Title
Mechanisms and kinetics of the dose-dependent sulfoconjugation of salicylamide in the dog

Author
Waschek, James Arvid

Publication Date
1984

Peer reviewed|Thesis/dissertation

MECHANISMS AND KINETICS OF THE DOSE-DEPENDENT SULFOCONJUGATION OF SALICYLAMIDE IN THE DOG

by

JAMES ARVID WASCHEK

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PHARMACEUTICAL CHEMISTRY

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

[redacted]

Date University Librarian

Degree Conferred: . . . . DEC 31 1984 . . . . . .

DEDICATED TO

PAULINE AND JOANNA

ACKNOWLEDGEMENTS

With sincere gratitude, I thank:

Dr. Thomas N. Tozer, my research advisor, for the many, many hours spent with me discussing the work summarized in this dissertation and for constant encouragment while the work was being carried out.

I also thank him for reviewing this dissertation and giving me many helpful suggestions. Most of all, I thank him for teaching me how to approach and solve scientific problems.

Dr. Susan M. Pond, for her valuble contribution to the design of the experiments described here. I also thank her for the many hours spent in reviewing the contents of this dissertation and for constant support throughout the project.

Dr. Sven Øie, for reviewing this dissertation and for many helpful suggestions.

At the time of this writing, two papers, listed below, had been published, and contain portions of the research summarized in this dissertation.

1. Fielding RM, Waschek JA, Rubin GM, Pond SM and Tozer TN: Analysis of Salicylamide and Its Metabolites in the Blood and Urine by HPLC. J. Liq. Chromatog. 7:1221-1234, 1984

2. Waschek JA, Rubin GM, Tozer TN, Fielding RM and Pond SM: Dose-dependent Bioavailability and Metabolism of Salicylamide in Dogs. J. Pharmacol. Exp. Ther. 230:89-93, 1984

This work was supported by Grant GM 26556 from the National Institutes of Health.

MECHANISMS AND KINETICS OF THE DOSE-DEPENDENT SULFOCONJUGATION OF SALICYLAMIDE IN THE DOG

ABSTRACT

The mechanisms of the dose-dependent sulfoconjugation observed with several phenolic substrates is investigated by studying the metabolism and pharmacokinetics of salicylamide (SAM) in the dog. At doses of up to 40-mg/kg, the sulfoconjugate of SAM accounts for more than 85% of the urinary metabolites. When SAM is given intravenously to anesthetized dogs, dose-dependent sulfoconjugation is found to occur in at least three extrahepatic sites - the kidneys, lungs and forelimb.

By co-injecting inorganic [35S]-sulfate and SAM, the the plasma inorganic sulfate pool is shown to provide the sulfate used in the reaction and equilibrate with the metabolically-active sulfate pool within two to three minutes. Depletion of plasma sulfate, however, does not play an important role in the dose-dependent sulfoconjugation of SAM. Pronounced dose-related changes in SAM kinetics are observed under a variety of experimental conditions despite constant plasma sulfate concentrations.

When the kinetics are followed over a sufficient period of time after oral administration of a 40-mg/kg dose of SAM,

log plasma SAM concentration vs. time curves bend downward, suggesting Michaelis-Menten kinetics. However, a comparison of these curves with those after smaller doses of SAM indicates that the rate of decline depends on the dose administered rather than the concentration of SAM in the plasma. Thus, neither depletion of inorganic sulfate in the plasma, rate-limited uptake or activation of inorganic sulfate, nor typical Michaelis-Menten kinetics, fully explain the dose-dependent kinetics of SAM in the dog. Other time-dependent factors related to SAM dose are suggested.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

Page

LIST OF FIGURES

Page

Page

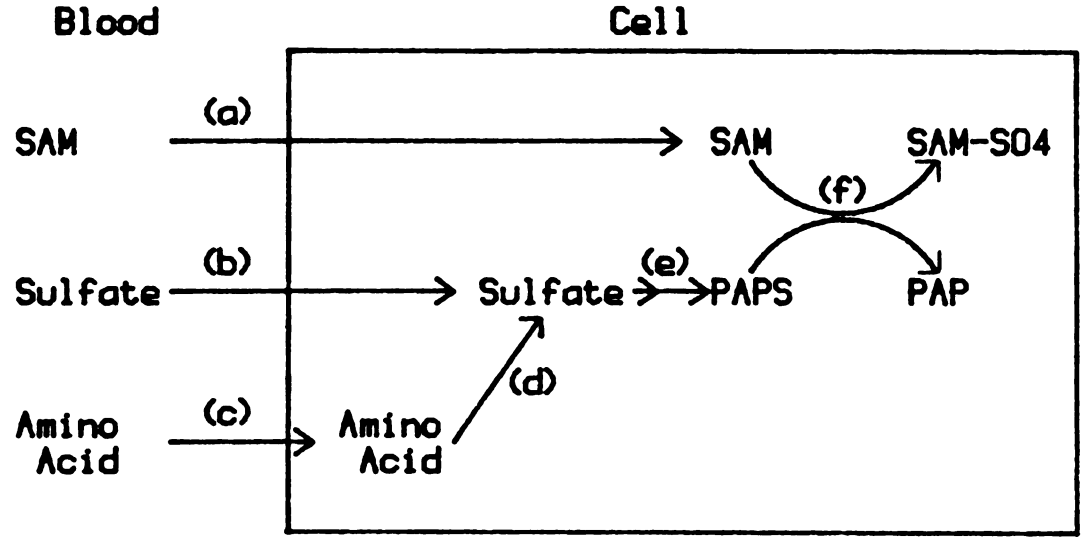
ABBREVIATIONS

ANOVA	analysis of variance
APS	adenosine 5'-sulfatophosphate
ATP	adenosine triphosphate
AUC	area under the concentration vs. time curve
HPLC	high performance liquid chromatography
IV	intravenous
Km	concentration to reach one-half of the maximum velocity of a reaction
PAP	adenosine 3', 5'-biphosphate
PAPS	adenosine 3'-phosphate, 5'-sulfatophosphate
PPi	inorganic phosphate
SAM	salicylamide
SD	standard deviation
TBA	tetrabutylammonium (ion)
Vmax	maxium velocity of a reaction

MECHANISMS AND KINETICS OF THE DOSE-DEPENDENT SULFOCONJUGATION OF SALICYLAMIDE IN THE DOG

INTRODUCTION

After administration of a phenolic substrate, the amount of sulfoconjugate formed in may cases fails to increase in direct proportion to dose (Mulder 1981a). Many studies *in vivo* suggest that depletion of limited body sulfate stores is the primary mechanism for this dose-dependence (Levy and Matsuzawa, 1967; Galinsky and Levy, 1981). Studies with isolated cells and purified enzymes (Chapter 1), however, suggest a variety of other potential mechanisms, including Michaelis-Menten saturation of metabolizing enzyme, rate-limited cellular uptake or activation of inorganic sulfate, and inhibition of metabolizing enzymes by products of the sulfoconjugation reaction. A summary of possible rate-limiting steps for salicylamide sulfoconjugation is shown in Fig. I-1. The goal of the research described in this dissertation was to examine the mechanisms of dose-dependent sulfoconjugation *in vivo*.



(a) Uptake of SAM into cell.

(b) Uptake of sulfate into cell.

(c) Uptake of sulfur-containing amino acids into cell.

(d) Conversion of sulfur-containing amino acids to sulfate.

(e) Formation of active sulfate, PAPS.

(f) Transfer of sulfate from PAPS to SAM.

Fig. I-1. Schematic view of various processes believed to be involved in sulfoconjugation (shown for the substrate salicylamide (SAM). Amino acid = sulfur-containing amino acid, e.g. cysteine or methionine; PAPS = adenosine 3' phosphate 5' sulfatophosphate; PAP = adenosine 3' 5' biphosphate; SAM-SO4 = sulfate conjugate of SAM.

Salicylamide (SAM) in the dog was used to study the mechanism of dose-dependent sulfoconjugation because preliminary studies had indicated that SAM shows pronounced dose-dependence in its kinetics and that the primary route of its elimination is sulfoconjugation. After establishing the SAM dosage range over which dose-dependent sulfoconjugation occurs, studies were carried out to determine the relationship between SAM sulfoconjugation and body sulfate stores, as estimated by the concentration of inorganic sulfate in plasma. Subsequently, the time course of change in SAM kinetics after SAM administration was studied. In these experiments, changes in SAM kinetics were examined in relation to the plasma concentrations of SAM and SAM-sulfate as well as inorganic sulfate.

In a further series of studies, the rate at which inorganic sulfate in the plasma becomes available for sulfoconjugation was investigated by infusing SAM and inorganic [35S]-sulfate and measuring the specific activity of SAM-[35S]-sulfate formed. The primary source of sulfate used for the reaction (inorganic sulfate vs. sulfur-containing amino acids) was also determined. Finally, because SAM clearance values suggested that sulfoconjugation of SAM occurs in several organs in the dog, metabolic extraction of SAM in the lungs, kidneys and limb tissues was determined in anesthetized dogs.

CHAPTER 1

BACKGROUND

I. INTRODUCTION

The goal of this dissertation research is to examine the mechanisms of the dose-dependent sulfoconjugation of phenolic substances by using salicylamide (SAM) in the dog. Sulfoconjugation, as defined in this dissertation, is the process by which a sulfate group is covalently attached to a substrate, which is usually rendered less toxic and more easily excreted in the urine or bile. In this chapter, the current knowledge of drug sulfoconjugation is summarized.

Because the literature contains little information with respect to drug sulfoconjugation in the dog, most of this review summarizes studies done in the rat and other species. Included are sections on 1) the biochemistry of the sulfoconjugation reaction, 2) sulfate homeostasis and 3) *in vivo* examples of dose-dependent sulfoconjugation. A fourth section reviews the mechanisms for the dose-dependence that have been proposed on the basis of studies with purified enzymes, isolated hepatocytes, perfused livers and intact animals.

II. BIOCHEMISTRY OF SULFOCONJUGATION

An example of a sulfoconjugation reaction is shown below (Fig. 1-1). Substrates for the reaction include phenols, aromatic amines, aliphatic alcohols and amines.

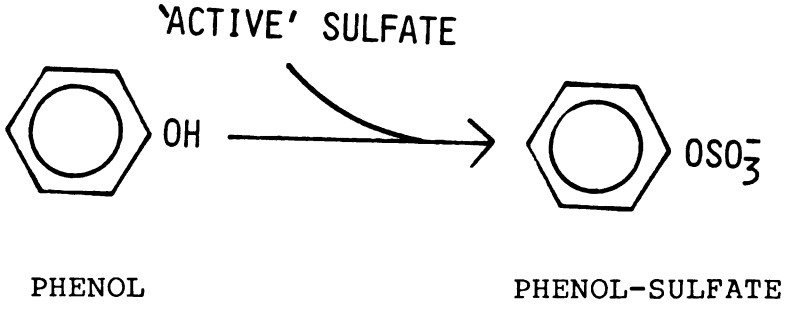


Fig. 1-1. Sulfoconjugation of phenol.

A. Activation of inorganic sulfate (Mulder, 1981b).

The primary sources of sulfate for the sulfoconjugation reaction are inorganic sulfate and sulfur-containing amino acids provided by diet. After inorganic sulfate is formed in or taken up by the metabolizing cell (discussed in section III of this chapter) activation must occur before sulfoconjugation can take place. This activation occurs in two steps, each utilizing a different enzyme and requiring a molecule of adenosine triphosphate (ATP). This activation system has been found in most mammalian tissues. The first activation step (Fig. 1-2), catalyzed by the soluble enzyme ATP sulfurylase (EC 2.7.7.4), results in the formation of adenosine 5' sulfatophosphate (APS). The equilibrium of the reaction greatly favors the reactants. Apparent Km values (concentrations giving half-maximum velocity) for sulfate for enzymes purified from rat liver range from 0.1 to 3.2 mM (Mulder, 1981b)

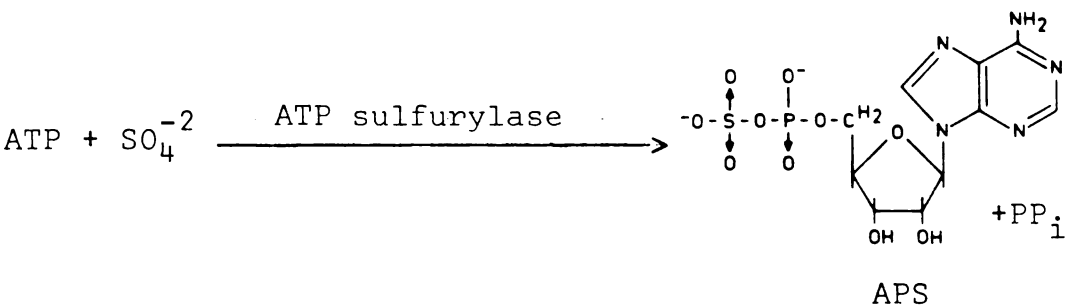
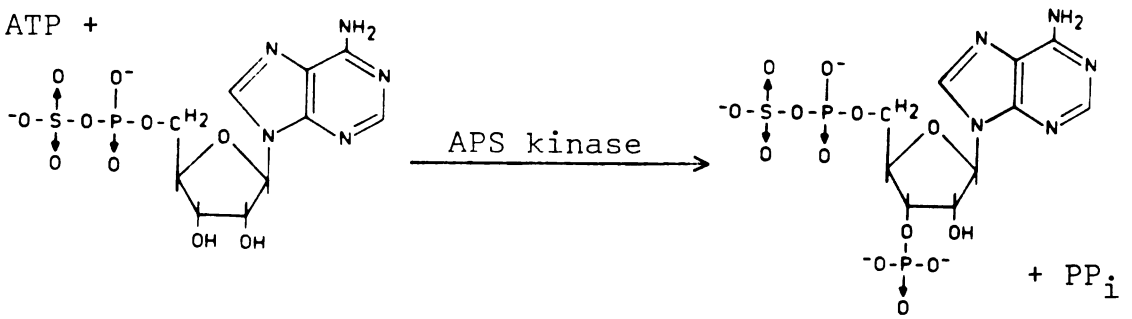


Fig. 1-2. Formation of adenosine 5'-sulfatophosphate (APS).

The second activation step (Fig. 1-3), catalyzed by APS kinase (EC 2.7.7.5), results in formation of adenosine 3'-phosphate, 5'-sulfatophosphate (PAPS) in an almost irreversible reaction. This enzyme has not been purified from mammalian sources but has been purified from Baker's yeast and spinach leaf. Values of Km for APS with these enzymes are both below 5 uM (Mulder, 1981b).



APS PAPS

Fig. 1-3. Formation of adenosine 3'-phosphate, 5'-sulfatophosphate (PAPS)

B. Transfer of sulfate to substrate (Mulder 1981c).

An example of sulfate transfer to substrate is shown shown below (Fig. 1-4).

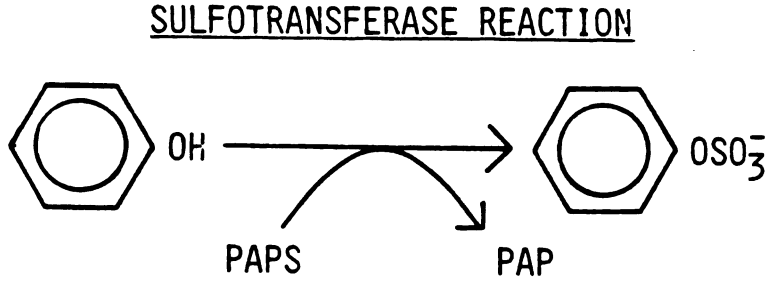


Fig. 1-4. Transfer of sulfate from PAPS to phenol forming phenol-sulfate and adenosine 3', 5'-biphosphate (PAP).

Studies in various mammalian species show that phenolic substances may undergo sulfoconjugation not only in the liver, but also in the kidney (Watrous and Fujimoto, 1971; Quebbmann and Anders, 1973; Jones et al., 1979), intestinal wall (Merits, 1976; Sakai et al., 1980), lungs (Hook and Bend, 1976; Mehta and Cohen, 1979; Cassidy and Houston, 1980), brain (Pennings, 1978) and other organs (Wengle, 1966; Bostrom and Wengle, 1967; Foldes and Meek, 1974). In the dog, sulfotransferase activity has been found in the liver, kidney, small intestine and lung (Wong and Yeo, 1982).

Numerous enzymes capable of transferring sulfate are found within a single species. These exist not only for the purpose of detoxification of foreign

substances, but also for metabolism of steroids, biogenic amines and other hormones and for biosynthesis of sulfated proteins, lipids, and glycosaminoglycans. At least four enzymes capable of transferring sulfate to phenolic substrates (phenol sulfotransferases) are found in the soluble fraction of liver cell homogenates (Sekura and Jakoby, 1979; 1981). As a result, a given phenolic substrate may be metabolized by several forms of sulfotransferase.

Kinetic studies utilizing rat brain phenol sulfotransferase (Pennings and Van Kempen, 1982) indicate that the reaction proceeds first by binding of the cosubstrate, PAPS, followed by binding of the substance to be metabolized. After the transfer of sulfate, the newly formed sulfoconjugate and then PAP leave the enzyme. With sulfotransferase enzymes purified from rat liver and brain, Km values for PAPS range from 6 to 23 uM (Sekura and Jakoby, 1979 and 1981; Pennings and Van Kempen, 1982).

III. SULFATE HOMEOSTASIS

The rate at which the sulfoconjugation reaction proceeds must depend, at least in part, on the concentration of PAPS at the metabolic site. The production of PAPS may, in turn, depend on the concentration of inorganic sulfate within the cell. Inorganic sulfate is provided to the cell primarily by the oxidation of cysteine or methionine or by the transport of inorganic sulfate from the blood (Cooper 1983). Transport of inorganic sulfate into rat hepatocytes has been shown to occur via a facilitated carrier system (Cheng and Levy, 1980; Schwarz, 1982). Apparent Km values for sulfate transport in rat hepatocytes range from 0.3 - 6.5 mM, depending on the experimental conditions (Cheng and Levy, 1980; Schwarz, 1982). At low extracellular sulfate concentrations (less than 0.2 mM), hepatocytes may concentrate inorganic sulfate within the cell (Schwarz 1982). In vivo studies, in which inorganic [35S]-sulfate and phenol were co-injected intravenously to rats indicate that inorganic sulfate in the plasma is available for sulfoconjugation within minutes (Mulder and Scholtens, 1978).

Much data indicate that inorganic sulfate is well absorbed from the gastrointestinal tract of most mammals (O'Conner and Summerfield, 1976; Krijgsheld et al., 1979). A saturable carrier system has been proposed on the basis of studies utilizing the rat ileum (Cardin and Mason, 1975).

The volume of distribution of inorganic sulfate in the dog is reported to be about 20% of body weight (Douglas

et al., 1969). Total body stores of inorganic sulfate in the dog, calculated as the product of volume and plasma sulfate concentration (1.4 mM, O'Conner and Summerfield, 1976) are about 0.28 mmol/kg.

Renal clearance of inorganic sulfate in the dog, calculated from literature values of sulfate excretion rate and normal plasma concentrations (O'Conner and Summerfield, 1976), is about 0.3 ml/min-kg. However, renal clearance may be be sulfate concentration dependent. In the rat, saturable reabsorption appears likely, based on the large dependence of clearance on plasma sulfate concentration (Lin and Levy, 1983).

IV. DOSE-DEPENDENT SULFOCONJUGATION IN VIVO.

Studies demonstrating dose-dependent sulfoconjugation of phenolic substances in various mammalian species are summarized in Table 1-1. In most of these studies, conclusions are based either on metabolite excretion rates or on the relative composition of total metabolites excreted. When data are based on metabolite excretion rates, dose-dependent effects on metabolite formation cannot be distinguished dose-dependent effects on metabolite excretion. When data are based only on the relative composition of total metabolites excreted, a decrease in metabolism in one pathway cannot be distinguished an increase in metabolism in another pathway.

Studies of acetaminophen in the rat, however, also utilized plasma drug concentration (Galinsky and Levy, 1981; Watari et al., 1983). This allowed authors to calculate clearance of acetaminophen to individual metabolites and thus more clearly distinguish between effects on individual metabolic pathways. Both studies clearly demonstrated pronounced dose-dependence of acetaminophen metabolism specifically in the sulfoconjugation pathway. Galinsky and Levy (1981) also used plasma metabolite data to show that plasma acetaminophen-sulfate concentrations did not increase in proportion to acetaminophen dose.

Table 1-1. Studies in which dose-dependent sulfoconjugation of phenolic substances in mammalian species has been demonstrated.

SPECIES	SUBSTRATE	REFERENCE
rabbit	phenol	Williams et al, 1938
rabbit	p-hydroxy-benzene sulphonamide	Sammons et al, 1941
rat	phenol	Weitering et al, 1979
rat	acetaminophen	Buch et al, 1968 Galinsky and Levy, 1981 Watari et al, 1983 Hjelle and Klassen, 1984
rat, mouse hamster	acetaminophen	Jollow et al, 1974
primates	1- naphthol and phenol	Mehta et al, 1978
man	salicylamide	Levy and Matsuzawa, 1966 and 1967

V. POSSIBLE MECHANISMS OF DOSE-DEPENDENT SULFOCONJUGATION.

A. In enzyme preparations.

Low Km values are reported for many phenolic substances with various sulfotransferase preparations (Mulder et al., 1981c). A value of 0.009 mM for salicylamide was determined with a sulfotransferse enzyme partially purified from rat hepatocytes (Davis 1975). Saturation of sulfotransferase activity could occur if concentrations of substrate at the metabolic site exceed the Km value of that substrate. However, the enzymes used for determination of Km values were frequently not purified to homgeneity. Furthermore, Km values for sulfotransferase are reported to be sensitive to the pH and ionic strength of the assay mixture (Sekura and Jakoby, 1981) and oxidation state of the enzyme (Dodgson, 1977).

One product of the sulfotransferase reaction, adenosine 3', 5' -biphosphate (PAP, Fig. 1-4), inhibits phenol sulfotransferase enzymes (Sekura and Jakoby, 1981; Pennings and Van Kempen, 1982). The inhibition is competitive with PAPS. Sulfotransferase activity of two enzymes purified from rat liver was inhibited by 50% by PAP concentrations of 29 and 50 uM (Sekura and Jakoby, 1979; 1981). ATP sulfurylase is also reported to be inhibited by PAP (Pennings and Van Kempen, 1982). It is not known if PAP may accumulate in metabolizing cells. Enzymatic hydrolysis of PAP within hepatocytes

has been demonstrated (Brunngraber, 1958; Lewis and Spencer, 1959).

Inhibition of sulfotransferase activity by high concentrations of substrate has been demonstrated with several phenolic substances (Pennings and Van Kempen, 1982; Sekura and Jakoby, 1979; 1981).

B. In isolated hepatocytes and perfused livers.

The rate of sulfoconjugation of acetaminophen (Moldeus et al., 1979), SAM (Koike et al., 1981) and other phenolic compounds (Mulder and Keulemans, 1978; Mulder, 1981d) is sensitive to concentrations of inorganic sulfate in the medium or perfusate. The sulfate concentration that results in half of the maximum rate of sulfoconjugation (Km for sulfate) varies with the substrate and experimental procedure. Reported Km values range from 0.2 to 3 mM.

C. In vivo.

Coadministration of sulfate donors with phenolic substrates in many cases results in an increased percentage of a dose that appears in the urine or bile as the sulfate conjugate (Table 1-2). The dose-dependence of acetaminophen in rats (Galinsky and Levy, 1981) and salicylamide in man (Levy and Matsuzawa, 1966; 1967) is lessened or abolished when sulfate donors are coadministered. This suggests that the dose-dependence is

Table 1-2. Examples of studies in which the rate of sulfoconjugation of a phenolic substrate has been increased by coadministration of sulfate donors.

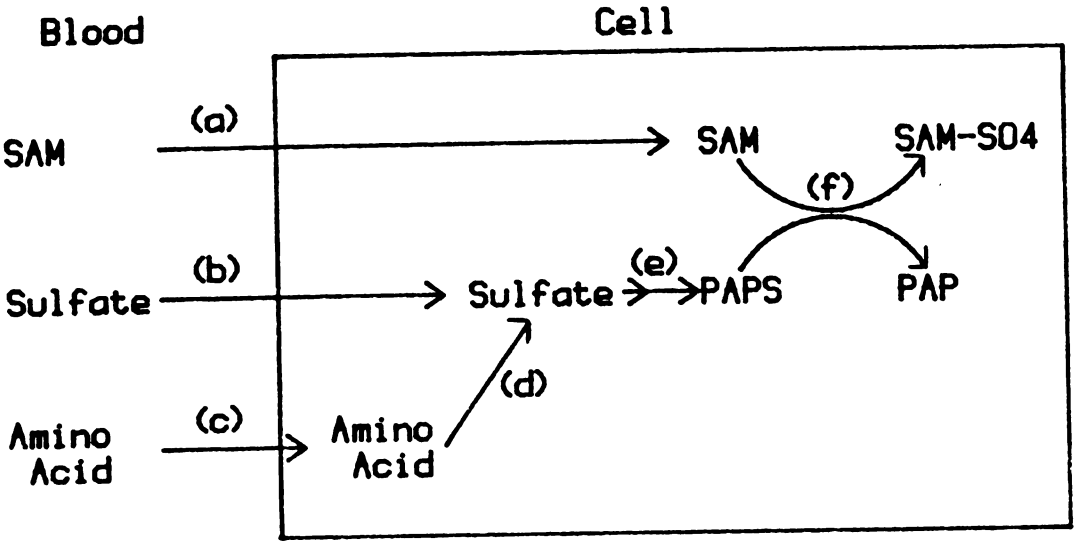
SPECIES	SUBSTRATE	SULFATE DONOR	REFERENCE
man	tyramine	inorganic sulfate	Smith and Mitchell, 1974
man	salicylamide	l-cysteine	Levy and Matsuzawa, 1966; 1967
rabbit	various	various	Bray et al, 1952
rats	acetaminophen	various	Buch, 1968 Galinsky and Levy, 1979; 1981

caused by decreased body stores of inorganic sulfate or readily available sulfate precursors after administration of higher doses of substrate. When acetaminophen was infused intravenously into rats, plasma acetaminophen concentrations did not reach steady-state in the time period predicted by the first-order kinetics of a small IV dose (Galinsky and Levy, 1981). To determinine if the prolonged rise of acetaminophen concentration was due decreased clearance as a result of depletion of plasma sulfate, the experiment was repeated, but sodium sulfate was also infused. The kinetics of acetaminophen under these conditions were typically first-order, indicating that when sulfate depletion is prevented, the dose-dependence is abolished. Others have questioned the importance of sulfate depletion because dose-dependence has been observed with some substrates at doses that do not appreciably lower plasma inorganic sulfate (Weitering et al, 1979). Plasma concentrations of inorganic sulfate in rats appear to reflect readily available body stores of sulfate because the rate of sulfoconjugation of harmol correlates with plasma inorganic sulfate concentration (Krijgsheld et al., 1982). When inorganic [35S]-sulfate is co-injected with phenol to rats, the specific activity of phenol-[35S]-sulfate in the bile reaches a constant value within a few minutes, indicating that rapid equilibrium occurs between PAPS and plasma sulfate (Mulder and Scholtens, 1978).

Depletion of plasma inorganic sulfate may only partially explain the dose-dependence observed in studies of acetaminophen in rats. Sodium sulfate supplementation increased clearance of acetaminophen to the sulfate conjugate but log plasma acetaminophen concentration vs. time plots after sulfate supplementation showed downward curvature (Galinsky and Levy, 1981). Downward curvature was also observed when acetaminophen was given to rats without sulfate supplementation in another study (Watari et al., 1983) Although this downward curvature could be due to saturation of another metabolic pathway, it is possible that Michaelis-Menten saturation of sulfotransferase activity occurred.

V. CONCLUSIONS

Although most *in vivo* studies indicate that depletion of body stores of sulfate is an important cause of dose-dependent sulfoconjugation, a variety of other potential mechanisms have been demonstrated *in vitro*. Several steps involved in providing active sulfate (PAPS) to the metabolizing cell could become rate-limiting at high substrate doses, when demand for PAPS is high (Fig. 1-5). Additionally, saturation of the metabolizing enzyme with substrate (typical Michaelis-Menten kinetics) is a potential mechanism for the dose-dependence. Product-inhibition by PAP is also a possibility based on data obtained with several purified sulfotransferase enzymes. Because the dose-dependent sulfoconjugation of some substrates is not completely explained by depletion of inorganic sulfate in the plasma, these alternative mechanisms deserve consideration.



(a) Uptake of SAM into cell.

(b) Uptake of sulfate into cell.

(c) Uptake of sulfur-containing amino acids into cell.

(d) Conversion of sulfur-containing amino acids to sulfate.

(e) Formation of active sulfate, PAPS.

(f) Transfer of sulfate from PAPS to SAM.

Fig. 1-5. Schematic view of various processes believed to be involved in sulfoconjugation (shown for the substrate salicylamide (SAM). Amino acid = sulfur-containing amino acid, e.g. cysteine or methionine; PAPS = adenosine 3' phosphate 5' sulfatophosphate; PAP = adenosine 3' 5' biphosphate; SAM-SO4 = sulfate conjugate of SAM.

CHAPTER 2

GENERAL METHODS AND MATERIALS

I. INTRODUCTION

Methods and materials described here are those used repeatedly in experiments described in subsequent chapters. Methods and materials unique to individual experiments are described in the methods sections of the respective chapters.

II. DESCRIPTION AND GENERAL CARE OF DOGS

Male mongrels dogs weighing between 21 and 27 kg were used in all experiments. All were from random sources except for one dog (#35255), who was colony raised at the University of California - Davis. Random source dogs received internal parasite medication, were immunized against canine distemper, hepatitis and leptospirosis, and were kept at the University of California - San Francisco (UCSF) animal facility located at Hunters Point for observation for at least one month prior to transferring to the animal care facility located at UCSF.

General care included daily observation and feeding by Animal Care Facility personnel. The dogs were fed Field and Farm Chow (Ralston, St. Louis, MO) at approximately 1 PM daily. This chow contained at least 21% crude protein and was composed primarily of the following: yellow corn, soybean meal, wheat, meat and bone meal, and animal fat.

III. SALICYLAMIDE AND [14C]-SALICYLAMIDE

Salicylamide (SAM) was obtained from Eastman Organic Chemicals (Rochester, NY, Lot A8B). Radiolabeled SAM, labeled on the carboxyl carbon, was custom synthesized by ICN (Irvine CA, Lot 1207109, specific activity 50 mCi/mmol). Radiochemical purity, performed by thin layer chromatography by ICN in May, 1982, was greater than 99%. A 50 uCi/ml [14C]-SAM in 95% ethanol injection solution was prepared for the experiments. Radiochemical purity, tested in our laboratory two months prior to completion of experiments was greater than 98%. The high performance liquid chromatography (HPLC) assay for plasma SAM concentration described in Section V-A was used for the purity analysis.

IV. GENERAL EXPERIMENTAL PROTOCOL

Before all experiments, the dogs were fasted overnight. In the morning, the dogs were placed upright on a table in a sling where they could rest comfortably. Catheters (Angiocath # 28183, Deseret Co., Sandy, UT), one each for drug infusion and drug sampling, kept patent throughout the study with normal saline (25-50 ml/hr), were placed in a cephalic vein of the forelimb and a saphenous vein of the hindlimb. Intragastric administration of SAM was accomplished with a stomach tube (K-10, Kaslow size 16, Pharmaseal Inc., Toa Alta, Puerto Rico), which was reinforced by inserting polyethylene tubing on the inside. Blood samples, 3 to 8 ml, collected into tubes containing oxalate/fluoride (Vacutainer #6428 Becton and Dickinson,

Rutherford, NJ), were kept on ice during experiments. The bladder was catheterized (Swan-Ganz #93-111-7F, Edwards Laboratory, Irvine, CA) and urine was collected over ice. At the end of the experiment, the bladder was flushed with 20 ml of normal saline, allowed to empty and total volume was recorded. Plasma, obtained from blood by centrifugation, and portions of urine were stored at -20^{o}C until analyzed.

V. ASSAYS

A. Salicylamide and metabolites in the plasma and urine (Fielding et al., 1984)

1. Materials

a. Reagents and chemicals

Methanol, ethyl acetate, acetonitrile and dichloromethane (all HPLC grade) were obtained from Burdick and Jackson Laboratories (Muskegon, MICH). Tetrapentylammonium chloride and tetrabutylammonium (TBA) hydrogen sulfate were obtained from Eastman Kodak Co. (Rochester, NY) and Aldrich Chemical Co. (Milwaukee, Wisc), respectively. Salicylic acid methyl amide (N-methylsalicylamide) was purchased from ICN Pharmaceuticals (Plainview, NY). Phenol reagent solution (2N) was obtained from Fisher Scientific Co (Pittsburg, PA). Bacterial B-glucuronidase and aryl sulfatase, types VII and VI, respectively, and myristic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Ethyl aminobenzoate (Benzocaine, U.S.P.) was obtained from Merck & Co. (Rahway, NJ).

Gentisamide (melting point 214-215^{O}C) was synthesized from gentisic acid (Sigma Chemical Co., St. Louis, MO) using the methods described by Bray et al. (1948) and Raistrick and Simonart (1933).

