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Absorption enhancement of dextran sulfate after enteral administration in a dispersion

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Abstract

Dextran sulfate sodium, a polyanion with a relative molecular mass of approximately 8000, is poorly absorbed after oral or enteral administration of aqueous solutions. When given to rats without absorption enhancers, no drug was found in serum using an analytical method with a detection limit of 0.08 μg/ml. Delivery of the drug in a dispersion formulation containing 10 mM monoolein and 20 mM sodium taurocholate increased the absorption of dextran sulfate from the small intestine of rats. Drug was present in serum after administration of the formulation locally to the duodenum, jejunum and the ileum. The ileum was selected for further study. Data from 12 rats showed variability in the maximum drug concentration and the time at which it occurred. Negligible absorption enhancement occurred when the formulation was given orally by gavage. When intestinal contents were present, absorption was reduced. Direct administration of the formulation to the absorptive site produced significant blood levels of the drug. This indicates intestinal absorption enhancement of dextran sulfate can be achieved.

Keywords: Dextran sulfate; Dispersion; Enteral absorption enhancement or promotion; Lipid-surfactant mixed micelles; Monoolein; Sodium taurocholate

1. Introduction

Dextran sulfate sodium is a polysaccharide with antiviral (Ueno et al., 1987; Baba et al., 1988a,b,c), antilipemic (Uchida et al., 1975) and radioprotective (Ross and Peeke, 1986; Vacek et al., 1988) activity (Jeanes, 1974). It has a long history of oral administration as an antilipemic. Although material with a relative molecular mass (M_r) of approximately 8000 was shown to be active in vitro against HIV-1 and underwent clinical trials (AIDS Clinical Trials Information Service, 1990), we are not aware of any studies indicating in vivo efficacy. Although dextran sulfate was well tolerated after oral and intravenous administration (Uchida et al., 1975; Abrams et al., 1989), virtually no drug was found in plasma after oral dosing (Wada et al., 1976; Lorentsen et al., 1989; Hartman et al., 1990). Many researchers reported the drug was poorly absorbed (Tomizawa and Kagawa, 1971; Fukawa et al., 1978; Lorentsen et al., 1989; Foster et al., 1990). This study was undertaken to determine if an

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increase in dextran sulfate levels in blood could be accomplished, thereby increasing the likelihood of in vivo efficacy.

Emulsions, microemulsions and mixed micellar formulations have been used to improve the bioavailability of poorly absorbed drugs (Muranishi, 1990; Kararli et al., 1992; Constantinides et al., 1994). Lipid-surfactant mixed micelles containing fatty acids or mono- and diglycerides with bile salts have been used extensively to promote drug absorption. These formulations have been administered nasally, orally and to specific regions of the gastrointestinal tract (Muranishi et al., 1979b; Kararli et al., 1992; Shao and Mitra, 1994; Wüthrich et al., 1994). Such delivery systems have increased blood levels of heparin (Muranishi et al., 1977; Tokunaga et al., 1978; Taniguchi et al., 1980), which is structurally similar to dextran sulfate. Mixed micellar formulations have been used for the enteral administration of aminoglycoside antibiotics delivered as complexes with dextran sulfate with molecular mass of 500 000 to 1 million (Yoshikawa et al., 1981, 1983a,b,c). The high molecular mass dextran sulfate improves targeting of the complex to lymph.

