Sulfamethazine and Its Metabolites in Pork: Effects of Cooking and Gastrointestinal Absorption of Residues

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Cooking-related changes in residues of [^{14}C]sulfamethazine (SMZ) and its metabolites were measured in pork products obtained from an animal given a single dose of the drug. In addition, the gastrointestinal absorption of residues after ingestion of cooked pork by dogs was assessed by analysis of blood and urine for SMZ and its metabolites. No loss of SMZ could be detected in pork loin, liver, and cured ham as a result of cooking by various methods. Levels of the N^4-acetyl metabolite of SMZ were not altered by cooking but the N^4-glucoside metabolite exhibited cooking-related declines of up to 70%. Liver, which contained high concentrations of the glucoside, exhibited an increase in SMZ as a result of cooking, and this could be explained by heat-related hydrolysis of SMZ-glucoside. Analysis of dog serum indicated slow but complete absorption of SMZ present in meat. Urine analysis showed the N-acetyl metabolite was also absorbed. The results indicate that cooking does not destroy and may increase SMZ in pork products and that ingestion of cooked pork can result in the absorption of drug and metabolite residues.

INTRODUCTION

The presence of drug residues in food producing animals is a continuing problem due to potential and perceived health hazards arising from ingestion of residue-containing meat products. Government regulatory considerations are directed toward concentrations of drugs in uncooked meat, but assessment of potential risks to the health of consumers requires knowledge of the extent of drug residues in cooked meat. Risk estimation also requires knowledge of the gastrointestinal absorption of drug residues after the ingestion of cooked meat. Similar information is required for drug metabolites present in meat because it is possible that products of drug biotransformation can possess potentially harmful biological activity that is similar to or different from the unchanged drug. The present study was conducted to examine the effects of cooking on residues of sulfamethazine (SMZ) and its metabolites in pork products and to examine the absorption of the drug in dogs after ingestion of cooked meat.

The antibacterial drug SMZ was studied because it is found in about 5% of tested swine carcasses at levels exceeding federal standards (Sundlof, 1989; Van Dresser and Wilcke, 1989). There is a limited amount of information available regarding the effects of cooking on SMZ residues in meat. Using a microbiological assay, O'Brien et al. (1981) found a small loss (e.g. 7%) of drug-related antibacterial activity occurred during the cooking of beef. Epstein et al. (1988) found a greater loss of SMZ (up to 40%) in a cured pork product. No reports are available regarding the effects of cooking on SMZ metabolite residues in meat. Metabolites of SMZ should be considered because they may account for some of the adverse effects of the drug including allergic reactions that are common to sulfonamides (Huber, 1986). Consideration of human exposure to SMZ residues is hampered because there have been no studies of the gastrointestinal absorption of the drug after ingestion of residue-containing meat.

The metabolism and elimination kinetics of SMZ have been studied in swine, and the following metabolites are known to be present in tissues of animals given the ^{14}C-labeled drug: SMZ-N^4-glucoside, N^4-acetyl-SMZ, desamino-SMZ (as shown in Figure 1) and an unknown metabolite fraction (Mitchell and Paulson, 1986). In the present study, these products were determined in cooked and uncooked pork obtained from an animal given [^{14}C]-SMZ and in the serum and urine of dogs fed residue containing meat. The results provide information useful in estimating potential exposure to SMZ residues from ingestion of cooked meat containing the drug and its metabolites.

EXPERIMENTAL PROCEDURES

Chemicals. Sulfamethazine (phenyl ring, uniformly labeled with ^{14}C), 8.2 mCi/mmol, and unlabeled sodium sulfamethazine were purchased from Sigma Chemical Co., St. Louis, MO. Each product was greater than 99% pure by TLC and HPLC. The sulfamethazine metabolites N^4-acetyl, N^4-glucoside, and desaminosulfamethazine were synthesized and purified as previously described (Paulson et al., 1981).