SAM-sulfate was prepared from the urine of a 31 year old human male using a modification of an

ion-pair extraction method used for extraction of steroid conjugates from aqueous solution (Mattox et al., 1972a, 1972b). Modifications included optimization of molar amounts of extraction and counter-extraction ions. Following a 2 gm oral dose of SAM, approximately 200 ml of urine was collected over three hours. The urine and 25 ml of a 420 mM solution of TBA hydrogen sulfate in dichloromethane were added to a separatory flask. An additional 175 ml of dichloromethane was added and the contents were shaken and allowed to stand. After separation into phases, the aqueous phase was discarded. To the organic phase was added 25 ml of aqueous myristic acid (500 mM) and 200 ml of aqueous sodium bicarbonate (500 mM). After shaking and separation of phases, the organic phase was discarded and the aqueous phase was lypophilized. A portion of this was redissolved in water and subjected to preparative HPLC. For this an Altex Ultasphere 5 micron ODS (10 x 250 mm) column and an Altex Ultrasphere 5 micron Octyl precolumn (4.6 x 45 mm) were employed. Mobile phase was made of the following:

200 mM tetrapentylammonium sulfate	90 ml
100 mM phosphate buffer pH 6.2	100 ml
methanol	180 ml

isopropanol	30 ml
acetonitrile	400 ml
distilled water	700 ml

Mobile phase was pumped at a flow rate of 160 ml/hr. Injections of 125 ul (containing about 400 ug of SAM-sulfate) were made and peaks were monitored at 237 nm. The retention time of the collected peak was approximately 10 minutes. Fractions corresponding to this peak were collected after each of 24 injections. These fractions were pooled and a few samples were reinjected and monitored at 237 and 200 nm to insure that no other peaks were present. The remainder of the pooled fractions was extracted with an equal volume of dichloromethane. The aqueous phase was removed and the organic phase was washed with an equal volume of water. After drying over anhydrous sodium sulfate, the organic phase was evaporated under reduced pressure to dryness. The melting point of the resulting crystals was 105 to 110°C. The identity of the purified TBA salt was confirmed by liquid secondary ion mass spectrometry at University of California-Berkeley through the services of the Biorganic Mass Spectrometry Resource (Fig. 2-1).

b. Liquid Chromotography Instrumentation

Mobile phase was delivered by a model A-60-S

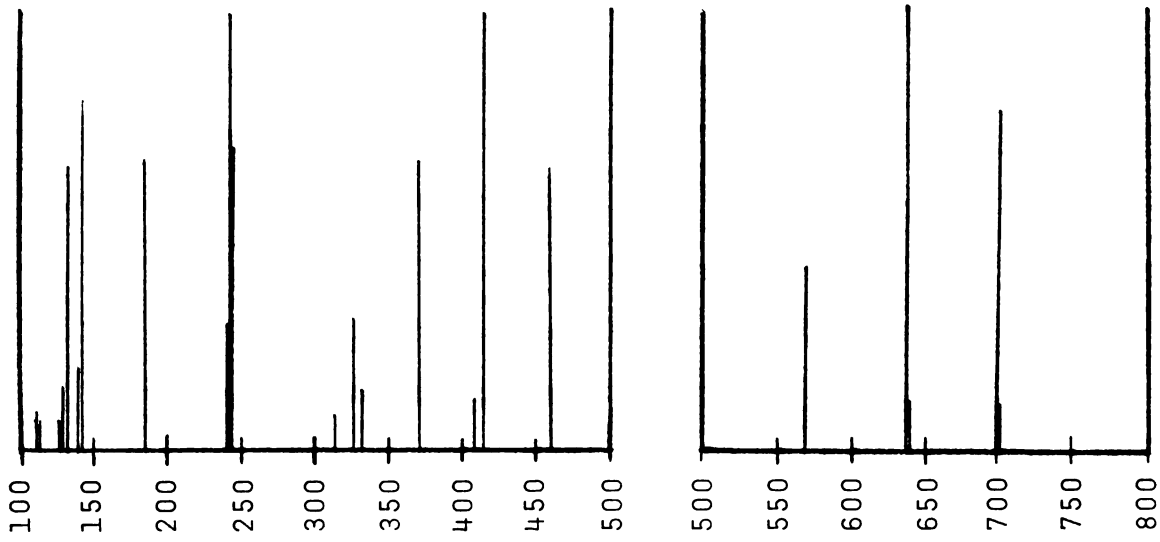


Fig. 2-1. Mass spectrograms of purified SAM-sulfate (as the tetrabutylammonium (TBA) salt) obtained by liquid secondary ion mass spectrometry using a Cs+ primary beam (Kratos MS 50 S). Scan on left, run at 30 sec/decade with signal magnified by 71.4 above a mass of 300, shows the TBA ion with a mass of 242 and the protonated TBA-SAM-sulfate molecular ion with a mass of 459. Scan on right, run at 100 sec/decade, shows the (TBA_2SAM-sulfate)+ ion with a mass of 700.

pump (Eldex Laboratories, Menlo Park, CA) or a model 6000A pump (Waters Assoc., Milford, MA). The injector was a WISP 710B (Waters Assoc.). The column effluent was monitored with a model SF770 detector (Kratos Analytical Instruments, Westwood, NJ) and its output signal recorded and integrated by a model 3390A integrator (Hewlett-Packard, Avondale, PA).

2. Assay of salicylamide and gentisamide in plasma

a. Stability of SAM

The stability of SAM in freshly drawn blood and in plasma stored at -20°C is described in Section V-B.

b. Preparation of samples

Frozen samples were thawed at room temperature and gently mixed. A volume of 1.0, 1.5, or 2.0 ml of each sample was transferred to a 16 x 100 mm test tube and the total volume was brought to 2.0 ml with water. Each sample was extracted twice with 6 ml of ethyl acetate and the pooled organic layers were evaporated to dryness under a gentle stream of nitrogen at 40°C. Methanol (20 ul), to redissolve the residue in each tube; and then 0.2 to 2.0 ml mobile phase containing internal standard salicylic acid methylamide (SAMA) were added. By selecting the volumes of plasma extracted and mobile phase added, peak heights were maintained within the range

of the calibration curve. Each prepared sample was filtered through a 0.45 micron centrifugal microfilter (#SS009, Schleicher & Schuell, Keene NH) before injection. Injections (40 ul) of each sample were made in duplicate and the plasma concentrations of SAM and gentisamide were calculated by comparing their corrected average peak height ratios to a calibration curve. The calibration curve was prepared by spiking blank plasma with SAM at several concentrations between 0.075 and 16.0 mg/L and then preparing calibration samples in the same manner as the unknown samples.

c. Chromatographic conditions

A 150 mm x 2.1 mm I.D. column packed with Rosil Phenyl, 5 micron particles, was employed (Alltech Assoc., Deerfield, IL). The mobile phase, 10 mM pH 2.2 phosphate buffer in water, was delivered at a rate of 0.3 ml/min. Column effluent was monitored at 296 nm.

d. Assay precision and sensitivity

Within-run coefficients of variation were less than 3% (n=10) over the range of the SAM calibration curve; inter-assay variation was less than 10% (n=6).

3. Assay of salicylamide conjugates in the plasma

a. Sample preparation

To 1.0 ml samples, 0.1 ml of 0.2 M phosphate

buffer (pH 5.2) was added. Buffered samples were then extracted twice with 1:1 hexane/ethyl acetate. The organic layer was discarded and the aqueous phase was allowed to stand for a few minutes to evaporate any residual organic phase. Acetonitrile, 3.0 ml, containing 8 mg/L of the internal standard, ethyl aminobenzoate, was added. Samples were centrifuged for 5 minutes at 1000 x g. The supernate was evaporated to dryness under a gentle stream of nitrogen in a water bath maintained at 40 C. The residue was redissolved in 0.3 ml of mobile phase containing 40% methanol described below). After filtration through a 0.45 micron centrifugal microfilter, duplicate 30 ul injectons were made of each prepared sample and the concentration of each metabolite determined by comparing its average peak height ratio with a standard curve prepared from urine samples of known metabolite concentration. See section V-A-4 for method of preparation of urine containing known concentrations of metabolites.

b. Chromatographic conditons

A modified Alltech 605 RP column was employed. The column, packed with 5 micron C18 particles, with an inner diameter (I.D.) of 4.6 mm, was cut to a length of 120 mm to reduce column pressure and improve peak separation. A 40 mm x 2.1 mm I.D. precolumn packed with CO:PELL ODS (Whatman Inc.,

Clifton, NJ) was attached directly to the main column. Injected samples were eluted over 30 minutes by a two-stage process. For the first five minutes, the mobile phase consisted of 40% methanol, 12 mM tetrapentylammonium chloride and 10 mM pH 6.2 phosphate buffer. For the last 25 minutes, mobile phase contained 50% methanol but was otherwise identical that used during the first stage. The flow rate was 1.0 ml/min throughout the two-stage process. Switching of mobile phase was accomplished with a solid state timing device attached to an in-line solvent switching valve (Model 5300 and 5302, Rheodyne, Inc., Cotati, CA). Column effluent was monitored at 230 nm.

c. Identification of chromatographic peaks

Two peaks appeared in chromatograms of dog plasma under these conditions. Effluent fractions corresponding to each of the peaks were collected and subjected to the following treatments: acid hydrolysis (in 2N hydrochloric acid for 20 hours at 65^{o}C), incubation with bacterial B-glucuronidase (from 1 to 20 hours at 37^{o}C and pH 7.0) and colorimetric determination of gentisic acid derivatives (Becher et al., 1952). The earliest fraction, identified as SAM-glucuronide, was converted to SAM by glucuronidase or acid hydrolysis, gave no color in the gentisic acid test

and was unaffected by treatment with sulfatase. The next fraction, identified as SAM-sulfate, was converted to SAM by either sulfatase or acid hydrolysis but not glucuronidase and gave a negative gentisic acid test. The HPLC retention time of the SAM-sulfate peak was the same as that of the peak corresponding to the purified SAM-sulfate (Section V-A-1-a).

4. Assay of salicylamide and metabolites in the urine

a. Sample preparation

Frozen samples were thawed and gently mixed. One ml of each sample was mixed with 3 ml of methanol, centrifuged for 5 min at 1000 x g and the supernate filtered through a 0.2 micron disposable syringe filter assembly (#4192, Gelman Sciences, Ann Arbor, MICH). Duplicate 15 ul injections of each prepared sample were made and the amount of metabolite was determined by comparing its peak height with a calibration curve, prepared from urine samples of known metabolite concentration as follows: An adult male mongrel dog (#36002) was given orally 1.5 mmol of SAM containg 40 uCi of [14C]-SAM and urine was collected for six hours. One ml of this urine was prepared as described above and separated by the HPLC procedure described in Section V-A-3. The amount of conjugate in a volume of prepared urine was calculated by dividing the

amount of [14C] radioactivity collected under each peak by the specific activity of the administered drug. A calibration curve was prepared by diluting the urine obtained as described above with various amounts of blank urine obtained from another dog.

b. Chromatographic conditions

Chromatographic conditions were the same as for assay of SAM conjugates in the plasma (Section V-A-3).

c. Identification of chromatographic peaks

Two chromatographic peaks appeared in the urine of dogs. These were identified to be SAM-glucuronide and SAM-sulfate by using the enzymatic and chemical hydrolysis methods described in Section V-A-3-c.

d. Assay precision

The coefficient of variation over the range of the calibration curve (0.07 to 0.3 gm/L for SAM-glucuronide and 1.0 to 4.0 gm/L for SAM-sulfate) were 5% for SAM-glucuronide, (n=6) and 7% for SAM-sulfate, (n=6) within runs and 6.5% for both conjugates, (n=7) between runs.

B. Unchanged [14C]-SAM concentration in the plasma.

1. Materials

Hexane, and ethyl acetate were obtained from Burdick and Jackson (Muskegon, MICH) and Aquasol from New England Nuclear (Boston, MA). Potassium phosphate, dibasic and monobasic, were obtained from Baker Chemical Co. (Phillipsburg, NJ).

2. Stability of SAM

a. Metabolism in freshly drawn whole blood

Metabolism of SAM in whole blood was studied because phenol sulfotransferase activity in human platelets had been reported (Bonham-Carter et al., 1983). Immediately after drawing about 5 ml of blood from a dog, 200 ul of [14C]-SAM (22600 dpm/ml) was added. After mixing for a few seconds, two equal portions were poured into blood collection tubes of the type used in dog experiments (Section IV). One tube was put immediately on ice and the other was incubated at 37 C for 30 minutes. Plasma from both samples, obtained by centrifugation, was extraced and counted as described in Section V-B. The [14C]-SAM concentration in plasma obtained from blood incubated for 30 minutes at 37^{o}C was not different from that from blood placed on ice for 30 minutes (3367 +/- 168 vs. 3430 +/- 172 dpm/ml, respectively). Because enzymatic reactions are usually temperature-sensitive (Giese, 1962), it was

concluded that detectable metabolism of SAM in freshly drawn blood does not occur.

b. Stability in plasma stored at -20°C

Measured concentrations of [14C]-SAM in plasma standards stored at -20°C were unchanged over several months.

3. Procedure

To 0.5 ml plasma was added an equal volume of potassium phosphate buffer, 200 mM (pH 6.0) and six ml of 4:1 mixture of hexane-ethyl acetate. Samples were then vortexed for a few seconds and centrifuged at 2,250 x g for ten minutes. Four ml of the organic phase was then added to 10 ml of Aquasol and counted for ten minutes in a scintillation counter (Beckman model LS 9800). The assay was found to be linear over a range of 0.06 ug/L to 8 mg/L (determined by adding various amounts of labeled and unlabeled SAM). The above extraction procedure, developed utilizing the HPLC assay procedure described above (section V-A-2), minimized interferences from other radiolabeled metabolites. Less than 5 % of gentisamide (not an observed metabolite in the dog) and less than 1% of salicylamide conjugates are extracted. Because the assay was consistently linear and slope of the standard curve unchanged between runs, four to five identical 0.5 ml samples of a serum sample spiked with [14C]-SAM, 12,000 dpm/ml, were used for standards. Counting

efficiency was approximately 90%. The mean extraction and counting efficiency in these sample was used to calculate [14C]-SAM concentration in unknown samples. Coefficients of variation were less than 2%.

C. [14C]-SAM metabolites in the urine

Total drug and metabolites (total radioactivity) was measured by adding 100 ul of urine to 10 ml of Aquasol and counting (efficiency 90%). Individual metabolites of SAM in the urine were measured by the HPLC assay procedue described in Section V-A-4. Two-minute HPLC fractions were added to 15 ml Aquasol and counted (efficiency = 90%). Only one mobile phase was used as an eluent (methanol concentration of 42%).

D. Inorganic Sulfate in the plasma

1. Materials

Trichloroacetic acid, ammonium sulfate and dextran, clinical grade, average molecular weight 71,000, were obtained from Sigma Chemical Co. (St. Louis, MO); barium chloride was obtained from Allied Chemical Co. (Morristown, NJ).

2. Procedure

The turbidimetric assay of Krijgsheld et al. (1979) was modified as follows: Two ml of trichloroacetic acid was added to 200 ul plasma. After vortexing, samples were centrifuged at 2,250 x g for ten minutes and 1.3 ml of the supernate was pipetted into a capped plastic centrifuge tube. Further centrifugation at 12,800 x g for three minutes removed any remaining visible particles from the supernate. One ml was then added with minimal mixing to 250 ul of an aqueous solution of 2% barium chloride and 1% dextran. After 10 minutes, the contents were carefully transferred to a 2 ml quartz curvette. One minute later, absorbance was read at 360 nm against a distilled water blank one minute later using a Cary-118 spectrophotometer. Samples containing sulfate concentrations greater than 1.6 mM were assayed in the same manner except that four ml of 5% trichloroacetic acid were added to 100 ul of plasma

in the first step. All aqueous reagents were prepared with deionized water. Plasma for the standard curves was obtained from a dog in whom plasma sulfate had been depleted twelve hours earlier with an oral dose of SAM, 0.58-mmol/kg. The sulfate concentration in this sample (0.11 mM) was kindly assayed by ion chromatography (Girard and Glatz, 1981) by Marilyn Morris in the laboratory of Gerhard Levy, School of Pharmacy, State University of New York at Buffalo, Amherst, NY. Standard curves were prepared by adding either deionized water or standard aqueous solutions of ammonium sulfate to this plasma sample.

CHAPTER 3

DOSE-DEPENDENT BIOAVAILABILITY AND SULFOCONJUGATION OF SALICYLAMIDE IN DOGS

I. INTRODUCTION

Previous studies in dogs indicate that a large portion of on orally administered 30-mg/kg dose of salicylamide (SAM) appears in the urine as the sulfoconjugate (Gugler et al., 1975). Furthermore, significant metabolism occurs during the first-pass through the intestinal wall and liver. In man, pronounced dose-dependence in SAM kinetics has been observed (Barr et al., 1973).

The goal of the experiments described in this chapter was to establish SAM in the dog as an experimental model to study dose-dependent sulfoconjugation. SAM was given by the oral route to obtain information on both first-pass and overall metabolism. Because previous work in rats indicated that depletion of inorganic sulfate in the plasma is associated with the dose-dependent metabolism of acetaminophen (Galinsky and Levy, 1981), plasma sulfate concentrations were measured.

II. METHODS

A. Tracer Methodology

To allow calculation of average values of the pharmacokinetic parameters after a single oral dose of SAM, an IV tracer dose of [14C]-SAM was concurrently administered. Concurrent administration of isotopes by different routes was first used by Strong et al. (1975) for bioavailability estimation to minimize the error that occurs when clearances of the IV reference and oral doses are different. The error that occurs when clearance changes within the elimination of a single dose may also be reduced by this method. To allow for a delay in the absorption of SAM, IV doses were given four minutes after each oral dose.

B. Experimental protocol

Six dogs were each given 5, 10, 20, and 40 mg/kg of SAM on separate occasions at least two weeks apart. The order of oral dose administration varied between dogs. After obtaining blank blood and urine samples, SAM, dissolved in 10 ml of an 4:1 solution of propylene glycol/ethanol, and 100 ml of water were successively administered via a gastric tube. Four minutes later, 40 uCi of [14C]-SAM was administered intravenously over one minute. The injection port was then flushed with 10 ml of normal saline. Five- to eight-ml blood samples were

collected at 0, 2, 4, 8, 12, 16, 20, 30, 45, 60, 75, 90, 120, 180, 240, and 360 minutes. After 360 minutes, all catheters were removed and the dog was transferred to a metabolic cage and urine was collected for another 18 hours.

Three of the dogs were also given single oral and intravenous tracer doses (40 uCi) of SAM on separate occasions. These studies were carried out in a manner similar to that described above, except that additional blood samples were collected at 1 and 3 min.

C. Assays

1. Plasma and urine SAM and metabolites - Chapter 2, Section V-A
2. Plasma [14C]-SAM - Chapter 2, Section V-B
3. Urinary [14C]-SAM metabolites - Chapter 2, Section V-C
4. Plasma inorganic sulfate - Chapter 2, Section V-D

D. Treatment of data

Because SAM in the plasma decayed exponentially over the time period studied, clearance and half-life were calculated rather than Michaelis-Menten parameters. Clearance (Cl) was calculated as Dose(IV)*/AUC*, where Dose(IV)* is the dose of [14C]-SAM and AUC* refers to the area under the plasma concentration vs. time curve of unchanged [14C]-SAM from time 0 to infinity. The area up

to the first measurement was estimated as C1 x t, where C1 is the concentration of the first measurement and t is the time elapsed from the midpoint of the SAM injection to the time of the first measurement. The AUC between concentration time points was calculated by the trapezoidal rule. The area after the last measurement, C(last), was estimated as C(last)/k, where k, the slope of the terminal log concentration-time values, was determined using a least squares regression program written in this laboratory. A concentration-time value was considered in the terminal phase when absorption and distribution phases appeared to be complete. Volume of distribution was determined as Cl/k. Half-life was estimated from 0.693/k using IV data. Clearance of SAM to each of the metabolites, SAM-sulfate and SAM-glucuronide, was calculated as M*/AUC* where M* is the amount of drug recovered in the urine as the radiolabeled metabolite and AUC* again refers to radiolabeled unchanged drug in the plasma. Bioavailability was calculated as Clx(AUC)/Dose(oral) where AUC refers to the area for unlabeled SAM.

One-way analysis of variance (ANOVA) with Hartley's post tests (Dixon and Massey, 1983) was carried out to determine statistical significance of differences of parameters at various dose levels. For parameters obtained at only two oral dose levels, Student t-tests were applied.

III. RESULTS

Bioavailability increased from with dose, from 0.24 +/- 0.14 (mean +/- SD) after the 5-mg/kg dose to 0.76 +/- 0.20 after the 40-mg/kg dose ($P < 0.05$, ANOVA, Table 3-1). Bioavailability of the tracer dose (approximately 0.005 mg/kg) was estimated, but in a different manner, because the drug had to be given orally and intravenously on separate occasions. When the tracer dose was given orally, plasma [14C]-SAM concentrations were always less than 40 dpm/ml above background, a value near the sensitivity of the assay. When tracer was given intravenously, peak plasma concentrations of 1,500-6,000 dpm/ml were found. Therefore, bioavailability of the oral tracer dose was probably less than 0.02 in the three dogs studied.

Typical Michaelis-Menten curvature in log plasma SAM concentration vs. time curves was not observed over the time period studied (Fig. 3-1). The terminal half-lives of the labeled and unlabeled drug were nearly the same [(half-life, unlabeled)/(half-life, labeled) = 0.93 +/- 0.17]. Clearance decreased with dose, from 3.4 +/- 1.0 L/min after the 5-mg/kg dose to 0.6 +/- 0.1 L/min after the 40-mg/kg dose ($P < 0.01$, Fig. 3-2 and Table 3-1). Clearance of the intravenous tracer dose, when given alone, was even higher (13.7 +/- 6.0 L/min). Increases in clearance were accompanied by decreases in SAM half-life (Table 3-1). Volume of distribution did not change consistently with dose.

The reduction of plasma inorganic sulfate concentrations

Table 3-1. Pharmacokinetic parameter values (mean(SD)) after four doses of salicylamide given to six dogs.

Parameter	Oral SAM dose (mg/kg)				F ratio(a)
	5	10	20	40	
Bioavailability	0.24 (0.14)	0.42 (0.24)	0.62 (0.38)	0.76 (0.20)	4.8(b)
Clearance (l/min)	3.4 (1.0)	2.1 (0.6)	1.3 (0.4)	0.60 (0.11)	23.8(c)
Half-life (min)	5.0 (1.2)	9.3 (1.6)	16.2 (4.5)	23.5 (6.1)	25.6(d)
Volume of Distribution (l/kg)	1.1 (0.3)	1.1 (0.2)	1.3 (0.2)	0.86 (0.14)	4.0(e)

(a) ANOVA value
(b) Value at 5-mg/kg dose differs ($P<.05$) from those at the 20- and 40-mg/kg doses.
(c) Values at all dose levels differ ($P<.05$) from each other.
(d) Values at all dose levels differ ($P<.05$) except for the values at the 5- and 10-mg/kg dose levels, which do not differ from each other.
(e) Value at the 20-mg/kg dose differs ($P<.05$) from that at the 40-mg/kg dose.

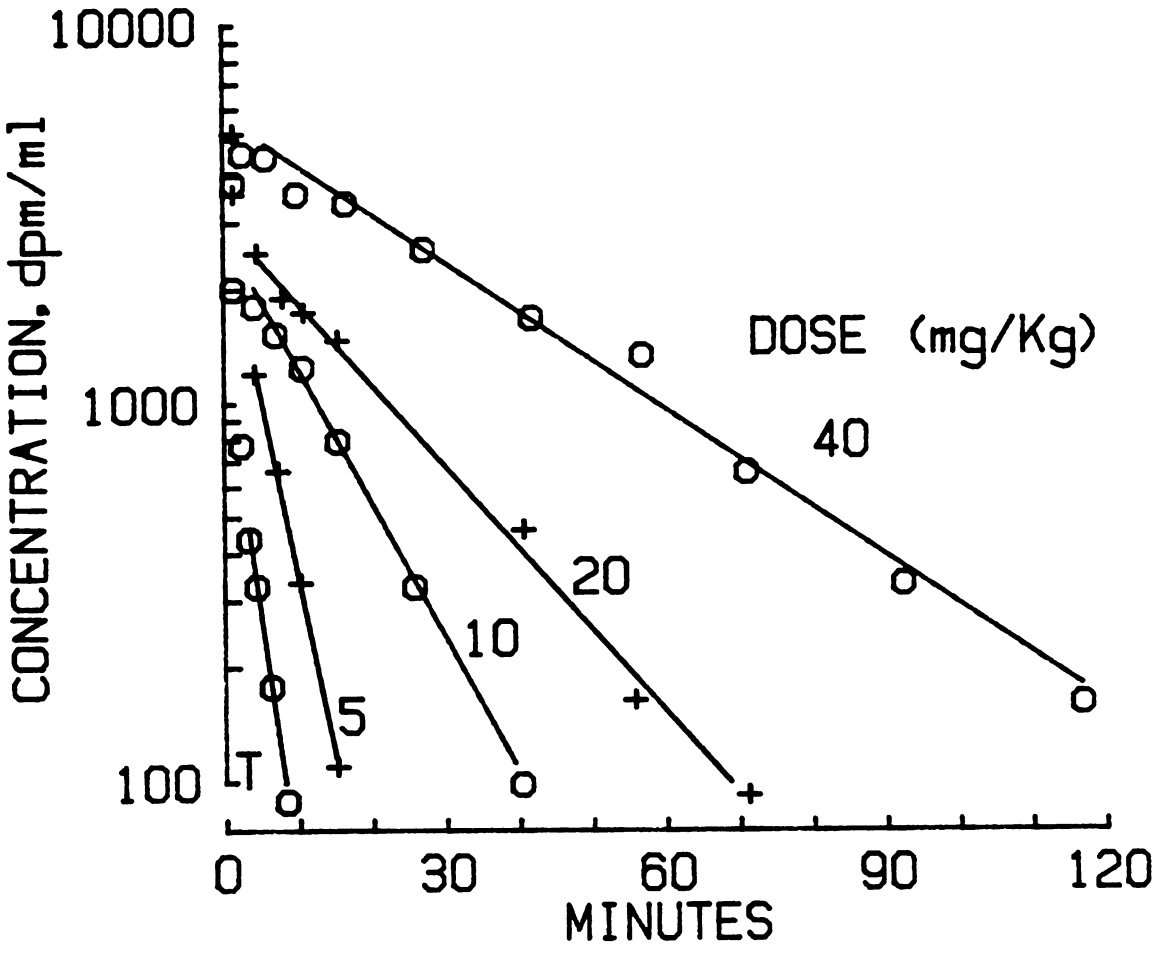


Fig. 3-2. Unchanged [14C]-salicylamide concentration in plasma vs. time profiles in a dog given 40 uCi of [14C]-salicylamide alone (T) or four minutes after each of the indicated oral doses of unlabeled SAM.

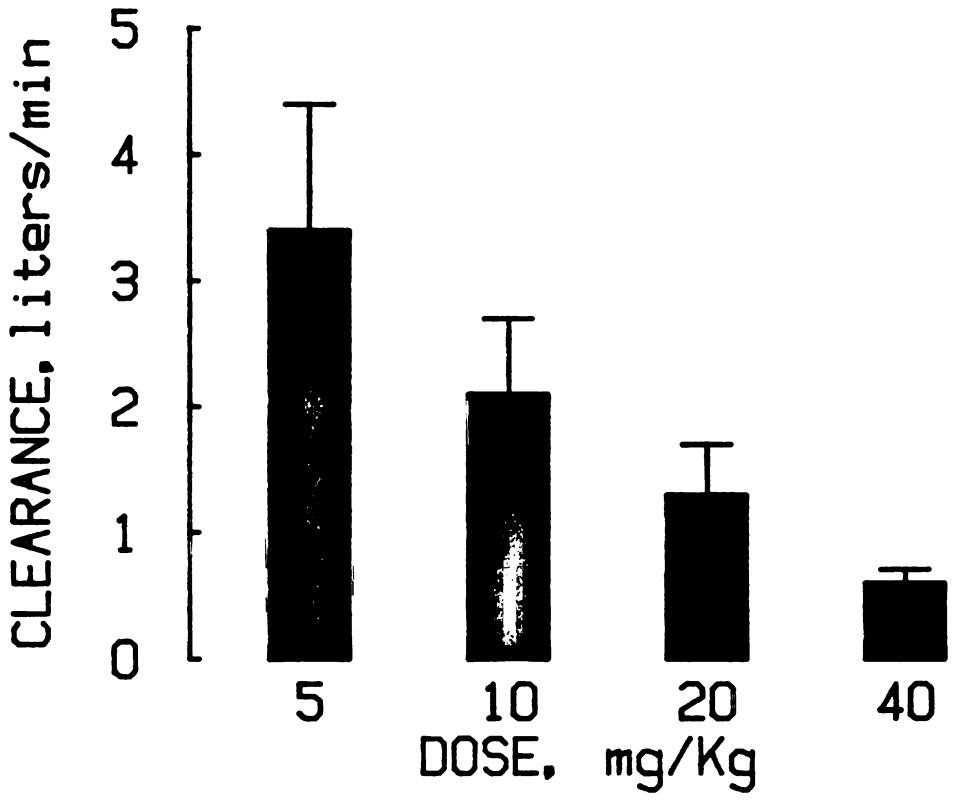


Fig. 3-1. Clearance values (Dose/AUC) of salicylamide (mean +/- SD) for six dogs determined from an intravenous tracer dose of [14C]-salicylamide administered four min after each of the oral doses indicated.

was also dose-dependent (Table 3-2). The low concentrations reached at 2 hours after each of the SAM doses were sustained at 4 hours.

More than 80% of the administered radiolabeled SAM was recovered in the urine at all dosage levels (Table 3-3). An apparent trend towards lower recovery of unlabeled drug as SAM-sulfate or SAM-glucuronide at higher doses was not statistically significant. Linear and log-linear regression analysis of dose vs. recovery of unlabeled drug gave correlation coefficients of 0.17 and 0.16, respectively.

After the high (40-mg/kg) and low (5-mg/kg) oral doses and after the intravenous tracer dose alone, more than 99% of the radioactivity recovered in the urine consisted of SAM-sulfate or SAM-glucuronide. The sulfate conjugate consisted of 97% of the total conjugates excreted in the urine after the 5-mg/kg SAM dose. After the 40-mg/kg dose, this value was 85%. The sum of unchanged SAM and gentisamide (labeled or unlabeled) was less than 1% of of the recovered SAM dose. Counting of HPLC fractions showed no other peaks of radioactivity.

Clearance of SAM to SAM-sulfate was significantly higher at the 5-mg/kg level than that at the 40-mg/kg level ($P < 0.05$, Table 3-4) whereas clearance of SAM to SAM-glucuronide was the same. When intravenous tracer was given alone to three dogs, clearance of SAM to SAM-sulfate was even higher (15.1 +/- 7.4 liters/min) than that at the 5-mg/kg dose level. Clearance of SAM to SAM-glucuronide

Table 3-2. Plasma concentrations of inorganic sulfate (mM), (mean(SD)) after oral doses of salicylamide (SAM) in six dogs.

SAM Dose Level (mg/kg)	Time after Dosing (min)			
	0	60	120	240
5	0.92 (0.31)	0.76 (0.28)	0.76 (0.28)	0.75 (0.30)
10	0.86 (0.11)	0.64 (0.16)	0.66 (0.18)	0.65 (0.16)
20	0.90 (0.13)	0.60 (0.13)	0.55 (0.16)	0.55 (0.16)
40	0.88 (0.17)	0.47 (0.16)	0.27 (0.13)	0.27 (0.10)

Table 3-3. Urinary recovery (%) (mean(SD)) of radiolabeled and unlabeled salicylamide (SAM) and metabolites after oral doses of SAM given to six dogs.

	Oral SAM dose				F ratio (a)
	5	10	20	40	
Labeled SAM	85 (13)	88 (5)	87 (12)	80 (12)	0.49(b)
Unlabeled SAM(c)	95 (21)	85 (22)	78 (17)	71 (12)	1.8(b)

(a) ANOVA value
(b) $P > 0.05$
(c) Determined from sum of molar equivalents of sulfate and glucuronide conjugates of SAM excreted in urine in 24 hours.

Table 3-4. Clearances (l/min) (mean(SD)) of salicylamide (SAM) to its sulfate and glucuronide conjugates after two doses of SAM given to six dogs.

	Oral SAM dose (mg/kg)		t value
	5	40	
Clearance to glucuronide	0.08 (0.05)	0.08 (0.04)	0.21(a)
Clearance to sulfate	2.8 (0.8)	0.45 (0.12)	6.9(b)

(a) $P > 0.1$
(b) $P < 0.001$

after the IV tracer (0.11 +/- 0.10 liters/min) was not significantly different from that at the 5- and 40-mg/kg dose levels.

Detailed data for individual dogs are presented in Appendix 1.

IV. CONCLUSIONS AND DISCUSSION

These studies demonstrate that bioavailability and clearance of SAM in the dog are dose-dependent and that SAM is almost completely eliminated by sulfoconjugation. Furthermore, whereas the dose-dependence occurs in the sulfoconjugation pathway, glucuronidation is unaffected. SAM in the dog is therefore a potentially useful experimental model to study the mechanisms of dose-dependent sulfoconjugation.

An additional finding of interest was that, despite the dose-dependence observed, plasma [14C]-SAM concentrations declined exponentially with time at all dose levels, with no apparent Michaelis-Menten curvature. Increases in half-life with dose were associated with progressively greater depletion of plasma sulfate. Similar kinetics were observed when several doses of acetaminophen were given by intravenous bolus doses to rats (Galinsky and Levy, 1981). However, when sodium sulfate was infused to prevent the sulfate depletion, downward cuvature of log actetaminophen concentration vs. time curves was observed. This suggests that the downward curvature of log acetaminophen concentration-time curves is prevented by the opposing effect of sulfate depletion in the absence of exogenous sulfate supplementation. It is not clear from their data if the downward curvature was due to saturable metabolism in the sulfoconjugation or glucuronidation pathway or due to another time-dependent

effect.

Unlike acetaminophen in the rat, dose-dependent changes in SAM clearance were observed at doses that caused only minimal sulfate depletion. Clearance of the tracer and 5-mg/kg doses 13.7 +/- 6.0 L/min and 3.4 +/- 1.0 L/min, respectively, yet plasma sulfate after the 5-mg/kg dose was reduced by less than 20%. This suggests that the mechanism of dose-dependent sulfoconjugation of SAM is not entirely due to depletion of plasma inorganic sulfate. Then effects of co-administration of sodium sulfate on the kinetics of SAM in the dog are examined in the next chapter. Subsequent chapters further investigate the relationship between plasma sulfate concentrations and SAM clearance changes.

Also noted in these studies were clearance values that greatly exceed published values of cardiac output in the dog (Altman and Dittmer, 1971). This suggests that metabolism of SAM may occur in several organs. This issue is addressed in Chapter 7.

