Tissue Distribution of [^{14}C]Sucrose Octasulfate following Oral Administration to Rats

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Purpose. Aluminum sucrose octasulfate (SOS) is used clinically to prevent ulcers. Under physiologic conditions, the sodium salt of this drug can be formed. Our objective was to determine whether sodium SOS was absorbed when administered orally. In addition to furthering our understanding of aluminum SOS, this study also aimed to clarify how other polyanionic drugs, such as heparin and low-molecular-weight heparins, are absorbed.

Methods. [^{14}C]-labeled and cold sodium SOS (60 mg/kg) were given to rats by stomach tube. Radioactivity was counted in gut tissue, gut washes, and nongut tissue (i.e., lung, liver, kidney, spleen, endothelial, and plasma samples) at 3 min, 6 min, 15 min, 30 min, 60 min, 4 h, and 24 h, and in urine and feces accumulated over 4 h and 24 h.

Results. Peak radioactivity was found in the tissue and washes of the stomach, ileum, and colon at 6 min, 60 min, and 4 h, respectively, showing progression through the gut. Gut recovery accounted for 84% of the dose at 6 min but only 12% of the dose at 24 h, including counts from feces. Radioactivity was recovered from nongut tissue (averaging 8.6% of the dose) and accumulated urine (18% of the dose at 24 h). When total body distribution was considered, the recovery of radioactivity was greater for the endothelium than for plasma (peak percentage of the dose was 65% at 15 min, 20% at 3 min, 5% from 20 to 240 min for the vena cava, aortic endothelium, and plasma, respectively).

Conclusions. Results indicate that sodium SOS is absorbed, agreeing with previous studies demonstrating the oral absorption of other sulfated polyanions. Endothelial concentrations must be considered when assessing the pharmacokinetics of these compounds. The measured plasma drug concentrations reflect the much greater amounts of drug residing with the endothelium.

KEY WORDS: gastrointestinal absorption; sucrose octasulfate; oral; endothelium; rats.

INTRODUCTION

The water-insoluble aluminum salt of sucrose octasulfate (SOS) sucralfate (Carafate, Aventis Pharmaceuticals, Kansas City, MO) is used clinically to treat and prevent gastrointestinal ulcers (1,2). Its mechanism of gastroprotection is believed to be due to the stimulation of wound healing at the site of the ulcer through binding, protection, and activation of basic fibroblast growth factor (3,4).

SOS is a negatively charged polyanion, and its aluminum salt is insoluble, forming a gel in the gastrointestinal tract, and thus is believed not to be absorbed. Under physiologic conditions, however, aluminum ions can exchange with sodium ions to form the water-soluble sodium salt of SOS. Despite its high water solubility, sodium SOS is also not believed to be absorbed from the gastrointestinal tract because of its highly charged nature. One goal of this study was to assess whether sodium SOS is orally bioavailable.

A number of studies have shown that negative polyanions are minimally absorbed following oral administration (5–7). Orally administered heparins show little evidence of bioavailability when the activated partial thromboplastin time or anti-factor Xa activity is used as a pharmacodynamic marker (8). Contrary to current opinion, recent studies by our group and others have suggested that negative polyanions, such as unfractionated and low-molecular-weight heparins, sulodexide, pentosan polysulfate, and dextran sulfate (average molecular weight, 8000 Da) are absorbed following oral administration (9–13). Orally administered heparin and dextran sulfate have been demonstrated to be effective antithrombotic agents in a rat jugular vein thrombosis model. Considerable distribution to the endothelium occurs following oral administration, although only low levels (less than 1% of the administered dose) are found in plasma (9,14,15).

This study was undertaken to better understand enteral absorption of the water-soluble sodium salt of SOS and to determine whether this compound showed the same distribution as that reported for other sulfated polyanionic compounds following oral administration. Furthermore, sodium SOS is monodisperse and homogeneous, making it an excellent model for the study of more complex, polydisperse, and heterogenous drugs, such as unfractionated and low-molecular-weight heparins.

MATERIALS AND METHODS

Materials

Sodium SOS standard was a generous gift from Bukh Meditec (Copenhagen Denmark). Uniformly [^{14}C]-labeled sucrose was from Amersham Life Sciences (Buckinghamshire, United Kingdom). Dowex resin was from Aldrich Chemical Company (Milwaukee, Wisconsin). Bio-Gel resin was from Bio-Rad Laboratories (Hercules, California). Cellulose acetate membrane was from Gelman Sciences Inc. (Ann Arbor, Michigan). Enzeco (R) alkaline protease "L" was from Biddle Sawyer Corp. (New York, New York). All other reagents, chemicals, and solvents were from Aldrich Chemical Co.

