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Sulfamethazine Residue in Calf Tissues

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Sulfamethazine prolonged release bolus was orally administered to calves in a single therapeutic dose of 225 mg per lb of body weight. Sulfamethazine residues in edible tissues were measured. The average biological half-life of the drug in various tissues fell within a narrow range of 0.87 to 1.05 days. Tissue residues in muscle, fat, liver, and kidney fell to control levels by 16 days. Data are presented which validate the method used in the range of 100–10 000 ppb. Tissue residue levels are not adversely affected if the tissues are frozen for up to 40 days prior to analysis.

In the development of sulfamethazine prolonged release bolus (Hava-span, Bayvet Corporation, Kansas City, Mo.) it was necessary to determine the clearance of the drug in four edible tissues—liver, kidney, muscle, and fat. Messersmith et al. (1967), administered sulfamethazine in combination with antibiotics to swine over a 14-week period. They found that the residue of sulfamethazine in edible tissues was less than 0.1 ppm 7 days after treatment was discontinued. Righter et al. (1971) administered sulfamethazine as a drench at a dose of 99 mg per kg per day and reported that sulfamethazine levels in calf tissues were reduced to 0.1 ppm by the eighth day after withdrawal of the drug.

Of the several methods (Fellig and Westheimer, 1968; Mooney and Pasarela, 1964; Righter et al., 1972) available, the procedure of Tishler et al. (1968) was chosen. The method described below is a modification of this procedure. Method validation data at both high and low levels of sulfamethazine are presented.

MATERIALS AND METHODS

Reagents and Chemicals. Sulfamethazine, USP, was obtained from B. L. Lemke and Company. All other reagents and solvents used were either analytical reagents or of the highest available purity.

Glassware. All glassware used in this study was washed with 6 N hydrochloric acid, 20% methanolic potassium hydroxide, detergent, distilled water, and acetone, in that order.

Experimental Design. Five groups of two steers and two heifers weighing between 400 and 600 lb were orally administered a single clinical dose (1 bolus/100 lb) of sulfamethazine prolonged release bolus. One group of calves was sacrificed at each of the following intervals: 2, 5, 10, 16, and 21 days. In addition, one steer and one heifer weighing 600 lb each were sacrificed and their liver, kidney, muscle, and fat collected and analyzed to determine sulfamethazine residues in control animals. All animals were kept on concrete floor and on a sulfa-free diet for 60 days prior to dosing and thereafter until sacrificed.

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Five pounds of adipose tissue was taken from the greater omentum and the large deposits on the body walls surrounding the kidneys. Both kidneys were collected and freed of fat and connective tissue. The entire liver was collected. Five pounds of striated muscle was taken from the neck region of the carcass and the fat removed.

The kidney and liver were each cut into 2- to 5-g pieces. The fat and muscle were each cut into chunks and ground in an electric grinder. Each tissue was then mixed thoroughly and sampled randomly. The tissue samples were analyzed immediately as described below. Weighed portions of the tissue samples were stored at −20 °C for studying the effect of freezing on sulfamethazine levels.

Analytical Methods. (a) *Liver, Kidney, or Muscle.* Tissue was weighed into a VirTis blender jar (200 mL) and homogenized with 90 mL of a mixture of acetone–chloroform (1:1). The extract was decanted through a glass wool plug into a 1-L round-bottomed flask. The extraction was repeated twice, each time decanting the extract through the glass wool plug into the same flask. The VirTis jar and the funnel were washed with several portions of the solvent mixture and the washes were collected in the round-bottomed flask. The solvent was removed from the combined extracts on a rotary evaporator at 70 °C. The residue was dissolved in 50 mL of hexane and transferred quantitatively to a 250-mL separatory funnel by washing with two 25-mL portions of hexane, two 3-mL portions of acetone, and two 25-mL portions of hexane, in that order. The organic phase was extracted into 10 mL of 1 N HCl, by vigorous shaking for 1 min, and the acid phase was drawn off into a 50-mL graduated cylinder. The organic phase was extracted three more times with 4-mL portions of 1 N HCl and then discarded. The acid phases were combined and diluted to 30 mL (in a graduated cylinder) with 1 N HCl and filtered through a 30-mL fine sintered glass funnel into a 125-mL Erlenmeyer flask. The graduated cylinder was washed with 2 mL of 1 N HCl and the washings filtered through a sintered glass funnel into the Erlenmeyer flask. Sulfamethazine content of the acid extract was determined by Bratton Marshall reaction.