CHAPTER 4

DOSE-DEPENDENT SULFOCONJUGATION OF SALICYLAMIDE IN DOGS: EFFECT OF SULFATE DEPLETION OR ADMINISTRATION

I. INTRODUCTION

Administration of sulfate donors has been shown to increase the sulfoconjugation of several phenolic substrates in rabbits (Bray et al., 1952), rats (Buch et al., 1968; Galinsky and Levy, 1979; 1981) and man (Levy and Matsuzawa, 1966; 1967; Smith and Mitchell, 1974). Krijgsheld et al. (1982) has shown further that a relationship exists between plasma inorganic sulfate concentration and the sulfoconjugation of harmol in the rat. By injecting phenol and inorganic [35S]-sulfate intravenously into rats and collecting metabolites excreted in the bile, Mulder and Scholtens (1978) demonstrated that inorganic sulfate in the plasma is available for conjugation within minutes.

In Chapter 3, however, it was found that clearance of salicylamide (SAM) in the dog is highly dose-dependent, even at doses that only slightly reduce plasma inorganic sulfate concentrations. In the experiments presented here, the relationship between plasma sulfate and sulfoconjugation of SAM was studied further. Plasma sulfate concentrations were raised or lowered prior to SAM administration, and the kinetics were compared to control experiments.

Because intestinal first-pass metabolism of SAM in the dog appears to occur (Gugler et al., 1975), active sulfate

precursors were co-administered orally with SAM and the effect on bioavailability determined.

II. METHODS

A. Experimental protocol

1. Control experiments

Dogs were given SAM, 5 mg/kg (n=5) or 20 mg/kg (n=6). After obtaining blank blood and urine samples, SAM, dissolved in 10 ml of propylene glycol/ethanol 4:1, was administered through a gastric tube. Immediately after SAM administration, 100 ml of either water or an aqueous solution of sodium sulfate or N-Acetylcysteine was administered through the gastric tube. Four minutes later, a 40 uCi tracer dose of [14C]-SAM was administered intravenously over one minute. Blood samples, 8 ml, were collected at 0, 2, 4, 6, 8, 12, 15, 20, 25, 30, 45, 60, 75, 90 and 120 minutes after the oral dose. Following the acute phase of the study, all catheters were removed and the dog was transferred to a metabolic cage and urine was collected for an additional 24 hours. Experiments in individual dogs were separated by at least two weeks.

2. Depletion of plasma sulfate.

To deplete plasma inorganic sulfate concentrations, five dogs were given orally 80 mg/kg of SAM, dissolved in 20 ml of propylene glycol/ethanol 4:1, at 10 PM, on the evening before the study. The following morning, a 5-mg/kg dose of SAM was orally administered in the manner described in control experiments. The kinetics of SAM, 5 mg/kg, with and

without sulfate depletion were compared.

3. Exogenous Precursor Dosing.

Sodium sulfate (anhydrous, Mallinckrodt, St. Louis, Mo) was administered to six dogs by intravenous infusion, to raise plasma inorganic sulfate concentrations, or through a gastric tube, to increase the sulfate supply to the intestinal lumen. The parenteral dose of sodium sulfate, 50 mg/kg in 100 ml normal saline, was infused over 30 minutes, beginning 20 minutes before the oral administration of SAM, 20 mg/kg. The oral dose of sodium sulfate, 100 mg/kg in 100 ml of water, was given immediately after the administration of SAM, 20 mg/kg. N-Acetylcysteine (Mead-Johnson, Evansville, Ind), 50 mg/kg dissolved in 100 ml of water, was also given orally to four dogs in the same manner as oral sodium sulfate. The kinetics of SAM in the presence of exogenously administered precursors were compared to those of SAM without precursor supplementation.

B. Assays.

1. Plasma and urine SAM and metabolites - Chapter 2, Section V-A
2. Plasma [14C]-SAM - Chapter 2, Section V-B
3. Urine [14C]-SAM metabolites - Chapter 2, Section V-C
4. Plasma inorganic sulfate - Chapter 2, Section V-D

C. Treatment of Data.

Clearance, bioavailability, half-life, and clearance of SAM to the metabolites, SAM-sulfate and SAM-glucuronide were calculated as described in Chapter 3, Section II-D.

Statistical analyses were carried out using the the One-way analysis of varience (ANOVA) and Student's t-tests (Dixon and Massey, 1983).

III. RESULTS

A. Depletion of Plasma Sulfate

Twelve hours after the administration of an 80-mg/kg dose of SAM, plasma inorganic sulfate concentrations had decreased to less than 0.30 mM, the lower limit of assay reliablity. Clearance and half-life appeared to be decreased and increased, respectively, although not significantly at the $p < 0.05$ level (Table 4-1). The lack of statistical significance may have been due to values obtained in one dog in which depletion of sulfate had no apparent effect. In other dogs, clearance measured twelve hours after depletion was 20 to 60% of control values. Bioavailability and volume of distribution were unchanged by sulfate depletion.

Clearance of SAM to the sulfate conjugate was reduced from a control value of 2.9 +/- 0.8 L/min to 1.6 +/- 0.7 L/min (mean +/- SD, $p < 0.05$, Table 4-2). This effect may have been exaggerated, however, because accurate calculation of this parameter requires complete recovery of radiolabeled metabolite. The lowest recovery in any single experiment (57%) occurred in a depletion experiment. Clearance of SAM to the glucuronide conjugate was unchanged.

B. Exogenous precursor dosing

Sixty minutes after the intragastric administration

Table 4-1. Pharmacokinetic parameter values of salicylamide (mean(SD)) in five dogs given 5 mg/kg orally in control and sulfate depleted (a) conditions.

	Total Clearance (l/min)	Half-life (min)	Bioavailability	Volume of Distribution (l/kg)
5 mg/kg SAM control	3.7 (1.0)	4.4 (0.8)	0.22 (0.15)	1.1 (0.27)
5 mg/kg SAM after depletion	2.0 (1.1)	9.4 (6.2)	0.19 (0.18)	0.9 (0.6)
t value	2.5(b)	1.6(c)	0.2(c)	0.9(c)

(a) Plasma inorganic sulfate was depleted by administering an 80-mg/kg oral dose of salicylamide twelve hours prior to the experiment.
(b) $P = 0.06$, Student's t-test
(c) $P > 0.1$, Student's t-test

Table 4-2. Clearances (l/min) (mean (SD)) of salicylamide (SAM) to its sulfate and glucuronide conjugates in five dogs given 5 mg/kg orally in control and sulfate depleted (a) conditions.

	Clearance of SAM to SAM-sulfate	Clearance of SAM to SAM-glucuronide
5 mg/kg SAM control	2.9 (0.8)	0.095 (0.047)
5 mg/kg SAM after depletion	1.6 (0.7)	0.078 (0.029)
t value	4.4(b)	0.7(c)

(a) Plasma inorganic sulfate was depleted by administering an 80-mg/kg oral dose of SAM twelve hours prior to the experiment.
(b) $P < 0.01$
(c) $P > 0.1$

of the control 20-mg/kg dose of SAM, mean plasma concentrations of inorganic sulfate were reduced to about 70% of baseline values ($P < 0.05$, Student's t-test, Fig 4-1). When this dose of SAM was given 20 minutes after the beginning of a 30-minute intravenous infusion of sodium sulfate, plasma concentrations of inorganic sulfate were always 60 to 100% higher than those seen when SAM was administered alone ($P < 0.05$). When sodium sulfate was administered through the gastric tube immediately after the SAM dose, plasma concentrations of inorganic sulfate were significantly higher than those seen when SAM was administered alone ($P < 0.05$). N-Acetylcysteine, administered orally with SAM, had no effect on the reduction of plasma sulfate concentrations produced by SAM (see Appendix 2).

Administration of exogenous precursors by either the oral or parenteral route had no effect on the general appearance of the log radiolabeled plasma SAM concentration vs. time curves, i.e., SAM concentrations decayed exponentially with time (Fig 4-2). Clearance, half-life, and volume of distribution of SAM were not significantly affected ($P > 0.1$, ANOVA, Fig. 4-3). Intragastric administration of sodium sulfate reduced the bioavailability of SAM in each dog, but the difference in mean values was of borderline statistical significance (0.63 ± 0.38 vs 0.25 ± 0.14, $P = 0.06$, Fig. 4-3). Intragastric administration of N-Acetylcysteine had no

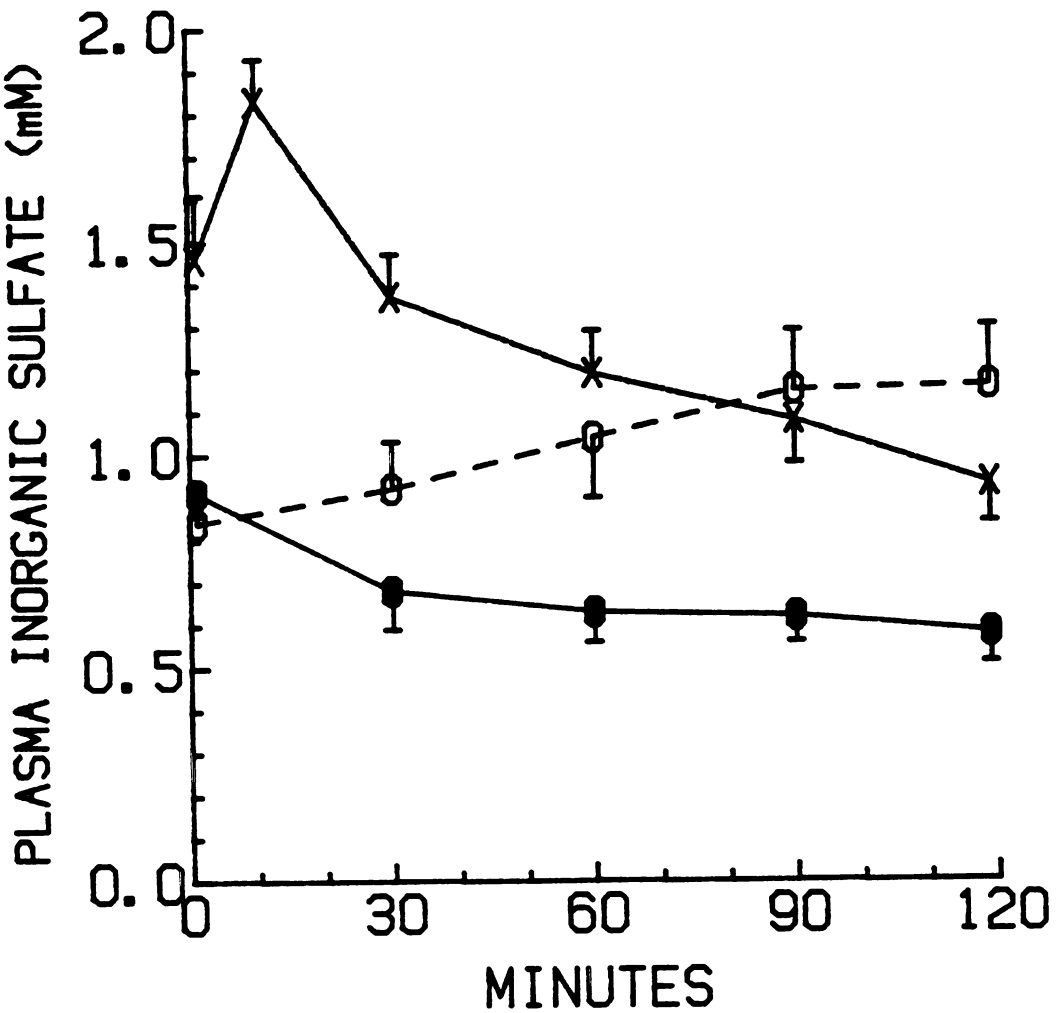


Fig. 4-1. Plasma concentrations of inorganic sulfate (mean +/- SD) in six dogs after oral administration of 20 mg/kg of salicylamide (SAM): (●) SAM given alone; (X) SAM given 20 min after the beginning of a 30-min, 50-mg/kg IV infusion of sodium sulfate; (O) SAM given concurrently with an 100-mg/kg oral dose of sodium sulfate.

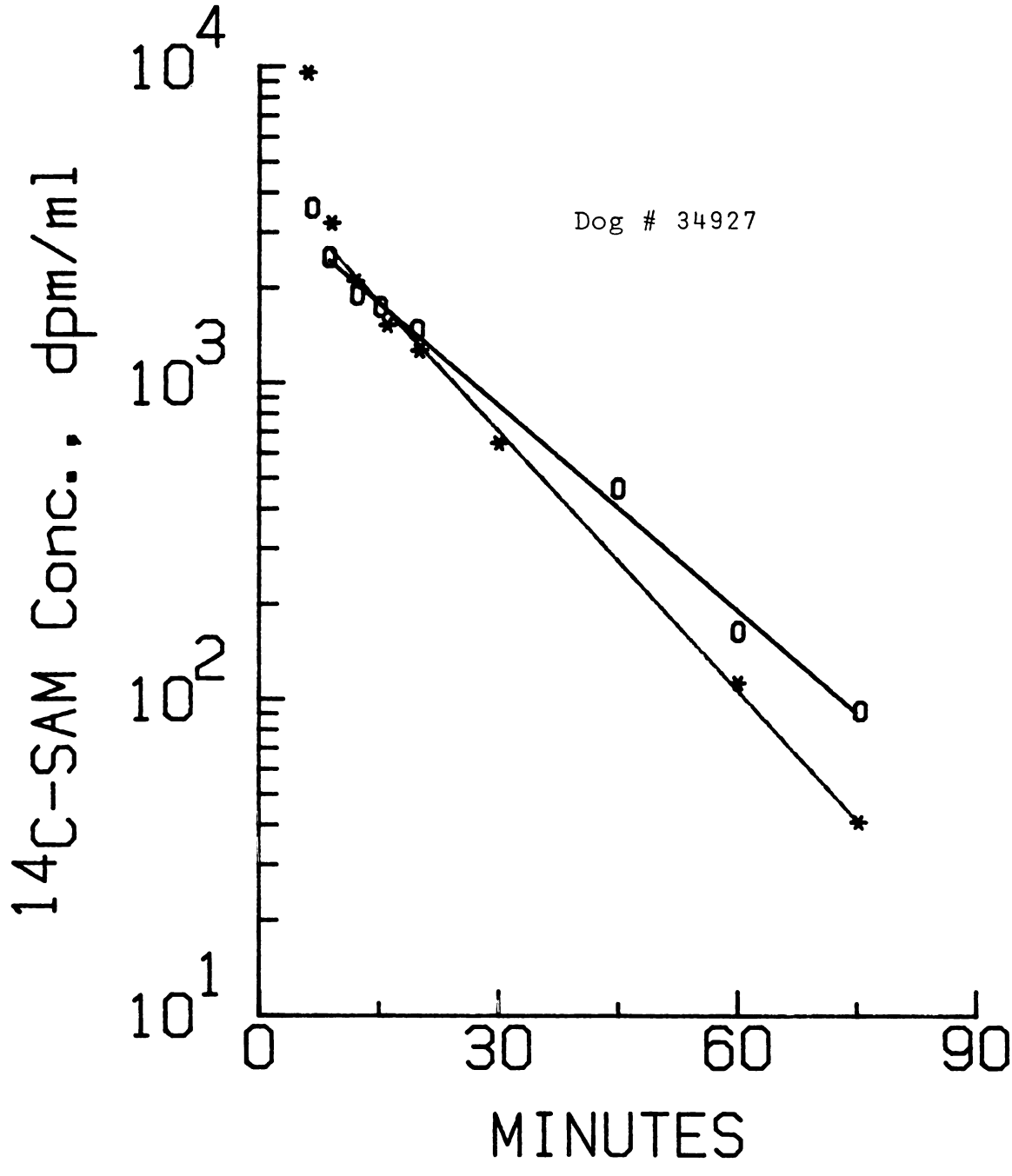


Fig. 4-2. Unchanged [14C]-SAM concentration in plasma vs. time in a dog given 40 uCi IV [14C]-SAM 5 min after an oral 20-mg/kg dose of SAM: (O) control; (★) 20 min after the beginning of a 30 min, 50-mg/kg IV infusion of sodium sulfate.

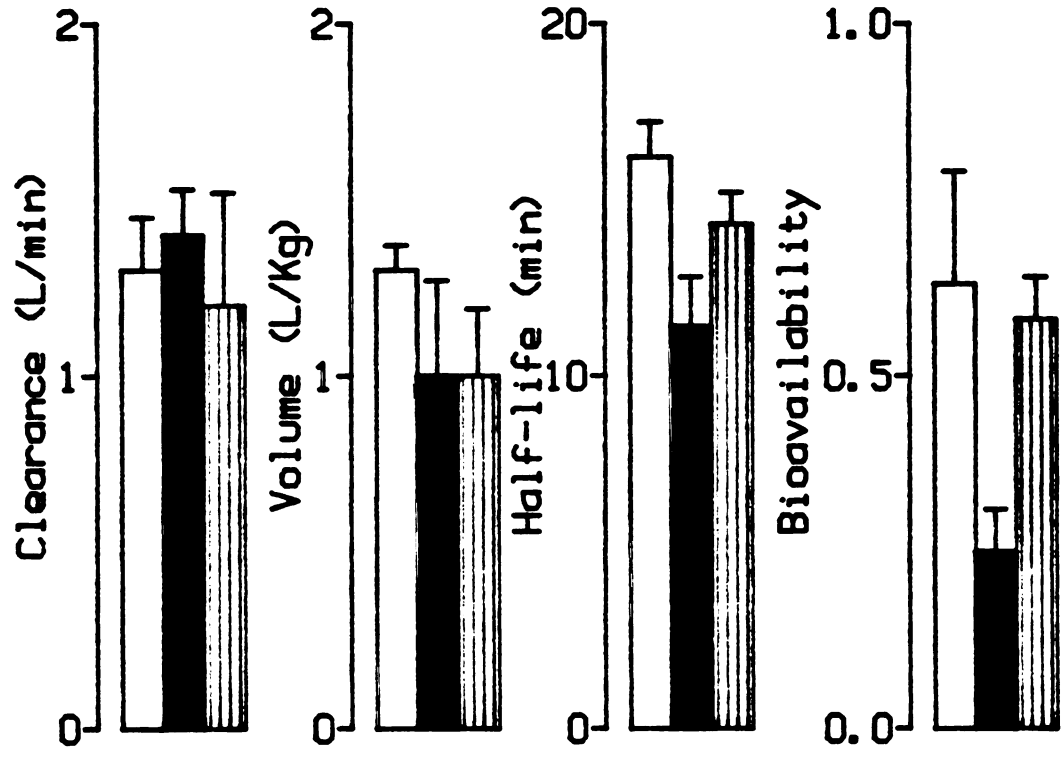


Fig. 4-3. Pharmacokinetic parameter values (mean +/- SD) of salicylamide (SAM) in six dogs after administration of an oral 20-mg/kg dose of SAM; (open bars) control; (solid bars) given concurrently with orally administered sodium sulfate, 100 mg/kg; (bars with vertical lines) given 20 min after the beginning of a 30-min, 50-mg/kg intravenous infusion of sodium sulfate.

effect on bioavailability (Appendix 2). The percentage of the oral SAM dose recovered as drug and metabolites in the urine was similar in control and sulfate experiments ($P > 0.1$, Table 4-3).

Detailed data for individual dogs are presented in Appendix 1 (controls experiments) and Appendix 2 (sulfate depletion and precursor supplementation experiments).

Table 4-3. Urinary recovery (%) (mean+/-SD) of radiolabeled and unlabeled SAM and metabolites after administration of 20 mg/kg salicylamide (SAM) with and without sulfate precursors.

	Labeled SAM	Unlabeled SAM (a)
20 mg/kg SAM control	87 +/- 12	78 +/- 17
20 mg/kg SAM + IV sodium sulfate	94 +/- 9	78 +/- 9
20 mg/kg SAM + oral sodium sulfate	85 +/- 8	73 +/- 7
20 mg/kg SAM + oral N-Acetylcysteine	92 +/- 10	95 +/- 8
F value (b)	1.00(c)	0.94(c)

(a) determined from the sum of molar equivalents of sulfate and glucuronide conjugates of SAM excreted in 24 hours.
(b) ANOVA value
(c) $P > 0.1$

IV. CONCLUSIONS AND DISCUSSION

The results of these studies suggest that plasma concentrations of inorganic sulfate play a relatively unimportant role in the dose-dependent sulfoconjugation of SAM in the dog over the dosage range studied. Infusion of sodium sulfate prior to and after administration of a 20-mg/kg dose of SAM raised and maintained plasma sulfate concentrations well above normal but did not restore SAM clearance towards normal values. When plasma inorganic sulfate concentrations were reduced below 0.30 mM, a possible reduction of clearance was observed. Further depletion of sulfate by the 5-mg/kg dose of SAM, however, must be considered as a reason for the apparent reduction of clearance. In Chapter 5, similar experiments include administration of an IV tracer alone to see if the effect was due to further depletion.

In humans, dose-dependent sulfoconjugation of SAM can be overcome by co-administration of large oral doses of the sulfate precursor, L-cysteine with SAM (Levy and Matsuzawa, 1966, 1967). The differences between dogs and man in responsiveness to exogenous precursors dosing may arise because normal plasma inorganic sulfate concentrations are lower in man (0.3 mM, Krijgsheld et al., 1980) than in the dog. Larger doses of SAM, such as the 80-mg/kg dose used in the depletion studies, may be needed to lower the sulfate concentrations in the dog to the range in which SAM

sulfoconjugation is sensitive.

Although the reduction of bioavailability by administration of sodium sulfate with SAM was not statistically significant by ANOVA, the value in each dog after sodium sulfate administration was lower than the corresponding control value. Nonparametric statistical tests (Wilcoxin Signed-Rank, Dixon and Massey, 1983) indicate that the reduction of bioavailability was significant at the $P < 0.05$ level. The fact that recovery of orally administered SAM was the same in control and sulfate supplementation experiments suggests that the reduction of bioavailability, if it occurred, was not the result of decreased absorption of SAM. First-pass metabolism of SAM in the dog has been shown to occur in the intestinal wall and liver (Gugler et al., 1975). A reduction of bioavailability could have occurred due to increased concentrations of sulfate in first-pass organs.

Our results with SAM in the dog are similar to those reported in studies of phenol metabolism in rats (Weitering et al, 1979; Mulder and Meerman, 1978). In those studies, dose-dependent effects were clearly detected at phenol doses that lowered plasma inorganic sulfate concentrations by less than 25%. Intravenous infusion of sodium sulfate did not restore the decreased sulfoconjugation of phenol observed after high phenol doses. These and our results suggest that depletion of inorganic sulfate in the plasma may only partially explain the dose-dependent sulfoconjugation some

substrates. The possibility that depletion of PAPS occurs inside the metabolizing cells after high SAM doses, due to rate-limited uptake or activation of inorganic sulfate, will be examined in Chapter 6.

It was of interest that sodium sulfate co-administration had no effect on log plasma SAM concentration vs. time curves, i.e. SAM concentrations declined exponentially, in a manner similar to control experiments. In contrast to this observation, when sodium sulfate was co-infused with acetaminophen in rats, downward curvature was observed, implying that when sulfate stores are adequate, typical Michaelis-Menten kinetics are operative (Galinsky and Levy, 1981). Included in the next chapter are studies in which plasma SAM concentrations and half-life are followed over an extended period of time. The changes in kinetics are studied as a function of plasma concentrations of SAM, SAM-sulfate and inorganic sulfate.

CHAPTER 5

TIME-DEPENDENT, SULFATE-INDEPENDENT KINETICS OF SALICYLAMIDE IN DOGS

I. INTRODUCTION

Results of experiments in Chapters 3 and 4 suggest that plasma sulfate depletion only partially explains the dose-dependent sulfoconjugation of salicylamide (SAM) in dogs. From studies of acetaminophen metabolism in rats, a possible alternative mechanism is suggested. Like SAM in dogs, acetaminophen in rats is metabolized to the sulfoconjugate in a dose-dependent manner. When acetaminophen is given as an IV bolus (Watari et al., 1983) or as a bolus during a sodium sulfate infusion (Galinsky and Levy, 1981), downward curvature of log plasma acetaminophen concentration vs. time curves is observed. This curvature indicates that, after drug administration, the kinetics change with time. This change in kinetics with time could be related to drug concentration in a Michaelis-Menten manner, or to another time-dependent factor. Investigated here, in detail, is the change in SAM kinetics in the dog with time after both oral administration and intravenous infusion of SAM.

In Chapter 4, a sulfate-depleting 80-mg/kg SAM dose resulted in depression of SAM clearance twelve hours later. In those studies, clearance was measured with a 5-mg/kg SAM dose. Similar studies are carried out here, but the

clearance is measured with an IV tracer dose of [14C]-SAM. This allows the change in SAM kinetics to be measured without the possibility of further depletion of plasma sulfate by the 5-mg/kg test dose. Also studied are the changes in SAM kinetics with time after a less depleting 40-mg/kg dose of SAM. The changes in SAM kinetics after this dose are studied in relation to plasma concentrations of inorganic sulfate, SAM and SAM-sulfate. A third set of experiments assess changes in kinetics when SAM is given by infusion at different rates.

II. METHODS

A. SAM, 80-mg/kg oral dose

Clearance twelve hours after an 80-mg/kg SAM dose was measured here in two ways, with a 5-ug/kg (tracer) SAM dose and with a 5-mg/kg SAM dose. The clearance values were compared to control values obtained with the same doses twelve hours before administration of the 80-mg/kg SAM dose. Three dogs were used in these experiments.

At 10 AM on day one, a 40 uCi intravenous tracer dose of [14C]-SAM was given. Blood samples, 4 to 5 ml, were collected at 0, 1, 2, 3, 4, 5, 6, 7, 9, 11 and 13 minutes. Thirty minutes later, a 5-mg/kg dose of SAM, dissolved in 10 ml of a 4:1 solution of propylene glycol/ethanol, and 100 ml of water were administered successively through a gastric tube. To determine clearance during the elimination of the 5-mg/kg dose of SAM, another tracer dose of [14C]-SAM was given intravenously five minutes after the 5-mg/kg oral dose. Blood samples were collected at 0, 2, 4, 6, 9, 12, 15, 20, 27, 35, 42, 50, 60, 75 and 120 minutes following the 5-mg/kg dose. After collection of these samples, catheters were removed and the dogs were allowed access to food and water for 2 hours. At 10 PM, an 80-mg/kg dose of SAM was administered orally to the dogs. At 10 AM of the following morning, studies were repeated, i.e., a tracer dose was given alone, followed 30 minutes later by a 5-mg/kg oral dose and another tracer.

B. SAM, 40-mg/kg oral dose

The kinetics of SAM after a 40-mg/kg dose were studied in three dogs over four hours because preliminary studies indicated that clearance is initially depressed, but returns to normal values over this time period. In this experiment, changes in SAM kinetics were studied as a function not only of plasma sulfate concentration, but also of plasma concentrations of SAM and SAM-sulfate.

To establish baseline clearance values, a 40 uCi tracer dose of [14C]-SAM was administered intravenously over one minute. Thirty minutes later, 40 mg/kg of SAM, dissolved in 10 ml of a 4:1 solution of propylene glycol/ethanol, and 100 ml of water were administered successively through a gastric tube. Separate tracer doses, 40 uCi, of [14C]-SAM were administered at 5, 120 and 240 minutes after the oral dose. Following each tracer dose, four-ml blood samples were collected at time intervals of approximately one-half of the anticipated half-life.

C. SAM infusion studies

In these studies, SAM was infused in a step-up, step-down manner. Plasma sulfate concentrations were kept constant by co-infusing equimolar amounts of sodium sulfate. Because the SAM infusion rate was the same in the first and final infusion periods, Michaelis-Menten kinetics would predict that plasma SAM concentrations and clearance at the end of these periods would be the same if

steady state were reached. Thus, the protocol potentially allowed demonstration of changes in SAM clearance due to Michaelis-Menten kinetics or other causes while normal plasma sulfate concentrations were maintained.

Studies were conducted in three dogs. An equimolar solution of SAM and sodium sulfate was prepared for each dog by adding SAM and sodium sulfate (0.38 mmol/kg) to a flask and bringing to total volume 100 ml with 0.1 N sodium hydroxide. A solution of [14C]-SAM, 1.3 uCi/ml in normal saline, was also prepared. The solution of SAM and sodium sulfate was infused intravenously at 0.58 umol/min-kg for 60 minutes (Period I). The rate was increased 2.5-fold for 90 minutes (Period II) and then reduced to the original rate for an additional 90 minutes (Period III). These infusion times, designed to allow steady-state to be reached, were based on plasma SAM half-lives determined in previous single dose studies (Chapter 3). The total SAM doses infused in Periods I, II and III, described above, were 4.8, 18.0 and 4.8 mg/kg, respectively.

Radiolabeled SAM was infused into a separate forelimb vein at a fixed rate (0.4 uCi/min) throughout all three study periods. Three-ml blood samples were collected at 5 to 15 minute intervals throughout each of the study periods. Plasma concentrations of [14C]-SAM and inorganic sulfate were determined.

D. Assays

1. Plasma SAM and SAM-sulfate - Chapter 2, Section V-A

2. Plasma [14C]-SAM - Chapter 2, Section V-B

3. Plasma inorganic sulfate - Chapter 2, Section V-D

E. Treatment of data

In single dose studies, a two-compartment body model was computer-fit to plasma [14C]-SAM concentration-time values to obtain estimates of clearance and terminal half-life. A modification of the COMPT program (Pfeffer, 1973) was used for this purpose.

III. RESULTS

A. SAM, 80-mg/kg oral dose

Plasma sulfate concentrations and clearance (Dose/AUC) of tracer and 5-mg/kg doses of SAM were determined twelve hours before and twelve hours after an 80-mg/kg dose of SAM. Administration of the 80-mg/kg dose of SAM resulted in reduction of plasma sulfate concentrations from 0.82 +/- 0.03 mM (mean +/- SD) to below the level of assay sensitivity (< 0.30mM) in each dog. Clearance of the tracer dose was reduced from control values by 36% (11.1 +/- 1.7 vs. 7.1 +/- 2.6 l/min); Clearance of the 5-mg/kg dose was reduced by 47% (3.2 +/- 0.8 vs. 1.7 +/- 1.0 l/min, Fig. 5-1). Thus, the clearance remained depressed at 12 hours as measured by either method.

B. SAM, 40-mg/kg oral dose

Administering tracer doses of SAM before and five minutes after the 40-mg/kg dose of SAM showed that clearance values after the 40-mg/kg dose were reduced from predosing values of 11.4 +/- 2.0 l/min (mean +/- SD) to 0.60 +/- 0.30 l/min (Fig. 5-2, period I vs. II). Clearance increased to 3.8 +/- 2.1 l/min after the third tracer dose, given at 120 minutes (Period III), and had returned to predosing values after the fourth tracer dose, given at 240 minutes (period IV). This return to baseline values occurred despite persistently-depressed plasma inorganic sulfate concentrations (Table 5-1)

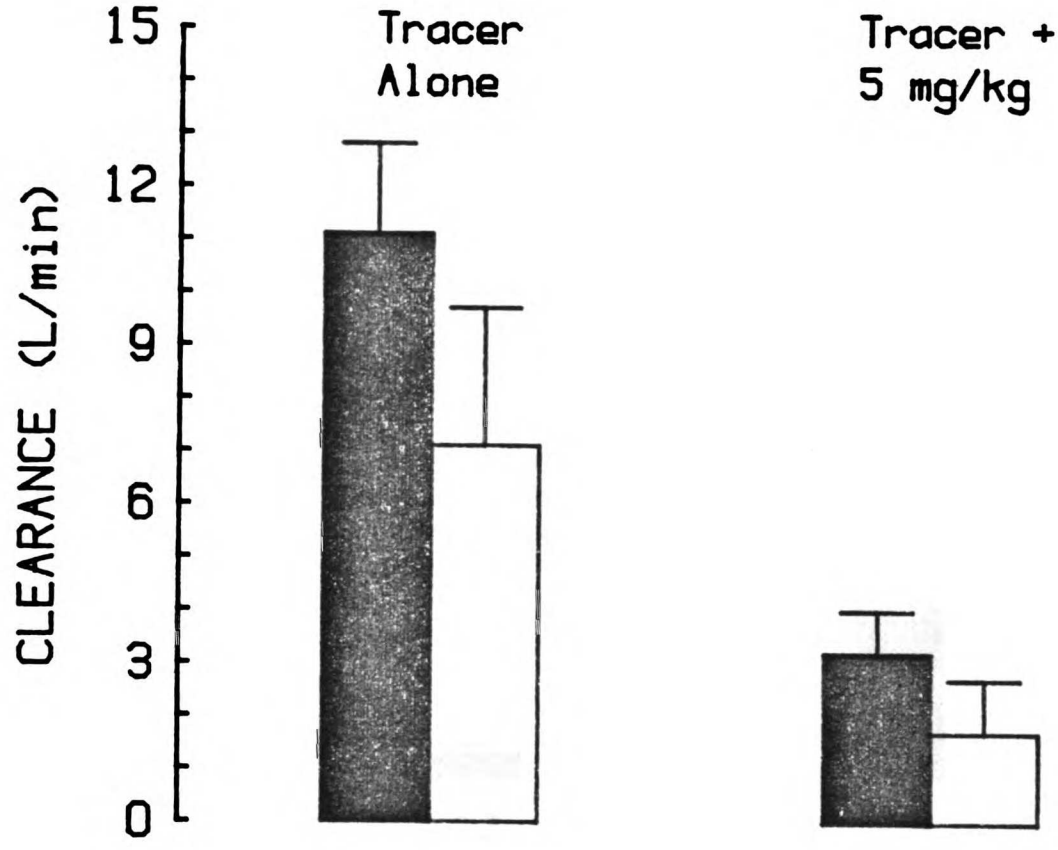


Fig. 5-1. Salicylamide (SAM) clearance (+/- SD) in dogs 12 hr before (closed bars) and 12 hr after (open bars) depletion of plsma inorganic sulfate with an 80-mg/kg dose of SAM. Clearance values (Dose/AUC) were measured using an intravenous tracer of [14C]-SAM given alone (on left) or five minutes after a 5-mg/kg dose of SAM (on right).

