood concentrations of the parent drug and the acetyl metabolite. In one study (2), the steady-state blood level was measured following multiple-dose administration.

The first complete pharmacokinetic study in humans was conducted by Kaplan *et al.* (6) in which sulfisoxazole was administered intravenously, intramuscularly, and orally. Pharmacokinetic parameters were determined and availability was assessed. The present study compared sulfisozaxole pharmacokinetics in dogs, swine, and humans.

EXPERIMENTAL

Materials—A 12.5% sulfisoxazole[1] solution was prepared with lithium hydroxide. The solution was filtered and placed in sterile 50-ml ampuls before use. Sulfisoxazole acetyl[2] was used as a reference standard for serum and urinary metabolite assay. All chemicals and solvents used in the high-performance liquid chromatographic (HPLC) assay were high purity solvents[3] and were filtered before use.

Experimental Model—The trial consisted of three female dogs (~2

[1] Hoffmann–La Roche, Nutley, N.J.
[2] Acetyl-N^4-sulfisoxazole, Hoffmann–La Roche, Nutley, N.J.
[3] Burdick & Jackson solvents, Bodman Chemical Co., Doraville, Ga.

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Table I—Two-Compartment Pharmacokinetic Parameters in Dogs Administered Sulfisoxazole as a Single Intravenous Dose

Subject	$t_{1/2,\alpha}$, hr	$t_{1/2,\beta}$, hr	V_1, liters	V_2, liters	k_{12}, hr^{-1}	k_{21}, hr^{-1}	k_{10}, hr^{-1}	Cl_T, liters/hr
1	4.61	35.18	9.03	2.95	0.0068	0.0208	0.1424	1.29
2	5.02	33.97	11.04	5.07	0.0103	0.0224	0.1258	1.39
3	3.70	36.28	9.55	6.87	0.0151	0.0210	0.1701	1.62
4	3.74	33.48	10.07	6.34	0.0143	0.0227	0.1689	1.70
5	3.92	29.87	14.07	10.77	0.0206	0.0269	0.1525	2.15
6	3.50	33.64	9.84	7.29	0.0169	0.0228	0.1791	1.76
Mean	4.08	33.74	10.60	6.55	0.0140	0.0228	0.1565	1.65
SD	0.60	2.17	1.83	2.59	0.0049	0.0022	0.0200	0.30

years old, 20.0 ± 1.0 kg), six female pigs (~3 months old, 20.0 ± 1.0 kg), and six male human volunteers (25–30 years old, 70–80 kg). The dogs were administered 100 mg of sulfisoxazole/kg by intravenous and oral routes in two replicates for each route with a 30-day rest period between administrations. The pigs also were administered 100 mg of sulfisoxazole/kg by intravenous and oral routes with a 21-day washout period. All animals were housed in metabolism cages and were maintained on a commercial diet[4] with *ad libitum* access to water.

Sulfisoxazole was administered both orally and intravenously as a 12.5% solution to dogs and swine. The human volunteers received 2.0 g of sulfisoxazole in a single oral dose. Due to the potential toxicity of intravenous administration of sulfisoxazole and its removal from the drug market, the Human Research Committee of the University of Florida would not allow intravenous administration to humans.

Sampling Schemes—For dogs and swine, blood samples were taken at 0.5, 1, 2, 3, 4.5, 6, 9, 12, 22, 32, 44, 56, and 72 hr after intravenous administration and at 0, 1, 2, 4, 6, 8, 10, 12, 14, 23, 32, 44, 56, 76, and 96 hr after oral administration. Total urine was collected daily by natural voiding for up to 4 days. Human blood samples were collected at 0, 1, 2, 4, 6, and 8 hr after oral administration. Urine was not collected in the human trial. Blood samples were taken from the cephalic vein in dogs and humans and *via* the anterior vena cava in swine. Serum and urine samples were frozen at −4° for up to 14 days before analysis.