Published studies indicate monoolein-sodium taurocholate absorption enhancers temporarily alter gastrointestinal permeability without causing acute damage. In a review of absorption enhancers, Muranishi (1990) found the time required for the complete recovery of intestinal barrier functions was usually 15–20 min after exposure to lipid-surfactant formulations. After treatment of rat rectal mucosa with 10 mM oleic acid in micellar form, microscopic examination showed the mucous layer was removed, but the structure of normal epithelial cells remained intact (Muranishi, 1990, Fig. 3B). In a comparative study of several absorption enhancers, reversibility, histological evaluation and assessment of biochemical markers were used to evaluate intestinal damage (Swenson et al., 1994). Sodium taurocholate, when used alone, was found to cause minimal damage which was rapidly reversed. When co-administered with lipids, however, bile salts have been reported in numerous studies to be non-damaging (van Hoogdalem et al., 1989). In a study using equimolar monoolein and sodium taurocholate (40 mM), examination of reversibility, histology and protein release showed the formulations to be non-damaging to the small intestine of rats (Tokunaga et al., 1978). No morphological changes were found in the colon of rats after administration of equimolar (40 mM) monoolein and sodium taurocholate (Hastewell et al., 1994).

Another study included a 1-h pretreatment of small intestines of rats with mixed micelles containing 40 mM each of monoolein and sodium taurocholate (Muranishi et al., 1979a). Drugs administered in the presence of this formulation were absorbed, but no absorption enhancement occurred when the drugs were administered immediately after this pretreatment. Similar adjuvants were tested in the small and large intestines of rats and gave the same results. In two other studies, the large intestines of rats were pretreated for 2 h by exposure to lipid-surfactant mixed micelles of linoleic or oleic acid and polyoxyethylated hydrogenated castor oil (Yoshikawa et al., 1985, 1986). Again, absorption enhancement was seen only when drug was co-administered with the enhancers. The authors concluded mucosal damage did not occur.

In this investigation an aqueous formulation containing monoolein or glyceryl monooleate with sodium taurocholate and dextran sulfate was administered to male Sprague–Dawley rats. The purpose of the experiments was to determine the feasibility of increasing the absorption of dextran sulfate of relative molecular mass 8000 by delivering it with a lipid-surfactant formulation. Oral and intestinal administration were studied, and absorption from different regions of the small intestine was examined.

2. Materials and methods

2.1. Materials and reagents

Monoolein was obtained from TCI America (Portland, OR); glyceryl monooleate was supplied by Pfaltz and Bauer, Inc. (Waterbury, CT); the compounds have the same empirical formula and differ in the placement of the ester linkage by one

carbon position. Sodium taurocholate, ~98% purity, was purchased from Sigma (St. Louis, MO). The animal anesthetics were purchased as follows: ketamine HCl, 100 mg/ml as ketamine from Fort Dodge Laboratories, Inc. (Fort Dodge, IA); xylazine, 20 mg/ml from Ben Venue Laboratories, Inc. (Bedford, OH, USA); acepromazine maleate, 10 mg/ml from Vedco, Inc. (St. Joseph, MO). Dextran sulfate, sodium salt, relative molecular mass 8000, 17.7% sulfur content, was donated by Ueno Fine Chemicals Industry (Sanda, Hyogo, Japan). All other chemicals were reagent grade and were purchased from Fisher Scientific (Pittsburgh, PA). Simulated gastric fluid and simulated intestinal fluid were prepared as per the United States Pharmacopoeia. Water was purified by reverse osmosis. Male Sprague–Dawley rats were purchased from Ace Animals, Inc. (Boyertown, PA). The Branson sonifier model 450 (Shelton, CT) was used with a 3/4-inch tip at output setting 9 and 50% duty cycle to give an output power of 165 W. A Coulter particle sizer model N4MD (Coulter Electronics, Inc., Hialeah, FL) was used to determine the size and distribution of droplets comprising the discrete phase of the formulation. Serum was separated from clotted blood in a clinical centrifuge, IEC Model CL (International Equipment, Needham Heights, MA). Serum was filtered through a 0.45-micron nylon filter, 4 mm diameter (Gelman Sciences, Ann Arbor, MI).