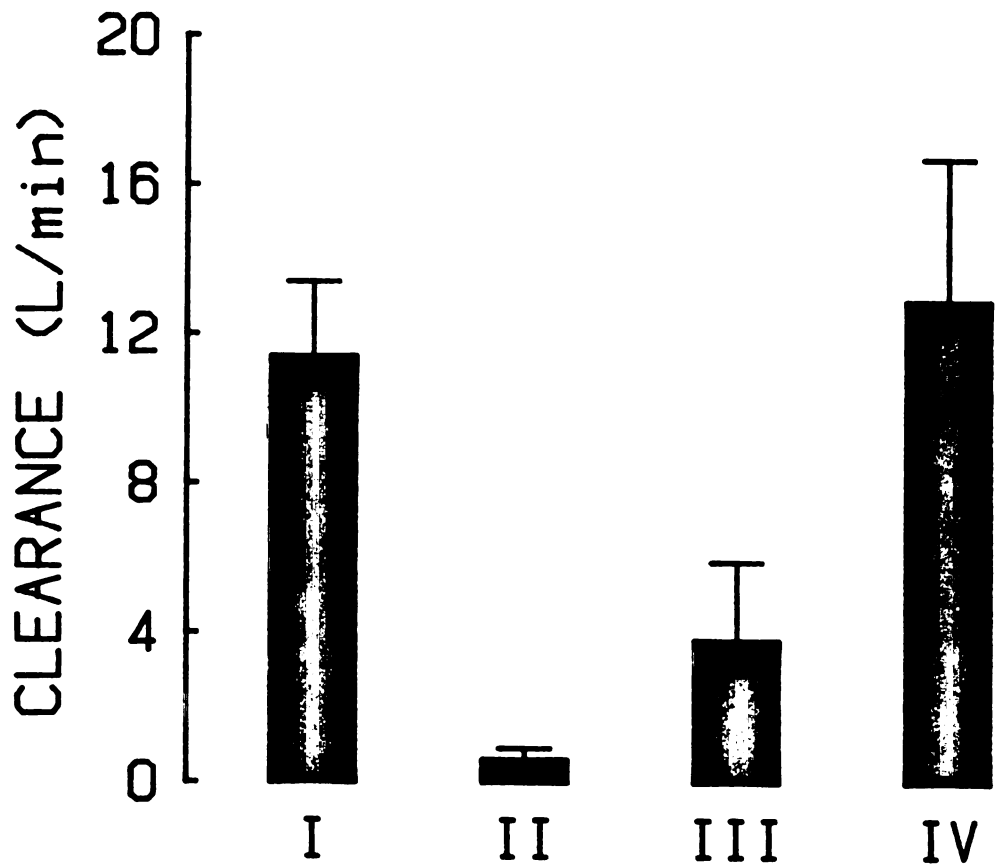


Fig. 5-2. Salicylamide clearance (mean +/- SD) in dogs before and during various time periods after oral administration of a 40-mg/kg dose of salicylamide. Clearance values were determined from Dose/AUC after each of the intravenous tracer doses. Period I = prior to oral dose, Periods II, III and IV = 5-120, 120-180 and 240-270 min after the oral dose, respectively.

Table 5-1. Plasma inorganic sulfate concentrations (mean +/- SD) vs. time in three dogs after a 40-mg/kg oral dose of salicylamide (SAM).

Time after dose	Plasma Inorganic Sulfate Concentration (mM)
Baseline (a)	1.08 +/- .09
120 min	0.40 +/- .04
240 min	0.40 +/- .08

(a) 30 minutes prior to the 40-mg/kg SAM dose.

Semilogarithmic plots of unlabeled SAM concentration vs. time (Fig. 5-3) appeared to curve downward. Plasma [14C]-SAM data demonstrated a decrease in half-life with time and thereby confirmed this downward curvature. At 240 minutes, plasma SAM concentrations were undetectable in two dogs (Fig. 5-4). In the third dog, plasma SAM concentration at 240 minutes was detectable (approximately 0.025 mg/l) but below the reliable limit of the assay sensitivity (0.05 mg/l). Plasma SAM-sulfate concentrations at 254 minutes were 4.1 +/- 2.9 mg/l (Fig. 5-4).

C. SAM infusion studies

Plasma inorganic sulfate concentrations did not change throughout the three study periods (Fig. 5-5). Plasma [14C]-SAM concentrations increased in period II, when the SAM infusion rate increased, and then decreased in Period III, when the SAM infusion rate was decreased. Because the [14C]-SAM infusion rate was unchanged throughout periods I to III, the changes in plasma [14C]-SAM concentrations in Periods II and III infer that clearance changes inversley with infusion rate. Because steady-state conditions were not clearly reached by the end of each infusion period, accurate values of clearance could not be calculated.

Detailed data for individual dogs are presented in Appendix 3.

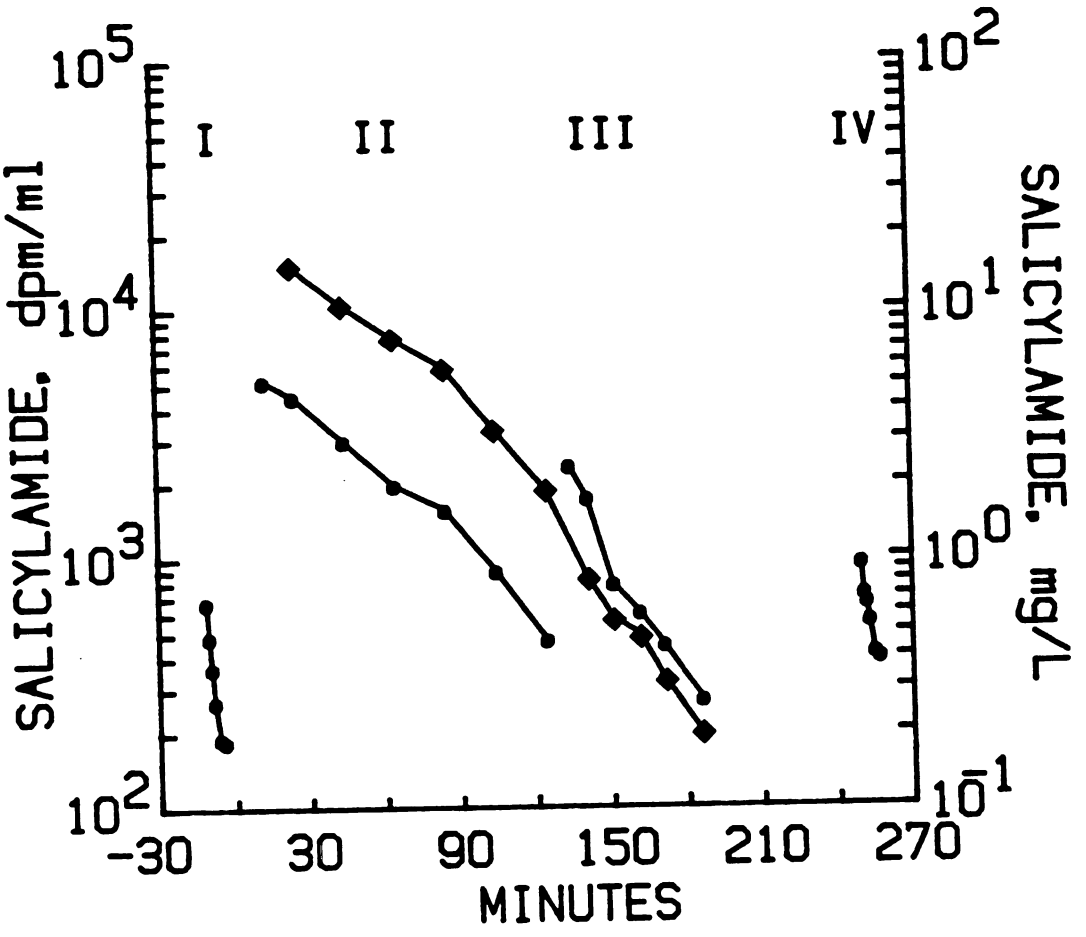


Fig. 5-3. Plasma [14C]-salicylamide (●) and unlabeled salicylamide (SAM) (◆) concentrations vs. time in a dog given 40 mg/kg of SAM orally (at time = 0). Periods I-IV correspond to the time periods over which clearance values in Fig. 5-2 were determined with intravenous tracer doses of [14C]-SAM. To show only the post-distributional decay of plasma [14C]-SAM concentration, data within the first three miniutes after each tracer dose were omitted.

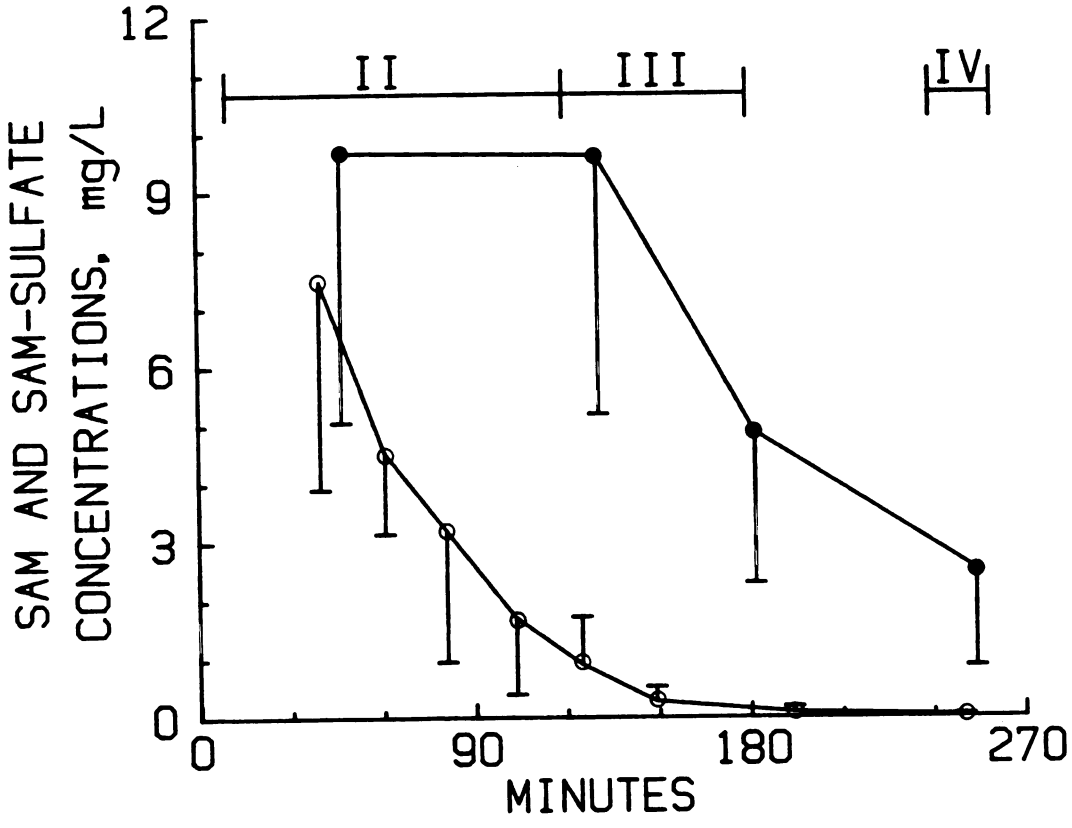


Fig. 5-4. Salicylamide (SAM) (o) and SAM-sulfate (●) concentrations (mean +/- SD) in plasma vs. time in dogs after the oral administration of a 40-mg/kg dose of SAM. Periods II-IV correspond to time periods over which clearance values in Fig. 5-2 were determined. Only post-absorptive data are shown. SAM-sulfate concentrations are shown as molar equivalents of SAM.

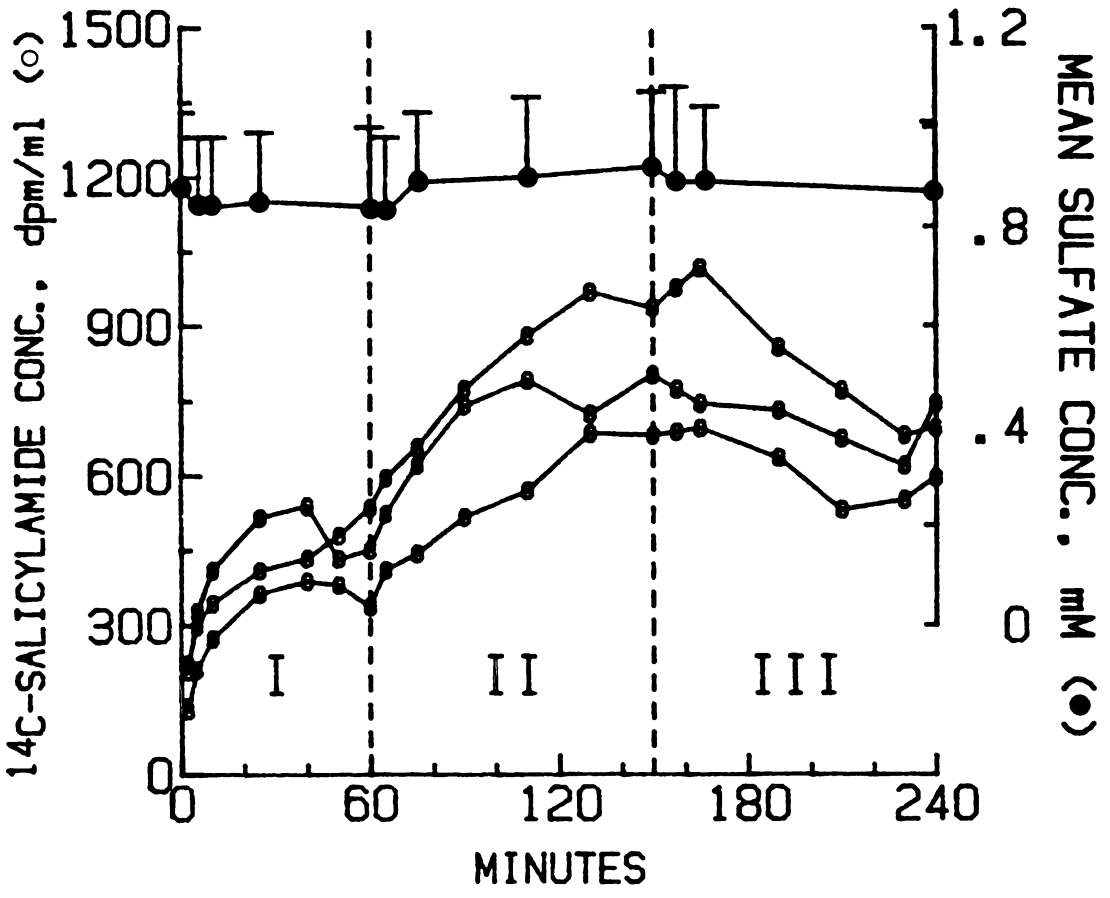


Fig. 5-5. Plasma [14C]-salicylamide (SAM) concentrations (O) vs. time in individual dogs during the intravenous infusion of [14C]-SAM at a fixed rate (0.4 uCi/min). Unlabeled SAM, together with equilmolar amounts of sodium sulfate, were co-infused at the following rates: 0.58 umol/min-kg (Period I), 1.45 umol/min-kg (Period II) and 0.58 umol/min-kg (Period III). Plasma inorganic sulfate concentrations (●) (mean +/- SD) are also shown.

IV. CONCLUSIONS AND DISCUSSION

These studies demonstrate that the changes in SAM kinetics with dose are not closely related to plasma concentrations of inorganic sulfate or SAM.

The results of the 80-mg/kg SAM dose experiment are in agreement with earlier observations (Chapter 4) that SAM clearance is reduced twelve hours after an 80-mg/kg dose of SAM. In studies reported here, clearance of both the tracer and 5-mg/kg dose of SAM were reduced twelve hours after the 80-mg/kg SAM dose. The reduction of clearance of the tracer SAM dose indicates that the kinetics of even a very small dose of SAM (tracer dose = approximately 5 ug/kg) appear to be depressed twelve hours after an 80-mg/kg dose of SAM. The lack of return of clearance to normal occurred in association with plasma inorganic sulfate concentrations in each dog that were below 0.30 mM.

Studies in which SAM was given as a 40-mg/kg oral dose and by intravenous infusion, however, demonstrate that pronounced clearance changes may occur independent of plasma inorganic sulfate concentrations. Ten minutes after the 40-mg/kg oral dose of SAM, clearance was greatly depressed, but returned to baseline values in 240 minutes despite persistently low plasma inorganic sulfate concentrations. In SAM infusion studies, clearance changed even while plasma inorganic sulfate concentrations were normal and constantly maintained.

The relationship of SAM kinetics to plasma SAM

concentrations is less clear. SAM infusion studies were designed to test a Michaelis-Menten hypothesis. Because it is uncertain if steady-state conditions were reached by the end of each infusion period, it is not possible to confirm or rule out simple Michaelis-Menten enzyme saturation. The kinetics may be complex because multiple forms of sulfotransferase capable of metabolizing phenolic substrates appear to exist (Sekura and Jakoby, 1979; 1981). Extrahepatic SAM metabolism and blood flow limitations of metabolism are additional potential complexities.

In the 40-mg/kg dose experiment, log plasma SAM concentration vs. time curves appeared to bend downward. This curvature was confirmed by plasma [14C]-SAM data after administration of intravenous tracer doses of radiolabeled SAM. Although this curvature suggests Michaelis-Menten kinetics, the slope of the log plasma SAM concentration vs. time curves appears to be unrelated to plasma SAM concentration. This becomes apparent if plasma SAM concentration data from previous studies (Chapter 3) are superimposed on data from the same dogs in the experiments reported here (Fig. 5-6). At a given plasma SAM concentration, the subsequent decline in SAM concentration is greater after lower doses of SAM.

A number of time-dependent effects related to dose other than plasma drug concentration could explain the results. One possibility is inhibition of metabolism by the conjugation reaction products, adenosine 3', 5'-biphosphate

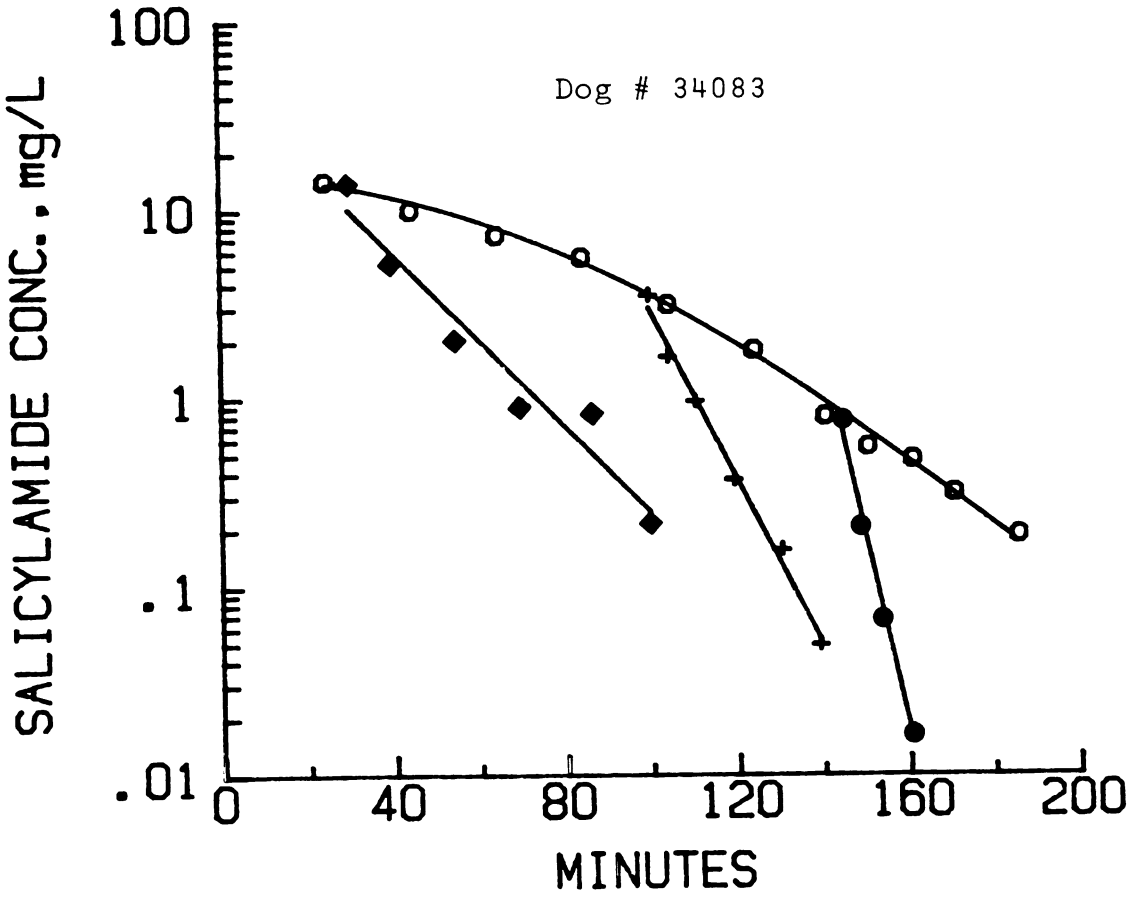


Fig. 5-6. The decline of plasma salicylamide (SAM) concentration after a single 40-mg/kg SAM dose (O) is slower than that observed after 20 (◆), 10 (+) or 5 (●) mg/kg SAM doses. The data for the 20-, 10- and 5-mg/kg doses are superimposed over the 40-mg/kg data by placing the first declining concentration-time point for that dose over the corresponding concentration after the 40-mg/kg SAM dose. The line for the 40-mg/kg dose was drawn with a french curve; other lines were obtained by log-linear least squares regression.

(PAP) and SAM-sulfate. Inhibition by SAM-sulfate, if it occurs, must be associated with plasma concentrations higher than those observed 240 minutes after the 40-mg/kg oral SAM dose (4.1 mg/l) because clearance had returned to baseline values at that time. Inhibition of purified sulfotransferase by PAP has been demonstrated (Sekura and Jakoby, 1979; 1981). Another potential time-dependent effect is depletion of intracellular active sulfate (PAPS). This could occur if demand for PAPS is greater than the ability to mobilize or activate inorganic sulfate. This possibility in the dog is examined in the next chapter.

CHAPTER 6

RATE OF CONJUGATION OF SALICYLAMIDE WITH PLASMA [35S]-SULFATE IN DOGS

I. INTRODUCTION

Salicylamide (SAM) infusion studies described in the preceding chapter indicate that sulfoconjugation of SAM in the dog is dose-dependent even when plasma sulfate concentrations are normal and constantly maintained. A possible explanation is that transport or activation of inorganic sulfate limits the rate of sulfoconjugation after high SAM doses, and that depletion of active sulfate in metabolizing cells occurs. Reported here are studies designed to approximate the rate of sulfate uptake, activation and conjugation with SAM in the dog. The plasma inorganic sulfate pool is labeled with inorganic [35S]-sulfate and rate of incorporation of the radiolabeled sulfate determined from the specific activities of inorganic sulfate in the plasma and SAM-sulfate in the urine. Because urinary SAM-sulfate is measured, it was necessary to determine if the metabolite, once formed, undergoes hydrolysis and reconjugation before excretion in the urine.

II. METHODS

A. Rate of generation of SAM-sulfate in the plasma

A 10 ml 0.9% NaCl aqueous solution containing [14C]-SAM, 7.3 umol/kg, (specific activity 275 uCi/mmol) was intravenously administered to a dog over one minute. Blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, and 22 minutes. Plasma concentrations of [14C]-SAM and [14C]-SAM-sulfate were determined.

B. Fate of administered SAM-[35S]-sulfate.

To prepare SAM-[35S]-sulfate, a 45 ml 0.9% NaCl solution containing ammonium [35S]-sulfate (Amersham, Arlington Heights, ILL, specific activity 1070 mCi/mmol, Oct 17, 1983, lot 153BA) and 32 umol/kg SAM was administered intravenously to a dog over one minute. SAM-[35S]-sulfate was extracted from the 6-hour pooled urine collection and counterextracted as described in Chapter 2, Section V-A-1-a. The specific activity of the recovered SAM-[35S]-sulfate was 6.3 uCi/mmol.

The prepared SAM-[35S]-sulfate (containing 40 umol SAM-sulfate) was then given intravenously over 17.5 minutes to a different dog. Urine was collected for six hours. The specific activity of the SAM-[35S]-sulfate extracted from the urine was compared with that of the administered compound.

C. Rate of incorporation of [35S]-sulfate into SAM-sulfate (Condition I).

A ten ml 0.9% NaCl solution containing unlabeled SAM, 7.3 umol/kg and inorganic [35S]-sulfate, 10 uCi, was given intravenously to three dogs over 30 seconds. Blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 9, 11, 14, 18, 22, 26 and 30 minutes and urine was collected for six hours. The specific activity of SAM-[35S]-sulfate in the urine was compared with that of inorganic sulfate in the plasma.

D. Rate of incorporation of [35S]-sulfate into SAM-sulfate (Condition II).

Inorganic [35S]-sulfate, 10 uCi, was given intravenously over 30 seconds to three dogs to prelabel thesulfate pools. Fifteen minutes later, a 172-ml aqueous solution containing sodium sulfate, 1.13-mmol/kg, and unlabeled SAM, 7.3-umol/kg, was infused into two forelimb veins over 1.2 minutes. Blood and urine samples were collected and data were analyzed as described in the preceding section.

E. Assays.

1. Plasma [14C]-SAM - Chapter 2, Section V-B
2. Plasma [14C]-SAM-sulfate

Plasma, 100 ul, was added to 15 ml of Aquasol for

counting of total [14C] activity. The concentration of [14C]-SAM-sulfate was calculated by subtracting the [14C]-SAM concentration from total radioactivity. Studies reported in Chapter 3 indicate that at SAM doses of 36 umol/kg or less, the sulfate conjugate constitutes greater than 97% of the radioactivity appearing in the urine. Therefore, other radiolabeled metabolites were unlikely to be present at interfering concentrations in the plasma.

3. Unlabeled SAM-sulfate in the urine and in the purified SAM-sulfate solution - Chapter 2, section V-A
4. SAM-[35S]-sulfate in the urine and in the purified SAM-sulfate solution

Concentrations were measured in the same manner as was described for [14C]-SAM-sulfate in the urine (Chapter 2, Section V-C). To ensure that no other [35S]-labeled endogenous substances appeared under the SAM-sulfate peak, 10 uCi of inorganic [35S]-sulfate was administered to a dog and urine was collected for six hours. A portion of this was subjected to the above analysis.

5. Plasma unlabeled SAM - Chapter 2, Section V-A
6. Plasma inorganic sulfate - Chapter 2, Section V-D
7. Plasma inorganic [35S]-sulfate

Plasma, 100 ul, was added to 15 ml of Aquasol for counting of total [35S] activity (efficiency

approximately 90%). Because molar concentrations of SAM-sulfate in the plasma after a 7.3-ummol/kg SAM dose (Fig. 6-1) were less than 1% of molar concentrations of inorganic sulfate concentrations in the plasma (about 1.0 mM), the contribution of SAM-[35S]-sulfate to total plasma radioactivity was assumed to be less than 1%. Corrections were therfore not made. It was assumed that interfering amounts of [35S]-labeled endogenous substances were not formed during the time over which SAM-[35S]-sulfate was measured.

F. Treatment of Data

Clearance of SAM was calculated as Dose/AUC, where AUC is the area under the plasma SAM concentration vs. time curve from 0 to infinity. Up to the last measurement, C(last), the AUC was calculated by the trapezoidal rule. The remaining area under the curve was calculated as C(last)/k, where k is the first-order rate constant estimated by least squares regression analysis of terminal time points. Half-life was calculated as 0.693/k.

IV. RESULTS

A. Rate of generation of SAM-sulfate in the plasma.

The disappearance of SAM and appearance of SAM-sulfate in the plasma demonstrated that SAM, 7.3 umol/kg, is rapidly metabolized to SAM-sulfate and that SAM-sulfate quickly leaves the cells in which it was formed (Fig. 6-1a). Concentrations of SAM-sulfate in the plasma reached a peak at 12 minutes, then declined with a half-life of about 75 minutes (Fig. 6-1b).

B. Fate of administered SAM-sulfate.

During the six-hour collection period after administration of SAM-[35S]-sulfate, 87% of the dose was excreted into the urine as SAM-sulfate. If the administered SAM-sulfate had hydrolyzed and reconjugated with unlabeled sulfate in the body (approximately 6.4 mmoles, Chapter 1), the specific activity of SAM-sulfate excreted in the urine would have decreased to 0.04uCi/mmol, i.e., less than 1% of that of the metabolite injected. The specific activity of the metabolite collected, 6.5 uCi/mmol, however, was 102% of that of the SAM-[35S]-sulfate administered. Therefore, the metabolite did not undergo detectable hydrolysis and reconjugation before its excretion in the urine.

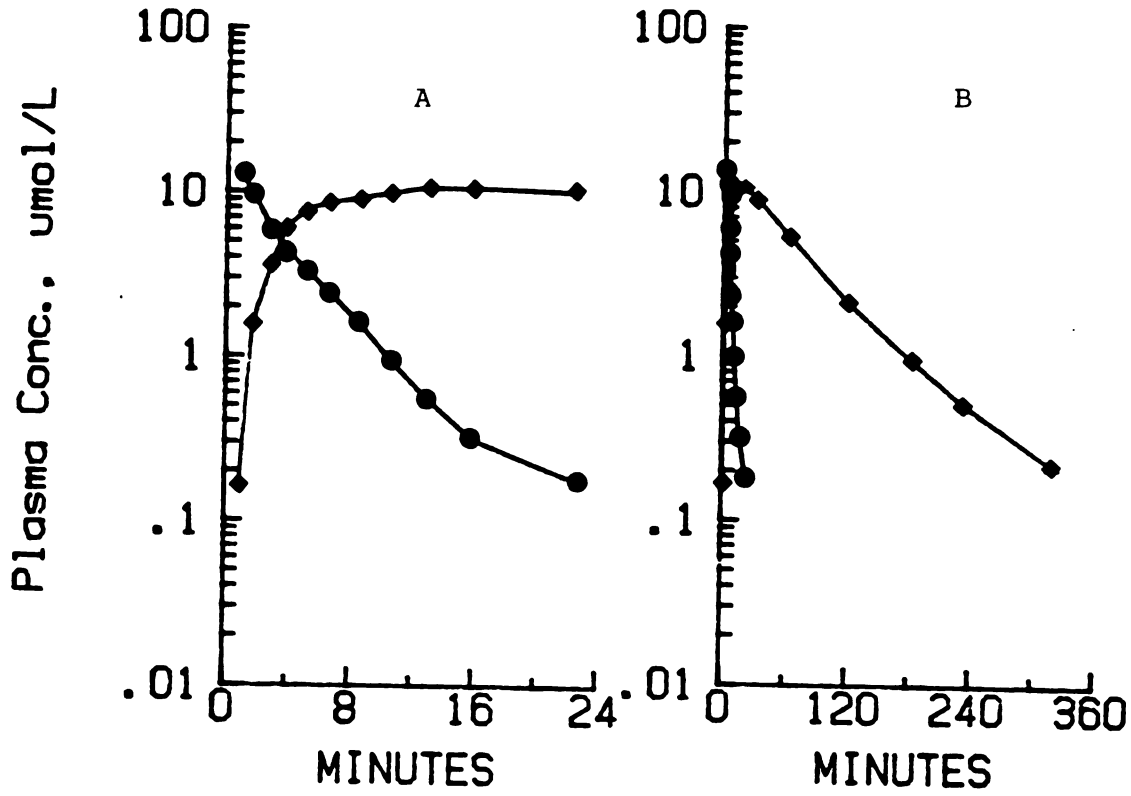


Fig. 6-1. Plasma concentrations of salicylamide SAM (●) and SAM-sulfate (◆) in a dog after an intravenous injection of SAM, 7.3 umol/kg. Molar concentrations were calculated from concentration of drug or metabolite (dpm/ml) divided by the specific activity of the administered drug. Panel A: 0 to 24 min; panel B: 0 to 360 min.

C. Rate of incorporation of [35S]-sulfate into SAM-sulfate (Condition I).

SAM, injected simultaneously with inorganic [35S]-sulfate, disappeared within minutes; half-life and clearance were 4.0 +/- 2.2 min and 2.2 +/- 0.4 l/min respectively (mean +/- SD). Plasma inorganic sulfate concentrations were 0.96 +/- 0.16 mM before and 0.89 +/- 0.11 mM 20 minutes after co-injection of SAM and inorganic [35S]-sulfate. The specific activity of inorganic [35S]-sulfate in the plasma declined over the time period during which SAM-sulfate was being formed as the tracer distributed throughout the body (Fig. 6-2).

The six-hour urinary recovery of the SAM dose as SAM-sulfate was 81 +/- 2%. The specific activity of the recovered SAM-sulfate, 2.36 +/- 0.18 uCi/mmol, was in the range of that of inorganic sulfate in the plasma observed minutes after SAM injection (Fig. 6-2).