Assay for Sulfisoxazole and Sulfisoxazole Acetyl—Sulfisoxazole and sulfisoxazole acetyl in plasma and urine were analyzed by a reversed-phase HPLC technique developed in these laboratories (7), using a 60% distilled water–40% methanol liquid phase with acetate buffer added to reduce the pH to 4.00. The detection limit for this assay was 10 ng/ml for both sulfisoxazole and the acetyl metabolite at 254 nm[5].

Serum samples were injected directly into the HPLC system, which was fitted with an in-line precolumn[6]. Unbound, unmetabolized sulfisoxazole and sulfisoxazole acetyl were extracted by cooling 25.0 ml of urine to 4° in an ice bath; sufficient 6 *M* HCl was added to reduce the pH to 3.0. After 5 min, 15.0 ml of chloroform[3] was added, the solution was removed from the ice bath, and extraction was completed in 5 min by swirling the solution once per minute. The chloroform was removed and evaporated under nitrogen, and the residue was reconstituted with methanol[3]. The peak height ratio was used to calculate the concentration of unbound, unmetabolized sulfisoxazole and sulfisoxazole acetyl with sulfathiazole as the internal standard.

Both serum and urine samples were spotted on TLC plates (8) to determine if other metabolites were present. All spots were accounted for without the elution of other metabolites.

Plasma Protein Binding—Human plasma samples, prepared at concentrations of 25, 50, 100, 200, 300, 400, and 500 μg of sulfisoxazole/ml, were dialyzed against 2.0 ml of pH 7.4 phosphate buffer. The dialysate was collected and analyzed for sulfisoxazole content. Protein binding of plasma samples collected from dogs, swine, and humans at various time intervals also was determined by equilibrium dialysis. The fraction of the drug bound was defined as C_f/C_t, where C_f is the drug concentration in the dialysate and C_t is the drug concentration in the plasma at the completion of equilibrium dialysis.

Data Analysis—A two-compartment open body model was applied to the analysis of plasma sulfisoxazole data after intravenous administration using a NONLIN program (9). Pharmacokinetic parameters, including α, β, k_{12}, k_{21}, k_{10}, V_1, and V_2, were defined from the curve fitting. Plasma clearance was defined from the model as $k_{10}V_1$, where V_1 is the distribution volume of the central compartment. Renal clearance was defined as f_eCl_t, where f_e is the fraction of sulfisoxazole excreted unchanged in the urine. The volume of the peripheral compartment (V_2) was defined from the model $k_{12}V_1 = k_{21}V_2$.

Bioavailability of sulfisoxazole following solution administration to dogs and swine was determined by the urinary excretion method. The unchanged sulfisoxazole excreted in the urine in 72 hr was compared for oral and intravenous administration. The *AUC* (area under the plasma concentration–time curve) method was not applied to the determination of bioavailability in this investigation due to the discrepancies in terminal half-lives observed following intravenous and oral administration. This discrepancy could be due to the erratic absorption of the oral dose. The half-life of sulfisoxazole following oral administration to humans was determined from the log-linear slope of the plasma concentration–time curve. No attempt was made to assess the oral bioavailability in humans.

RESULTS

In the 72-hr period following intravenous administration in the dog, sulfisoxazole levels showed biexponential decay (Fig. 1). The β-phase half-life in these animals had a mean of 33.74 hr, while the α-phase half-life ranged from 3.5 to 5.0 hr (mean 4.08). Mean values for the volume of the central pool and the steady-state volume of distribution were 10.60 and 17.15 liters, respectively. Serum sulfisoxazole clearance ranged from 1.39 to 2.15 liters/hr (mean 1.65). The fraction of the dose eliminated unchanged was 42.2%. Renal clearance averaged 0.7 liter/hr. Pharmacokinetic parameters of the two-compartment model in dogs following intravenous sulfisoxazole administration are summarized in Table I.