2.2. Formulation preparation and droplet size determination

Sodium taurocholate (NaTC), 275 mg, was dissolved in 24.8 ml of purified water in a cylindrical glass shell vial (29 × 94 mm). Monoolein (MO) was liquefied by warming the container in ~40°C tap water, and 89 mg MO was added to the vial. The mixture was sonicated for 10 min to form a slightly hazy solution. Dextran sulfate (DS), 400 mg, was diluted to 10 ml with the solution. The composition of the formulation was 10 mM MO, 20 mM NaTC and 40 mg/ml DS. A placebo formulation was prepared without dextran sulfate. All formulations were prepared daily and used within 6 h.

Literature reports of MO-NaTC formulations describe them as clear mixed micellar solutions. Experiments were performed on our formulations to minimize the droplet size, resulting in clear solutions. Sonication conditions and formulation composition were varied. The Coulter particle size analyzer was used in size distribution profile (SDP) mode for evaluating the droplet size of each formulation. Particle size is correlatable with appearance (Bennett et al., 1968). The turbidity of each solution was assessed by a standardized visual comparison.

2.3. Animal procedures

Male Sprague–Dawley rats weighing 350–400 g were fasted for 18 h prior to administration of the formulation; free access to water was allowed. A dose of 40 mg/kg of dextran sulfate was used; the volume of formulation given was 0.35–0.40 ml. In one set of experiments the drug formulation was administered orally by gavage to conscious and anesthetized animals. The anesthesia consisted of intraperitoneal administration of a mixture of ketamine, xylazine and acepromazine in concentrations of 45, 4.5, and 0.68 mg/kg, respectively. In the enteral experiments, the formulation was delivered directly to a specific region of the small intestine. The intestines were exposed through a midline incision and a segment was isolated with the blood supply intact. Drug solution was placed at the proximal end of the loop, and the intestines were reposited in the body cavity. Initial studies utilized a formulation containing glyceryl monooleate. The monoolein formulation was then given orally to an additional group of rats and used in all subsequent intestinal administration experiments.

Despite fasting, at times intestinal contents were seen in the isolated segment. The effect of this material on drug absorption was examined by administering the drug formulation to untreated and rinsed intestines. Rinsing was performed by gently flushing the test segment with normal saline and carefully expelling it with a small volume of air prior to ligation of the distal end. An in vitro experiment was performed to determine whether drug was adsorbed onto, or interacted with, intestinal contents. An aqueous solution of dextran sulfate was combined with material pooled from two rats. The samples were allowed to stand for 10 min. Four ratios of intestinal contents to drug

were tested. The samples were centrifuged for 1 min, and the supernatants were analyzed for drug content. Moisture content of intestinal contents was determined by loss on drying. Drug concentrations determined were corrected for dilution.

Control animals for all experiments were handled identically to the test animals. The formulation was an aqueous solution of dextran sulfate without the absorption enhancers. In all experiments blood was collected from the lateral tail vein. A sample of 0.25 ml was taken every 10 min for up to 1 h; the initial blood sample was taken immediately prior to drug administration.

2.4. Analytical method

Serum levels of dextran sulfate were determined by size-exclusion chromatography utilizing a method developed in this laboratory (Maderich and Sugita, 1993). The original method was modified by increasing the injection volume from 10 μl to 40 μl. This reduced the detection limit to 0.08 μg/ml and the quantitation limit to 2 μg/ml. Briefly, blood was allowed to clot at room temperature and centrifuged to separate serum. Samples were filtered and analyzed by direct injection onto the chromatographic system; dextran sulfate was monitored at 525 nm as a complex with a metachromatic dye. The method is suitable for quantitation of dextran sulfate and its lower molecular mass metabolites.

3. Results and discussion

3.1. Drug administration without absorption enhancers

Control animals for the intestinal experiments were given an aqueous solution of dextran sulfate without absorption enhancers. Six rats were dosed. The entire small intestine was used in two rats and the ileum in four. No drug was found in the blood at any time point. This finding is in agreement with earlier studies which found little or no intact dextran sulfate in blood or urine after oral or enteral administration (Tomizawa and Kagawa, 1971; Wada et al., 1976; Lorentsen et al., 1989; Foster et al., 1990; Hartman et al., 1990).