Preparation of Residue-Containing Meat. A 100-kg market hog (U.S. No. 1) was given a 3 mg/kg iv dose of [^{14}C]SMZ (as the sodium salt) containing 130 μCi of radioactivity. The specific activity of the dose was 875 dpm/μg. At 4.4 h after the dose, a blood sample was obtained and the animal was slaughtered. A sample of bladder urine was obtained and frozen. The liver, tongue, boneless loin (NAMP 413), shoulder, boneless Boston butt (NAMP 406A), and boneless ham (NAMP 402C) were removed, weighed, and sealed in Cryovac bags (NAMP, 1988). The meat was held for 24 h at 4 °C, then the liver was sliced into consecutive 1-cm slices, and 1 cm of meat was removed from the end of the tongue. For the loins, 1.0–1.4-kg roasts were taken from each end and the center portion was sliced into consecutive

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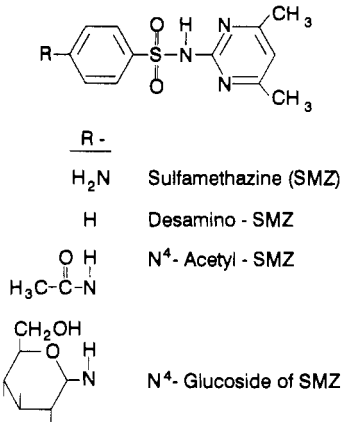

Figure 1. Chemical structures of sulfamethazine and metabolites included in analysis of pork products.

chops 1 cm thick. Alternating slices of meat were used for cooking and raw samples, as needed.

To prepare sausage, boneless shoulders were ground using a KitchenAid Food Grinder, Model FG-A, and a commercial pork sausage seasoning (Heller's) containing salt, ground red pepper, sage, ground black pepper, and sugar was added (20 g/kg of ground meat). The sausage was reground and 75-g sausage patties, 1 cm thick, were formed by hand.

Hams were pumped to 120% of green weight with a brine containing 9.9% salt, 3.4% sugar, 1.5% phosphate, 0.13% ascorbate, 0.08% nitrate, and 8.4% commercial water-soluble flavor liquid smoke (Zestic, Hickory Specialties). The hams were cured by cooking at 140 °F for 12 h, 160 °F for 2 additional h, after which the temperature was increased to 180 °F until the hams reached an internal temperature of 152–155 °F. This and all other cooking procedures were carried out in an approved hood using the guidelines of the Office of Biological, Chemical and Radiation Safety, Michigan State University. Roasts (1.0–1.4 kg) were removed from the shank and butt portion of both hams, and the center was sliced into 1-cm slices. All meat was sealed and frozen in Cryovac bags until cooked. After cooking the meat was wrapped in foil, placed in plastic bags, and refrozen until used for analysis of SMZ and its metabolites.

Cooking. The procedures of Stachiw et al. (1988) were used to charbroil pork loin to an internal temperature of 75 °C and to roast pork loins, ham shank, and butt pieces at 163 °C to internal temperature of 75 °C. Liver slices and pork loin chops were braised to internal temperatures of 80 and 75 °C, respectively, as described by Maul et al. (1971). The equipment and procedures outlined by Funk et al. (1971) were used to pan-fry liver slices to 80 °C as well as to pan-fry sausage patties, pork loin chops, and ham to 75 °C. Tongue was pressure cooked for 40 min at 15 psi following the procedures of Smith et al. (1977).

Internal end-point temperature in the geometric center of the meat was monitored as described by Stachiw et al. (1988). Cooking-related weight losses were determined for each cut of meat in order to correct SMZ and metabolite concentration values. Cooking losses ranged from 17% (braised liver) to 28% (pan-fried pork loin chops). Corrected values for concentration of SMZ and metabolite levels in cooked meat are reported as ppm (micrograms of SMZ or for metabolites, SMZ equivalents per gram wet weight of uncooked meat). This permitted a direct comparison of concentrations in cooked and uncooked meat.