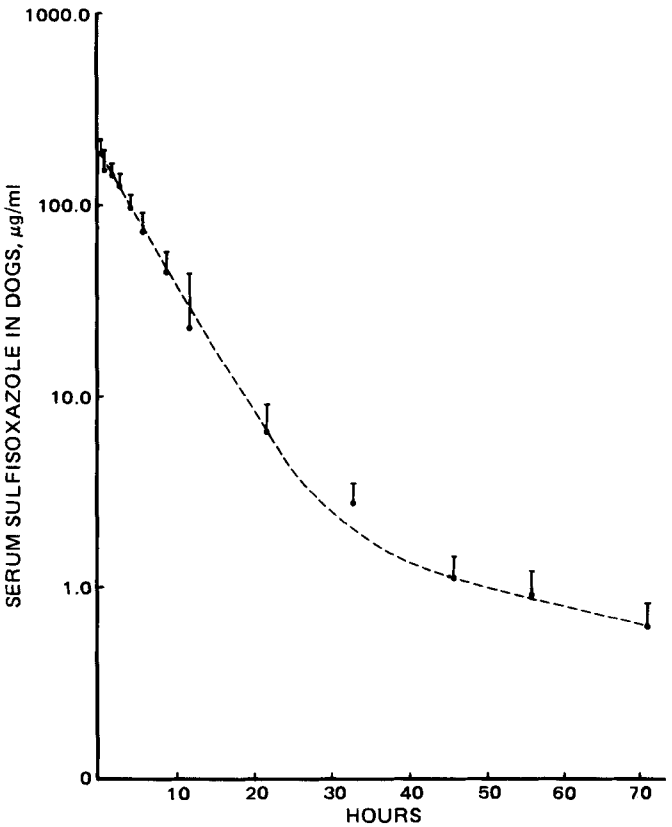

Figure 1—*Serum concentrations of unbound, unmetabolized sulfisoxazole in dogs.*

[4] Purina Dog Chow, Ralston-Purina Co., St. Louis, Mo., and 18% protein swine feed, University of Florida Swine Unit, Gainesville, Fla.

[5] Model 440 absorbance detector, Waters Associates, Milford, Mass.

[6] Corasil, Waters Associates, Milford, Mass.

Table II—Two-Compartment Pharmacokinetic Parameters in Swine Administered Sulfisoxazole as a Single Intravenous Dose

Subject	$t_{1/2,\alpha}$, hr	$t_{1/2,\beta}$, hr	V_1, liters	V_2, liters	k_{12}, hr^{-1}	k_{21}, hr^{-1}	k_{10}, hr^{-1}	Cl_T, liters/hr
1	1.21	31.64	12.76	11.98	0.0214	0.0228	0.5509	7.03
2	1.36	36.67	15.65	24.65	0.0317	0.0202	0.4782	7.48
3	1.18	56.34	8.20	15.70	0.0247	0.0129	0.5631	4.62
4	1.31	64.77	9.58	33.12	0.0401	0.0116	0.4876	4.67
5	1.32	42.11	7.57	15.73	0.0293	0.0141	0.4931	3.73
6	1.40	46.82	9.09	17.49	0.0304	0.0158	0.4647	4.22
Mean	1.30	46.39	10.48	19.76	0.0296	0.0162	0.5063	5.29
SD	0.09	12.39	3.11	7.75	0.0064	0.0044	0.0406	1.56

The plasma levels in swine following intravenous administration also were biexponential (Fig. 2) and were analyzed by a two-compartment model. The α- and β-phase half-lives in swine had means of 1.30 (range 1.18–1.40) and 53.33 (range 31.64–64.77) hr, respectively. The mean volumes were 10.48 and 30.24 liters for the central and steady-state pools, respectively. Plasma clearance ranged from 3.73 to 7.48 liters/hr (mean 5.29). Based on the percent of dose excreted unchanged (18.2%), the average renal clearance was 0.96 liter/hr. Pharmacokinetic constants of the two-compartment model in six swine following intravenous sulfisoxazole administration are recorded in Table II.