Preparation of Unlabeled and [^{14}C]-Labeled SOS

Sulfonation of Sucrose

SOS was prepared through the sulfonation of sucrose by the procedure shown in Scheme 1. Briefly, sucrose (1.0 mg;

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ABBREVIATIONS: DMF, dimethylformamide; dpm, disintegrations per minute; EtOH, ethanol; $NEt_3 \cdot SO_3$, triethylamine sulfur trioxide complex; PAGE, polyacrylamide gel electrophoresis; SOS, sucrose octasulfate.

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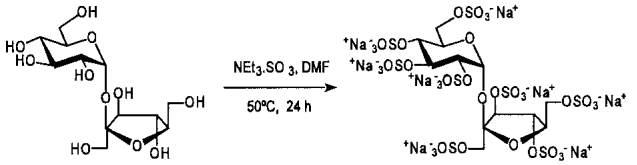

0.003 mmol) was dissolved in an ethanol (EtOH)/H_2O mixture (1.25 ml H_2O containing 3% EtOH). The solution was evaporated to dryness *in vacuo*. At 50°C and under anaerobic conditions (nitrogen), the residue was dissolved in anhydrous dimethylformamide (DMF; 0.5 ml), sucrose (9.0 mg; 0.026 mmol) was added followed by the addition of a triethylamine sulfur trioxide anaerobic ($NEt_3 \cdot SO_3$) complex (1.5 eq/OH) slowly over a 10-min period. This reaction mixture was stirred under conditions (nitrogen) at 50°C for 12 h. An additional portion of DMF (0.5 ml) and $NEt_3 \cdot SO_3$ complex (1.5 eq/OH) was added, and the reaction mixture was stirred at 50°C under nitrogen for another 12 h. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in water and eluted using Dowex-1-chloride (Dowex 1 × 2-200) column. The column was initially washed with water to remove the unreacted sucrose. The sulfated products then were eluted with aqueous 2 M sodium chloride. This fraction was concentrated by evaporation *in vacuo* and was desalted by elution with water on a P-2 column. Then, the residue was dissolved in water and eluted from a Dowex 50 (Na^+) ion exchange resin to yield the final product. The yield of the sodium salt of SOS was 65% from sucrose. Purity was assured by ion exchange chromatography, thin layer chromatography, gradient polyacrylamide gel electrophoresis (PAGE), and proton and carbon nuclear magnetic resonance spectroscopy.

Sulfonation of [^{14}C]-Labeled Sucrose

For the preparation of [^{14}C]-labeled SOS, the identical procedure as that used to prepare unlabeled SOS was used. In this reaction, 250 μCi of uniformly [^{14}C]-labeled sucrose (1.7 mCi /mg dissolved in EtOH/H_2O mixture, 1.25 ml H_2O containing 3% EtOH) and 9 mg of unlabeled sucrose were combined and persulfonated, and the product was purified (as above). The sample purity was confirmed by ion-exchange chromatography, thin layer chromatography, and gradient PAGE analysis.

The [^{14}C]-labeled SOS (21 mg) had a total radioactivity of 59.5 μCi (specific activity of 6.5×10^6 disintegrations per minute (dpm) /mg). Cold SOS was dissolved in water at a concentration of 100 mg/ml to which was added 33 nCi/mg [^{14}C]-labeled SOS for use in the animal experiments. The [^{14}C] label is directly incorporated into the carbon backbone of the sucrose rings, making it stable to all but complete catabolic transformation of the sodium [^{14}C]-labeled SOS.

Pharmacokinetic Studies

Animals

Twenty male Wistar rats, with a mean (±SD) weight of 284 ± 24 g, were handled and housed according to the Principles of Animal Care set by the Canadian Federation of Biological Societies. Animals were fasted overnight prior to treatment and were anesthetized with barbital and methoxyflourane for experimental procedures.