D. Rate of incorporation of [35S]-sulfate into SAM-sulfate (Condition II).

Under these experimental conditions, in which a large bolus of sodium sulfate was given with SAM, half-life and clearance were 5.4 +/- 2.3 min and 4.5 +/- 0.6 l/min (mean +/- SD), respectively. The rapid infusion of inorganic sulfate increased plasma inorganic sulfate concentrations from 0.74 +/- 0.16 mM just before the infusion to 7.3 +/- 0.9 mM one minute after the infusion. Concentrations of

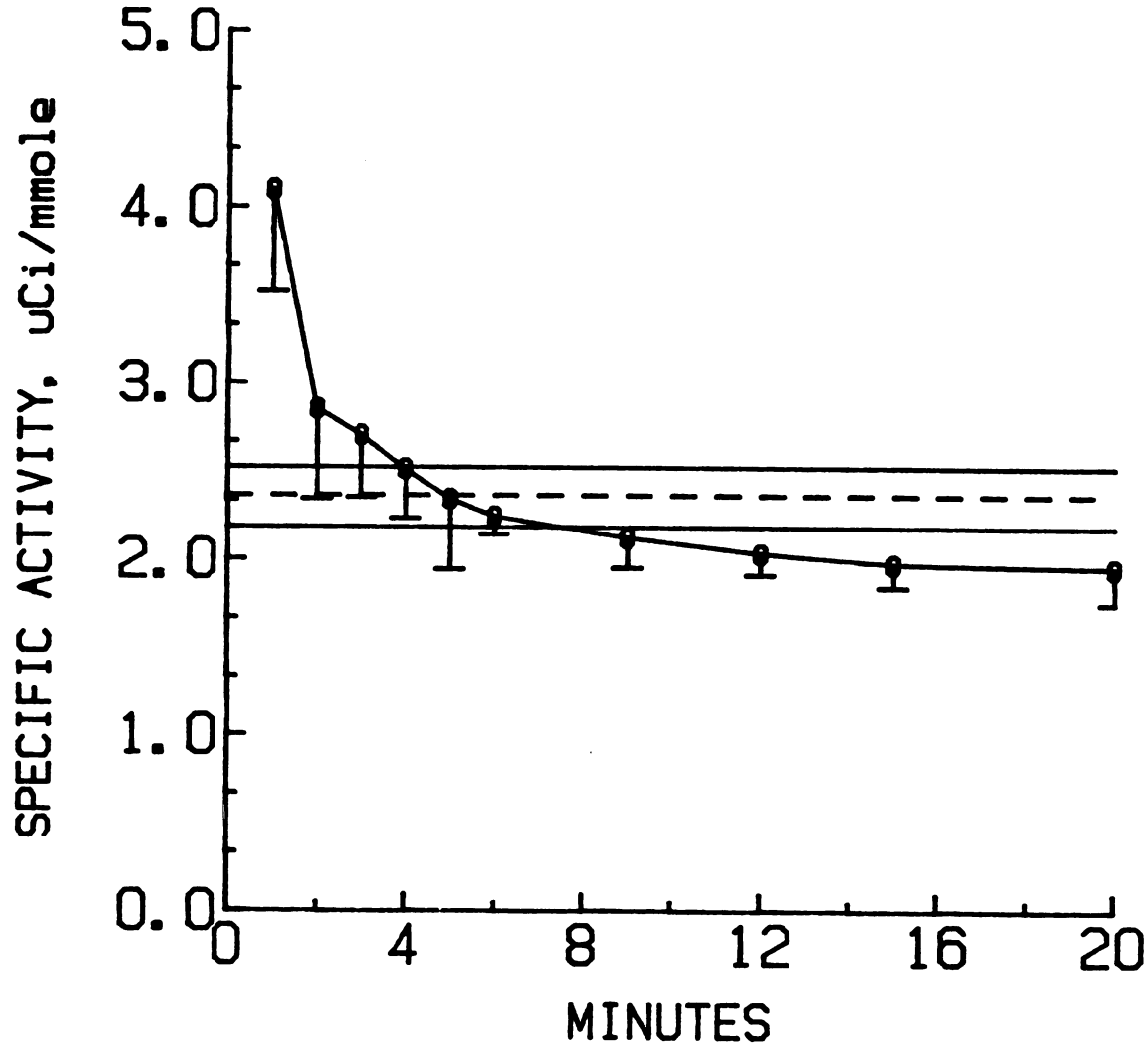


Fig. 6-2. The specific activity (mean +/- SD) of plasma inorganic [35S]-sulfate vs. time in dogs after intravenous co-injection of inorganic [35S]-sulfate, 10 uCi, and salicylamide, 7.3 umol/kg. The horizontal dashed line and enclosed area indicate the specific activity (mean +/- SD) of salicylamide-sulfate excreted in the urine.

sulfate declined, on average, to 60% of this value over the next 20 minutes. The specific activity of inorganic [35S]-sulfate in the plasma dropped from 2.03 +/- 0.07 uCi/mmol just before SAM and sodium sulfate co-injection to 0.21 +/- 0.03 uCi/mmol one minute after the injection. The specific activity increased gradually over the next 20 minutes to a value of 0.31 +/- 0.05 uCi/mmol at 20 minutes.

The six-hour urinary recovery of SAM as SAM-sulfate was 98 +/- 5 %. The specific activity of the SAM-sulfate recovered in the urine could not be accurately quantified because fractions collected from the HPLC column contained only near background levels of radioactivity. A specific activity of 0.4 uCi/mmol would have been clearly detected. Even if the actual value was this high, it would be only slightly greater than that of inorganic [35S]-sulfate in the plasma after dilution with unlabeled sodium sulfate (0.21 to 0.31 uCi/mmol).

Detailed data for individual dogs are presented in Appendix 5.

IV. CONCLUSIONS AND DISCUSSION

These studies demonstrate that the metabolically-active sulfate (PAPS) pool is in rapid equilibrium with inorganic sulfate in plasma. The data also demonstrate that the sulfate used for the reaction originates almost exclusively from inorganic sulfate in the plasma or from sulfate stores that are in rapid equilibrium with the plasma sulfate pool. These conclusions are deduced from the fact that the specific activity of SAM-sulfate excreted in the urine was not greatly different than that of inorganic sulfate in the plasma during the time that the metabolite was being formed. SAM-sulfate, when exogenously administered, was found not to undergo detectable hydrolysis and reconjugation before excretion into the urine.

If it is assumed that SAM equilibrates between the plasma and metabolizing site instantaneously, and that the conjugation rate is proportional to concentrations of SAM and PAPS, then the data can be analyzed in a different manner. If equilibrium between sulfate pools is also instantaneous, then the predicted specific activity of SAM-sulfate formed is calculated as follows:

$$SA = \frac{\Sigma(SAMi \times S^{*}i \times \Delta t)}{\Sigma(SAMi \times Si \times \Delta t)}$$

where SA is specific activity of metabolite formed and S*i, Si and SAMi are average plasma concentrations within a time interval of radiolabeled inorganic sulfate, unlabeled inorganic sulfate, and SAM, respectively. The calculated value for SAM-sulfate formed, 2.82 +/- 0.06 uCi/mmol, is about 20% higher than the measured value. Thus, up to 20% of the total amount of SAM-sulfate may been formed before inorganic [35S]-sulfate in the plasma was available for sulfoconjugation. It can be estimated from plasma SAM-sulfate data in Fig. 6-1 that SAM-sulfate formation is 20% complete within two to three minutes. This time interval represents the greatest amount of time that could have elapsed before inorganic [35S] sulfate from the plasma was available for metabolism in the activated form. In contrast, if there had been a five-minute delay in sulfate mobilization, over 75% of SAM would have been metabolized and the specific activity of the SAM-sulfate formed would be approximately 0.5 to 0.6 uCi/mmol, a value that is much lower than the measured value of about 2.4 uCi/mmol.

Although the dose of SAM used in these studies was small (7.3 umol/kg), dose-dependent effects were clearly apparent because clearance after smaller doses of [14C]-SAM (36 nmol/kg) were 10-15 l/min (Chapters 3 and 4) in contrast to values of 2 to 5 l/min observed here. The rapid equilibrium of inorganic [35S]-sulfate in the plasma with the metabolically-active sulfate pool argues against the dose-dependent effects being due to sulfate uptake or

activation. However, an alternative interpretation of the data is that the entire PAPS pool size is only a small fraction of the 7.3 umol/kg SAM dose administered. Then, if PAPS consumption occurs at a greater rate than its formation, the unlabeled PAPS pool could become depleted before a significant amount of the SAM dose is metabolized. Subsequently formed SAM-sulfate would then, by necessity, reflect the specific activity of the less available inorganic sulfate in plasma. Mulder and Scholtens (1978), however, argue against a small PAPS pool, reasoning that an ability to provide PAPS on demand, while keeping the PAPS pool small, would require inefficient rapid recycling of PAPS in the absence of substrate. Negative feedback control of PAPS formation could, however, keep the PAPS pool small. Concentrations of PAPS in various tissues, including dog hepatocytes, are reported to be low or undetectable (Wong and Yeo, 1979; Glazenburg, 1983), however, PAPS is labile and other data are necessary to resolve this question.

When the effective PAPS pool was prelabeled with [35S]-sulfate and SAM was given with a large bolus of sodium sulfate, rapid equilibrium of sulfate pools was again demonstrated. The specific activity of SAM-sulfate excreted in the urine reflected the rapid dilution of specific activity of inorganic sulfate in the plasma by the large bolus of sodium sulfate.

Although the number of dogs studied was small and the study protocols were not strictly comparable, clearance of

SAM, when given with a the large bolus of sodium sulfate, was higher (4.5 +/- 0.6 l/min) than when SAM was given with only a tracer dose of inorganic sulfate 2.2 +/- 0.4 l/min). This suggests that greatly increased plasma sulfate concentrations can drive the sulfoconjugation reaction. When sodium sulfate was administered in doses that maintained plasma sulfate concentrations above, but not greatly above normal, SAM dose-dependent effects were not lessened or reversed (Chapter 4).

Incorporation of inorganic [35S]-sulfate into other phenolic substrates has been studied. After IV co-injection of inorganic [35S]-sulfate and harmol to rats, the specific activity of harmol-[35S]-sulfate appearing in the bile reached a constant value within a few minutes (Mulder and Scholtens, 1978). In similar studies with phenol in mice, Herbai (1970) demonstrated that the specific activity of phenol-[35S]-sulfate in the blood reached a constant value at 15 minutes, the first time point measured. Because we measured the specific activity of plasma sulfate as well as the metabolite formed, we were able to compare the specific activity of the metabolite formed directly with that of plasma sulfate. As a result, we have been able to demonstrate not only that the effective PAPS pool equilibrated rapidly with plasma sulfate but also that the sulfate used for the conjugation reaction originated almost exclusively from inorganic sulfate in the plasma or from sulfate stores that are in rapid equilibrium with plasma

sulfate.

Information was also obtained on the possible inhibition of sulfoconjugation by the metabolite, SAM-sulfate. After the 7.3 uM/kg SAM dose, clearance was depressed, but plasma SAM-sulfate concentrations were never greater than 10 uM. In Chapter 5, normal clearance values were observed 240 minutes after a 40-mg/kg dose of SAM, a time when plasma SAM-sulfate concentrations were almost 30 uM. The depressed clearance after the 7.3 uM/kg dose, therefore, must not have been due to inhibition by elevated concentrations of SAM-sulfate in the plasma.

CHAPTER 7

DOSE-DEPENDENT EXTRAHEPATIC METABOLISM OF SALICYLAMIDE IN DOGS

I. INTRODUCTION

In Chapters 3 and 4, the observed values of plasma clearance after IV tracer doses of salicylamide (SAM) were greater than 10 l/min. Because the blood to plasma SAM concentration ratio in the dog is approximately one (experiments described herein), these plasma clearance values approximate those of the blood. When blood clearance values approach or exceed cardiac output, metabolism in several organs is suggested. Presented here are studies designed to determine if metabolism of SAM occurs in selected extrahepatic sites in the anesthetized dog. Metabolism in the lungs and the tissues upstream from the sampling site (the forelimb) are studied because metabolism in these organs can produce clearance values greater than cardiac output. Metabolism in the kidney is also studied because data in the literature indicate that renal sulfoconjugation in the dog may occur (Wong and Yeo, 1982).

Previous studies (Chapter 4 and 5) suggest that SAM clearance is depressed when concentrations of plasma sulfate decrease below 0.30 mM. To determine the responsiveness of extrahepatic metabolizing tissues to plasma sulfate concentrations, SAM was infused in some studies for a period of time to allow sulfate depletion to occur. The SAM infusion was then continued, but sodium sulfate was also

infused. Concentrations of inorganic sulfate and SAM were measured before and after the sulfate infusion and the kinetics of SAM were compared.

II. METHODS

A. Partitioning of SAM into red blood cells

Freshly drawn whole blood was obtained from a dog and six ml were placed into each of two heparinized tubes. To 1.5 ml samples of blood and plasma, obtained from blood by centrifugation, were added 20 ul of water or an equal volume of aqueous unlabeled SAM solution, 1.5 g/l. Samples were then spiked with 100 ul of [14C]-SAM, 9000 dpm/ml and mixed. After 30 minutes at room temperature, the blood samples were centrifuged and plasma obtained. Samples were then extracted and the concentration of [14C]-SAM determined as described in Chapter 2, Section V-B.

B. Metabolic extraction studies

1. Experimental protocol

Ketamine HCL (150 mg) and acepromazine maleate (25 mg) were administered subcutaneously one-half hour prior to sodium pentobarbital anesthesia (150 mg intravenously). Additional sodium pentobarbital was administered as necessary during the experiment to maintain anesthesia. A superficial vein of the right forelimb was catheterized for the infusion of salicylamide and sodium sulfate solutions (Angiocath # 2818, Deseret Co., Sandy, UT). A vein of the left forelimb was similarly catheterized for the withdrawal

of peripheral venous blood and for the administration of anesthetic. The left femoral artery was catheterized percutaneously (Angiocath # 2818). A pulmonary arterial catheter (Swan-Ganz Monitoring Catheter, model 93-123-6F, American Edwards Labs, Irvine, CA) and a renal venous catheter (Torcon Green Catheter, #6.5-C2-10333, Cook, Inc., Bloomington, IN) were placed via the external jugular vein under fluoroscopic guidance with the aid of a contrast agent (Angiovist 282, Schering AG, Berlin, West Germany). Patency of catheters was maintained by flushing with 2 ml of heparinized normal saline (10 units/ml) after each sample and, in the case of the left forelimb vein and on some occasions the renal vein and pulmonary artery, by the slow infusion of normal saline (1-2 ml/min) into the line between blood samples.

Aqueous solutions of sodium SAM were prepared by adding approximately 8 ml of 1.0 N NaOH and 16 ml of water to each gram of salicylamide, adjusting the pH to 10 with phosphoric acid, and diluting to the required volume with water. Radiolabeled SAM solutions were made by the dilution of the stock solution of [14C]-SAM with 0.9% NaCl. All solutions were prepared within 48 hours of administration and were refrigerated. Before infusion to dogs, solutions of SAM and sodium sulfate were filtered into sterile empty vials through 0.2 micron sterile filters (ACRODISC #4192, Gelman

Sciences, Ann Arbor, MI).

Two experiments, separated by at least three weeks, were carried out on each of four dogs. The approximate infusion times to reach steady-state were estimated as desribed in infusion studies in Chapter 5. In the first experiment, SAM and [14C]-SAM were infused at 3.0 ug/min-kg and 2.5 uCi/min, respectively, for 15 minutes (0.5 ml/min). Twenty minutes later, SAM and [14C]-SAM were infused at 500 ug/kg-min and 0.5 uCi/min, respectively, for 180 minutes (0.4 ml/min). After 120 minutes at this rate, sodium sulfate was co-infused at 5.64 mg/min-kg (1.2 ml/min) for ten minutes, and then at 0.75 mg/min-kg (0.16 ml/min) from 130 to 180 minutes.

In the second experiment SAM and [14C]-SAM were infused at 0.083 mg/min-kg and 0.2 uCi/min, respectively, for 40 minutes (0.16 ml/min). These rates were increased 2.5-fold for the next 140 minutes. After 80 minutes at this higher infusion rate, sodium sulfate was co-infused for ten minutes at 2.4 mg/min-kg (1.2 ml/min) and at 0.32 mg/kg-min (0.16 ml/min) from 90 to 140 minutes.

Blood samples were simultaneously withdrawn from the femoral artery, renal vein, pulmonary artery and forelimb vein catheters at 5- to 10-minute intervals for measurement of plasma SAM concentrations. To mimimize the effects of catheter dead-space, 2 ml of

blood were withdrawn through each catheter and discarded immediately before taking each blood sample. Absorption of drug by catheters was tested by comparing of [14C]-SAM concentration of spiked control plasma samples with spiked samples that had been drawn through a catheter. Adsorption was found to be negligible even at the lowest concentration (about 10 ug/l).

2. Assays

   a. Plasma [14C]-SAM - Chapter 2, Section V-B

   b. Plasma inorganic sulfate - Chapter 2, Section V-D

3. Treatment of Data

   Steady-state clearances were calculated from:

$$CL=R/Css$$

where CL= clearance, R= rate of drug infusion, Css= steady-state plasma concentration.

After the highest infusion rate, when steady state was not necessarily reached, non steady-state clearances were approximated by:

$$CL=[R-(V\times\Delta C/\Delta t)]/Cav$$

where V= volume of distribution, previously determined to be 1.0 l/kg for the dog (Chapter 3), $\Delta C/\Delta t$= the change of plasma concentration with time during the last interval of the infusion and Cav= average plasma concentration during the same interval. This calculation assumes that volume of distribution is constant. Previous studies (Chapter 3) indicate that that the volume of distribution is constant at SAM doses of 5 to 40 mg/kg.

Metabolic extraction was calculated using the plasma SAM concentrations entering and leaving these organs at steady-state. Plasma SAM concentration in the femoral artery was assumed to be the same as that leaving the lungs and entering the forelimb and kidney. Plasma SAM concentrations at other sites were measured directly. Extraction ratios (ER) for the kidney, lung and forelimb were calculated as follows:

$$ER(kidney) = 1 - (Crv/Cfem)$$

$$ER(lung) = 1 - (Cfem/Cpulm)$$

$$ER(forelimb) = 1 - (Cfl/Cfem)$$

where Crv, Cfem, Cpulm and Cfl are plasma SAM concentrations in the renal vein, femoral artery, pulmonary artery and forelimb vein, respectively.

III. RESULTS

A. Partitioning of SAM into red blood cells.

The concentration of radiolabel in spiked plasma was not different than that in plasma obtained from spiked whole blood. These values were unaltered by addition of unlabeled SAM (final concentration about 20 mg/l). Therefore, the concentrations in the blood and plasma are approximately the same and the ratio of concentrations does not change with SAM concentration.

B. Metabolic extraction studies

Plasma SAM concentrations appeared to reach steady-state at the lowest three infusion rates (Figs. 7-1 and 7-2). Significant dose-dependent extraction of SAM was observed in the lung, kidney and forelimb tissues (Fig. 7-3). At the lowest infusion rate, renal extraction was the highest, exceeding 80%; extraction in the lung and forelimb were about 35 and 45%, respectively. These values were dose-dependent and approached zero at the highest infusion rate.

Plasma inorganic sulfate concentrations fell significantly at the highest two SAM infusion rates, from 0.75 +/- 0.07 mM to 0.41 +/- 0.15 mM during the 208 ug/min-kg infusion and from 0.70 +/- 0.05 to less than 0.30 mM during the the 500 ug/min-kg infusion (Fig. 7-4 and Appendix 5). Sulfate concentrations returned to

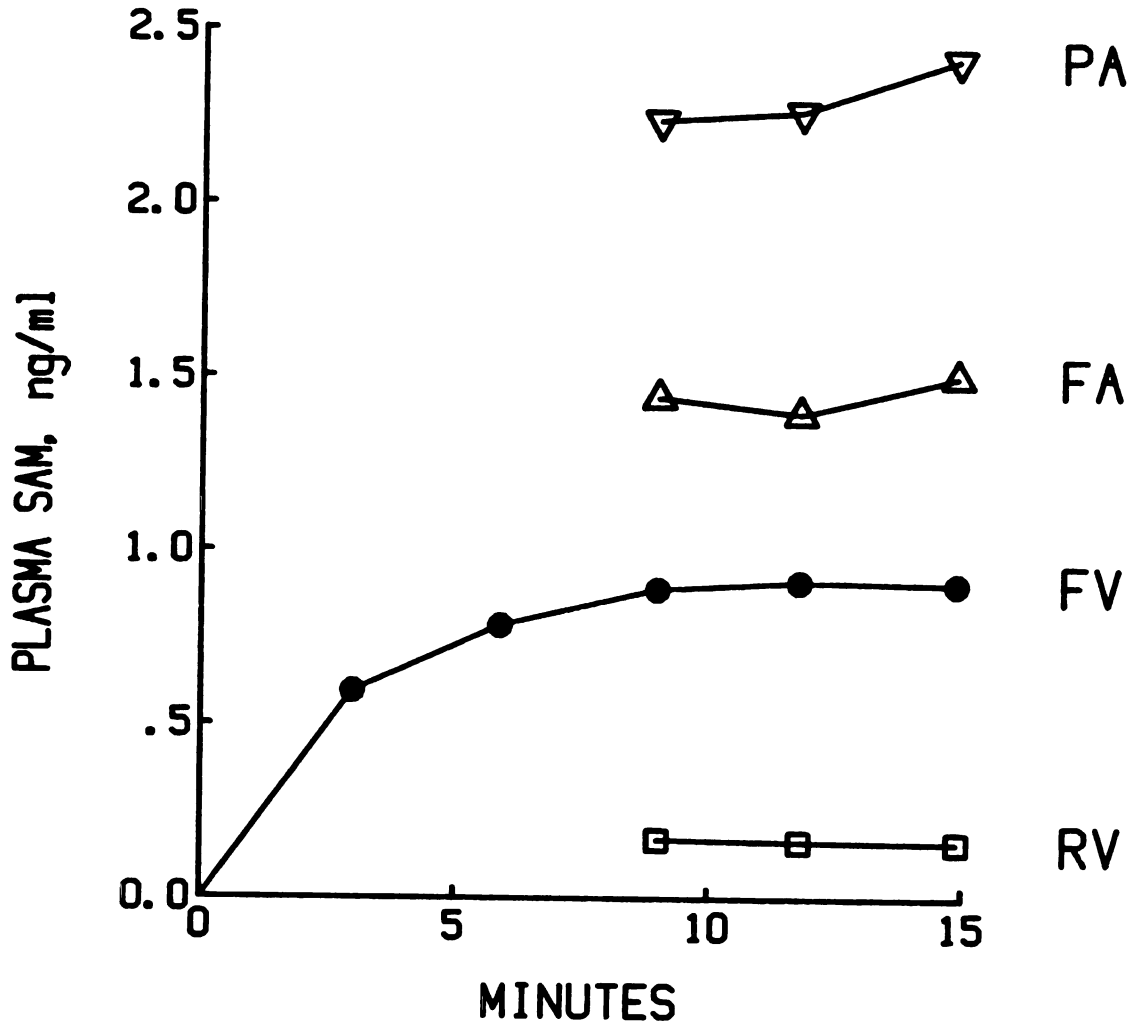


Fig. 7-1. Plasma concentrations of salicylamide (SAM) during the intravenous infusion of [14C]-SAM at 0.3 ug/min-kg (2.5 uCi/min). PA, FA, FV and RV refer to concentrations in the pulmonary artery, femoral artery, forelimb vein and renal vein, respectively.

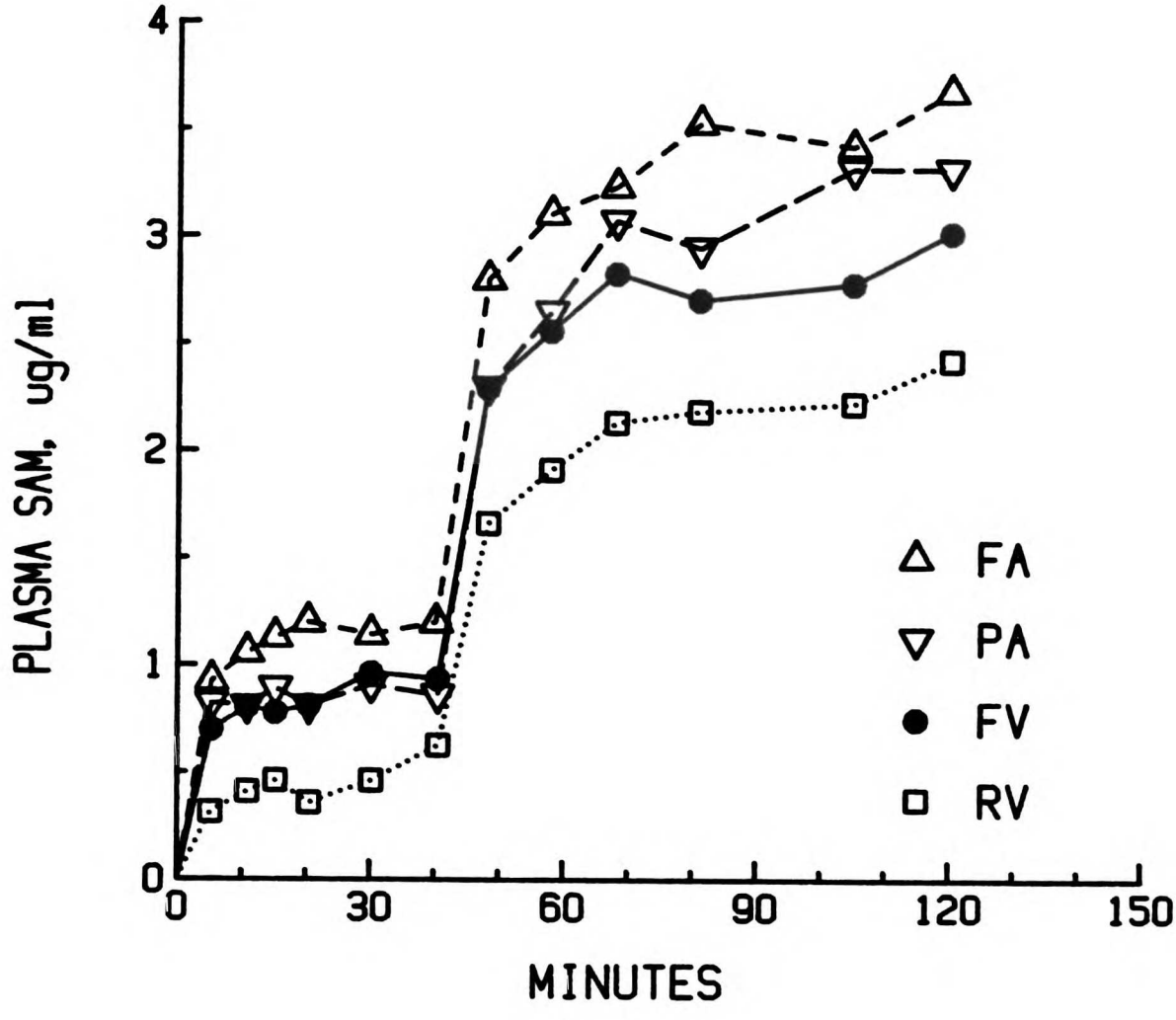


Fig. 7-2. Plasma concentrations of salicylamide (SAM) in a dog during the intravenous infusion of [14C]-SAM at two rates: 83 ug/min-kg (0.2 uCi/min) from 0 to 40 min; 208 ug/min-kg (0.5 uCi/min) from 40 to 120 min. PA, FA, FV and RV refer to concentrations in the pulmonary artery, femoral artery, forelimb vein and renal vein, respectively.

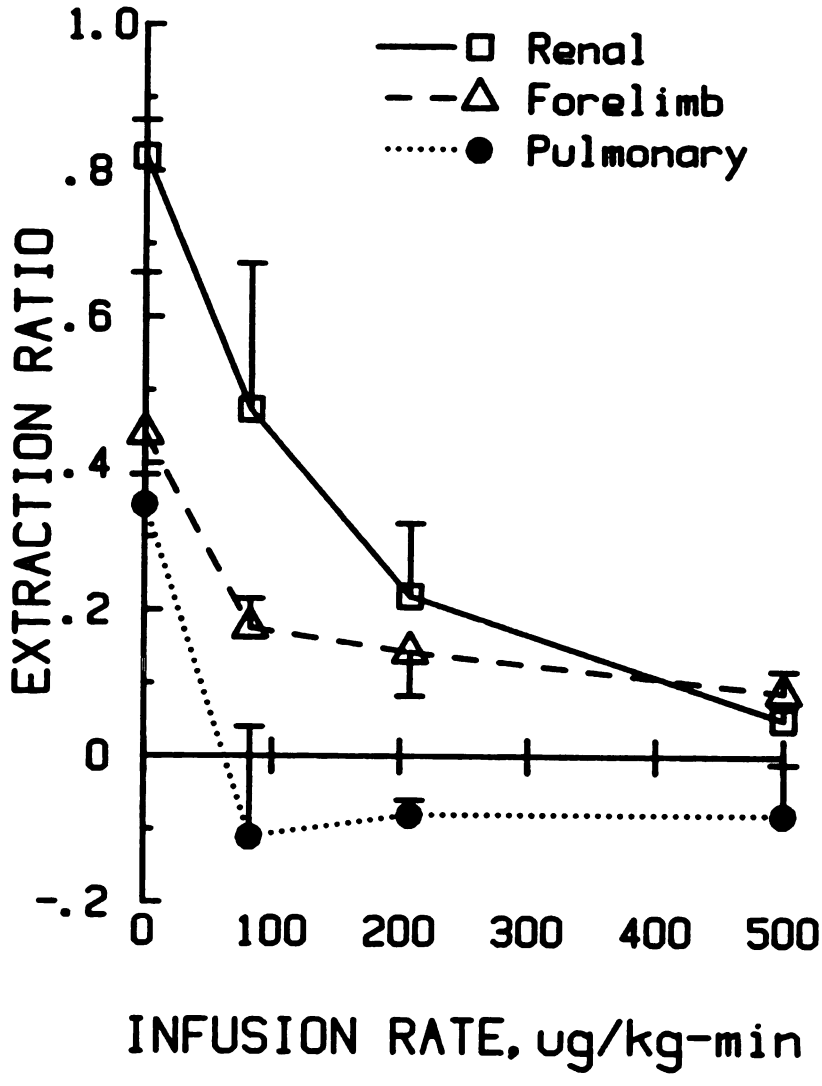


Fig. 7-3. Dose-dependent metabolic extraction of salicylamide by three organs during the infusion of salicylamide at four different infusion rates. Mean extraction ratio (+/- SD) from experiments in four dogs is shown.

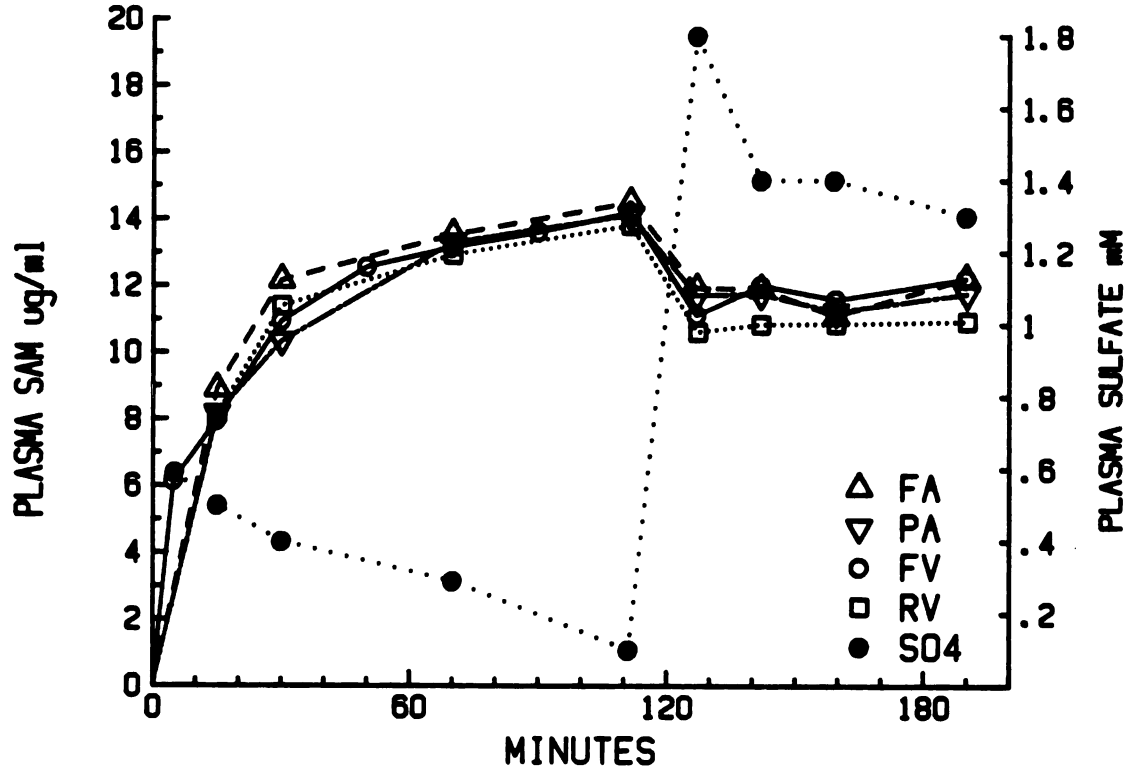


Fig. 7-4. Plasma salicylamide (SAM) and inorganic sulfate concentrations in a dog during infusion of SAM, 500 umol/min-kg, before and during co-infusion of sodium sulfate. Sodium sulfate was infused at 39.8 umol/min-kg from 112 to 124 min and at 4.8 umol/min-kg from 124 to 190 min. PA, FA, FV and RV refer to SAM concentrations in the pulmonary artery, femoral artery, forelimb vein and renal vein, respectively.

between one and two times the baseline values during sulfate co-infusion (Fig. 7-4 and Appendix 5). SAM clearance (using femoral artery plasma SAM concentration) increased from 0.5 +/- 0.1 to 0.65 +/- 0.1 l/min ($P < 0.05$, Students paired t-test) during sulfate infusion at the highest SAM infusion rate (500 ug/kg-min) but did not change significantly at the lower SAM infusion rate (208 ug/kg-min). Sulfate co-infusion had no significant effect on any of the extrahepatic extraction ratios measured (Table 7-1).

Detailed data for individual dogs are presented in Appendix 5.

Table 7-1. Effect of plasma inorganic sulfate concentrations on extrahepatic extraction of SAM during two constant-rate infusions in four dogs.

Plasma Sulfate Concentration (mean(SD))		Change in Mean Extraction Ratio(b)		
Before(a) mM	After(a) mM	Kidney	Lung	Forelimb
208 ug/min-kg SAM infusion				
0.41 (0.15)	0.94(c) (0.23)	+0.0015(d)	-0.044(d)	-0.044(d)
500 ug/min-kg SAM infusion				
0.10 (0.02)	1.70(c) (0.45)	+0.030(d)	+0.038(d)	-0.06(c)

(a) Inorganic sulfate concentrations measured before and after co-infusion of sodium sulfate.
(b) Mean difference between extraction ratio observed before and after sodium sulfate co-infusion.
(c) $P > 0.1$, Student's t-test.
(d) $P < 0.05$, Student's t-test.

IV. CONCLUSIONS AND DISCUSSION

Dose-dependent metabolism of SAM in the lung, kidney and the forelimb is demonstrated here in the intact anesthetized dog. Because greater than 99% of [14C]-radioactivity recovered in the urine after a tracer dose of [14C]-SAM is the sulfate conjugate (Chapter 3), the extrahepatic metabolism must involve the sulfoconjugation pathway. Infusion of sodium sulfate does not change the metabolic extraction in individual organs and affects overall metabolism only at high infusion rates, when plasma sulfate concentrations are greatly decreased.