Following oral administration of sulfisoxazole to dogs, the peak plasma concentrations, ranging from 122.3 to 165.0 μg/ml, occurred at 1 hr. The peak time for swine occurred at 1 hr with much lower peak concentrations of 32.8–100.6 μg/ml. Sulfisoxazole acetyl reached a maximum of 12.1–20.0 μg/ml in 2–4 hr in all six pigs. The β-phase half-life of the acetyl metabolite was estimated to range from 23.2 to 37.9 hr with a mean of 31.0 ± 5.3 hr in pigs. Dogs either do not metabolize many aromatic amine compounds through acetylation or else possess a deacetylase that precludes analysis of the acetyl derivative (10). Sulfisoxazole acetyl was not observed in the urine or serum of dogs administered sulfisoxazole.

Following oral sulfisoxazole administration to humans, the plasma data were analyzed by a one-compartment model. Two-compartment analysis was not possible due to the lack of sampling in the 1st hr. The peak concentration of the unmetabolized sulfisoxazole occurred in <1 hr following oral administration, although the exact time was not known (Fig. 3). The rapid absorption of sulfisoxazole following oral administration was due to the drug being given as a solution. The β-phase half-life of sulfisoxazole ranged from 6.0 to 8.8 hr with a mean of 7.41 ± 0.59 hr. The half-life of sulfisoxazole in humans was not comparable to either the α-phase or the β-phase half-life in dogs and swine; however, it was consistent with the β-phase half-life reported in a previous intravenous study in humans (6). The acetyl metabolite peaked at 4 hr and exhibited a longer β-phase half-life than the parent compound (Fig. 3). The mean biological half-life of sulfisoxazole acetyl was 14.1 ± 2.2 hr.

Table III records the percent of dose excreted as unchanged sulfisoxazole in the urine for the 96-hr period. Dogs and swine excreted 42.2 and 18.2% of the intravenous dose, respectively, during the trial period. In the oral trial, dogs excreted 29.4% of the dose compared to 18.3% excreted in swine.

Sulfisoxazole bioavailability (Table IV), 69.8 and 100% in dogs and swine, respectively, was determined from the unchanged drug excreted in the urine in 72 hr. Bioavailability of the drug in humans, although not available from this study due to a lack of corresponding intravenous data, was demonstrated to be complete (6).

In dogs, the fraction of the drug bound to plasma protein ranged from 30 to 50% in the sulfisoxazole concentration of 0–240 μg/ml (Table IV). In the same concentration range, sulfisoxazole was found to bind swine plasma protein from 40 to 60%. In humans, the bound fraction ranged from 25 to 40% in the concentration range of 80–200 μg/ml during the 8 hr following oral sulfisoxazole dosing. The *in vitro* binding of sulfisoxazole to blank human plasma in the range of 25–500 μg/ml was higher (38.9–63.0%) than *in vivo* binding and showed a concentration dependency. These *in vivo* plasma protein binding data were quite different from the