Oral Administration

The drug preparation was introduced into the stomach using a stomach tube in a measured quantity averaging 0.16 ml to give 60 mg/kg of unlabeled SOS plus 1978 nCi/kg [^{14}C]-labeled SOS. This was followed by 0.2 ml of saline solution to give a total administered volume of approximately 0.4 ml. Three control animals were given 0.4 ml of saline solution. Rats were killed at 3 min, 6 min, 15 min, 30 min, 60 min, 4 h, and 24 h following oral SOS administration with two animals being killed at each time period. To insure that none of the observed absorption was due to aspiration in the lung, three additional rats that had been given similar amounts of cold and [^{14}C]-labeled SOS in gelatin capsules, which were swallowed, were killed at 6 min, 15 min, and 24 h after the drug administration.

Tissue Collection

Animals to be killed at 4 h and 24 h after drug administration were placed in metabolic cages, and urine and feces were collected. At the designated times, animals were deeply anesthetized, blood samples of approximately 8 ml (9 parts blood to 1 part 3.8% sodium citrate) were taken from the abdominal aorta, and plasma was prepared. As a source of endothelium, the thoracic aorta and superior vena cava were removed and placed in saline solution. Liver, lung, spleen, and kidney were collected. Stomach, duodenum, jejunum, ileum, and colon were removed and washed with distilled water, and the contents were collected. In the three rats in which drugs had been administered by gelatin capsules, additional tissue was harvested including tongue, esophagus, thymus, heart, brain, bladder, ureters, trachea, scrotum, bile ducts, thoracic aorta and superior vena cava (minus endothelium), bone marrow from the tibia of the right hind limb, four areas of muscle, including the diaphragm, the cleidobrachialis in the neck, vastus medialis of the left hind limb, and the biceps of the right forelimb, three areas of skin, including the ear, the center and caudal areas of the back, and hair samples near the mouth. A measured quantity of approximately 100 mg of each tissue, 250 μl of the lumenal contents, and 100 μl of plasma were counted for radioactivity. The remaining gut wash fluid and tissues were frozen for later extraction and analysis of polyanions. For counting the tissue, samples were treated with 1 ml of tissue solubilizer NCSII prior to the addition of 5 ml of cocktail for counting aqueous samples. Samples were counted on a Beckman Scintillation Counter (Beckman Coulter, Inc., Fullerton, California).

SOS Extraction from Endothelium

The endothelium was removed from blood vessels according to the method of Hiebert and Jaques (16). Vessels were slit open, pinned to dental wax lumen side up, and rinsed in Locke's solution. Cellulose acetate paper was applied to the lumenal surface, and, when lifted, the endothelium was removed. The length and width of the imprint was measured to the nearest millimeter. The mean (± SD) areas for aortic and vena caval endothelium were 2.6 ± 0.3 and 0.8 ± 0.2 cm^2, respectively. Endothelium derived from the vena cava was

counted immediately following removal by cellulose acetate paper, whereas the aortic endothelium was further processed. Cellulose acetate paper was removed from the aortic endothelium by dissolving it in cold acetone followed by centrifugation and the discarding of the supernatant. The process was repeated to give a dry tissue powder.

SOS Extraction from Tissues and Plasma

Tissues were minced and defatted with acetone and isopropanol/petroleum ether. Tissue and plasma were digested with ENZECO (R) alkaline protease ′L′ (Biddle Sawyer Corp.) in 100 mM Tris-Cl buffer (pH 9.5) at 60°C. Extracts then were dialyzed against water using 1000 molecular weight cut-off (MWCO) dialysis tubing. [^{14}C]-labeled SOS and that derived from extracted tissue were run on an agarose gel electrophoresis system with toluidine blue staining (17), where the presence of chemical SOS in tissue extracts was determined by the migration and staining properties of samples by comparison to an SOS standard.

Analysis

Results are expressed as mean ± SEM where three or more data points are available. A one-way analysis of variance, followed by the Tukey multiple comparison test, was used to compare the percentage of the dose recovered in gut tissue and gut washes between various levels of the gut as well as that recovered from the endothelium and plasma. A logarithmic transformation was used prior to analysis to ensure similar variances between groups. To determine the percentage of the dose present in the endothelium, the total surface area of the endothelium was estimated based on the reported figure of 7000 cm^2 per 100 g of muscle (18). Accounting for less vascular tissue in the body, the corrected value used was 6360 cm^2 of endothelial surface per 100 g of tissue. To determine the percentage of the dose in plasma, the plasma volume was assumed to be 40.4 ml/kg body weight, as previously reported for rats (19). A paired *t* test was used to determine differences in recovery between gut tissues and washes.