Lack of appearance of dextran sulfate in serum has been interpreted in some cases to indicate the drug is poorly absorbed or not absorbed intact, and in others that it is absorbed but rapidly metabolized. Hartman et al. (1990) found an apparent bioavailability of 6.8% after oral administration of [^{3}H]DS at 1.02 mg/kg to rats. However, the radiolabel was found in plasma solely on material with a relative molecular mass below 200. The authors concluded the drug was almost totally degraded in the gastrointestinal tract when given orally (Mitsuya et al., 1990). Lorentsen et al. (1989) used a competitive binding assay and two bioassays to determine concentrations of dextran sulfate in plasma after oral administration of 1800 mg to adult males. Virtually no drug was found, and they concluded it was poorly absorbed. Foster et al. (1990) also monitored [^{3}H]DS, and they fractionated plasma and urine by column chromatography. The drug was given to rats at a dose of 20 mg/kg. Only a trace amount of intact drug was found in plasma at 3 and 24 h after oral dosing, but a significant amount of tritiated water was found. This group concluded some DS is absorbed intact from the gastrointestinal tract and is rapidly metabolized or degraded prior to entering the systemic circulation. Wada et al. (1976) monitored ^{35}S in plasma for 24 h after oral administration of $\sim$ 100 mg/kg of labeled drug to rats. They found maximum radioactivity in plasma during the first 5 h; the level never exceeded 0.03% of the dose per milliliter of plasma. Tomizawa and Kagawa (1971) found very low blood levels of ^{35}S at 3–4 h after duodenal administration of labeled DS. In our laboratory intact drug was monitored, thus avoiding some of the ambiguity in data interpretation which was problematic in other studies.

Jaques et al. (1991) found very little dextran sulfate in plasma after oral administration of aqueous solutions to rats. After intravenous (i.v.) dosing, the highest blood levels were found at the first time point of 2.4 min. The concentration was 0.7 mg/ml, which was half the theoretical value based on a plasma volume of 40.4 ml/kg. After both oral and i.v. drug administration, dex-

Table 1
Comparison of endothelial uptake results

Route of administration	Tissue analyzed	Dextran sulfate concentration[a]			
		Units	Jaques et al. (1991)[b]	Maderich et al. (present study)[c]	
				Procedure 1[d]	Procedure 2[e]
Intravenous	Aorta	(mg/cm^3)	24.80 ± 11.85	53.02	84.1 ± 19.7
	Vena cava	(mg/cm^3)	35.56 ± 25.23	77.23	N.A.
	Plasma	(mg/ml)	0.35 ± 0.08	N.A.	N.A.
	Serum	(mg/ml)	N.A.	0.55 ± 0.05	N.A.
Gavage	Aorta	(mg/cm^3)	11.726 ± 4.171	Undetected	
	Vena cava	(mg/cm^3)	16.125 ± 9.252	Undetected	
	Plasma	(mg/ml)	0.001 ± 0.0004	N.A.	
	Serum	(mg/ml)	N.A.	0.003 ± 0.001	

N.A.: not analyzed.
[a]Samples taken 6 min after dosing.
[b]Mean ± S.E. of 3–7 rats.
[c]Mean ± S.E. of 4–6 rats.
[d]Procedure 1: Blood vessels pooled for analysis.
[e]Procedure 2: Individual aortas analyzed.

tran sulfate was found in the endothelium of the aorta and vena cava at 2.4 and 6 min. The authors proposed the drug is rapidly absorbed from the stomach and immediately taken up by the endothelium (Jaques et al., 1991).