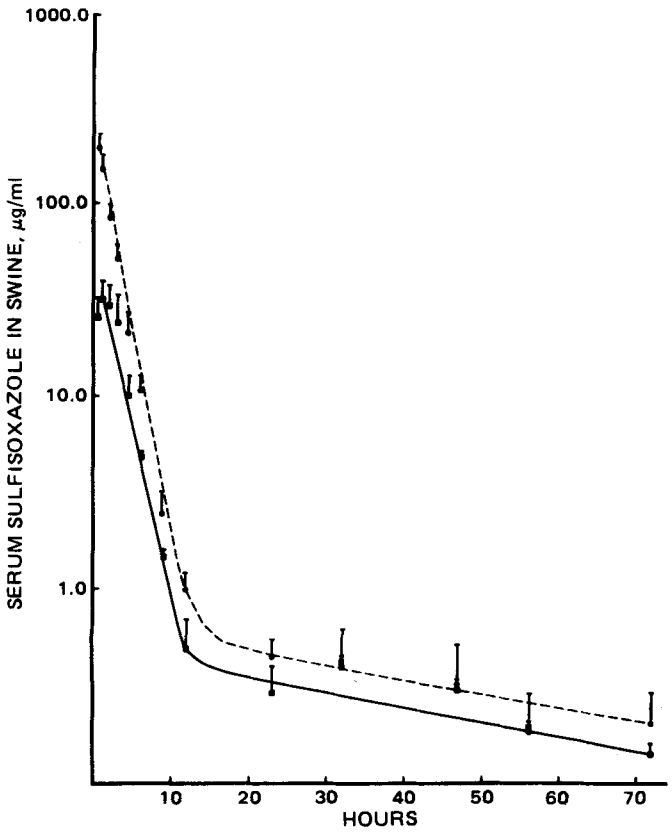

Figure 2—*Serum concentrations of unbound, unmetabolized sulfisoxazole (---) and sulfisoxazole acetyl (—) in swine.*

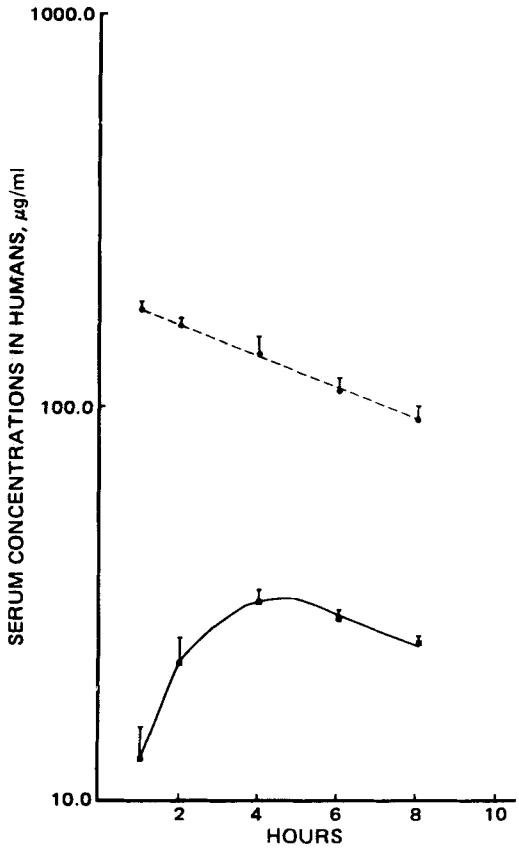

Figure 3—*Serum concentrations of unbound, unmetabolized sulfisoxazole (---) and sulfisoxazole acetyl (—) in humans.*

Table III—Comparison of the Percentage of Sulfisoxazole Dose Excreted Unchanged in the Urine in Dogs and Pigs in a 4-Day Period

Hours	Dogs		Pigs	
	Intravenous	Oral	Intravenous	Oral
24	39.6	26.2	16.1	15.2
48	41.8	29.1	18.1	17.7
72	42.2	—	18.2	—
96	—	29.4	—	18.3

85% previously reported by Struller (11) but were consistent with the Randall *et al.* (2) report of 25%.

Table IV lists some mean pharmacokinetic parameters of sulfisoxazole in dogs, swine, and humans. Most of these parameters are significantly different from one another among species and can be used to evaluate the pharmacokinetic closeness of one species to another.

DISCUSSION

The mean volume of the central compartment (V_1) was approximately the same in dogs (10.6 liters) and swine (10.5 liters). However, this value may become quite different when expressed in terms of lean body weight because of the much greater fat content in swine. This condition may explain the difference in the extrapolated initial concentration (C_0) of 192.0 μg/ml in dogs and 245.5 μg/ml in swine, although both species were given the same total dose. The V_1 value in humans was not available from this study; however, it could be derived from the Kaplan *et al.* (6) data as 7.7 liters, ~30% smaller than that of dogs. The C_0 was reported as 259.0 μg/ml in humans, a value 30% greater than that of dogs. The discrepancies in V_1 and C_0 are surprisingly consistent and compensated for each other in dogs and humans. This observation is in agreement with the dog being closer to humans with regard to fat content.