RESULTS

[^{14}C]-Labeled SOS in Gastrointestinal Tissue and Washes

The recovery of radioactivity as percentage of the dose administered is shown in Fig. 1. Peak radioactivity was found in stomach tissue at 6 min, in ileum tissue at 60 min, and in colon tissue at 4 h. Stomach tissue radioactivity dropped to one third of the original value by 30 min. Recovery from stomach tissue was significantly greater than all other gut tissue at 3 and 6 min, and was greater than that in the ileum and colon at 15 min. Recovery from duodenal tissue was constant from 15 min to 24 h, and in jejunal tissue from 15 min to 24 h. Radioactivity recovered from the duodenum at 15 min, the jejunum at 30 min, and the ileum at 60 min was significantly different than that recovered from colon tissue at these times. The peak radioactivity recovered from washes was obtained at 6 min, 30 min, 15 min, 60 min, and 4 h, respectively, for the stomach, duodenum, jejunum, ileum and colon. Radioactivity recovered from stomach washes was significantly greater than that from all washes at 3 and 6 min, and was greater than that recovered from ileum and colon washes at 15 min and from colon washes at 30 and 60 min. Recovery from jejunum washes at 30 and 60 min was greater than that from the colon. Recovery from colon washes was greater than that from stomach washes at 24 h. In general, recovery from gut washes was slightly higher than that from tissues (Fig. 1; Table 1) and approached significance when the stomach was considered ($P = 0.07$). The total radioactivity recovered from gastrointestinal tissues and washes decreased with time, with approximately 10% of radioactivity present in washes and tissue at 24 h after drug administration (Table 1). Interestingly, concentrations were lower when the drug was administered by gelatin capsule in the 24-h group. Chemical SOS also was found in gut washes and tissue following extraction and analysis with agarose gel electrophoresis, as shown in Fig. 2, A and B. Only trace amounts were found in duodenal tissue.

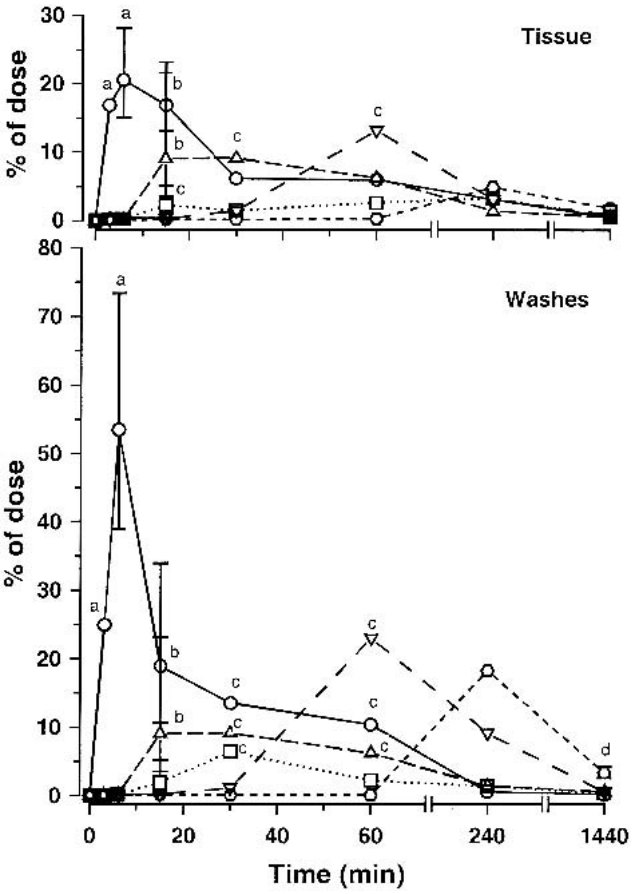

Fig. 1. Concentration and percentage recovery of radioactivity from tissue and washes of different parts of the gut following the administration of [^{14}C]-labeled SOS ($4.5 \pm 0.02 \times 10^6$ dpm/kg) and cold SOS (60 mg/kg) by stomach tube or gelatin capsule. Shown are the mean ± SEM of three rats per group at 6 min, 15 min, and 24 h, and the mean of two rats at all other times from different areas of the gut, stomach (circle), duodenum (square), jejunum (upward triangle), ileum (downward triangle), and colon (hexagon). To have equal variances between groups, a logarithmic transformation was used prior to analysis using a one-way analysis of variance. a, significantly greater than all other tissues and washes; b, significantly greater than the ileum and colon; c, significantly greater than the colon; and d, significantly greater than the stomach. The percentage of radioactivity recovered in all tissues and washes was significantly greater than that from untreated control tissues or washes ($P < 0.05$).