(b) *Fat.* The fat was weighed into a 500-mL Erlenmeyer flask and to it was added 350 mL of acetone–chloroform (1:1). The mixture was stirred with a magnetic stirrer until

Table I. Amount of Sulfamethazine Added to Tissues for Recovery Studies

Sulfamethazine level in tissue, ppb	Wt of tissue, g	Vol of sulfamethazine added
0	50 ± 0.1	
100	50 ± 0.1	5 mL of 100 μg/100 mL
1 000	25 ± 0.1	5 mL of 500 μg/100 mL
10 000	10 ± 0.1	5 mL of 2000 μg/100 mL

the fat dissolved and only the connective tissue remained. The resulting solution was filtered through a glass wool plug into a 1-L round-bottomed flask. The flask and the funnel were washed with several portions of acetone–chloroform (1:1) and each wash filtered into the round-bottomed flask. Sulfamethazine content was determined by following the procedure as in analysis of liver starting with "The solvent was removed. . .".

(c) *Sulfamethazine Standards.* Sulfamethazine standard was accurately weighed (100 mg) and dissolved in 1 N HCl and diluted to 100 mL with 1 N HCl, and stored under refrigeration (stock standard). Working standard (1.25 μg/mL) was prepared from stock standard by appropriate dilutions with distilled water.

Two, 4, 8, and 20 mL of working standard were pipetted into 50-mL graduated cylinders. Five milliliters of 1 N HCl was added to each cylinder and diluted to 30 mL with distilled water. The contents of each cylinder were transferred to a 125-mL Erlenmeyer flask. Each graduated cylinder was washed with 2 mL of distilled water and the wash was added to the Erlenmeyer flask. Sulfamethazine content of the acid extract was determined by Bratton Marshall reaction.

(d) *Bratton Marshall Reaction.* One milliliter of 0.2% aqueous sodium nitrite was added to the acid extract, mixed well, and allowed to stand for 3 min. This was followed by adding 1 mL of 1% aqueous ammonium sulfamate and mixing well, and allowing the solution to stand for 2 min. Two 15-mL aliquots from each standard or sample were pipetted into separate 50-mL Erlenmeyer flasks. One milliliter of 0.1% aqueous *N*-(1-naphthyl)-ethylenediamine dihydrochloride was added to one of the aliquots. The reaction mixture was mixed well and allowed to stand for 15 min. One milliliter of distilled water was added to the second aliquot and mixed (this was the sample blank). The absorbance of each sample was determined against its own blank at 545 nm in a 5 cm cell using a Cary 15 spectrophotometer.

METHOD VALIDATION

For method validation studies, a known amount of sulfamethazine was added to the four tissues and analyzed according to the above procedures in our laboratory and by Harris Laboratories in Lincoln, Nebr.

Recovery from Liver, Kidney, or Muscle (Table I). The tissue was homogenized with 90 mL of acetone–chloroform (1:1) for 1 min. To it was added 5 mL of aqueous sulfamethazine and the mixture was homogenized for another 30 s. The samples were analyzed as in analysis of liver starting with "The extract was decanted. . .". Samples containing 10 000 ppb of sulfamethazine were diluted tenfold prior to color development.

Recovery from Fat (Table I). The fat was dissolved in 350 mL of acetone–chloroform (1:1) by stirring. When the solution was clear, 5 mL of aqueous sulfamethazine was added and the mixture was stirred for an additional 5 min. The samples were analyzed as in analysis of fat starting with "The resulting solution was filtered. . .". Samples

Table II. Comparative Statistics of Method Validation Data Obtained on Edible Calf Tissues

Amt added, ppb	Lab	Liver				Kidney				Muscle				Fat			
		Av % recovery[a]	Range	SD	SE[b]	Av % recovery[a]	Range	SD	SE[b]	Av % recovery[a]	Range	SD	SE[b]	Av % recovery[a]	Range	SD	SE[b]
100	Cutter[c]	84.5	79.9–87.5	3.3	2.3	70.5	64.4–73.6	3.6	2.6	84.5	75.9–92.1	7.7	5.5	95.5	93.9–99.2	2.1	1.5
	Harris[d]	96.0	86.1–109.5	6.7	4.8	95.0	91.3–97.1	2.4	1.7	70.5	58.7–76.5	6.4	4.5	92.0	75.1–101.5	8.2	5.8
1 000	Cutter[c]	91.5	86.8–99.3	4.8	3.4	85.0	78.9–93.2	6.1	4.3	84.0	77.8–86.7	3.5	2.5	97.0	94.9–99.4	2.3	1.6
	Harris[d]	90.5	88.6–97.4	3.4	2.4					79.5	75.3–82.6	2.8	2.0	88.5	84.6–90.4	1.8	1.3
10 000	Cutter[c]	87.5	77.7–97.7	9.4	6.7	80.5	68.4–93.7	10.3	7.3	70.0	57.3–76.9	7.5	5.3	96.0	93.5–98.1	1.8	1.3
	Harris[d]																
Blank	Cutter[c]	4.0[e]	1.5–6.9[e]	2.1	1.5	11.0[e]	4.6–19.2[e]	5.5	3.9	1.0[e]	0–2.3[e]	0.9	0.6	0[e]	0–0[e]		
(0)	Harris[d]	11.8[e]	9.5–13.8[e]	1.5	1.1	24.5[e]	20.4–30.7[e]	4.0	2.8	15.0[e]	3.6–25.5[e]	8.4	5.9	4.0[e]	0.7–8.7[e]	2.5	1.7

[a] Average values have been rounded off to the nearest 0.5 and have been corrected for the tissue blank. [b] Standard error calculated based on $N = 2$. [c] Average of five replicates. [d] Average of ten replicates, except kidney (average of five replicates). [e] Values are in parts per billion.