Absorption Experiments. Three mongrel dogs weighing from 17 to 19 kg were allowed only water for 24 h prior to an oral dose of an aqueous solution of [^{14}C]SMZ (sodium salt) or ingestion of ground cooked pork (roasted pork picnic). The oral solution was prepared by taking a sufficient amount of the remaining dose solution prepared for the pig (described above) and diluting it with distilled water to produce a dose of approximately 100 μg/kg of [^{14}C]SMZ having a specific activity identical to that for the dose given to the pig. The exact dose administered to each dog was calculated based on scintillation analysis of an aliquot of the dose solution. The oral dose was administered to the dogs from a needleless syringe.

Approximately 1 month after the administration of an oral dose of [^{14}C]SMZ, the same dogs were given cooked ground pork containing an amount of [^{14}C]SMZ identical to that administered in solution. The amount of meat provided to the dogs was based on their body weight and the results of an analysis of the ground meat for unchanged SMZ. The concentrations of radioactive metabolites in the meat were not considered part of the SMZ dose. The dogs were fasted for 24 h, and each animal ingested nearly all of the approximately 500 g of meat. The exact dose in each dog was calculated based on the weight of the meat minus the weight of a small amount (less than 10%) of uningested meat.

Blood samples were obtained from the animals at appropriate intervals over a 48-h period, and urine was collected in metabolism cages for the same period of time. Urine and cage washings were collected at frequent intervals for determination of total radioactivity. Excretion of radioactivity was less than 0.03% of the dose/h at the end of 48 h, at which time elimination of drug and metabolites was considered essentially complete. Serum, urine, and cage washing samples were frozen until analysis.

Analysis of Samples. Duplicate samples of cooked and uncooked meat (5.0 g), serum (5.0 mL), and urine (0.2 mL) were extracted and analyzed by HPLC and scintillation analysis according to the procedure of Paulson et al. (1985). The volumes of the extraction and sample preparation procedures used here were the same as those reported for a 10-g tissue sample in the published procedure. All values are reported as ppm SMZ or, in the case of metabolites, as SMZ equivalents. The limit of detection for the method was 0.02 ppm. Total radioactivity in meat samples was determined by dissolving accurately weighed samples (approximately 0.1 g) in NCS solubelizer and scintillation analysis using an external standard for quench correction. Unidentified metabolites in samples were calculated by adding concentrations (SMZ equivalents) determined by HPLC analysis for unchanged SMZ, and each of its known metabolites (SMZ-acetyl, SMZ-glucoside, and desamino-SMZ) and subtracting the sum from total radioactivity in the sample.

Data Analysis. Calculation of areas under serum level curves, first-order absorption rate constants, and half-life were according to standard procedures (Gibaldi and Perrier, 1982). These parameters were determined from data obtained in each dog and mean values computed for a particular treatment. Differences between treatments (i.e. methods of SMZ administration, meat vs solution) were compared statistically using Student's t test. Mean values for the concentrations of SMZ and its metabolites in uncooked meat and meat cooked by various methods were also compared using Student's t test. The level of significance was $P < 0.05$.

SMZ-Glucoside Degradation. Solutions containing 2.3 nmol/mL pure SMZ-glucoside were prepared in 0.1 N HCl, 1 mM acetate buffer, pH 5.0, and 1 mM phosphate buffer, pH 7.0. The solutions (1 mL) contained in sealed glass vials were placed in a water bath at 95 °C or kept at room temperature (approximately 20 °C) for 6 h. At regular intervals, 100-μL aliquots of the solutions were removed and mixed with an equal volume of pH 7 phosphate buffer (0.2 M), and 10 μL was injected into the HPLC using previously published conditions (Paulson et al., 1985) for quantitative analysis of SMZ-glucoside and SMZ. Peak heights were compared to those obtained from standard solutions containing 2.3 nmol/mL of the metabolite and SMZ for quantitation of these materials.