Table I. Recovery of Radioactivity from Nongut Rat Tissue at Various Times following Administration of [^{14}C]-Labeled SOS (4.5 ± 0.02 × 10^6 Dpm/kg) and Cold SOS (60 mg/kg) by Stomach Tube or Gelatin Capsule[a]

Time min	Spleen (dpm × 10^3/g)	Liver (dpm × 10^3/g)	Lung (dpm × 10^3/g)	Kidney (dpm × 10^3/g)	Plasma (dpm × 10^3/ml)	Urine (dpm × 10^3/ml)
3	**0.0**	**0.0**	**89.0**	**1.1**	**3.9**	
	0.0, 0.0	0.0, 0.0	172.2, 5.7	0.7, 1.5	3.7, 4.2	
6	**0.5**	**0.9**	**74.3**	**2.6**	**2.8**	**0.0**
	0.4, 0.0, 1.0[b]	0.6, 0.4, 1.8[b]	34.9, 149.1, 39.0[b]	1.1, 4.1, 2.5[b]	1.3, 4.7, 2.4[b]	0.0[b]
15	**1.1**	**4.6**	**87.8**	**5.0**	**8.3**	**44.7**
	0.6, 1.9, 0.8[b]	2.6, 9.7, 1.5[b]	5.1, 256.8, 1.4[b]	6.5, 6.1, 2.4[b]	6.9, 16.2, 1.8[b]	44.7[b]
30	**0.9**	**3.7**	**1.9**	**5.6**	**5.6**	
	1.4, 0.5	4.7, 2.7	1.5, 2.3	4.8, 6.3	6.7, 4.6	
60	**1.2**	**7.0**	**22.3**	**7.2**	**7.6**	
	0.9, 1.5	7.9, 6.0	5.0, 39.7	7.3, 7.1	9.4, 5.9	
240	**1.9**	**4.7**	**3.3**	**4.9**	**5.5**	**12.7**
	2.7, 1.1	6.4, 3.1	2.8, 3.8	5.6, 4.3	5.9, 5.2	7.5, 17.8
1440	**1.2**	**3.5**	**2.9**	**3.1**	**2.7**	**40.8**
	1.4, 1.8, 0.3[b]	4.6, 3.9, 2.0[b]	5.7, 2.3, 0.8[b]	3.7, 4.1, 1.5[b]	3.2, 4.0, 0.9[b]	42.6, 74.3, 5.6[b]

[a] Bold numbers show averages. Numbers below these in standard type show individual values being averaged.
[b] Doses administered by gelatin capsule; all others administered by stomach tube.

[^{14}C]-Labeled SOS in Nongut Tissue

Radioactivity was found in lung, kidney, and plasma as early as 3 min following drug administration, and was found in spleen and liver at 6 min (Table 2). Concentrations were similar in plasma throughout the 24-h time period. In spleen, liver and kidney, concentrations reached a plateau at 15 min, which was maintained for 24 h. Radioactivity was found in urine at 15 min, 4 h, and 24 h, but not at 6 min, after drug administration. The highest concentrations were observed in the lung at 3, 6, and 15 min after drug administration. Recovery from the lung was quite variable and was evident even when the drug was administered by gelatin capsule. Average lung radioactivity concentrations dropped significantly by 4 h but were still found at 24 h after drug administration. Considerable chemical SOS was observed in lung and urine, as shown in Fig. 2C. Only trace amounts were recovered from kidney and liver.

Total [^{14}C] Recovery

The amount of radioactivity recovered averaged 74% (Table 1). Up to 4 h, 80.4% of the total administered amount of the drug was accounted for, whereas at 24 h only 34% of the drug was recovered. Only trace amounts were recovered in the feces, which were accumulated up to 24 h (0.001%). An average of 8.6% was recovered from the lung, liver, kidney, spleen, and plasma, with less recovered at 24 h than at previous times. A gelatin capsule was used to preclude the possibility that any of the observed absorption was due to aspiration into the lung when the stomach tube was used. Amounts recovered at 24 h were less when given by gel capsule vs. stomach tube, suggesting a decreased absorption when a gelatin capsule was used.