Clearance values after the tracer infusion of SAM, based on venous drug concentration, were 8.2 +/- 0.7 l/min (mean +/- SD), and were greater than published values of cardiac output in the dog (about 4 l/min, Sapirstein, 1958; Altman and Dittmer, 1971). However, if the SAM infusion rate is corrected for the first-pass extraction in the lungs, and if clearance values are based on arterial concentration, then clearance values of 2.9 +/- 0.3 l/min are obtained. Blood flow to the kidney and non-visceral tissues of the dog are approximately 0.5 and 1.3 l/min, respectively. If the extraction ratio across the non-visceral tissues of the animal is the same as that across the forelimb tissues, then the sum of the clearances in the kidney and non-visceral tissues (product of blood flow and extraction ratio in each organ) is about 1.0 l/min or about 35 % of arterial clearance. Unless appreciable metabolism occurs in the blood

or blood vessel walls, then visceral organs receiving blood in parallel with the kidneys must account for the remaining 1.9 l/min of arterial clearance. Because this value is in excess of liver blood flow (about 1.0 l/min), then it is likely that metabolism occurs in extrahepatic sites in addition to those studied.

With the knowledge of dose-dependent metabolism in the lungs and the tissue upstream from the blood sampling site, clearance values in previous chapters can be reassessed. For example, if extraction across the lungs and limb tissues after tracer administration by IV bolus is the same as determined after IV infusion, then clearance values after IV tracer bolus (Chapter 3) can be adjusted to a value of 4.9 +/- 2.1 l/min. Clearance values after the highest dose of SAM (40 mg/kg) do not need to be corrected because extraction in the lungs and limb tissue at higher doses appears to be negligible (Fig. 7-3). Although the range of clearance values is not as great when values are adjusted in this manner, the dose-dependent metabolism is still quite apparent.

The extrahepatic dose-dependent sulfoconjugation of SAM has other interesting implications regarding the overall pharmacokinetics of SAM. Previous studies (Chapters 3 and 5) indicated that the log SAM concentration vs. time plots curved downward after a 40-mg/kg SAM dose, suggesting Michaelis-Menten kinetics. This curvature was apparent only when concentrations are followed over a time period greater

than 120 minutes. Metabolism occuring in different organs may be characteristically different, and result in different apparent Vmax and Km values. Because some organs of elimination, such as the intestinal wall, liver and lungs are anatomically arranged in series with respect to blood flow, the concentrations of drug entering these organs may be different. These possibilities as well as the possibility of multiple forms of sulfotransferase (Chapter 1) preclude the use of a simple Michealis-Menten model to explain drug concentrations vs. time profiles, even if such a mechanism occurs.

The concentration of inorganic sulfate in plasma had only a small effect on total drug clearance at the highest SAM infusion rate and had no obvious effect on the extrahepatic extraction observed at low infusion rates. The lack of effect of plasma sulfate on SAM metabolism after small doses of SAM and the definite, although not dramatic, effect of plasma sulfate concentration on clearance after a large dose of SAM is consistant with earlier observations.

CONCLUSIONS

Greater insight into the mechanisms of dose-dependent sulfoconjugation have been gained by studying the metabolism and pharmacokinetics of salicylamide (SAM) in the dog. The most prominent findings of the research are summarized below.

1. SAM is primarily metabolized by sulfoconjugation and the conjugate is subsequently excreted in the urine.

At all doses studied, recovery of drug and metabolites in the urine was greater than 70% (Chapter 3). SAM-sulfate accounted for more than 85% of the urinary metabolites. The formation of SAM-sulfate is essentially irreversible (Chapter 6). SAM-glucuronide was the only other detectable metabolite formed.

2. SAM sulfoconjugation shows pronounced dose-dependence.

Bioavailability increased and clearance of SAM (Dose/AUC) decreased with dose. Clearance was particularly dose-dependent, ranging from 13.7 +/- 6.0 L/min (mean +/- S.D.) after the IV tracer dose to 0.60 +/- 0.11 L/min after the 40-mg/kg dose (Chapter 3). When clearance was broken

down into its individual metabolic components, it was found that the dose-dependent clearance was due to a specific effect on the sulfoconjugation pathway. Glucuronidation did not change with dose.

3. Dose-dependent metabolism of SAM occurs in several organs.

Dose-dependent metabolism of SAM in the kidneys, lungs and forelimb was demonstrated in the anesthetized dog by infusing SAM to steady state at four different dosing rates. First-pass metabolism in the lung and metabolism in the tissue just upstream from the sampling site at least partially explains the apparent clearance values greater than cardiac output after low doses of SAM.

4. The sulfate moiety consumed in the sulfoconjugation reaction comes from inorganic sulfate in the plasma or from sulfate stores that are in rapid equilibrium with plasma sulfate. Inorganic sulfate in the plasma is available for conjugation with SAM within two to three minutes.

The source of sulfate for the reaction and the rate of incorporation of plasma sulfate into SAM-sulfate was

determined by co-injecting SAM and inorganic [35S]-sulfate (Chapter 6). Because the specific activity of metabolite formed was nearly the same as that of inorganic sulfate in the plasma, it was concluded that the sulfate used for the reaction originates from sulfate in the plasma or from sulfate stores that are in rapid equilibrium with plasma sulfate. A delay in sulfate uptake or activation, if any, must be less than two to three minutes.

5. The rate of SAM sulfoconjugation is not closely related to plasma inorganic sulfate concentration.

It was found in initial studies that clearance changed substantially over a SAM dosage range that did not greatly lower plasma inorganic sulfate concentrations (Chapter 3). The greatly depressed clearance after the 40-mg/kg dose returned to normal in 240 minutes, but plasma sulfate concentrations were persistently depressed (Chapter 5).

When sodium sulfate was infused before and after oral administration of a 20-mg/kg SAM dose, clearance was depressed to the same extent as that after a control 20-mg/kg dose (Chapter 4). When SAM was co-infused at varying rates with equimolar amounts of sodium sulfate, normal plasma sulfate concentrations were maintained, but SAM clearance changed inversely with infusion rate (Chapter 5).

There was some evidence that plasma sulfate concentrations, if greatly increased or decreased, may affect the kinetics of SAM. After a 80-mg/kg SAM dose, clearance was reduced for at least 12 hours in association with plasma sulfate concentrations that were below the level of assay reliability (0.3 mM, Chapter 4). When plasma sulfate concentrations were increased 10-fold above normal values, the clearance of a 1-mg/kg SAM dose was about twice that observed in the normal situation (Chapter 6).

6. The dose-dependent kinetics of SAM are not explained by typical Michaelis-Menten kinetics.

If plasma SAM concentrations are measured over a sufficient period of time, downward curvature of log plasma SAM concentration vs. time curves is observed, suggesting Michaelis-Menten kinetics (Chapter 5). However, when plasma concentration data after different SAM doses in the same dogs are compared, the rate of decline of SAM concentration is not necessarily related to the plasma SAM concentration. At a given concentration, the fractional rate of decline is slower after larger than after smaller doses of SAM. Infusion studies suggest that a time-related factor may be involved in the dose-dependence.

7. Decreased SAM clearance does not appear to be associated with elevated concentrations of SAM-sulfate in the plasma.

After a 1-mg/kg IV SAM dose, clearance was depressed, however, plasma SAM-sulfate concentrations attained were less those associated with normal SAM clearance values (Chapter 6).

In summary, the dose-dependent sulfoconjugation of SAM in the dog is not fully explained by one simple mechanism. Inorganic sulfate in the blood is the primary source of sulfate for the reaction and is rapidly available for conjugation. However, depletion of inorganic sulfate in the plasma appears to play a role in the dose-dependence only when concentrations are greatly reduced. Typical Michaelis-Menten enzyme saturation also does not appear to explain the results because the kinetics are not closely related to plasma concentrations of SAM. It is suspected that a time-dependent factor other than depletion of inorganic sulfate in the plasma may be involved in the dose-dependent sulfoconjugation.

BIBLIOGRAPHY

Altman, P. and Dittmer, D.: Respiration and Circulation, Federation of American Societies for Experimental Biology, Bethesda, MD, 1971

Barr, B. et al.: Dose-Dependent Drug Metabolism During the Absorptive Phase. Rev. Can. Biol. 32:31-41, 1973

Bonham-Carter et al.: Human-Platelet Phenol Sulfotransferase M and P: Substrate Specificities and Correlation with in vivo Sulfoconjugation of Paracetamol and Salicylamide. B. J. Clin. Pharmacol. 15:323-330, 1983

Bostrom, H. and Wengle, B.: Studies on Ester Sulphates, Acta Endocrinol. 56:691-704, 1967

Bray, H. et al.: Kinetic Studies of the Metabolism of Foreign Organic Compounds. Biochem. J. 52:419-423, 1952

Brunngraber, E.: Nucleotides Involved in the Enzymatic Conjugation of Phenols with Sulfate. J. Biol. Chem. 233:472-477, 1958

Buch, H. et al.: Ausscheidung Freien und Konjugierten Sulfates bei Ratte und Menschen nach Verabreichung von N-Acetyl-p-Aminophenol. Naunyn-Schmiedebergs Arch. Pharmak. Path. 259:276-289, 1968

Cardin, C. and Mason, J.: Sulfate Transport by the Rat Ileum: Effect of Molybdate and other Anions. Biochim. Biophys. Acta 394:46-54, 1975

Cassidy, M. and Houston, J.: In vivo Assessment of Extrahepatic Conjugative Metabolism in First-Pass Effects using the Model Compound Phenol. J. Pharm. Pharmacol. 32:57-59, 1980

Cheng, S. and Levy, D.: Characterization of the Anion Transport System in Hepatocyte Plasma Membranes. J. Biol. Chem. 255:2637-2640, 1980

Cooper, J., Biochemistry of Sulfur-Containing Amino Acids, Ann. Rev. Biochem. 52:187-222, 1983

Davis, D.: Radioisotopic Assay for Rat Liver Sulfotransferase Activity, Biochem. Pharmacol. 24:975-978, 1975

Dixon, W. and Massey, F.: Introduction to Statistical Analysis, 4th Ed., McGraw-Hill, New York, 1983

Dodgson, K.: Conjugation with Sulfate, in Drug Metabolism From Microbe to Man. p. Parke, D. and Smith, R. Ed. Taylor and Francis, London, 1977

Douglas, G. et al.: Evaluation of Radiosulfate as a Measure of Functional and Total Extracellular Fluid. Surgical Forum 20:35-36, 1969

Fielding R. et al.: Analysis of Salicylamide and its Metabolites in Blood and Urine by HPLC, J. Liq. Chromotog, 7: 1221-1234, 1984

Foldes, A. and Meeks, J.: Occurence and Localization of Brain Phenolsulphotransferase, J. Neurochem. 23:303-307, 1974

Galinsky, R. and Levy, G.: Effect of N-Acetylcysteine on the Pharmacokinetics of Acetaminophen in Rats. Life Sciences 25:693-700, 1979

Galinsky, R. and Levy, G.: Dose- and Time-dependent Elimination of Acetaminophen in Rats: Pharmacokinetic Implications of Cosubstrate Depletion, J. Pharmacol. Exp. Ther. 219:4-20, 1981

Giese, A.: Cell Physiology, pp 203-204, Saunders, Philadelphia, 1962

Girard, J. and Glatz, J.: Ion Chromatography With Conventional HPLC Instrumentation. American Laboratory 26, 1981

Glazenburg, E. et al.: Effects of Variation in the Dietary Supply of Cysteine and Methionine on Liver Concentration of Glutathione and "Active Sulfate" (PAPS) and Serum Levels of Sulfate, Cystine, Methionine and Taurine: Relation to the Metabolism of Acetaminophen. J. Nutr. 113:1363-1373, 1983

Gugler, P. et al.: Effect of Portacaval Shunt on the Disposition of Drugs with and without First-Pass Effect. J. Pharmacol. Exp. Ther. 195:416-423, 1975

Herbai, G.: A Double Isotope Method for the Determination of the Miscible Inorganic Sulfate Pool of the Mouse Applied to *in vivo* Studies of Sulfate Incorporation into Costal Cartilage. Acta Physiol. Scand. 80:470-491, 1970

Hjelle, J. and Klaassen, C.: Glucuronidation and Biliary Excretion of Acetaminophen in Rats. J. Pharmacol. Exp. Ther. 228:407-413, 1984

Hook, G. and Bend, J.: Pulmonary Metabolism of Xenobiotics, Life Sci. 18:279-290, 1976

Jollow, D. et al.: Acetaminophen-Induced Hepatic Necrosis. 6. Metabolic Disposition of Toxic and Non-Toxic Doses of Acetaminophen. Pharmacol. 12:251, 1974

Jones, D. et al.: Use of Isolates Kidney Cells for Study of Drug Metabolism, Biochem. Pharmacol. 28:929-935, 1979

Koike, M. et al., Sulfoconjugation and Glucuronidation of Salicylamide in Isolate Rat Hepatocytes, J. Pharm. Sci. 70:308-311, 1982

Krijgsheld, K. et al.: Absorption, Serum Levels and Urinary Excretion of Inorganic Sulfate after Oral Administration of Sodium Sulfate in the Concious Rat, Biochim. Biophys. Acta 586:492-500, 1979

Krijgsheld, K. et al.: Serum Concentration of Inorganic Sulfate in Mammals: Species Differences and Circadian Rhythm. Comp. Biochem. Physiol. 67A:683-686, 1980

Krijgsheld, K., et al.: The Dependence of the Rate of Sulphate Conjugation on the Plasma Concentration of Inorganic Sulphate in the Rat in vivo. Biochem. Pharmacol. 31:3997-4000, 1982

Levy, G. and Matsuzawa, T.: Role of Sulfate Formation in Biotransformation of Salicylamide in Man.: J. Pharm. Sci. 55:222-223, 1966

Levy, G. and Matsuzawa, T.: Pharmacokinetics of Salicylamide Elimination in Man. J. Pharmacol. Exp. 156:285-293, 1967

Lewis, M. and Spencer, B.: The Enzymatic Degradation of Nucleotide Sulphatophosphate Anhydrides. Biochem. J. 85:18P, 1959

Lin, J. and Levy, G.: Renal Clearance of Inorganic Sulfate in Rats: Effect of Acetaminophen-Induced Depletion of Endogenous Sulfate. J. Pharm. Sci. 72:213-217, 1983

Mattox, V. et al.: Extraction of Steroidal Glucosiduronic Acids from Aqueous Solutions by Anionic Liquid Ion-exchangers, Biochem. J. 126:533-543, 1972a

Mattox, V. et al.: Recovery of Steroidal Glucosiduronic Acids from Organic Solvents Containing Anionic Liquid Ion-exchangers, Biochem. J. 126:545-552, 1972b

Mehta R. et al.: The Influence of Dose on the Pattern of Conjugation of Phenol and 1-Naphthaol in Non-Human Primates. Xenobiotica 8:445-452, 1978

Mehta, R. and Cohen G.: Major Differences in the Extent of Conjugation with Glucuronic Acid and Sulphate in Human Peripheral Lung, Biochem. Pharmacol. 28:2479-2484, 1979

Merits, I.: Formation and Metabolism of [14C]-Dopamine 3-O-Sulfate in Dog, Rat and Guinea Pig. Biochem. Pharmacol. 25:829-833, 1976

Moldeus, P., et al.: Regulation of Glucuronidation and Sulfate Conjugation in Isolated Hepatocytes, Drug Metabolism and Dispostion 7:416-419, 1979

Mulder, G.: Sulfation of Drugs and Related Compounds, pp 158-161, CRC Press, Boca Raton, FL, 1981a

Mulder, G.: Sulfation of Drugs and Related Compounds, pp 56-70, CRC Press, Boca Raton, FL, 1981b

Mulder, G.: Sulfation of Drugs and Related Compounds, pp 90-98 CRC Press, Boca Raton, FL, 1981c

Mulder, G.: Sulfation of Drugs and Related Compounds, pp 147-153, CRC Press, Boca Raton, FL, 1981d

Mulder, G. and Keulemans, K.: Metabolism of Inorganic Sulphate in the Isolated Perfused Rat Liver, Biochem. J. 176:959-965, 1978

Mulder, G. and Meerman, J.: Glucuronidation and Sulphation in vivo and in vitro: Selective Inhibition of Sulphation by Drugs and Deficiency of Inorganic Sulphate, in Conjugation Reactions in Drug Biotransformation. Aitio, A. Ed. Elsevier/North-Holland Biomedical Press, New York, 1978

Mulder, G. and Scholtens, E.: The Availability of Inorganic Sulphate in Blood for Sulphate Conjugation of Drugs in Rat Liver in vivo. Biochem. J. 172:247-251, 1978

Mulder, G. et al.: UDP Glucuronidyltransferase and Phenolsulfotransferase from Rat Liver in vivo and in the Perfused Liver. Biochem. Pharmacol. 24:103-107, 1975

O'Connor, W. and Summerfield, R.: Sulphate Excretion by Dogs Following Ingestion of Ammonium Sulphate or Meat, J. Physiol. 260:597-607, 1976

Pennings, E. et al.: Kinetics and Mechanism of Rat Brain Phenol Sulfotransferase Reaction, Biochem. J. 173:299-307, 1978

Pennings, J. and Van Kempem, G.: Enzyme Kinetics of the Sulfoconjugation Reaction, in Sulfate Metabolism and Sulfate Conjugation, Mulder G., Ed, Taylor and Francis, London, 1982

Pfeffer, M.: COMPT, A Time-sharing Program for Non-linear Regression Analysis of Compartmental Analysis of Drug Distribution. J. Pharmacokin. Biopharm. 1:137-163, 1973

Quebbmann, A and Anders, M.: Renal Tubular Conjugation and Excretion of Phenol and p-Nitrophenol in the Chicken: Differing Mechanisms of Renal Transfer. J. Pharmacol. Exp. Ther. 184:695-708, 1973

Sakai, K. et al.: Vascularly Perfused Rat Small Intestine: A Research Model for Drug Absorption. Jpn. J. Pharmacol. 30:231-241, 1980

Sammons, H. et al.: Studies in Detoxification. The Conjugation and Oxidation of p-Hydroxybenzenesulphonamide in the Rabbit. The Characterization of p-Sulphonamidophenylglucuronide. Biochem. J. 35:557-563, 1941

Sapirstein L.: Regional Blood Flow by Fractional Distribution of Indicator. Am. J. Physiol. 193:161-168, 1958

Schwarz, L.: Uptake and Accumulation of Inorganic Sulfate in Isolated Hepaocytes and the Correlation to Initial Rates of Sulfation, in Sulfate Metabolism and Sulfate Conjugation, Mulder. G. Ed. Taylor and Francis, London, 1982

Sekura, R. and Jakoby, W.: Phenol Sulfotransferases, J. Biol. Chem. 254: 5658-5663, 1979

Sekura, R. and Jakoby, W.: Aryl Sulfotransferase IV from Rat Liver. Arch. Biochem. Biophys. 211:352-359, 1981

Smith, I. and Mitchell, P.: The Effect of Oral Inorganic Sulphate on the Metabolism of 4-Hydroxyphenethylamine (Tyramine) in Man: Tyramine O-Sulfate Measurement in Human Urine. Biochem. J. 142:189-191, 1974

Strong, J. et al.: Absolute Bioavailability in Man of N-acetylprocainamide determined by a Novel Stable Isotope Technique. Clin. Pharmacol. Ther. 18:377-390, 1975

Watari, N. et al.: Pharmacokinetic Study of the Fate of Acetaminophen and its Conjugates in Rats. J. Pharmacokin. Biopharm. 11:245-272, 1983

Watrous W. and Fujimoto, J.: Inhibition of Morphine Metabolism by Catechol in the Chicken Kidney, Biochem. Pharmacol. 20:1479-1491, 1971

Weitering, J. et al.: The Availability of Inorganic Sulphate as a Rate-Limiting Factor in the Sulphate Conjugation of Xenobiotics in the Rat? Biochem. Pharmacol. 28:757-762, 1979

Wengle, B.: Distribution of Some Steroid Sulfokinases in Foetal Human Tissues. Acta Endocrinol. 52:607-618, 1966

Williams, R.: CXVIII. Studies in Detoxification. The Influence of (a) dose and (b) o-, m- and p-Substitution on the Sulphate Detoxification on Phenol in the Rabbit. Biochem. J. 32:878-887, 1938

Wong, K and Yeo, Y.: Assay of Adenosine 3'-Phosphate 5'-Sulphatophosphate in Hepatic Tissues. Biochem. J. 181:107-110, 1979

Wong, K. and Yeo, T.: Importance of Extrahepatic Sulfoconjugation, Biochem. Pharmacol. 31:4001-4003, 1982

APPENDIX 1

Table A1-1. Salicylamide (SAM) clearance (l/min) in dogs after administration of four oral doses of SAM and an IV tracer dose of [14C]-SAM.

Dog	SAM Dose (mg/kg)				
	Tracer	5	10	20	40
34927	16.7	4.4	2.4	1.4	0.49
34084	6.8	2.4	1.5	1.0	0.66
34083		4.1	2.7	1.1	0.62
34928	17.7	2.5	1.6	0.9	0.45
36002		4.7	2.6	1.4	0.96
35255		2.8	1.5	1.9	0.63
mean	13.7	3.4	2.1	1.3	0.60
SD	6.0	1.0	0.6	0.4	0.11

Table A1-2. Salicylamide (SAM) half-life (min) in dogs after administration of four oral doses of SAM.

Dog	SAM Dose (mg/kg)			
	5	10	20	40
34927	3.2	8.4	13.1	22.3
34084	4.2	9.0	23.0	19.6
34083	6.0	8.8	15.7	17.6
34928	6.4	9.8	19.4	35.2
36002	4.3	7.4	15.6	23.1
35255	5.4	12.2	10.4	23.1
mean	5.0	9.3	16.2	23.5
SD	102	1.6	4.5	6.1

Table A1-3. Salicylamide (SAM) volume of distribution (l/kg) in dogs after administration of four oral doses of SAM.

Dog	SAM Dose (mg/kg)			
	5	10	20	40
34927	0.8	1.2	1.1	0.68
34084	0.7	0.9	1.7	0.90
34083	1.5	1.4	1.1	0.67
34928	1.0	1.1	1.2	0.98
36002	1.2	1.2	1.2	1.00
35255	1.1	1.1	1.3	0.91
mean	1.1	1.1	1.3	0.86
SD	0.3	0.2	0.2	0.14

Table A1-4. Salicylamide (SAM) bioavailability in dogs after administration of four oral doses of SAM.

Dog	SAM Dose (mg/kg)			
	5	10	20	40
34927	0.02	0.21	0.32	0.67
34084	0.38	0.33	0.92	0.57
34083	0.26	0.50	0.64	0.73
34928	0.34	0.20	0.54	1.02
36002	0.10	0.46	1.18	0.58
35255	0.33	0.83	0.15	1.00
mean	0.24	0.42	0.62	0.76
SD	0.14	0.24	0.38	0.20

Table A1-5. Clearance of salicylamide (SAM) to the sulfate conjugate in dogs after administration of two oral doses of SAM and an IV tracer dose of [14C]-SAM.

Dog	SAM Dose (mg/kg)		
	Tracer	5	40
34927	19.4	2.9	0.40
34084	6.6	2.0	0.53
34083		3.4	0.35
34928	19.3	2.2	0.34
36002		4.0	0.65
35255		2.1	0.43
mean	15.1	2.8	0.45
SD	7.4	0.8	0.12

Table A1-6. Clearance of salicylamide (SAM) to the glucuronide conjugate in dogs after administration of two oral doses of SAM and an IV tracer dose of [14C]-SAM.

Dog	SAM Dose (mg/kg)		
	Tracer	5	40
34927	0.11	0.04	0.07
34084	0.06	0.09	0.07
34083		0.09	0.06
34928	0.17	0.04	0.08
36002		0.17	0.14
35255		0.08	0.06
mean	0.11	0.08	0.08
SD	0.06	0.05	0.04

EXPERIMENT: 5 mg/kg control

DOG: 34927 DATE: 1-20-83 TIME: 11:30 AM

WT: 24 kg

ORAL DOSE: 120 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.5-5.5 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			1.27
3.0		< 0.01	
6.15	5197	0.05	
9.3	1199	< 0.01	
12.15	665	0.061	
15.15	336	0.028	
20.05	110	< 0.01	0.90
32.3		< 0.01	0.85
43.6		< 0.01	
63.1		< 0.01	1.07
120.7			1.12
180.5			1.14
244			1.24

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 92 mg

SAM-GLUCURONIDE: 11 mg

TOTAL: 103 mg 86 % OF DOSE ADMINISTERED

[14C] RECOVERY: 95.1 million dpm 108 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 96.6 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 1.4 million dpm

EXPERIMENT: 5 mg/kg control

DOG: 34084 DATE: 1-13-83 TIME: 10:45 AM

WT: 21 kg

ORAL DOSE: 105 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.9-5.3 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			
2.4		0.023	0.99
4.3		0.54	
6.1	22862	0.63	
8.5	4468	1.08	
11.2	2438	1.39	
14.7	1227	0.78	
20.6	352	0.216	
27.3	155	0.043	0.66
34.9	< 75	0.017	0.79
61			0.74
121			0.84
179			0.74
239			0.72
361			0.72

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 54 mg

SAM-GLUCURONIDE: 9 mg

TOTAL: 63 mg 60 % OF DOSE ADMINISTERED

[14C] RECOVERY: 92.2 million dpm 105 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 93.4 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 4.3 million dpm

EXPERIMENT: 5 mg/kg control

DOG: 34083 DATE: 4-13-83 TIME: 9:55 AM

WT: 23 kg

ORAL DOSE: 115 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			1.10
4.4		0.466	
6.8	2818	0.538	
9.2	1703	0.897	
12.2	1023	0.772	
16.4	450	0.211	
21.1	221	0.068	
28	70	0.017	
37.3	62		
61			0.93
120			0.97
253			0.94

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 94 mg

SAM-GLUCURONIDE: 8 mg

TOTAL: 102 mg 89 % OF DOSE ADMINISTERED

[14C] RECOVERY: 90.4 million dpm 103 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 85.6 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 2.6 million dpm

EXPERIMENT: 5 mg/kg control

DOG: 34928 DATE: 1-6-83 TIME: 10:40 AM

WT: 22.0 kg

ORAL DOSE: 110 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-5.2 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.90
2.2		1.36	1.07
4.1		1.23	
6.3		0.83	
7.5	4324	0.64	
12.4	1196	0.35	
16.2	731		
20.9	458	0.20	
30	166	0.057	0.80
36	92		0.92
40.7			
46.7			
51.3			0.92
61.3			0.73
119.5			0.74
180			0.77
240			0.74
356			0.79

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 77 mg

SAM-GLUCURONIDE: 49 mg

TOTAL: 126 mg 114 % OF DOSE ADMINISTERED

[14C] RECOVERY: 101 million dpm 115 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 101.0 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 1.9 million dpm

EXPERIMENT: 5 mg/kg control

DOG: 36002 DATE: 1-11-83 TIME: 9:30 AM

WT: 24.9 kg

ORAL DOSE: 124.5 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.8-5.5 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.70
2.4		< 0.010	
4.5		0.055	
7.3	2625	0.127	
10.3	1482	0.237	
13.7	633	0.115	
17.4	307	0.039	
23.2	152	0.019	
29.0	84	0.011	
60			0.57
120.4			0.55
180.4			0.43
240			0.52
355			0.54

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 96 mg

SAM-GLUCURONIDE: 34 mg

TOTAL: 130 mg 104 % OF DOSE ADMINISTERED

[14C] RECOVERY: 98.2 million dpm 112 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 101.3 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 4.3 million dpm

EXPERIMENT: 5 mg/kg control

DOG: 35255 DATE: 4-12-83 TIME: 9:50 AM

WT: 19.9 kg

ORAL DOSE: 99.5 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 5.6-6.6 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.39
2.0		0.016	
4.0		0.19	
6.7		0.93	
9.1	4239	1.12	
12.0	2022	0.801	
16.3	689	0.200	
20.9	302	0.096	
28	120	0.022	
36	58	0.018	
43.9		< 0.010	
60			0.31
120.1			0.36
240			0.34

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 94 mg

SAM-GLUCURONIDE: 9 mg

TOTAL: 103 mg 104 % OF DOSE ADMINISTERED

[14C] RECOVERY: 93.3 million dpm 106 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 90.5 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 3.6 million dpm

EXPERIMENT: 10-mg/kg control

DOG: 34927 DATE: 12-20-82 TIME: 10:00 AM

WT: 24.3 kg

ORAL DOSE: 243 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.2-5.2 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.98
2.65		0.02	
6.0	3968		
8.9	1873	0.49	
11.7	1583	0.88	
15.3	1288	0.94	
20.1	824	0.75	
30.5	339	0.25	0.93
45.0	102	0.07	
60.0		< 0.01	0.85
121			0.90
179.6			0.78
240.0			0.79
361.0			0.54

(a) Time after oral dose

Urine was lost

EXPERIMENT: 10-mg/kg control

DOG: 34084 DATE: 12-15-82 TIME: 9:55 AM

WT: 21.3 kg

ORAL DOSE: 213 mg

IV DOSE: 36 uCi TIME ADMINISTERED(a): 4.1-5.2 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.85
2.8		< 0.010	
5.8	9403	0.19	
8.9	1654	0.28	
11.8	1307	1.49	
15.7	1253	3.20	
20.0	1076	2.32	
30.1	483	0.40	0.71
44.4	131	0.14	
60.2	52		0.70
120.1			0.67
180			0.63
238			0.63
330			0.67

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 188 mg

SAM-GLUCURONIDE: 50 mg

TOTAL: 238 mg 112 % OF DOSE ADMINISTERED

[14C] RECOVERY: 73.1 million dpm 92 % OF DOSE ADMINISTERED

EXPERIMENT: 10-mg/kg control

DOG: 34083 DATE: 3-10-33 TIME: 10:40 AM

WT: 24 kg

ORAL DOSE: 240 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-4.5 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.95
2.3		< 0.01	
4.2		0.042	
5.9	2672	0.05	
8.5	1225	0.229	
12.0	1183	0.82	
15.9	1130	3.50	
20.3	870	1.65	
26.6	588	0.967	
35.4	265	0.371	
46.4	115	0.156	
55.2	60	0.049	
60	38	0.030	0.74
76.5		0.0072	
121			0.77
240			0.79

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 182 mg

SAM-GLUCURONIDE: 18 mg

TOTAL: 200 mg 83 % OF DOSE ADMINISTERED

[14C] RECOVERY: 80.3 million dpm 91 % OF DOSE ADMINISTERED

EXPERIMENT: 10-mg/kg control

DOG: 34928 DATE: 12-22-82 TIME: 10:15 AM

WT: 21.5 kg

ORAL DOSE: 215 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 5.2-6.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.84
4.3		0.85	
7.5	5872	0.77	
11.2	2849	0.56	
14.7	1686	0.57	0.74
19.2	987	0.77	0.74
30	545	0.37	
43	260	0.14	0.62
66	< 75		0.57
92			0.53
124			0.64
188			0.54
253			0.64
354			0.64

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 108 mg

SAM-GLUCURONIDE: 32 mg

TOTAL: 140 mg 65 % OF DOSE ADMINISTERED

[14C] RECOVERY: 70.0 million dpm 80 % OF DOSE ADMINISTERED

EXPERIMENT: 10-mg/kg control

DOG: 36002 DATE: 3-2-833 TIME: 11:10 AM

WT: 24 kg

ORAL DOSE: 220 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 6.5-7.5 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.88
3.3		< 0.01	
5.3		0.914	
7.5		3.89	
10.6	2795	0.801	
14.9	1109	0.725	
20.3	843	0.888	
38	166	0.152	
42.3		0.107	
46.5	91	0.101	
51.8	< 40	0.054	
60.7		0.062	
90			0.62
115			0.62
240			0.70

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 127 mg

SAM-GLUCURONIDE: 5 mg

TOTAL: 132 mg 60 % OF DOSE ADMINISTERED

[14C] RECOVERY: 74.7 million dpm 85 % OF DOSE ADMINISTERED

EXPERIMENT: 10-mg/kg control

DOG: 35255 DATE: 3-29-83 TIME: 8:45 AM

WT: 23.5 kg

ORAL DOSE: 235 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-4.8 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.68
2.8		7.60	
7.3	6006	4.50	
11.8	2129	3.51	
14.6	1552		
16.9	1178	2.60	
21.6	796	1.67	
29.6	416	1.13	
44.2	209	0.615	
60.6	110	0.349	0.40
75	50	0.155	
90.6		0.074	
120			0.35
255			0.36

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 232 mg

SAM-GLUCURONIDE: 10 mg

TOTAL: 242 mg 103 % OF DOSE ADMINISTERED

[14C] RECOVERY: 81.6 million dpm 93 % OF DOSE ADMINISTERED

EXPERIMENT: 20-mg/kg control

DOG: 34927 DATE: 11-18-82 TIME: 11:07 AM

WT: 23.2 kg

ORAL DOSE: 464 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			1.02
3.1		0.07	
6.6	3580	0.57	
8.8	2497	1.21	
12.3	1900	1.77	
15.2	1741	2.38	
19.8	1472	2.55	
29.6		2.52	0.83
45.0	464	1.138	
60.1	164	0.386	0.75
75.5	92	0.164	
90.3			0.78
115.3			0.70
181			0.71
246			0.70
343.5			0.66

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 419 mg

SAM-GLUCURONIDE: 44 mg

TOTAL: 463 mg 100 % OF DOSE ADMINISTERED

[14C] RECOVERY: 82.2 million dpm 93 % OF DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg control

DOG: 34084 DATE: 11-22-82 TIME: 10:00 AM

WT: 19.8 kg

ORAL DOSE: 396 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.75
3.2		5.33	
7.0	1631	6.42	
11.7	1852	8.81	
16.1	1958	10.43	
21.8	1892	8.50	
31.0	1436	4.04	0.56
46.2	831	1.66	
59.7	548	1.19	0.50
75.5	327	0.78	0.57
91	190	0.44	
120.5	100		0.49
181			0.44
240			0.49
362.5			0.53

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 176 mg

SAM-GLUCURONIDE: 32 mg

TOTAL: 208 mg 53 % OF DOSE ADMINISTERED

[14C] RECOVERY: 72.1 million dpm 82 % OF DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg control