RESULTS

Effects of Cooking. The extent of cooking-related change in concentrations of SMZ and its metabolites was tissue dependent. Uncooked liver contained the highest concentration of SMZ-glucoside of the tissues investigated, and cooking (braising) reduced the levels of the metabolite by 63% and correspondingly increased the concentrations of unchanged SMZ by a similar magnitude (Table I). The increase in SMZ caused by cooking was highest for liver compared to the other pork products studied. Unlike other tissues, uncooked and cooked liver did not contain detectable levels of the N^4-acetylated metabolite of SMZ (SMZ-acetyl). Unidentified radioactive metabolites ac-

Table I. Changes in Concentration (ppm) of SMZ and Metabolites Produced by Cooking Pork Liver

liver	SMZ	SMZ-glucoside	SMZ-acetyl	unidentified metabolites
uncooked[a]	1.66 ± 0.33	2.02 ● 0.19	0	2.62 ± 0.35
cooked[b]	2.77 ± 0.31[c]	0.74 ± 0.09[c]	0	2.56 ± 0.42
cooking change	+67%	-63%		-2%

[a] Mean ± SE, $N = 3$. [b] All samples cooked by braising; values (mean ± SE, $N = 4$) are corrected for weight loss during cooking. [c] Significantly different from uncooked ($P < 0.05$).

Table II. Changes in Concentration (ppm) of SMZ and Metabolites Produced by Cooking Pork Loin

loin	SMZ	SMZ-glucoside	SMZ-acetyl	unidentified metabolites
uncooked[a]	1.13 ± 0.06	0.26 ± 0.03	0.11 ± 0.02	1.34 ± 0.09
cooked[b]				
fried	0.95	0.07	0.10	1.24
braised	0.97	0.05	0.05	1.21
charbroiled	1.00	0.10	0.11	0.91
roasted	1.22	0.06	0.09	1.03
mean ± SE	1.04 ± 0.07	0.07 ± 0.01[c]	0.09 ± 0.02	1.10 ± 0.08
cooking change, %	-8	-73	-18	-18

[a] Mean ± SE, $N = 3$. [b] All values corrected for weight loss due to cooking. [c] Significantly different from uncooked ($P < 0.05$).

Table III. Changes in Concentration (ppm) of SMZ and Metabolites Produced by Cooking Cured Ham

cured ham	SMZ	SMZ-glucoside	SMZ-acetyl	unidentified metabolites
uncooked[a]	1.19 ± 0.09	0.10 ± 0.02	0.20 ± 0.03	1.85 ± 0.28
cooked[b]				
roasted	1.94	0.05	0.19	1.01
fried	1.34	0.08	0.14	1.58
fried	1.23	0.08	0.12	1.71
mean ± SE	1.50 ± 0.22	0.07 ± 0.01	0.15 ● 0.02	1.43 ± 0.21
cooking change, %	+26	-30	-25	-23

[a] Mean ± SE, $N = 3$. [b] All values corrected for weight loss due to cooking.

counted for about 40% of the total radioactivity in liver. The concentration of this unidentified fraction was higher in uncooked liver than in other tissues and it was not altered by cooking.

Cooked pork loin showed no changes in mean values for residues of SMZ, SMZ-acetyl, and unidentified metabolites (Table II). The mean values for cooked samples were somewhat lower but not statistically different from the mean values for uncooked samples. In contrast, concentrations of SMZ-glucoside in cooked loin were reduced 73% by cooking.

Concentrations of SMZ and its metabolites in cured ham are shown in Table III. Comparison of the values for uncooked ham with those found in uncooked pork loin (Table II) showed that the curing process did not alter residues except for a reduction (about 50%) in the levels of SMZ-glucoside. Cooking of the cured product did not result in significant changes in levels of SMZ and its metabolites (Table III). Individual samples of two other products, pork sausage and pork tongue, were cooked by frying and pressure cooking, respectively. Small increases in SMZ and large decreases in the glucoside metabolite (data not shown) similar to those presented for pork loin (Table II) were found in each of those pork products. Examination of all cooking-related data provided in Tables I–III gives no indication that a particular cooking method is more destructive of SMZ and SMZ metabolite residues.

Hydrolysis of SMZ-Glucoside. The conversion of SMZ-glucoside to SMZ was studied in aqueous solution at 95 °C and room temperature to provide information supporting the possibility that hydrolysis of that metabolite during cooking could account for a cooking-related increase of SMZ in some tissues. The results in Figure 2 indicate that SMZ is formed as a result of the hydrolysis of the metabolite and that this process is more rapid at higher temperatures and in an acidic environment.