Additional tissues were examined for the presence of the drug at 6 min, 15 min, and 24 h after drug administration by gelatin capsules. These additional tissues accounted for 4%, 4.5%, and 1.2% of the recovered radioactivity at 6 min, 15 min, and 24 h, respectively. Notable were the 1.3%, 0.75%, and 0.01% found in the esophagus, the 1%, 3.1%, and 0.07% found in the tongue, the 0.25%, 0.18%, and 0.01% found in the trachea, the 0.62%, 0.05%, and 0.23% found in the brain, and the 0.06%, 0.04%, and 0.46% found in bone marrow samples (25 mg) at 6 min, 15 min, and 24 h, respectively. Counts also were obtained from the thymus, scrotum, and aortic and vena caval walls minus the endothelium and in samples of muscle, skin, and hair. The amounts of radioactivity found in urine increased with time and accounted for 18.3% of the administered dose at 24 h.

Endothelial samples also were harvested from the aortic and vena caval endothelium. Results reported in terms of dpm per square centimeter are also expressed as the percentage of the dose that is estimated from the total body endothelium (Fig. 3). Counts were recovered from both the aortic and vena caval samples beginning at 3 min following oral administration. Peak amounts were observed in the aortic and vena caval endothelium at 3 and 15 min after drug administration, respectively. The label remained in the endothelium throughout the study period but dropped to near 0 at 24 h. Radioactivity in plasma, expressed as a concentration and as the percentage of the dose by considering total blood volume, never rose above 10% of the dose. The percentage of the dose recovered from endothelium vs. plasma was significantly greater when calculations were based on venal caval concentrations at 15, 30, 60, and 240 min after administration, and when they were based on aortic endothelial concentrations at 240 min. Significantly more radioactivity was associated with vena caval vs. aortic endothelium at 240 min.

DISCUSSION

Highly sulfated polyanionic compounds are considered to be poorly absorbed following oral drug administration. This study, however, supports the hypothesis that [^{14}C]-labeled SOS and cold SOS are absorbed in rats when administered by stomach tube. Evidence of this is radioactive or chemical SOS was found in urine, plasma, and nongut tissue. Radioactivity recovered from urine accounted for 18.3% of the administered dose at 24 h, whereas that from the lung, liver, kidney, and spleen accounted for an average of 8.6% of the dose. Radioactivity was found in plasma at all times up to