DOG: 34083 DATE: 5-3-83 TIME: 9:26 AM

WT: 22.4 kg

ORAL DOSE: 448 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-4.8 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			1.07
3.0		0.425	
6.0	3200	1.005	
9.0	2871	4.66	
12.0	2641	7.62	
15.0	2179	12.9	
20.0	2049	13.9	
30.0	1155	5.31	0.77
45.0	514	2.01	
60.0	238	0.92	0.71
76.7	201	0.84	
90.3	63	0.22	0.72
122.3			0.69
240			0.74

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE:	303 mg	
SAM-GLUCURONIDE:	40 mg	
TOTAL:	343 mg	77 % OF DOSE ADMINISTERED

[14C] RECOVERY: 85.7 million dpm 97 % OF DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg control

DOG: 34928 DATE: 12-8-82 TIME: 10:00 AM

WT: 21.5 kg

ORAL DOSE: 430 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 5.0-5.6 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.85
3.3		0.57	
7.1	3062	3.26	
10.2	3094	5.06	
13.6	2917	5.84	
16.9	2406	5.22	
22.1	1993	5.69	
30.3	1414	3.61	0.67
45.6	841	2.22	
61.0	466	1.37	0.56
74.8	284	0.71	0.65
90.1	248	0.52	0.52
120.3	49	0.10	0.30
180			0.32
243			0.48
359.6			

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 323 mg

SAM-GLUCURONIDE: 98 mg

TOTAL: 421 mg 98 % OF DOSE ADMINISTERED

[14C] RECOVERY: 86.9 million dpm 99 % OF DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg control

DOG: 36002 DATE: 2-3-83 TIME: 10:50 AM

WT: 24.5 kg

ORAL DOSE: 490 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 8.5-9.2 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.78
3.1		23.0	0.70
5.9		10.8	
9.2		11.3	
12.8	4726	5.39	
15.9	2447	3.50	
19.8	1582	6.17	
30.0	711	4.10	0.34
46.6	397	2.08	0.34
62.0	190		0.40
77.0			0.34
92.5	56		0.35
179			0.33
239			0.43

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE:	272 mg	
SAM-GLUCURONIDE:	20 mg	
TOTAL:	292 mg	68 % OF DOSE ADMINISTERED

[14C] RECOVERY: 57.3 million dpm 65 % OF DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg control

DOG: 35255 DATE: 2-1-83 TIME: 9:45 AM

WT: 21.5 kg

ORAL DOSE: 430 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-4.7 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			
3.0		0.072	0.95
6.0	6539	0.54	
8.8	2470	0.368	
12.0	1478	0.776	
15.0	1097	0.881	
20.3	679	1.25	
30.0	349	0.611	0.91
45.0	130	0.237	
60.0		0.059	0.66
75.0		0.020	
90.0			0.67
120.0			0.56
240.0			0.61

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 242 mg

SAM-GLUCURONIDE: 20 mg

TOTAL: 262 mg 61 % OF DOSE ADMINISTERED

[14C] RECOVERY: 79.0 million dpm 90 % OF DOSE ADMINISTERED

EXPERIMENT: 40 mg-kg control

DOG: 34927 DATE: 11-3-82 TIME: 10:00 AM

WT: 23.2 kg

ORAL DOSE: 928 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.15-5.00 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.97
3.6		1.94	
7.1	4092	6.58	
10.3	3944	13.91	
14.5	3386	17.76	
21.2	3504	21.82	
31.8	2641	18.20	0.74
46.4	1725	11.85	0.58
61.5	1388	8.25	0.62
75.7	682		
96.8	410	2.80	
121.4	178		
180			0.17
255			0.25
345			0.27

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 529 mg

SAM-GLUCURONIDE: 125 mg

TOTAL: 654 mg 70 % OF DOSE ADMINISTERED

[14C] RECOVERY: 89.4 million dpm 102 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 77.3 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 13.0 million dpm

EXPERIMENT: 40 mg-kg control

DOG: 34084 DATE: 2-8-83 TIME: 9:42 AM

WT: 21 kg

ORAL DOSE: 840 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 3.8-4.4 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.99
3.0		2.03	
6.0	10913	3.41	
9.0	4784	4.68	
12.0	3332	4.87	
15.0	2572	9.61	
20.0	1786	5.24	
32.4	1165	6.83	0.74
45.1	865	10.53	
61.6	556	7.48	0.62
75.3		5.38	
92	256	2.84	
119.6	718	0.72	0.35
180.1			0.37
257			0.35
353			0.42

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 436 mg

SAM-GLUCURONIDE: 103 mg

TOTAL: 539 mg 64 % OF DOSE ADMINISTERED

[14C] RECOVERY: 82.4 million dpm 94 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 73.0 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 10.0 million dpm

EXPERIMENT: 40 mg-kg control

DOG: 34083 DATE: 12-2-82 TIME: 11:50 AM

WT: 23.1 kg

ORAL DOSE: 924 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			1.01
2.6		13.49	
7.0	7227	17.99	
10.3	4199	15.71	
14.4	3309	15.05	
17.9	3049	16.14	
21.9	2630	18.53	
30.0	2029	23.56	0.73
44.5	1313	9.44	
59.6	698	5.36	0.52
75.4	405	2.95	
90	212	1.23	
126.6	< 100	0.214	0.45
186.1			0.33
243			0.33
300			0.42

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 473 mg

SAM-GLUCURONIDE: 139 mg

TOTAL: 612 mg 66 % OF DOSE ADMINISTERED

[14C] RECOVERY: 57.1 million dpm 65 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 52.8 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 9.8 million dpm

EXPERIMENT: 40 mg-kg control

DOG: 34928 DATE: 2-17-83 TIME: 10:55 AM

WT: 23.1 kg

ORAL DOSE: 924 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.3-5.9 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			
4.0		3.58	0.84
8.8	4755	22.11	
12.3	3742	48.32	
15.5	3138	31.06	
20.5	3077	32.09	
32.4	2525	22.13	0.55
42.6	2090	17.40	
59.7	1380	12.35	0.40
75		8.61	
91	874	8.44	
121	420	3.42	0.15
183			< 0.15
248			< 0.15
306			< 0.15

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 427 mg

SAM-GLUCURONIDE: 130 mg

TOTAL: 557 mg 60 % OF DOSE ADMINISTERED

[14C] RECOVERY: 92.0 million dpm 105 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 75.6 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 17.1 million dpm

EXPERIMENT: 40 mg-kg control

DOG: 36002 DATE: 11-4-82 TIME: 10:55 AM

WT: 25.5 kg

ORAL DOSE: 1020 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.55
3.0		0.619	0.73
5.6	921	3.03	
8.6	2365	7.01	
11.9	3570	18.06	0.59
20.0	2129	12.04	
34.2	1555	9.53	
45	1146	7.03	0.47
60	831	5.16	0.24
75	550	3.52	
90.5	350	2.27	0.33
120	113	0.66	0.11
180			0.27
240			0.04
345			0.24

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 682 mg

SAM-GLUCURONIDE: 279 mg

TOTAL: 961 mg 94 % OF DOSE ADMINISTERED

[14C] RECOVERY: 94.2 million dpm 107 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 79.0 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 17.4 million dpm

EXPERIMENT: 40 mg-kg control

DOG: 35255 DATE: 3-8-83 TIME: 11:30 AM

WT: 23.2 kg

ORAL DOSE: 928 mg

IV DOSE: 40 uCi TIME ADMINISTERED(a): 2.9-3.3 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.94
3.1		42.9	
6.2	4812	41.1	
9.4	4715	36.9	
12.8	3636	27.5	
16.5	2956	20.5	
20.2	2210	19.3	
30.4	1575	13.0	
47.1	1064	9.98	
61.7	663	6.33	0.38
75	515	4.96	
90		4.16	
126	98	0.80	0.23
190		0.02	
233			0.38
310			

(a) Time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 588 mg

SAM-GLUCURONIDE: 78.5 mg

TOTAL: 666.5 mg 72 % OF DOSE ADMINISTERED

[14C] RECOVERY: 71.9 million dpm 82 % OF DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 62.8 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 9.5 million dpm

EXPERIMENT: Tracer alone (IV)

DOG: 34927 DATE: 2-15-83 TIME: 10:33 AM

Wt: 23 kg

IV DOSE: 40 uCi

SAMPLING TIME (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		0.91
1.1	2087	
2.15	806	
3.15	455	
4.2	341	
6.15	185	
8.2	92	
10.0	59	
121.5		0.73

[14C] RECOVERY: 103 million dpm 117% of DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE: 103.7 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE: 0.6 million dpm

EXPERIMENT: Tracer alone (IV)

DOG: 34084 DATE: 4-26-83 TIME: 9:15 AM

Wt: 21 kg

IV DOSE: 40 uCi

SAMPLING TIME (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		0.77
1.0	5969	
2.0	1406	
3.05	755	
4.0	705	
6.0	227	
8.0	148	
11.0	58	
121		0.76

[14C] RECOVERY: 95 million dpm 108% of DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE: 88.9 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE: 0.8 million dpm

EXPERIMENT: Tracer alone (IV)

DOG: 34928 DATE: 3-16-83 TIME: 10:30 AM

Wt: 23 kg

IV DOSE: 40 uCi

SAMPLING TIME (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		0.67
1.15	1666	
2.4	841	
3.6	449	
4.8	293	
6.4	161	
8.4	109	
10.6	69	
120		0.63

[14C] RECOVERY: 96 million dpm 109% of DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE: 101.6 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE: 0.9 million dpm

EXPERIMENT: Tracer alone (oral)

DOG: 34927 DATE: 3-1-83 TIME: 10:00 AM

Wt: 23 kg

ORAL DOSE: 40 uCi

SAMPLING TIME (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		
2.0	0	
4.2	2	
5.2	2	
7.0	2	
8.8	3	
11.5	6	
14.5	7	
18.9	5	
31.3	4	

[14C] RECOVERY: 87 million dpm 99% of DOSE ADMINISTERED

EXPERIMENT: Tracer alone (oral)

DOG: 34084 DATE: 5-19-83 TIME: 9:20 AM

Wt: 21 kg

ORAL DOSE: 40 uCi

SAMPLING TIME (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		
2.7	3	
6.0	20	
9.0	32	
12.2	41	
15.2	41	
20.1	40	
30.0	35	
45.8	27	
60	26	
90	16	
120	27	

[14C] RECOVERY: 95 million dpm 108% of DOSE ADMINISTERED

EXPERIMENT: Tracer alone (oral)

DOG: 34928 DATE: 4-21-83 TIME: 9:40 AM

Wt: 20 kg

ORAL DOSE: 40 uCi

SAMPLING TIME (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		
2.2	0	
4.0	4.2	
6.0	6.8	
8.0	5.8	
11.0	8.1	
14.0	9.4	
17.2	6.7	
20.5	4.6	
25.5	4.5	
32.0	6.9	

[14C] RECOVERY: 90 million dpm 102% of DOSE ADMINISTERED

APPENDIX 2

Table A2-1. Salicylamide (SAM) clearance (l/min) in dogs adminisration of SAM, 5 mg/kg, in control (Chapter 3) and twelve hours after sulfate depletion with SAM, 80 mg/kg.

Dog	5 mg/kg SAM control	5 mg/kg SAM after depletion
34927	4.4	0.9
34084	2.4	1.1
34083	4.1	1.9
36002	4.7	2.9
35255	2.8	3.4
mean	3.7	2.0
SD	1.0	1.1

Table A2-2. Salicylamide (SAM) half-life (min) in dogs after administration of SAM, 5 mg/kg, in control experiments and twelve hours after sulfate depletion with SAM, 80 mg/kg.

Dog	5 mg/kg SAM control	5 mg/kg SAM after depletion
34927	3.2	15.2
34084	4.2	10.3
34083	4.5	15.5
36002	4.3	2.7
35255	5.6	6.1
mean	4.4	9.4
SD	0.8	6.2

Table A2-3. Salicylamide (SAM) volume of distribution (l/kg) in dogs after administration of SAM, 5 mg/kg, in control experiments and twelve hours after sulfate depletion with SAM, 80 mg/kg.

Dog	5 mg/kg SAM control	5 mg/kg SAM after depletion
34927	0.8	0.4
34084	0.7	0.8
34083	1.5	1.9
36002	1.2	0.6
35255	1.1	0.7
mean	1.1	0.9
SD	0.3	0.6

Table A2-4. Salicylamide (SAM) bioavailability in dogs after administration of SAM, 5 mg/kg, in control experiments (Chapter 3) and twelve hours after sulfate depletion with SAM, 80 mg/kg.

Dog	5 mg/kg SAM control	5 mg/kg SAM after depletion
34927	0.02	0.38
34084	0.38	0.21
34083	0.26	0.35
36002	0.10	0.01
35255	0.33	0.02
mean	0.22	0.19
SD	0.15	0.18

Table A2-5. Clearance of salicylamide (SAM) to the sulfate conjugate (l/min) in dogs after administration of SAM, 5 mg/kg in control experiments (Chapter 3) and twelve hours after sulfate depletion with SAM, 80 mg/kg.

Dog	5 mg/kg SAM control	5 mg/kg SAM after depletion
34927	2.9	0.7
34084	2.0	1.0
34083	3.4	1.7
36002	4.0	2.6
35255	2.1	1.7
mean	2.9	1.6
SD	0.8	0.7

Table A2-6. Clearance of salicylamide (SAM) to the glucuronide conjugate (l/min) in dogs after administration of SAM, 5mg/kg in control experiments (Chapter 3) and twelve hours after sulfate depletion with SAM, 80 mg/kg.

Dog	5 mg/kg SAM control	5 mg/kg SAM after depletion
34927	0.042	0.080
34084	0.091	0.065
34083	0.091	0.039
36002	0.170	0.086
35255	0.081	0.119
mean	0.095	0.078
SD	0.047	0.029

Table A2-7. Clearance (l/min) of salicylamide (SAM) after oral adminisration of SAM, 20 mg/kg, with sulfate donors.

Dog	SAM Dose (mg/kg)		
	IV sodium sulfate	oral sodium sulfate	oral N-Acetyl cysteine
34927	1.1	1.1	1.2
34084	0.6	1.3	0.5
34083	0.9	1.2	
34928	0.5	1.2	0.7
36002	2.7	1.9	
35255	1.2	1.7	2.2
mean	1.2	1.4	1.2
SD	0.8	0.3	0.7

Table A2-8. Salicylamide (SAM) half-life (min) after oral adminisration of SAM, 20 mg/kg, with sulfate donors.

Dog	SAM Dose (mg/kg)		
	IV sodium sulfate	oral sodium sulfate	oral N-Acetyl cysteine
34927	11.4	13.7	8.5
34084	15.0	14.9	20.2
34083	15.6	15.5	
34928	13.9	12.9	17.0
36002	10.5	5.7	
35255	19.4	7.2	14.6
mean	14.3	11.6	15.1
SD	3.1	4.1	4.9

Table A2-9. Salicylamide (SAM) volume of distribution (l/kg) after oral adminisration of SAM, 20 mg/kg, with sulfate donors.

Dog	SAM Dose (mg/kg)		
	IV sodium sulfate	oral sodium sulfate	oral N-Acetyl cysteine
34927	0.8	0.9	0.6
34084	0.6	1.3	0.5
34083	0.9	1.2	
34928	0.5	0.9	0.8
36002	1.6	0.6	
35255	1.4	0.8	2.0
mean	1.0	1.0	1.0
SD	0.4	0.3	0.7

Table A2-10. Salicylamide (SAM) bioavailability after oral adminisration of SAM, 20 mg/kg, with sulfate donors.

Dog	SAM Dose (mg/kg)		
	IV sodium sulfate	oral sodium sulfate	oral N-Acetyl cysteine
34927	0.28	0.28	0.14
34084	0.67	0.29	0.66
34083	0.51	0.35	
34928	0.74	0.40	0.47
36002	0.25	0.05	
35255	1.02	0.12	0.45
mean	0.58	0.25	0.43
SD	0.29	0.14	0.20

EXPERIMENT: 5 mg/kg, PRIOR DEPLETION

DOG: 34927 DATE: 9-9-83 TIME: 10:45 AM

Wt: 23.4 kg

ORAL SAM DOSE: 119.5 mg

IV SAM DOSE: 30 uCi TIME ADMINISTERED(a): 4.25-4.6 min

ORAL DEPLETION DOSE (SAM, 80 mg/kg) at 10:45 PM on 9-8-83

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-12 hr			0.76
Blank			0.25
2.2		0.0428	
4.0		4.16	
6.0	8725	4.79	
8.5	4737	3.62	
11.0	3509	3.35	
15	2139	0.191	
20	1500	0.127	
27.05	889		
36.7	617	0.111	
42.45	528		
50	373		
60.4	211		

[14C] RECOVERY: 64 million dpm 94% of DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 57 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 6.5 million dpm

EXPERIMENT: 5 mg/kg, PRIOR DEPLETION

DOG: 34084 DATE: 9-16-83 TIME: 10:48 AM

Wt: 22.4 kg

ORAL SAM DOSE: 112 mg

IV SAM DOSE: 30 uCi TIME ADMINISTERED(a): 4.3-4.6 min

ORAL DEPLETION DOSE (SAM, 80 mg/kg) at 11:15 PM on 9-15-83

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-12 hr			0.66
Blank			0.25
2.3		0.022	
4.0		0.728	
6.0	7915	1.24	
8.0	4977	2.17	
11.0	3456	1.47	
15	1998	0.494	
20	1226	0.267	
27	705	0.267	
35.4	455	0.158	
42.4	264	0.057	
50.1	181		
30	76		

[14C] RECOVERY: 71 million dpm 105% of DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 66 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 4.3 million dpm

EXPERIMENT: 5 mg/kg, PRIOR DEPLETION

DOG: 34083 DATE: 9-13-83 TIME: 10:30 AM

Wt: 22.7 kg

ORAL SAM DOSE: 113.5 mg

IV SAM DOSE: 30 uCi TIME ADMINISTERED(a): 15.5-15.9 min

ORAL DEPLETION DOSE (SAM, 80 mg/kg) at 10:45 PM on 9-12-83

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-12 hr			0.85
Blank			0.33
2.0			
4.0		0.759	
7.2		1.74	
9.15		1.85	
11.0		1.64	
15		0.251	
17.4	3558		
20	2533	0.429	
24.3	1263		
27	1101	0.045	
35	569	0.165	
41.9	329		
51.3	193		
60.1	152		
72.1	103		

[14C] RECOVERY: 68 million dpm 100% of DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 66 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 1.5 million dpm

EXPERIMENT: 5 mg/kg, PRIOR DEPLETION

DOG: 36002 DATE: 9-8-83 TIME: 9:45 AM

Wt: 27 kg

ORAL SAM DOSE: 135 mg

IV SAM DOSE: 30 uCi TIME ADMINISTERED(a): 4.6-4.9 min

ORAL DEPLETION DOSE (SAM, 80 mg/kg) at 10:00 PM on 9-7-83

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-12 hr			0.47
Blank			0.20
3.2		0.028	
5.8	10997	0.062	
7.8	1364	0.046	
9.8	558	0.046	
12.0	263	0.024	
15	143	0.010	

[14C] RECOVERY: 68 million dpm 99% of DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 65 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 2.1 million dpm

EXPERIMENT: 5 mg/kg, PRIOR DEPLETION

DOG: 35255 DATE: 9-14-83 TIME: 10:40 AM

Wt: 25.7 kg

ORAL SAM DOSE: 128.5 mg

IV SAM DOSE: 30 uCi TIME ADMINISTERED(a): 4.6-5.0 min

ORAL DEPLETION DOSE (SAM, 80 mg/kg) at 10:15 PM on 9-13-83

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-12 hr			0.42
Blank			0.14
2.1		0.038	
4.0		0.020	
6.0	8007	0.037	
8.0	1246	0.043	
11.0	511	0.062	
15.2	242	0.040	
20		0.016	

[14C] RECOVERY: 38 million dpm 57% of DOSE ADMINISTERED

RECOVERED AS [14C]-SAM-SULFATE 36 million dpm

RECOVERED AS [14C]-SAM-GLUCURONIDE 2.6 million dpm

EXPERIMENT: 20 mg/kg + SULFATE INFUSION

DOG: 34927 DATE: 5-10-83 TIME: 11:10 AM

Wt: 25 kg

ORAL SAM DOSE: 494 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-20.0			1.08
-5.2			1.50
-1.5			1.66
3.0		1.51	
6.0	9601	2.02	
9.0	3215	3.17	2.08
12.0	2105	3.71	
16.0	1527	5.73	
20	1268	5.66	
30	646	2.25	1.56
60	113	0.316	1.32
75.2	41	0.120	
91.3		0.060	1.30
120.7			1.21
180			1.13
245			1.10
345			0.98

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 359 mg

SAM-GLUCURONIDE: 40 mg

TOTAL: 399 mg 81 % OF DOSE ADMINISTERED

[14C] RECOVERY: 93 million dpm 103% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + SULFATE INFUSION

DOG: 34083 DATE: 5-24-83 TIME: 10:15 AM

Wt: 24 kg

ORAL SAM DOSE: 484 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.5-5.1 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-20.0			1.13
-3.7			1.78
3.0		2.00	2.10
6.0	9975	9.86	
9.0	4850	11.90	2.10
12.4	3642	16.95	
15.0	3043	12.41	
20	2255	9.5	
30	1324	4.61	1.78
45	571	1.34	
60	296	0.80	1.51
75	159	0.50	
90	76	0.25	1.45
120		0.060	
180			1.18

(a) time after oral dose

COLD RECOVERY (In SAM equivalents) (data missing)

SAM-SULFATE: mg

SAM-GLUCURONIDE: mg

TOTAL: mg % OF DOSE ADMINISTERED

[14C] RECOVERY: 89 million dpm 99% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + SULFATE INFUSION

DOG: 34084 DATE: 4-7-83 TIME: 11:20 AM

Wt: 22 kg

ORAL SAM DOSE: 440 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.5-5.6 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-19.1			0.78
-15.7			0.94
-11.2			1.01
-6.4			1.37
-1.4			1.54
3.2		2.81	
6.3	14521	6.21	
9.4	6129	11.0	1.93
12.4	4509	19.7	
15.3	3729	22.1	
20.0	2728	12.9	
30.3	1519	5.47	1.29
45	898	2.69	
60	536	1.74	1.28
79.5	182	0.38	
94	89	0.23	0.88
120		0.0.98	0.70
182			0.72
240			0.64
323			0.64

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 303 mg

SAM-GLUCURONIDE: 36 mg

TOTAL: 339 mg 77 % OF DOSE ADMINISTERED

[14C] RECOVERY: 78 million dpm 86% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + SULFATE INFUSION

DOG: 34928 DATE: 6-1-83 TIME: 9:45 AM

Wt: 20.9 kg

ORAL SAM DOSE: 418 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 6.1-6.9 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-20.3			0.74
-15.3			0.80
-9.8			1.17
-5.3			1.32
-0.3			1.32
3.0		17.4	
6.2		12.2	
9.7	14539	12.8	1.61
12.0	5572	7.01	
15.2	3614	8.67	
20.0	2472		
31.0	1797	6.28	1.30
45.8	1203	7.68	
60.3	758	3.40	1.21
77	248	1.36	1.10
89	228	0.954	
119.8		0.156	0.96
179.8			0.87
242			0.78
340.5			0.76

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 356 mg

SAM-GLUCURONIDE: 23 mg

TOTAL: 379 mg 91 % OF DOSE ADMINISTERED

[14C] RECOVERY: 86 million dpm 95% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + SULFATE INFUSION

DOG: 36002 DATE: 4-20-83 TIME: 9:50 AM

Wt: 25.5 kg

ORAL SAM DOSE: 510 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 5.0-5.5 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-17			0.77
-13.7			0.99
-7.8			0.96
-2.5			1.07
2.2		2.35	1.07
3.8		2.59	
6.2	12135	3.57	
8.3	3206	3.02	
11	1675	2.26	1.55
15	1031	1.61	
20	555	1.01	
31	258	0.545	1.18
45	99	0.231	
60		0.0832	0.92
76		0.0566	
900		0.0453	0.88
131			0.79

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 323 mg

SAM-GLUCURONIDE: 26 mg

TOTAL: 349 mg 69 % OF DOSE ADMINISTERED

[14C] RECOVERY: 82 million dpm 91% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + SULFATE INFUSION

DOG: 35255 DATE: 6-2-83 TIME: 10:15 AM

Wt: 23.9 kg

ORAL SAM DOSE: 478 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.3-4.8 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
-20.0			0.68
-1.7			1.30
3.1		27.03	2.10
7.3	6116	21.64	
10.2	3520	15.28	1.69
13	2606	11.01	
16	1924	9.90	
20	1647	8.56	
30	907	6.40	1.10
45	463	2.80	
61	334	1.77	0.89
80	141	0.763	
103		0.284	0.95
135.8			0.74

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 324 mg

SAM-GLUCURONIDE: 18 mg

TOTAL: 341 mg 71 % OF DOSE ADMINISTERED

[14C] RECOVERY: 86 million dpm 95% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL N-ACETYLCYSTEINE

DOG: 34927 DATE: 7-20-83 TIME: 12:30 AM

Wt: 24.1 kg

ORAL SAM DOSE: 482 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.2-4.8 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			1.07
3.2		1.348	
6.1	10763	2.428	
9.41	4593	2.626	
12.2	1916	2.127	
15	2261	2.000	0.82
20	1427	1.369	
34.8	474	0.385	0.82
46.4	203	0.205	
60		0.0679	0.65
90.9			0.60
120			0.58

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 270 mg

SAM-GLUCURONIDE: 21 mg

TOTAL: 291 mg 60 % OF DOSE ADMINISTERED

[14C] RECOVERY: 88 million dpm 98% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL N-ACETYLCYSTEINE

DOG: 34084 DATE: 7-22-83 TIME: 10:35 AM

Wt: 21.4 kg

ORAL SAM DOSE: 428 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-4.5 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.62
3.05		3.092	
6.0	13011	8.600	
9.05	7824	14.707	
12.05	6081	20.881	
14.9	4824	20.488	0.52
20	3658	14.733	
30	2128	6.859	0.42
45	1209	3.461	
60	757	1.968	0.36
75	610	1.661	
90	300	0.810	0.45
130.5	70	0.179	0.42

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 320 mg

SAM-GLUCURONIDE: 36 mg

TOTAL: 356 mg 74 % OF DOSE ADMINISTERED

[14C] RECOVERY: 54 million dpm 96% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL N-ACETYLCYSTEINE

DOG: 34928 DATE: 7-27-83 TIME: 10:00 AM

Wt: 22.2 kg

ORAL SAM DOSE: 444 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-4.5 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.88
3.0		0.397	
6.1	3912	2.27	
9.0	3767	4.75	
12.0	3403	6.80	
15.0	3235	7.39	0.76
20.05	3658	6.33	
30	2125	7.98	0.68
46	1352	3.05	
60	963	1.96	0.58
75	575	1.23	
90	329	0.165	0.60
120	85	0.616	0.60

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 243 mg

SAM-GLUCURONIDE: 26 mg

TOTAL: 169 mg 61 % OF DOSE ADMINISTERED

[14C] RECOVERY: 68 million dpm 76% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL N-ACETYLCYSTEINE

DOG: 35255 DATE: 8-2-83 TIME: 11:30 AM

Wt: 24 kg

ORAL SAM DOSE: 480 mg

IV SAM DOSE: 30 uCi TIME ADMINISTERED(a): 4.7-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			
3.05		0.122	0.80
6.0	4121	0.262	
9.15	1070	0.351	
12.0	638	0.552	
15.0	542	1.56	
20.05	497	3.53	
30.1	311	2.75	0.58
45.25	145	1.15	
64.7	60	0.241	0.48
75		0.117	
90		0.038	0.60
120			0.57

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 320 mg

SAM-GLUCURONIDE: 36 mg

TOTAL: 356 mg 74 % OF DOSE ADMINISTERED

[14C] RECOVERY: 54 million dpm 96% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL SULFATE

DOG: 34927 DATE: 11-30-82 TIME: 10:00 AM

Wt: 24.2 kg

ORAL SAM DOSE: 484 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.2-5.3 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.95
3.0		0.15	
6.7	4623	0.88	
10.1	2710	0.92	
12.5	2567	1.97	
15.8		2.84	
20.6	1738	2.78	
29.7	1178	2.19	1.12
46.2	564	1.01	
60.5	246		1.38
90.8		0.082	1.50
118.3		0.037	1.60
182			1.38
253			1.29
334			1.23

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 245 mg

SAM-GLUCURONIDE: 42 mg

TOTAL: 245 mg 60 % OF DOSE ADMINISTERED

[14C] RECOVERY: 60 million dpm 78% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL SULFATE

DOG: 34084 DATE: 3-15-83 TIME: 10:30 AM

Wt: 21 kg

ORAL SAM DOSE: 420 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 3.8-4.2 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.84
3.2		1.20	
7.2	3521	2.23	
9.0	4304	1.67	
12.8	2305	4.06	
15.7	1831	3.25	
20.4	1361	2.68	
30	816	1.18	0.80
48.4	234	0.280	
61.5	168	0.143	0.88
75.0	117	0.071	
89.6		0.010	0.98
119.8			1.12
179			1.29
234			1.21
320			1.14

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 275 mg

SAM-GLUCURONIDE: 28 mg

TOTAL: 303 mg 72 % OF DOSE ADMINISTERED

[14C] RECOVERY: 77 million dpm 88% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL SULFATE

DOG: 34083 DATE: 3-31-83 TIME: 9:50 AM

Wt: 22.4 kg

ORAL SAM DOSE: 448 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-4.8 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			1.04
3.1		0.70	0.98
6.7	6555	2.03	
9.1	3196	2.23	
12.4	1986	1.68	
15.3	1636	2.98	
20.0	1492	4.47	
30.0	907	2.62	1.16
45.1	358	0.80	
30.0	153	0.315	1.37
81.6	83	0.148	
94.1		0.092	1.48
120		0.016	1.34

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 314 mg

SAM-GLUCURONIDE: 26 mg

TOTAL: 340 mg 76 % OF DOSE ADMINISTERED

[14C] RECOVERY: 77 million dpm 86% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL SULFATE

DOG: 34928 DATE: 1-25-83 TIME: 9:31 AM

Wt: 23.1 kg

ORAL SAM DOSE: 462 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.8-5.9 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			
3.1		0.45	0.92
7.1	2698	1.41	
10.5	2224	1.58	
12.6	2086	1.85	
16.0	1807	2.58	
19.9	1702	3.70	
29.9	1301	3.70	0.94
45.0	787	1.70	
60.4	359	0.78	1.10
78.4		0.33	
95.4	53	0.14	
119.8		0.037	1.26
180			1.21
240			1.13
356			1.04

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 223 mg

SAM-GLUCURONIDE: 57 mg

TOTAL: 280 mg 61 % OF DOSE ADMINISTERED

[14C] RECOVERY: 68 million dpm 76% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL SULFATE

DOG: 36002 DATE: 5-31-83 TIME: 9:15 AM

Wt: 25.3 kg

ORAL SAM DOSE: 506 mg

IV SAM DOSE: 40 uCi TIME ADMINISTERED(a): 4.0-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.64
3.0		0.661	0.55
6.0	13124	0.882	
9.0	2265	0.824	
12.0	1100	0.696	
15	626	0.575	
20	196	0.453	
30.4	94	0.1225	0.46
60.1			0.51
90.4			0.55
120			0.58

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 369 mg

SAM-GLUCURONIDE: 25 mg

TOTAL: 394 mg 78 % OF DOSE ADMINISTERED

[14C] RECOVERY: 89 million dpm 99% of DOSE ADMINISTERED

EXPERIMENT: 20 mg/kg + ORAL SULFATE

DOG: 35255 DATE: 4-28-83 TIME: 10:00 AM

Wt: 22 kg

ORAL SAM DOSE: 440 mg

IV SAM DOSE: 38 uCi TIME ADMINISTERED(a): 4.0-5.0 min

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM CONC. (mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank			0.67
3.0		3.423	
6.0	9624	2.525	
9.0	2628	2.056	
15	990	1.410	
17.3	778	0.911	
20	481	0.534	
30.2	158	0.174	1.03
45	50	0.0583	
60			1.01
90			1.23
120			1.06

(a) time after oral dose

COLD RECOVERY (In SAM equivalents)

SAM-SULFATE: 303 mg

SAM-GLUCURONIDE: 20 mg

TOTAL: 323 mg 73 % OF DOSE ADMINISTERED

[14C] RECOVERY: 71 million dpm 83% of DOSE ADMINISTERED

APPENDIX 3

Table A3-1. Clearance (l/min) of tracer and 5-mg/kg dose of salicylamide (SAM) in dogs 12 hours before and 12 hours after sulfate depletion with SAM, 80 mg/kg.

Dog	Before depletion		After Depletion	
	Tracer	5 mg/kg	Tracer	5 mg/kg
34927	10.4	3.8	7.6	1.4
34084	9.8	3.5	9.4	2.8
34083	13.0	2.3	4.2	0.9
mean	11.1	3.2	7.1	1.7
SD	1.7	0.8	2.6	1.0

Table A3-2. Salicylamide (SAM) clearance (l/min) in dogs before and during various time periods after oral administration of a 40-mg/kg dose of SAM.

Dog	Time Period (a)			
	I	II	III	IV
34927	10.3	0.9	4.6	17.1
34084	13.7	0.6	5.3	12.0
34083	10.3	0.3	1.4	9.5
mean	11.4	0.6	3.8	12.9
SD	2.0	0.3	2.1	3.8

(a) Period I = prior to SAM dose, periods II, III and IV = 5-120, 120-180 and 240-270 minutes, respectively.