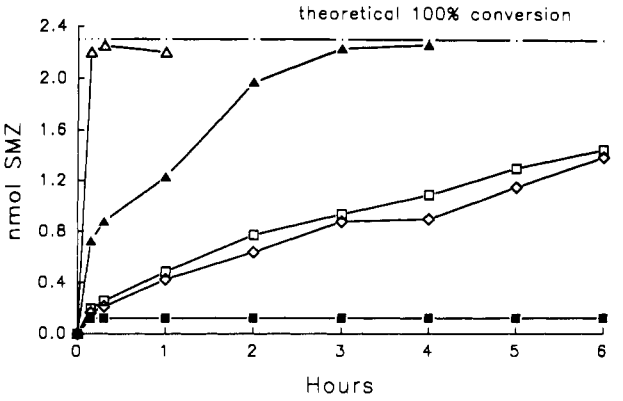

Figure 2. Effect of temperature and pH on conversion of SMZ-glucoside to SMZ in aqueous solution. Experimental conditions: pH 7.0, 95 °C (◇); pH 5.0, 95 °C (□); pH 5.0, room temperature (■); pH 1.0, 95 °C (△); pH 1.0, room temperature (▲).

Table IV. Doses of SMZ and SMZ Metabolites in Dogs Given the Drug in Aqueous Solution or as Residues in Cooked Pork[a]

ingested substance	oral solution	cooked meat
SMZ, μg/kg	103.2 ± 4.8	89.0 ± 7.5[b]
metabolites, μg eq/kg		
SMZ-glucoside	NA[c]	2.2 ± 0.1
SMZ-acetyl	NA	1.1 ± 0.5
SMZ-unidentified	NA	48.5 ± 2.3

[a] Calculation of doses in cooked meat are based on an HLPC analysis for SMZ and its metabolites (see Experimental Procedures). Values are mean ± SE, $N = 3$. [b] No statistical difference from mean dose given orally as a solution of SMZ (Student's t test, $P > 0.05$). [c] NA = not applicable; pure SMZ given orally.

Gastrointestinal Absorption of SMZ Residues. Serum levels of SMZ in dogs were measured after the labeled drug was given orally in aqueous solution and after ingestion of cooked pork containing labeled drug and its metabolites. The doses of drug-related substances ingested as meat residues were determined by an analysis of cooked loin meat for radiolabeled SMZ and its metabolites. The information in Table IV shows that the dose of unchanged SMZ ingested by dogs as a residue in cooked meat was not statistically different from the dose the animals received as pure SMZ in aqueous solution. The ingestion of cooked meat also resulted in substantial doses of SMZ metabolites as shown in Table IV.

The serum levels of SMZ displayed in Figure 3 show that peak concentrations of unchanged drug resulting from ingestion of meat residues were lower and delayed compared to levels resulting from oral administration of the pure drug. By 24 h after the dose, serum SMZ had fallen to below 0.05 μg/mL in each case, and by 48 h no SMZ could be detected in serum. Levels of individual SMZ metabolites in serum were below the limits for quantitative analysis throughout the duration of the experiment.

Pharmacokinetic values obtained from the serum level curves for SMZ are provided in Table V. The mean half-life for SMZ in dogs, obtained after an oral dose in aqueous solution, was 3.8 h. An insufficient number of data points in the postabsorptive phase of the serum level curve obtained after meat ingestion did not permit calculation of SMZ elimination half-life. The first-order rate constant for absorption of SMZ from ingested meat was calculated to be 0.125 h^{-1} ($T_{1/2} = 5.5$ h). The absorption rate constant

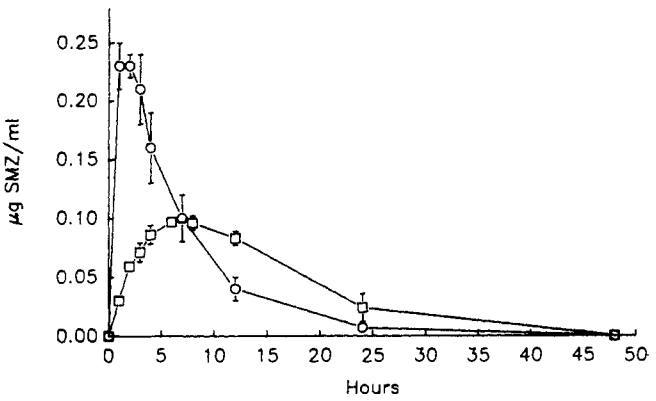

Figure 3. Serum levels of SMZ in dogs after administration of the drug in aqueous solution (circles) or as residues of the drug and its metabolites in cooked pork (squares).