The difference in C_0 also can be explained in part by the peripheral volume (V_2) of 6.6 and 19.8 liters in dogs and swine, respectively. Humans had a V_2 value (8.5 liters) closer to that of dogs. The larger V_2 in swine could be due to the greater volume of total body water in young animals (12). Three-month-old swine were used in this experiment to eliminate the large volume of body fat found in adult animals.

The distribution ratio defined as k_{21}/k_{12} was greatest in humans (2.3), followed by dogs (1.6) and swine (0.55), indicating that the drug returned from the peripheral compartment to the central compartment at a faster rate in humans than in swine (Table IV). This result was logically tied to the β-phase half-life of 7.4, 33.7, and 46.4 hr in humans, dogs, and swine, respectively, being in the reverse order to the k_{21}/k_{12} ratio previously discussed. The ratio of β/k_{10} (Table IV) was 0.03 in swine and 0.13 in dogs, and it was reported to be 0.66 in humans (6). This is in the same order as k_{21}/k_{12}, suggesting that more sulfisoxazole is available for elimination from the postdistributive phase in humans than in dogs and swine. This pattern also correlated reasonably with the β-phase half-life of the drug in these species.

By the end of the 72-hr intravenous trial in dogs, 42.2% of the dose was excreted in the urine as unchanged drug. At the end of 48 and 96 hr after oral administration, 29.1 and 29.4% of the dose were excreted, respectively, indicating that urinary excretion of the drug was complete at 72 hr. Therefore, oral sulfisoxazole bioavailability was determined by comparing the urinary excretion of the drug following these administration routes. Almost 70% of the dose was available to the general circulation after oral administration of the drug as a solution to dogs. The drug bioavailability in swine was determined in the same manner, and a value of almost 100% was obtained (Table IV). Dogs excreted 29.4% of the oral dose compared to 18.3% in swine, yet dogs actually exhibited less

Table IV—Comparison of Mean Pharmacokinetic Constants in Dogs, Swine, and Humans

Constant[a]	Dogs	Swine	Humans
k_{21}/k_{12}	1.63	0.55	2.30[b]
β/k	0.13	0.03	0.66[b]
F, %	69.8	100.0	97.5[b]
r_p, %	30–50	40–60	25–40

[a] F = bioavailability; r_p = percent bound. [b] Reference 6.

oral drug absorption. Since both dogs and swine excreted only up to 42.2 and 18.2% of the intravenous dose, respectively, during the trial period, other metabolites probably are present but are not detected by the assay method used. Kaplan *et al.* (6) reported that 52.9% of the dose was excreted as unchanged sulfisoxazole in humans. No urinary data from the present study were available for comparison since the intravenous study was not conducted.

Following oral sulfisoxazole administration to swine and dogs, terminal half-lives deviated significantly from those following intravenous administration in each crossover study, particularly in dogs. This finding may indicate erratic drug absorption in both species, especially in dogs. This erratic absorption may contribute to the incomplete drug absorption in dogs. Different physiology of the GI system also may contribute to the different extent of drug absorption in dogs and swine.

Differences did exist for both α- and β-phase biological half-lives, bioavailability, plasma protein binding, and urinary excretion among all three species. No conclusion can be drawn as to which animal model is pharmacokinetically closer to humans. However, dogs appeared to be a better model than swine for studying the bilirubin toxicity induced by sulfisoxazole administration. The oral dose was responsible for increasing the free, unbound bilirubin in dogs without an increase in total bilirubin. This result did not occur in humans and swine. This topic will be the subject of a separate report.

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