EXPERIMENT: TRACER AND 5 mg/kg, CONTROL

DOG: 34927 Wt: 23.3 kg

STUDY A: IV TRACER ALONE (30 uCi) GIVEN AT: 9:03 AM

TIME(a) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
Blank		0.86
1.04	3208	
2.08	827	
3.02	481	
4.0	297	
5.03	208	
5.97	147	

(a) Time after IV dose

STUDY B: ORAL 5 mg/kg DOSE + IV TRACER (30 uCi)

ORAL DOSE GIVEN AT 10:05 AM
TIME OF IV TRACER ADMINISTRATION(B): 4.55-4.75 min

TIME(b) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
6.0	5662	
9.0	1032	
10.98	562	
15.04	189	

(b) Time after oral dose

EXPERIMENT: TRACER AND 5 mg/kg, POST-DEPLETION

DOG: 34927 Wt: 23.3 kg DATE: 9-28-83

ORAL DEPLETION DOSE (SAM, 80 mg/kg) at 9:00 PM on 9-27-83

STUDY A: IV TRACER ALONE (30 uCi) GIVEN AT: 9:02 AM

TIME(a) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
Blank		0.06
1.0	4094	
2.08	1438	
3.0	731	
4.0	448	
5.0	319	
6.0	238	
7.0	192	

(a) Time after IV dose

STUDY B: ORAL 5 mg/kg DOSE + IV TRACER (30 uCi)

ORAL DOSE GIVEN AT 10:04 AM
TIME OF IV TRACER ADMINISTRATION(B): 4.55-4.90 min

TIME(b) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
6.0	7140	
8.07	3512	
9.0	3023	
11.0	2205	
15.12	1417	
20.0	1060	
27.04	559	
35.0	345	
42.0	286	
50.6	196	

(b) Time after oral dose

EXPERIMENT: TRACER AND 5 mg/kg, CONTROL

DOG: 34084 Wt: 23 kg

STUDY A: IV TRACER ALONE (30 uCi) GIVEN AT: 9:00 AM

TIME(a) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
Blank		0.80
1.03	3344	
2.0	980	
3.03	498	
4.0	306	
5.0	194	
6.03	155	

(a) Time after IV dose

STUDY B: ORAL 5 mg/kg DOSE + IV TRACER (30 uCi)

ORAL DOSE GIVEN AT 10:07 AM
TIME OF IV TRACER ADMINISTRATION(B): 4.50-4.95 min

TIME(b) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
6.0	7107	
8.13	1063	
11.0	416	
15.0	286	
20.0	150	

(b) Time after oral dose

EXPERIMENT: TRACER AND 5 mg/kg, POST-DEPLETION

DOG: 34084 Wt: 23 kg DATE: 10-5-83

ORAL DEPLETION DOSE (SAM, 80 mg/kg) at 10 PM on 10-4-83

STUDY A: IV TRACER ALONE (30 uCi) GIVEN AT: 9:00 AM

TIME(a) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
Blank		0.09
1.0	4109	
2.0	2037	
3.0	1310	
4.0	1033	
5.0	954	
6.0	828	
7.03	588	

(a) Time after IV dose

STUDY B: ORAL 5 mg/kg DOSE + IV TRACER (30 uCi)

ORAL DOSE GIVEN AT 10:03 AM
TIME OF IV TRACER ADMINISTRATION(b): 4.5-5.0 min

TIME(b) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
6.44	2654	
8.05	2526	
11.0	1145	
15.0	829	
20.03	551	
27.0	310	
35.45	171	
42.42	134	

(b) Time after oral dose

EXPERIMENT: TRACER AND 5 mg/kg, CONTROL

DOG: 34083 Wt: 23 kg

STUDY A: IV TRACER ALONE (30 uCi) GIVEN AT: 10:13 AM

TIME(a) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
Blank		0.81
1.0	2117	
2.0	868	
3.2	487	
4.0	397	
5.0	285	
6.0	234	
7.0	181	

(a) Time after IV dose

STUDY B: ORAL 5 mg/kg DOSE + IV TRACER (30 uCi)

ORAL DOSE GIVEN AT 11:16 AM
TIME OF IV TRACER ADMINISTRATION(B): 4.7-5.1 min

TIME(b) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
6.2	4992	
8.1	2866	
11.0	1817	
15.0	889	
20.0	546	
25.0	342	
35.0	151	

(b) Time after oral dose

EXPERIMENT: TRACER AND 5 mg/kg, POST-DEPLETION

DOG: 34083 Wt: 23 kg DATE: 9-30-83

ORAL DEPLETION DOSE (SAM, 80 mg/kg) at 10 PM on 9-29-83

STUDY A: IV TRACER ALONE (30 uCi) GIVEN AT: 10:35 AM

TIME(a) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
Blank		0.00
1.0	4109	
2.4	2037	
3.5	1310	
4.5	1033	
5.2	954	
6.0	828	
7.0	588	
9.0	413	
11.05	304	
15.05	214	

(a) Time after IV dose

STUDY B: ORAL 5 mg/kg DOSE + IV TRACER (30 uCi)

ORAL DOSE GIVEN AT 11:37 AM
TIME OF IV TRACER ADMINISTRATION(b): 4.5-4.9 min

TIME(b) (min)	SAM CONC. (dpm/ml)	SULFATE CONC. (mM)
6.0	6451	
8.0	3695	
11.03	2742	
15.0	2157	
20	1758	
27.05	1257	
35	907	
42	771	
50.4	544	
61.8	374	

(b) Time after oral dose

EXPERIMENT: 40 mg/kg, MULTIPLE TRACER

DOG: 34927 Wt: 25.3 kg DATE: 6-9-83

BASELINE KINETICS IV TRACER (40uCi) GIVEN AT: 9:25 AM

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		1.24
1.25	2122	
2.25	809	
3.25	450	
4.25	278	
5.35	241	
7.25	170	
9.25	114	
11.25	93	1.22

(a) Time after midpoint of IV dose

KINETICS AFTER 40-mg/kg ORAL DOSE

ORAL DOSE ADMINISTERED AT 9:48 AM

TIMES OF IV DOSE ADMINISTRATION (AFTER ORAL DOSE)

FIRST TRACER DOSE:	7.0-7.5 min	20 uCi
SECOND TRACER DOSE:	128.0-128.4 min	20 uCi
THIRD TRACER DOSE:	247.0-247.5 min	20 uCi

SAMPLING TIME(b) (min)	PLASMA SAM CONC. (mg/l)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM-SO4 CONC(mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
19.5		881		
38.2	3.53	590	11.5	
59.4	2.28	422		
79.7	2.02	266		
108.1	0.950	132		
124.3	0.618	107		0.42
134.5	0.361	344	16.6	
147.5	0.193	247		
159.0	0.266	113		
177.3		50		
197.5	0.030		4.74	
249.1		388		0.40
250.6		193		
256.6		47	3.74	
263.3		31		

EXPERIMENT: 40 mg/kg, MULTIPLE TRACER

DOG: 34084 Wt: 22.5 kg DATE: 6-7-83

BASELINE KINETICS IV TRACER (40uCi) GIVEN AT: 10:40 AM

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		1.00
1.25	4422	
2.25	963	
3.25	517	
4.25	305	
5.25	192	
6.25	141	
7.25	118	
9.25	77	
11.25	63	1.12

(a) Time after midpoint of IV dose

KINETICS AFTER 40-mg/kg ORAL DOSE

ORAL DOSE ADMINISTERED AT 11:02 AM

TIMES OF IV DOSE ADMINISTRATION (AFTER ORAL DOSE)

FIRST TRACER DOSE:	7.5-8.0 min	20 uCi
SECOND TRACER DOSE:	127.5-128.0 min	20 uCi
THIRD TRACER DOSE:	247.5-248.0 min	20 uCi

SAMPLING TIME(b) (min)	PLASMA SAM CONC. (mg/l)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM-SO4 CONC(mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
16.0		2810		
38.0	8.81	1005	11.3	
59.0	4.04	556		
78.0	1.85	244		
98.0	1.00	141		0.48
126.0	0.202	38		0.43
134.0		444	7.81	
148.0	0.065	93		0.56
163.0		36	6.40	
178.0	0.028	21		
198.0	0.012			
250.0	0.009	519		0.37

Table continued on the next page

continuation of table on previous page

SAMPLING TIME(b) (min)	PLASMA SAM CONC. (mg/l)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM-SO4 CONC(mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
251.5		230		
253	0.00	116	1.33	
255		66		

(b) Time after oral dose

EXPERIMENT: 40 mg/kg, MULTIPLE TRACER

DOG: 34083 Wt: 22.7 kg DATE: 7-29-83

BASELINE KINETICS IV TRACER (40uCi) GIVEN AT: 9:15 AM

SAMPLING TIME(a) (min)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		1.04
1.25	2995	
2.25	1445	
3.25	877	
4.25	679	
5.25	495	
6.25	373	
7.35	272	
9.35	195	
11.25	189	0.97

(a) Time after midpoint of IV dose

KINETICS AFTER 40-mg/kg ORAL DOSE

ORAL DOSE ADMINISTERED AT 9:45 AM

TIMES OF IV DOSE ADMINISTRATION (AFTER ORAL DOSE)

FIRST TRACER DOSE:	4.0-4.5 min	20 uCi
SECOND TRACER DOSE:	124.5-125.0 min	20 uCi
THIRD TRACER DOSE:	241.5-242.0 min	40 uCi

SAMPLING TIME(b) (min)	PLASMA SAM CONC. (mg/l)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM-SO4 CONC(mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
5.5		6623		
6.5		5353		
7.6		4323		
12.9		2621		
24.5	15.06	2268		1.04
44.5	10.56	1499		
64.4	7.75	991	24.2	
84.5	5.91	788		
104.5	3.29	446		
124.6	1.88	236	22.5	0.35
127.0		3224		0.45
128		2427		

Table continued on the next page

continuation of table on previous page

SAMPLING TIME(b) (min)	PLASMA SAM CONC. (mg/l)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA SAM-SO4 CONC(mg/l)	PLASMA INORGANIC SULFATE CONC. (mM)
129		1521		
134		1178		
141	0.835	872		
151	0.575	394		
161.5	0.494	303		
171	0.328	225		
186	0.196	135	12.7	
243		2232		0.35
248		1524		
249		1155		
250	0.027	713		
251		946	7.18	
252		657		
253		555		
255		414		
287		396		

(b) Time after oral dose

EXPERIMENT: SALICYLAMIDE AND SODIUM SULFATE INFUSION

DOG: 34927 WEIGHT: 24.5 kg

DATE: 3-15-84 TIME: 2:28 AM

INFUSION RATES (SALICYLAMIDE AND SODIUM SULFATE):
0.58 uMol/min-kg from 0 to 60.2 min
1.45 uMol/min-kg from 60.2 to 150.2 min
0.58 uMol/min-kg from 150.2 to 239.8 min

INFUSION RATE ([14C]-SALICYLAMIDE): 0.40 uCi/min

SAMPLING TIME (MIN)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (Mm)
Blank		0.71
2.6	206	
5.6	327	0.69
10.1	410	0.71
25.2	515	0.69
40.3	539	
51.6	432	
55	495	
60.1	450	0.67
65	523	0.67
75.6	621	0.73
90.1	739	
110	791	0.73
129.9	723	
141.5	864	
146.3	769	
150	801	0.77
155	822	0.69
165	744	0.73
180.3	834	
190.1	730	
210	673	
230.1	620	
235.7	680	
239.8	744	0.75

EXPERIMENT: SALICYLAMIDE AND SODIUM SULFATE INFUSION

DOG: 34084 WEIGHT: 21.2 kg

DATE: 3-13-84 TIME: 9:09 AM

INFUSION RATES (SALICYLAMIDE AND SODIUM SULFATE):
0.58 uMol/min-kg from 0 to 60.2 min
1.45 uMol/min-kg from 60.2 to 150.1 min
0.58 uMol/min-kg from 150.1 to 240.0 min

INFUSION RATE ([14C]-SALICYLAMIDE): 0.40 uCi/min

SAMPLING TIME (MIN)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (Mm)
Blank		0.94
2.7	221	
5.8	298	0.87
10	343	0.93
25	409	0.89
409	433	
50	479	
55	481	
60	535	0.87
65	595	0.87
75	656	0.93
90	773	
110	880	0.96
130	968	
140	1012	
145	965	
150	936	0.93
155	1001	0.93
165	1016	0.87
180	825	
190.1	856	
210	770	
230	680	
235	745	
240	695	0.85

EXPERIMENT: SALICYLAMIDE AND SODIUM SULFATE INFUSION

DOG: 34083 WEIGHT: 23.0 kg

DATE: 3-16-84 TIME: 10:38 AM

INFUSION RATES (SALICYLAMIDE AND SODIUM SULFATE):
0.58 uMol/min-kg from 0 to 60.5 min
1.45 uMol/min-kg from 60.5 to 150.2 min
0.58 uMol/min-kg from 150.2 to 240.0 min

INFUSION RATE ([14C]-SALICYLAMIDE): 0.40 uCi/min

SAMPLING TIME (MIN)	PLASMA [14C] SAM CONC. (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (Mm)
Blank		0.98
2.64	128	
5.6	210	0.96
10	273	0.98
25	362	0.96
40	388	
50	380	
55.2	290	
60	337	0.98
65.05	410	0.96
75.3	443	1.00
91.3	576	
110	569	1.00
130.3	684	
140.5	652	
145	678	
150	787	1.06
162.5	753	1.06
170.2	694	1.04
184	660	
191.5	635	
210.1	531	
230	551	
235	598	
240	595	1.02

APPENDIX 4

Table A4-1. Calculated average predicted (a) and actual measured value of specific activity of salicylamide-[35S]-sulfate in the urine after IV administration of salicylamide, 1 mg/kg, and inorganic [35S]-sulfate, 10 uCi.

Dog	Specific Activity (uCi/mmol)	
	Predicted	Actual Measured
34927	2.85	2.33
34084	2.75	2.20
34083	2.86	2.56
mean	2.82	2.36
SD	0.06	0.18

(a) See discussion, Chapter 6.

EXPERIMENT: SALICYLAMIDE, 1 mg/kg IV

DOG: 34927 WEIGHT: 24 kg

DATE: 3-22-83 TIME: 10:40 AM

SAMPLING TIME (a) (MIN)	PLASMA SAM CONC. (uMol/l)	PLASMA SAM-SULFATE CONC. (uMol/l)
0.93	12.9	0.16
1.7	9.51	1.56
2.92	5.91	3.68
3.9	4.39	6.16
5.2	3.34	7.84
6.6	2.47	8.59
8.6	1.60	9.24
10.5	0.98	9.91
12.9	0.57	10.80
15.9	0.32	10.73
22.6	0.18	10.37
33.1	0.08	8.96
64.3		5.30
120.3		2.1
182.2		0.96
235.3		0.51
320.3		0.22

(a) Time after midpoint of IV injection

EXPERIMENT: SALICYLAMIDE + [35S]-SULFATE

DOG: 34927 WEIGHT: 23 kg

DATE: 10-26-83 TIME: 11:06 AM

SAMPLING TIME (a) (MIN)	PLASMA SAM CONC. (mg/l)	PLASMA [35S] ACTIVITY (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (Mm)
Blank			0.93
1.25	2.15	8887	
2.2	1.66	6825	
3.2	1.403	5946	0.95
4.25	0.753	5226	
5.33	0.695	5374	
6.25	0.518	4979	0.99
9.2	0.188	4540	
12.5	0.083	4074	0.94
15.7	0.090	3770	
20.4	0.055	3718	0.96

(a) Time after midpoint of IV injection

SAM-SULFATE IN THE URINE

AMOUNT RECOVERED: 22.7 mg (in SAM equivalents)

SPECIFIC ACTIVITY: 2.33 uCi/mmol

EXPERIMENT: SALICYLAMIDE + [35S]-SULFATE

DOG: 34084 WEIGHT: 23 kg

DATE: 12-28-83 TIME: 10:01 AM

SAMPLING TIME (a) (MIN)	PLASMA SAM CONC. (mg/l)	PLASMA [35S] ACTIVITY (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (Mm)
Blank			0.88
1.2	2.50	6660	
2.2	1.10	5616	
3.2	0.66	4874	0.75
4.23	0.40	4321	
5.28	0.31	4250	
6.2	0.295	4212	0.83
9.23	0.11	3991	0.78
12.23	0.147	3955	0.83
15.2	0.148	3737	
20.2	0.087	3692	0.78

(a) Time after midpoint of IV injection

SAM-SULFATE IN THE URINE

AMOUNT RECOVERED: 23.5 mg (in SAM equivalents)

SPECIFIC ACTIVITY: 2.20 uCi/mmol

EXPERIMENT: SALICYLAMIDE + [35S]-SULFATE

DOG: 34083 WEIGHT: 22.5 kg

DATE: 11-3-83 TIME: 9:45 AM

SAMPLING TIME (a) (MIN)	PLASMA SAM CONC. (mg/l)	PLASMA [35S] ACTIVITY (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (Mm)
Blank			1.08
1.35	3.91	10743	
2.46	1.12	5561	1.09
3.36	0.852	5512	
4.35	0.66	5338	
5.43	0.413	4506	
6.45	0.301	4449	0.95
9.4	0.217	4077	
12.4	0.137	4144	0.96
14.4	0.089	4337	
20.4	0.040	3944	0.93

(a) Time after midpoint of IV injection

SAM-SULFATE IN THE URINE

AMOUNT RECOVERED: 21.2 mg (in SAM equivalents)

SPECIFIC ACTIVITY: 2.56 uCi/mmol

EXPERIMENT: [35S]-SULFATE, SALICYLAMIDE + SODIUM SULFATE

DOG: 34927 WEIGHT: 23 kg

DATE: 2-7-84 TIME: 11:17 AM

INORGANIC [35S]-SULFATE GIVEN AT 11:00 AM

SAMPLING TIME (a) (MIN)	PLASMA SAM CONC. (mg/l)	PLASMA [35S] ACTIVITY (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (Mm)
Blank		3018	0.65
1.75	0.792	3300	7.9
2.79	0.694	3354	7.2
3.95	0.444	3070	6.5
4.85	0.351	3148	
5.88	0.233	3095	
6.75	0.226	2999	6.2
9.85	0.173	2902	5.3
12.75	0.133	2915	5.0
15.75	0.049	2703	
20.75	0.029	2565	4.1
26.75	0.030	2300	
34.05	0.005	2116	

(a) Time after midpoint of IV injection of SAM and sodium sulfate

SAM-SULFATE IN THE URINE

AMOUNT RECOVERED: 17.6 mg (in SAM equivalents)

SPECIFIC ACTIVITY: < 0.31 uCi/mmol

EXPERIMENT: [35S]-SULFATE, SALICYLAMIDE + SODIUM SULFATE

DOG: 34084 WEIGHT: 17 kg

DATE: 2-8-84 TIME: 1:45 PM

INORGANIC [35S]-SULFATE GIVEN AT 12:57 PM

SAMPLING TIME (a) (min)	PLASMA SAM CONC. (mg/l)	PLASMA [35S] ACTIVITY (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		3370	0.65
2.19	0.973	3400	6.6
3.39	0.576	3326	5.3
4.34	0.486	3287	6.0
5.3	0.388	3324	
6.27	0.178	3278	
7.54	0.160	3345	4.9
9.64	0.174	3193	4.6
14.04	0.084	2965	4.3
16.9	0.057	2940	
20.84	0.044	2921	3.6
27.34	0.020	2612	

(a) Time after midpoint of IV injection of SAM and sodium sulfate

SAM-SULFATE IN THE URINE

AMOUNT RECOVERED: 18.1 mg (in SAM equivalents)

SPECIFIC ACTIVITY: < 0.33 uCi/mmol

EXPERIMENT: [35S]-SULFATE, SALICYLAMIDE + SODIUM SULFATE

DOG: 34083 WEIGHT: 23 kg

DATE: 2-13-84 TIME: 2:55 PM

INORGANIC [35S]-SULFATE GIVEN AT 2:39 PM

SAMPLING TIME (a) (min)	PLASMA SAM CONC. (mg/l)	PLASMA [35S] ACTIVITY (dpm/ml)	PLASMA INORGANIC SULFATE CONC. (mM)
Blank		3491	0.93
4.6	0.324	3382	5.5
6.0	0.209	3377	
7.7	0.128	3322	5.7
10.17	0.119	3191	5.9
13.35	0.075	2982	4.8
18.6	0.034	2952	4.9
25.3	0.018	2699	4.3

(a) Time after midpoint of IV injection of SAM and sodium sulfate

SAM-SULFATE IN THE URINE

AMOUNT RECOVERED: 16.8 mg (in SAM equivalents)

SPECIFIC ACTIVITY: < 0.56 uCi/mmol

APPENDIX 5

Table A5-1. Renal extraction ratios in dogs at various salicylamide infusion rates with and without sodium sulfate supplementaton.

	DOSING RATE (mg/min-kg)					
DOG #	0.0003	0.083	0.208	0.208 + SO4	0.5	0.5 +SO4
36804	.886	.165	.105	.028	.010	.110
36807	.845	.667	.207	.245	.051	.074
36851	.774	.575	.357	.321	.074	.105
35548	.772	.483	.203	.225	.063	.050
mean	.819	.473	.218	.095	.050	.085
SD	.050	.200	.100	.080	.020	.020

Table A5-2. Pulmonary extraction ratios in dogs at various salicylamide infusion rates with and without sodium sulfate supplementaton.

	DOSING RATE (mg/min-kg)					
DOG #	0.0003	0.083	0.208	0.208 + SO4	0.5	0.5 +SO4
36804	.373	-.109	-.066	-.056	.005	-.015
36807	.385	-.003	-.061	-.043	-.065	-.015
36851	.329	-.320	-.110	-.100	-.086	-.032
35548	.288	.009	-.079	-.055	-.157	-.087
mean	.344	-.110	-.080	-.060	-.081	.040
SD	.040	.150	.020	.030	.070	.030

Table A5-3. Forelimb extraction ratios in dogs at various salicylamide infusion rates with and without sodium sulfate supplementaton.

	DOSING RATE (mg/min-kg)					
DOG #	0.0003	0.083	0.208	0.208 + SO4	0.5	0.5 +SO4
36804	.375	.158	.086	.033	.103	.004
36807	.195	.213	.197	.137	.047	-.017
36851	.458	.201	.199	.115	.109	.088
35548	.730	.128	.085	.087	.089	.031
mean	.440	.175	.142	.093	.087	.027
SD	.220	.040	.060	.040	.030	.040

Table A5-4. Femoral clearance values (l/min) in dogs at various salicylamide infusion rates with and without sodium sulfate supplementaton.

	DOSING RATE (mg/min-kg)					
DOG #	0.0003	0.083	0.208	0.208 + SO4	0.5	0.5 +SO4
36804	4.76	1.52	0.98	1.25	0.45	0.78
36807	6.08	1.75	1.05	1.30	0.78	1.01
36851	4.68	1.83	1.46	1.39	0.76	0.96
35548	5.71	2.09	1.10	1.33	0.51	0.78
mean	5.31	1.80	1.15	1.32	0.62	0.88
SD	0.60	0.20	0.20	0.05	0.20	0.10

Table A5-5. Venous clearance values (l/min) in dogs at various salicylamide infusion rates with and without sodium sulfate supplementaton.

	DOSING RATE (mg/min-kg)					
DOG #	0.0003	0.083	0.208	0.208 + SO4	0.5	0.5 +SO4
36804	7.61	1.76	1.01	1.30	0.49	0.79
36807	7.57	1.91	1.34	1.42	0.80	1.02
36851	8.66	2.29	1.82	1.57	0.87	1.06
35548	9.11	2.39	1.22	1.46	0.55	0.83
mean	8.24	2.09	1.26	1.44	0.68	0.92
SD	0.70	0.30	0.20	0.10	0.20	0.10

EXPERIMENT: EXTRAHEPATIC EXTRACTION

DOG: 36807 WEIGHT: 23.2 kg

DATE: 1-19-84 TIME: 10:50 AM

INFUSION TIMES:

SAM	3.0 ug/min-kg from 0 to 15.7 min 500 ug/min-kg from 37.9 to 228.7 min
[14C]-SAM	2.5 uCi/min from 0 to 15.7 min 0.5 uCi/min from 37.9 to 228.7 min
SODIUM SULFATE	5.64 mg/min-kg from 150.1 to 162.1 min 0.75 mg/min-kg from 162.1 to 228.7 min

SAMPLING TIME (min)	PLASMA [14C]-SAM CONC (dpm/ml)				PLASMA SULFATE CONC. (mM)
	FORELIMB VEIN	PULMONARY ARTERY	FEMORAL ARTERY	RENAL VEIN	
Blank					0.67
2.95	575				
6.0	675				
9.0	685	1376	889	140	
12.2	778	1560	949	778	
15.4	719	1476	875	151	
37.8	25				0.52
42.8	568				0.59
52.9	735	753	822	750	0.50
68	1010	954	1123	1052	0.40
88	1160				
108	1215	1228	1250	1193	0.29
128	1257				
149.4	1311	1304	1339	1275	<0.20
165.1	1026	1081	1101	979	1.8
179.9	1108	1084	1099	1000	1.4
197.4	1070	1036	1024	1002	1.4
228.1	1128	1086	1129	1009	1.3

EXPERIMENT: EXTRAHEPATIC EXTRACTION

DOG: 36851 WEIGHT: 26 kg

DATE: 4-11-84 TIME: 11:18 AM

INFUSION TIMES:

SAM	3.0 ug/min-kg from 0 to 18.5 min 500 ug/min-kg from 33 to 228 min
[14C]-SAM	2.5 uCi/min from 0 to 18.5 min 0.5 uCi/min from 33 to 228 min
SODIUM SULFATE	5.64 mg/min-kg from 150.5 to 162.7 min 0.75 mg/min-kg from 162.7 to 228 min

SAMPLING TIME (min)	PLASMA [14C]-SAM CONC (dpm/ml)				PLASMA SULFATE CONC. (mM)
	FORELIMB VEIN	PULMONARY ARTERY	FEMORAL ARTERY	RENAL VEIN	
Blank					1.62
3.1	456				
6.0	583				
9.6	636	1759	1132	267	
13.2	648	1676	1120	240	
17.2	622	1810	1267	286	1.62
38.2	448				1.45
48.5	604	707	742	637	1.41
63	761	794	818	747	1.35
83.3	879				
103.5	967	1043	1107	1077	1.01
126.3	1125				
148.5	1143	1187	1314	1164	0.80
163.5	1110				2.86
167.6	1121	1149	1217	1077	2.73
182.7	1120	1120	1161	1084	2.79
198.6	1037	1150	1157	1016	2.79
226.7	970	1057	1113	968	2.79

EXPERIMENT: EXTRAHEPATIC EXTRACTION

DOG: 36804 WEIGHT: 23.2 kg

DATE: 1-12-84 TIME: 1:20 PM

INFUSION TIMES:

SAM	3.0 ug/min-kg from 0 to 15 min 500 ug/min-kg from 32.4 to 188 min
[14C]-SAM	2.5 uCi/min from 0 to 15 min 0.5 uCi/min from 3204 to 188 min
SODIUM SULFATE	5.64 mg/min-kg from 142.9 to 154.9 min 0.75 mg/min-kg from 154.9 to 188 min

SAMPLING TIME (min)	PLASMA [14C]-SAM CONC (dpm/ml)				PLASMA SULFATE CONC. (mM)
	FORELIMB VEIN	PULMONARY ARTERY	FEMORAL ARTERY	RENAL VEIN	
Blank					0.77
3.0	476				
5.9	628				
9.0	712	1789	1151	135	
11.8	729	1810	1114	131	
14.9	724	1931	1200	128	
29.4	20				0.70
37	407				
47.4	870	1005	1018	962	0.62
62.6	965	1097	1055	1067	0.45
82.5	1279				
102.3	1414	1542	1550	1556	0.25
123.5	1634				
141.6	1819	1908	1989	1857	<0.20
157.5	1554	1500	1547	1385	1.9
173.2	1471	1427	1464	1294	1.6
187.4	1384	1439	1419	1263	1.3

EXPERIMENT: EXTRAHEPATIC EXTRACTION

DOG: 36804 WEIGHT: 23.5 kg

DATE: 2-14-84 TIME: 10:38 AM

INFUSION TIMES:

SAM	0.083 mg/min-kg from 0 to 40 min 0.208 mg/min-kg from 40 to 187
[14C]-SAM	0.2 uCi/min from 0 to 40 0.5 uCi/min from 40 to 187
SODIUM SULFATE	2.4 mg/min-kg from 122 to 132.2 min 0.32 mg/min-kg from 132.2 to 187 min

SAMPLING TIME (min)	PLASMA [14C]-SAM CONC (dpm/ml)				PLASMA SULFATE CONC. (mM)
	FORELIMB VEIN	PULMONARY ARTERY	FEMORAL ARTERY	RENAL VEIN	
Blank					0.69
5.4	129	177	168	87	
9.5	180	189	202	149	
15.1	203	220	254	175	
21.3	220	233	278	192	0.60
31.5	252	260	290	269	
38.4	279	312	324	283	0.60
47.1	596	703	769	593	
56.1	712	882	880	821	
67.7	872	920	974	849	
80.4	929	1018	1078	893	0.42
100	1045	1066	1093	1031	
120	1087	1061	1179	1072	0.31
132.3	868	741	806	914	0.95
162.6	948	1072	1113	848	0.82
182.7	728	680	712	794	0.85

EXPERIMENT: EXTRAHEPATIC EXTRACTION

DOG: 36851 WEIGHT: 26 kg

DATE: 3-14-84 TIME: 11:13 AM

INFUSION TIMES:

SAM	0.083 mg/min-kg from 0 to 41 min 0.208 mg/min-kg from 41 to 180 min
[14C]-SAM	0.2 uCi/min from 0 to 41 min 0.5 uCi/min from 41 to 180 min
SODIUM SULFATE	2.4 mg/min-kg from 121 to 130 min 0.32 mg/min-kg from 130 to 180 min

SAMPLING TIME (min)	PLASMA [14C]-SAM CONC (dpm/ml)				PLASMA SULFATE CONC. (mM)
	FORELIMB VEIN	PULMONARY ARTERY	FEMORAL ARTERY	RENAL VEIN	
Blank					
5.4	149	175	197	67	1.45
10.9	171	172	225	87	
15.3	166	190	240	98	
20.5	173	172	225	76	
30.2	205	193	243	98	1.30
40.6	199	183	255	133	1.26
48.3	487	488	595	353	
58.1	544	563	660	406	
68.2	601	653	687	453	
81.2	575	626	750	464	1.03
105.1	591	705	727	472	
120.4	642	705	782	515	0.93
129.8	667	697	795	549	1.56
160.4	730	741	819	532	1.43
180.3	700	717	758	529	1.49

EXPERIMENT: EXTRAHEPATIC EXTRACTION

DOG: 36807 WEIGHT: 21.5 kg

DATE: 2-15-84 TIME: 10:44 AM

INFUSION TIMES:

SAM	0.083 mg/min-kg from 0 to 40.7 min 0.208 mg/min-kg from 40.7 to 186.5 min
[14C]-SAM	0.2 uCi/min from 0 to 40.7 min 0.5 uCi/min from 40.7 to 186.5 min
SODIUM SULFATE	2.4 mg/min-kg from 125.2 to 136.6 min 0.32 mg/min-kg from 136.6 to 186.5 min

SAMPLING TIME (min)	PLASMA [14C]-SAM CONC (dpm/ml)				PLASMA SULFATE CONC. (mM)
	FORELIMB VEIN	PULMONARY ARTERY	FEMORAL ARTERY	RENAL VEIN	
Blank					0.73
6.4	68				
12.9	139				
18.2	147				
21	126	231	249	62	0.64
25.4	172	229	255	76	
28.6	194	217	220	86	
39.9	230	289	284	90	0.61
47.6	545	635	712	438	
56.2	669	732	784	564	
67	760	892	959	700	
84.3	805	957	972	720	0.43
101	835	959	1037	828	
121.8		974	1056	881	0.34
135	849	946	1018	738	0.90
165	790	888	935	705	0.67
184.8	773	846	842	667	0.67

EXPERIMENT: EXTRAHEPATIC EXTRACTION

DOG: 35548 WEIGHT: 27.2 kg

DATE: 2-9-84 TIME: 10:36 AM

INFUSION TIMES:

SAM	500 ug/min-kg from 0 to 174.9 min
[14C]-SAM	0.5 uCi/min from 0 to 174.9 min
SODIUM SULFATE	5.64 mg/min-kg from 115 to 144 min 0.75 mg/min-kg from 144 to 174.9 min

SAMPLING TIME (min)	PLASMA [14C]-SAM CONC (dpm/ml)				PLASMA SULFATE CONC. (mM)
	FORELIMB VEIN	PULMONARY ARTERY	FEMORAL ARTERY	RENAL VEIN	
Blank					0.67
5.0	330				
16.2	712	581	805	728	0.51
30.4	926	805	1064	929	0.49
49.5	1245				
78.5	1358	1278	1463	1379	0.28
92	1492				
111	1556	1564	1691	1641	<0.20
131.7	1476	1361	1539	1440	2.6
149.2	1450	1304	1433	1363	2.8
162.6	1281	1300	1451	1348	2.3
174.9	1381	1302	1360	1309	2.2

EXPERIMENT: EXTRAHEPATIC EXTRACTION

DOG: 35548 WEIGHT: 25 kg

DATE: 4-25-84 TIME: 10:00 AM

INFUSION TIMES:

SAM	3.0 ug/min-kg from 0 to 12 min 0.083 mg/min-kg from 37 to 78 min 0.208 mg/min-kg from 78 to 216.6 min
[14C]-SAM	2.5 uCi/ min from 0 to 12 min 0.2 uCi/min from 37 to 78 min 0.5 uCi/min from 78 to 216.6 min
SODIUM SULFATE	2.4 mg/min-kg from 157 to 167 min 0.32 mg/min-kg from 167 to 216.6 min

SAMPLING TIME (min)	PLASMA [14C]-SAM CONC (dpm/ml)				PLASMA SULFATE CONC. (mM)
	FORELIMB VEIN	PULMONARY ARTERY	FEMORAL ARTERY	RENAL VEIN	
Blank					0.81
3	398				
6	507	1169			
8.8	584	1292	999	186	
10.4	600	1383	959	244	
11.5	628	1383	934	230	
37					0.79
42.6	147	153	156	92	
46.8	163	158	177	115	
52.6	137	190	176	105	
57.1	167	210	216	93	0.83
66.9	201	231	204	120	
78	185	198	214	115	0.72
85	503	560	576	454	
93.8	565	674	649	538	
106.1	669	698	723	619	
118.3	690	702	752	577	0.68
137.9	770	777	836	762	
156.6	821	832	907	750	0.60
167.2	780	788	855	645	0.90
196.3	250	827	790	651	0.90
216.6	733	736	832	624	1.80