Table V. Pharmacokinetic Values Obtained from Serum Level Curves Resulting from Ingestion of SMZ in Aqueous Solution or as a Residue in Cooked Meat[a]

	oral solution	cooked meat
half-life, h	3.8 ± 0.6	–[b]
time of peak serum level, h	1–2	6–8
absorption rate constant, h^{-1}	>12[b]	0.125 ± 0.02
area under serum level curve, μg h/mL	1.78 ± 0.24	1.72 ± 0.08

[a] Values are mean ± SE, $N = 3$. [b] Insufficient data points precludes calculation of accurate values.

for SMZ given in aqueous solution was estimated to be 100-fold greater than that resulting from meat ingestion. The estimation was based on the early attainment of peak plasma levels of SMZ after administration of SMZ in aqueous solution. An insufficient number of data points in the rapid absorptive phase resulting from an oral solution of SMZ precluded a more accurate analysis of the absorption rate constant. While absorption of the drug was much slower when ingested as a meat residue, the apparent extent of absorption was identical to that occurring after oral administration of the drug in solution as shown by the identical values for the areas under the serum level curves. This result indicates that the same amount of SMZ reached the blood after it was given in solution or ingested as a residue in meat.

Pooled (0–48 h) urine from dogs given [^{14}C]SMZ in pure form or as residues in meat was analyzed for total radioactivity and for SMZ and its metabolites to gain additional information on the disposition of ingested drug and metabolites. The radioactive material in urine reflected the total metabolism and excretion of ingested drug-related material because the time course for excretion of radioactivity indicated that it was essentially complete within 48 h (data not shown). The percent of the total radioactive dose recovered in urine over 48 h was the same whether the drug was administered in pure form or as residues in meat as shown in Table VI. Thus, correspondingly more radioactivity was excreted in urine after ingestion of meat because the radioactive dose was higher as it contained labeled SMZ metabolites in addition to unchanged [^{14}C]SMZ.

HPLC separation of urinary SMZ and its metabolites allowed recoveries of unchanged SMZ or metabolites (identified and unidentified) to be expressed as a percent of the known dose of [^{14}C]SMZ given to the animals either in solution or in meat. The results in Table VI show that 3 times more unchanged SMZ appeared in urine after ingestion of meat. Metabolite analysis showed that 69% of the dose of pure SMZ given in solution was recovered

Table VI. Recovery of [^{14}C]SMZ and Metabolites in Dog Urine (0–48 h)[a]

recovery	oral solution	cooked meat
SMZ,[b] % SMZ dose	5.3 ± 1.2	16.6 ± 2.0[d]
total metabolites,[b] % SMZ dose	68.5 ± 2.6	118.6 ± 11.2[d]
SMZ-glucoside,[b] % SMZ dose	0	0
SMZ-acetyl,[b] % SMZ dose	0	9.8 ± 1.4
SMZ-acetyl,[c] % acetyl-SMZ dose	NA	76.8 ± 5.8

[a] Values are mean ± SE, $N = 3$. [b] SMZ doses are given in Table IV. [c] Dose of SMZ-acetyl in cooked meat was 18.7 μg ± 2.8 (equivalents of SMZ), $N = 3$. [d] Significantly different ($P < 0.05$) from oral solution.