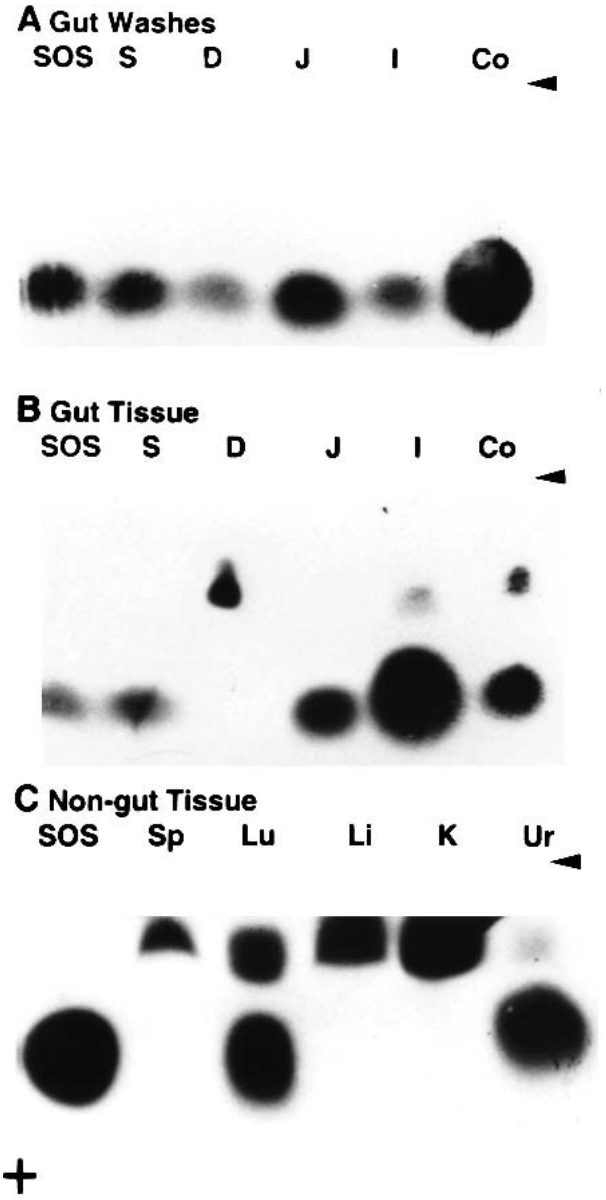

Fig. 2. Agarose gel electrophoresis of gut washes, tissues, and urine extracts from rats given [^{14}C]-labeled SOS and unlabeled SOS by stomach tube. Tissue and urine extracts were obtained from samples obtained near the times when radioactivity peaked; specifically at 3 min, 15 min, 1 h, 1 h, and 4 h, respectively, for stomach (S), duodenum (D), jejunum (J), ileum (I) and colon (Co) tissue and washes (A and B), and at 30 min, 15 min, 15 min, 30 min, and 24 h, respectively, for spleen (Sp), lung (Lu), liver (Li), kidney (K), and urine (Ur) (C). Radioactivity in tissue samples was counted and dissolved in water such that 2 μl contained 0.05 nCi. Following the application of samples (2 μl) where indicated by arrows, slides were subjected to electrophoresis, were fixed, and then were stained with toluidine blue and destained with 0.1% glacial acetic acid.

24 h, including as early as 3 min after administration, suggesting stomach absorption. However, the amounts recovered from plasma never rose above 10% of the administered dose.

We were able to recover chemical SOS from gut washes and tissues when examined at times when radioactivity peaked, although only trace amounts were recovered from duodenal tissue. This may be due to the small amount of tissue harvested from the duodenum relative to other gut tissue, thus making chemical extraction more difficult. We were also able to recover chemical SOS readily from lung tissue and urine, supporting the hypothesis that SOS is absorbed orally. Only trace chemical amounts were recovered from the liver and kidney, although this tissue was examined when peak radioactivity was found. This suggests that metabolizing of SOS may be occurring. This finding agrees with previous studies using [^{14}C]-labeled and unlabeled heparin in which the amount of chemical heparin recovered from the liver was much less than the amount of radioactivity, suggesting that the liver may be a site of metabolism (11).

Previous studies with sulfated polyanions have shown that these compounds readily bind to the endothelium. This binding, when all the body endothelium is considered, can account for a high percentage of the administered dose, as demonstrated when using unfractionated heparin or dextran sulfate (9). This study demonstrates the same effect with SOS, where the amounts recovered (reported as the percentage of the dose administered) in the vena caval and aortic endothelium were higher than those observed in plasma. The higher amounts found in vena caval vs. aortic endothelium agree with a similar trend observed with the low-molecular-weight heparin reviparin, in which concentrations in the vena caval endothelium were significantly higher than those from the aorta (10).

Results from gut tissue and gut washes showed the expected transit through the gastrointestinal tract with peak recoveries from both gut tissue and gut washes at 6, 60, and 240 min, respectively, for the stomach, ileum, and colon. At 24 h, only 35.3% of the total radioactivity could be accounted for, with 12.1% found in the gut and gut washes, including that recovered from the feces accumulated over the 24-h period. This suggests that SOS is widely distributed in the tissues. This is supported by observations that radioactivity could be found in other tissue throughout the body including muscle and bone marrow.

These results support those of previous studies with sulfated polyanionic compounds in which the effects of oral administration have been demonstrated. Dextran sulfate, unfractionated heparin, and low-molecular-weight heparin administered by the oral route have shown antithrombotic activity in a rat jugular vein model (9,10,20). Oral sulodexide, a mixture of dermatan sulfate and fast-migrating heparin, decreased fibrinogen concentrations following oral administration to patients with peripheral vascular disease (21). Oral pentosan polysulfate has been shown to be effective for the treatment of interstitial cystitis and prostatitis (12), whereas oral chondroitin sulfate has been suggested to be efficacious in the treatment of arthritis (22,23).