Table III. Effect of Storage at −20 °C on Sulfamethazine Residues in Calf Tissues

Days at −20 °C	Sulfamethazine residues, ppb			
	Liver	Kidney	Muscle	Fat
0[a]	5421	4175	1706	334
6[a]	5294	4178	1698	371
40[b]	4890	4082	1286	390

[a] Average of quadruplicates. [b] Average of duplicates.

containing 10 000 ppb of sulfamethazine were diluted tenfold prior to color development.

The data presented in Table II show that average control tissue levels and the average recoveries from all the tissues under investigation at 100-, 1000-, and 10 000-ppb levels. Recoveries from fat ranged from 88.5 to 97.0%. Recoveries from liver, kidney, and muscle ranged from 70.0 to 96.0%.

Consistently good recoveries were observed for fat and indicate the accuracy of the method. Adsorption of the drug to the connective tissue is assumed to be minimal since very little connective tissue remains after dissolution of the fat. The unrecovered sulfamethazine is considered to represent the inherent losses in the method. Recoveries from liver, kidney, and muscle are generally lower, probably due to greater adsorptive losses of the drug on these tissues. The precision of the method in both cases was acceptable.

Statistical evaluation of the data is also included in Table II. Standard deviation ranges from 1.8 to 10.3. However in most cases, it is less than 5.0. Standard error has been calculated for $N = 2$, since samples were to be analyzed in duplicate.

Effect of Storage at −20 °C on Sulfamethazine Residues in Calf Tissues. In order to investigate the effect of freezing on sulfamethazine residue levels, liver, kidney, muscle, and fat samples were analyzed initially (within 2 h of sacrificing the animal) and then at various time intervals after storage at −20 °C. These tissue samples were obtained from a calf which was sacrificed 5 days after oral administration of a therapeutic dose of a sulfamethazine prolonged release bolus. These results are recorded in Table III and are within the variation of the method. It is concluded that these tissues can be kept frozen for prolonged periods without adversely affecting the drug residue levels.

Calculations. Sulfamethazine concentration was calculated using a program for line of best fit by the method of least squares. Beer's law is obeyed for concentrations ranging from 50 to 1000 ppb.

RESULTS

Average sulfamethazine residues observed in various tissues of calves at 2, 5, 10, 16, and 21 days after oral administration of a single therapeutic dose are recorded in Table IV. Tissue levels increase after administration of the bolus and reach maximum in about 2 days.

In three out of four animals tissue residue levels were below 100 ppb by 10 days after dosing. By 16 days after dosing tissue residue levels fell to control level in four out of four animals.

Table IV. Sulfamethazine Residue in Calf Tissues after Oral Administration of a Single Therapeutic Dose of Sulfamethazine Prolonged Release Bolus

Days after adminis. of ther. dose	Concn of sulfamethazine in calf tissues, ppb[a]			
	Muscle	Fat	Liver	Kidney
2[b]	72 902	17 175	111 572	130 005
5[b]	2 948	738.9	7 440	6 350
10[b]	113.3	35.9	503.9	285.1
16[b]	12.0	10.4	17.7	21.2
21[b]	18.5	4.9	16.6	19.1
Control[c]	7.6	0.3	13.3	23.2

[a] All results reported are uncorrected for control levels. [b] Each result is an average of the results obtained on four animals. [c] Each result is an average of the results obtained on two animals.

Table V. Average Biological Half-Life of Sulfamethazine in Calf Tissues after Oral Administration of Sulfamethazine Prolonged Release Bolus

Calf tissue	k, days^{-1} [a]	Av biol half-life, days[b]
Muscle	0.7924	0.87
Fat	0.7544	0.92
Liver	0.6609	1.05
Kidney	0.7505	0.92

[a] k obtained by using a line of best fit on points of decay curve: $k = (2.303/t) \log (C_0/C)$. [b] $t_{1/2} = 0.693/k$; biological half-life is the time required to reduce to one-half that amount of unchanged drug which is in the body at the time equilibrium is established.

Average biological half-lives of the drug in tissues studied fell in a narrow range of 0.87 to 1.05 days (see Table V). This indicates that the rate of depletion of sulfamethazine from muscle, fat, liver, and kidney is practically the same.

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