in urine as total metabolites whereas after the drug was ingested in meat, 119% of the SMZ dose present in meat was recovered. The additional recovery of total metabolites after ingestion of meat was undoubtedly due to the absorption and excretion of radioactivity associated with labeled SMZ metabolites present in meat. Individual urinary metabolite analysis showed that the SMZ glucoside metabolite present in the ingested meat (approximately 35 μg) did not appear in the urine of dogs nor did this metabolite appear in urine after a dose of pure SMZ. The SMZ-acetyl metabolite was not found in dog urine after a dose of pure SMZ but was present in urine equivalent to about 10% of the SMZ dose after ingestion of meat containing SMZ and its metabolites. This result indicates that the SMZ-acetyl metabolite ingested in meat was absorbed from the GI tract and excreted in urine. As shown in Table VI, it was calculated that as much as 77% of the ingested SMZ-acetyl metabolite was absorbed and excreted.

DISCUSSION

The results of this study indicate that the cooking of pork products containing residues of SMZ and its metabolites does not result in destruction or loss of the unchanged drug; rather, in certain tissues the concentration of unchanged drug may increase. These results are consistent with those of O'Brien et al. (1981) who found essentially no cooking-related loss of SMZ-related microbiological activity in extracts of meat from drug-treated cattle. A limited study of a homogenized pork product showed a 40% loss of SMZ due to a curing process (Epstein et al., 1988). A similar loss due to curing (and cooking) could not be demonstrated in the present study which used a conventional curing and cooking process on intact meat.

Indirect evidence indicates that the cooking-related increase in unchanged SMZ which occurred in liver was due to the temperature-dependent hydrolysis of the glucoside metabolite to produce SMZ. The instability of the glucoside under strong acid conditions has been reported (Parks, 1984), and the present study documents a conversion of the metabolite to SMZ under neutral and more rapid conversion under weak acid conditions. Cooking significantly increased SMZ concentrations only in liver, a tissue containing high concentrations of the glucoside. The increase in SMZ in cooked liver (1.1 ppm) is accounted for by the nearly identical loss of SMZ-glucoside (1.3 ppm) during the cooking process. This relationship is consistent with the glucoside acting as a source of SMZ during cooking.

Tissues such as skeletal muscle which contain relatively low levels of the glucoside metabolite show a trend for higher levels of SMZ after cooking, but this increase was not statistically significant. Thus, a cooking-related increase in SMZ appears to be directly dependent on the relative concentration of glucoside metabolite in uncooked tissue. It was possible to observe a cooking-related loss

of SMZ-glucoside in every pork product examined, except cured ham. The lack of a cooking related decline in ham was probably due to a prior loss of glucoside during the curing process. It is not known how many other food-producing species will produce sufficient amounts of the glucoside metabolite to be a significant source of SMZ during cooking. It has been reported that chickens and sheep produce very little SMZ-glucoside compared to swine (Paulson et al., 1983).

It is known that SMZ-glucoside can be formed in liver under cold storage conditions, including freezing (Giera et al., 1982; Parks, 1984). The fraction of SMZ-glucoside in liver that was formed in the present study during frozen storage of the tissue is not known. However, the results presented here make it apparent that analysis of pork for SMZ residues should include analysis for the glucoside metabolite. This is necessary because it is a source of SMZ in cooked meat. In addition, the lability of the glucoside under acidic conditions make it likely that ingestion of meat containing the intact metabolite would result in hydrolysis of the conjugate to SMZ in the acidic conditions of the stomach.

Cooking-related changes in the acetyl metabolite of SMZ did not occur. The low levels of this metabolite in uncooked meat are consistent with another report of SMZ metabolite levels in swine tissue (Mitchell and Paulson, 1986). This metabolite was unexpectedly not detected in uncooked liver, and the reason for its absence from that tissue is not known. The metabolite was found to represent 33% of the urinary radioactivity in the pig used as a source of meat for this study. This result and the presence of SMZ-acetyl in loin and other products from the animal indicate that the metabolite was formed after the dose of SMZ. The hydrolysis of the acetyl metabolite during storage of tissue by amidases present in liver is a possible source of loss, but this was not investigated.