Previous studies examining the tissue distribution of [^{14}C]-labeled heparin and cold unfractionated heparin in the rat have shown a wide distribution of heparin in the tissues, a presence in the endothelium, and only small amounts in the feces (15), agreeing with the pattern of distribution for SOS that was observed in this study. However, radioactive counts found in accumulated urine were 1.5% of the administered dose at 24 h, and plasma counts were always less than 1% of the dose over the 24-h period. These amounts were considerably less than the radioactivity observed in this study in which 18.3% of the administered dose was observed in accumulated urine and less than 10% of the dose was observed in

Table II. Total Radioactivity in Gut, Gut Washes, and Nongut Tissue following Oral Administration of [^{14}C]-Labeled SOS and Cold SOS (60 mg/kg) by Stomach Tube or Gelatin Capsule[a]

Time (min)	Gut tissue (% of total)	Gut washes (% of total)	Nongut tissue & plasma (% of total)	Urine (% of total)	Recovery (%)
3	22.3	35.6	10.4	ND	68.3
6	23.7	60.1	13.7	0	97.5
15	31.1	50.4	20.4	0.06	102
30	17	35	5.1	ND	57.1
60	33	55.4	10.1	ND	98.5
240	18.5	33.5	5.3	2.2	59.5
1440	5.3	6.8[b]	5	18.3	35.3
Mean	21.5	39.5	8.6		74

[a] ND, not determined.
[b] Includes feces.

plasma over the 24-h period. The increased amounts found in urine and plasma with oral SOS as compared to unfractionated heparin may be due to an enhanced rate of absorption or to a decreased ability to bind to the endothelium. The enhanced rate of absorption of SOS may be due to its much smaller average molecular weight relative to unfractionated heparin. This is supported by our recent observations that showed effectiveness at much lower single doses for oral low-molecular-weight heparin vs. oral unfractionated heparin (24).

These results demonstrate that sodium SOS is absorbed following oral administration. It seems this drug is absorbed throughout the entire gastrointestinal tract with considerable absorption taking place in the stomach. Once absorbed, the sodium SOS binds primarily to the endothelium, with a small amount freely circulating in the plasma. Thus, endothelial concentrations must be considered when the pharmacokinetics of this compound are studied. The amounts found in plasma likely reflect a much greater concentration associated with the endothelium. These results also support the hypothesis that sulfated polyanions are absorbed following drug administration by the oral route. This is in contrast to the currently accepted view that polyanions are not bioavailable when administered orally (5,7,8). The possibility of the oral absorption of negative polyanions opens avenues for long-term use when systemic effects are desired. Furthermore, additional considerations are likely to be required regarding the pharmacokinetics and side effects of these polyanionic compounds.

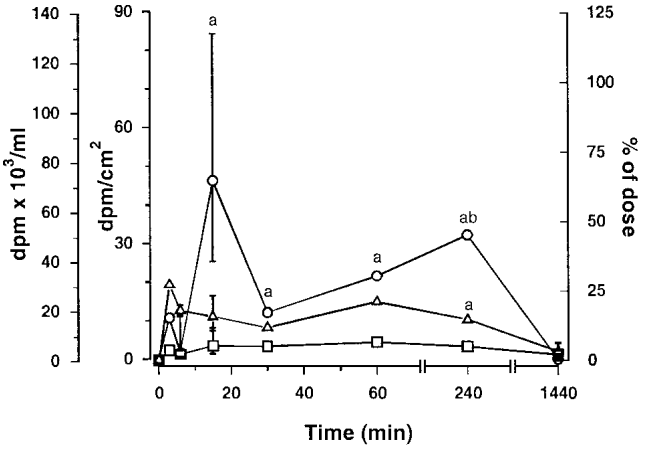

Fig. 3. The recovery of radioactivity, reported as the percentage of the administered dose, from plasma and endothelium following the administration of [^{14}C]-labeled SOS ($4.5 \pm 0.02 \times 10^6$ dpm/kg) and cold SOS (60 mg/kg) by stomach tube or gelatin capsule. The percentage of the dose assumes recovery from total endothelium when concentrations on vena caval endothelium (circle) and aortic endothelium (triangle) are considered, and from total plasma (square) when concentrations in plasma samples are considered. The calculation of the percentage of the dose assumed a total surface area of 6360 cm^2 per 100 g of tissue for the endothelium and a total plasma volume of 40.4 ml/kg body weight. Shown are the mean ± SEM of three rats per group at 6 min, 15 min, and 24 h, and mean of two rats at all other times. When comparing the percentages of the doses recovered, a logarithmic transformation was used prior to analysis using a one-way analysis of variance. a, significantly greater than recovery from plasma; b, significantly greater than recovery from aortic endothelium ($P < 0.05$).

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