Analysis of cooked and uncooked meat for the desamino metabolite of SMZ showed that it was not present in detectable amounts. This metabolite was reported to be produced in stomach contents after co-ingestion of SMZ and nitrite by swine and other animals (Paulson, 1987). The single, iv dose of SMZ given to the animal used in the present study apparently did not allow detectable amounts of desamino-SMZ to be formed in the stomach and distributed to tissues. Although the stability of desamino-SMZ during cooking remains undemonstrated, it is likely that this metabolite would be resistant to change. The desamino metabolite has a chemical structure that would be potentially more stable than SMZ because the metabolite has lost the reactive arylamino group of the unchanged drug.

Total ^{14}C associated with unidentified metabolic products of SMZ did not change as a result of cooking. These products were not characterized in the present study, and it is possible that cooking-related changes in chemical structure took place but did not result in a loss of ^{14}C from tissue. The present report confirms results from a previous study which indicated a similarly large fraction of unidentified products in the tissues of swine given [^{14}C]SMZ (Mitchell and Paulson, 1986). The present study showed that the unidentified metabolite fraction in tissues was greater than 90% extractable using methanol. This result suggests that very little SMZ is converted to metabolites which can covalently bind to tissue components. Incorporation into tissue of radiolabeled carbon derived from the metabolism of [^{14}C]SMZ could not reasonably account for unidentified radioactivity in tissues. The ^{14}C label in the drug used for this study was located in the phenyl ring, a relatively stable position to metabolism. In addition, the short time the drug was in the body prior to slaughter (4.4 h) would further reduce the chance of its metabolism to labeled one-carbon fragments that could be incorporated into endogenous tissue components. Other studies in swine using the same labeled compound indicated that greater than 90% of the drug could be recovered in urine and feces, leaving little possibility that a large amount of radioactivity entered the one-carbon pool to eventually be excreted as $^{14}CO_2$ (Paulson et al., 1981).

The bioavailability in dogs of SMZ ingested as residues in cooked pork was assessed using a technique often used for evaluating the relative extent of absorption of pharmaceutical dosage formulations (Gibaldi and Perrier, 1982). The identical areas under the SMZ serum level curves, after the drug was ingested either as a residue in meat or in the pure form in aqueous solution, indicated that the same amount of SMZ reached the blood in each case. Because the amount of SMZ in meat was the same as the amount administered in solution, it is reasonable to speculate that an identical amount was absorbed from meat and from solution. The slower rate of absorption of SMZ associated with ingestion of meat produced a 60% lower peak serum level of the drug. Putting this in toxicologic perspective indicates that a hypothetical adverse effect of SMZ would be lower after meat ingestion if the adverse effect were related to peak serum level of SMZ. Should the adverse effect be related to total SMZ exposure, the effect would be the same after ingestion of meat and after ingestion of the pure drug in solution.

Serum levels of SMZ after ingestion of meat may originate, in part, from conversion of ingested SMZ metabolites to SMZ. For example, SMZ-glucoside present in meat could be hydrolyzed to SMZ in the acidic contents of the stomach. It is unlikely, however, that hydrolysis of SMZ-glucoside made a large contribution to serum levels of SMZ after ingestion of meat because of the relatively small amounts of the glucoside metabolite in meat consumed by the animals in this study.

The analysis of pooled (0–48 h) urine from dogs given pure SMZ and meat containing the drug and its metabolites provided information on the fate of metabolites after ingestion. Since no SMZ-glucoside was found in the urine of dogs given pure SMZ, it is apparent that this species does not produce and excrete this metabolite. It is also evident that the small amounts of the glucoside metabolite ingested with meat were not absorbed and excreted intact. In contrast, the SMZ-acetyl metabolite present in meat was absorbed and excreted in urine because the data presented here show that the dog does not produce and excrete this metabolite after a dose of pure SMZ. This is consistent with the known deficiency of dogs to acetylate arylamines (Baggot, 1988). The metabolite excretion data in Table VI also indicated that more unidentified metabolites of SMZ were excreted after ingestion of meat, compared to excretion after a dose of the pure drug. This indicates absorption of unidentified metabolites of SMZ in meat can occur. A complete analysis of the risk involved from consumption of pork containing SMZ residues should address the identity and biological activity of the unknown metabolites in cooked meat, information that is not available at this time.

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