Our results do not support this conclusion. Table 1 shows a comparison of data from both studies. Following intravenous administration of aqueous dextran sulfate to six rats, drug levels in serum and the aortic endothelium at 6 min were correlated with the results reported by Jaques et al. (1991). However, when the solution was administered to four rats orally by gavage, very little drug was found in serum, and none was seen in the aorta or vena cava. These results, along with our finding of drug in plasma after enteral delivery with absorption enhancers, indicate dextran sulfate in aqueous solution is poorly absorbed from the gastrointestinal tract.

3.2. Droplet size analysis of the formulation containing absorption enhancers

The formulation consisted of 10 mM monoolein, 20 mM sodium taurocholate, and 40 mg/ml dextran sulfate. Using the sonication conditions described above, a nearly clear formulation was prepared consistently. Lipid-surfactant formulations prepared by sonication are typically called mixed micelles (Muranishi, 1985; van Hoogdalem et al., 1989; Muranishi, 1990). The appearance indicated a droplet size between 5 and 100 nm in diameter (Bennett et al., 1968), which is in the mixed micellar size range. Particle size analysis showed the disperse phase consisted of droplets with a bimodal size distribution. Addition of dextran sulfate appeared to increase the droplet size and distribution slightly, as shown in Table 2. However, the values are not statistically different at the 95% confidence interval. The distribution was bimodal and did not change after storage at room temperature for 18 h. The preliminary formulation containing 10 mM glyceryl monooleate in place of monoolein had a narrower size distribution. Before drug addition 98% of the droplets were 100 nm in diameter; the remaining 2% were 2400 nm. Although glyceryl monooleate gave a smaller droplet size system, monoolein was chosen for the final formulation. It has been more widely used as an absorption enhancer and more data are available in the literature for comparison.

Table 2
Droplet size of formulation before and after DS addition

DS (mg/ml)	MO (mM)	NaTC (mM)	Droplet size distribution				Dust (%)
			nm/S.D.	%	nm/S.D.	%	
0	10	20	58.2/10	68	582/150	32	0
40	10	20	68.2/18	57	704/120	43	0

Based on composition, droplet size, preparation procedure and stability, our formulation may be classified as an emulsion (Bennett et al., 1968; Martin et al., 1983; Hiemenz, 1986; Zografi et al., 1990). Based on the proportions of the phases, method of preparation, miscibility with water, and solubility of dextran sulfate, the formulation appeared to be an oil-in-water emulsion. In some respects the formulation was not a true emulsion. The oil used was monoolein or glyceryl monooleate. These compounds are polar monoglycerides; they interact with water to form various micellar and liquid crystalline phases (Wyatt and Dorschel, 1992). Emulsions typically are prepared using an oil phase which is immiscible with water. When used as drug delivery vehicles, oil-in-water emulsions usually contain a lipophilic drug in the disperse phase. Dextran sulfate is polyanionic and highly water soluble. It was presumed to be in the aqueous phase; its distribution in the formulation was not determined experimentally. The formulation may best be described as a drug solution in the presence of an absorption promoting disperse system (Muranishi et al., 1977). The formulation is a disperse system, or dispersion, but it does not fit neatly into any existing subcategory of disperse systems. However, this study has shown that lipid-surfactant formulations need not be in the mixed micellar size range to promote absorption.

3.3. Administration of drug with absorption enhancers by gavage

In the gavage experiments, 14 rats received the preliminary formulation containing glyceryl monooleate; four received the monoolein formulation. No dextran sulfate was found in the serum of these 18 rats. Lack of appearance of drug in the serum could have been due to various reasons described below.

The effect of pH on drug absorption from MO-NaTC formulations has not been established. Destabilization of the formulation may occur in the stomach, but it is not manifested by rapid breaking of the dispersion. In an in vitro experiment, 3-ml aliquots of the formulation were diluted to 5 ml and 10 ml with simulated gastric fluid. The droplet size of the formulation remained unchanged for at least 2 h after dilution.

Gastric fluid contains pepsin with a net positive charge. Dextran sulfate forms insoluble complexes with many cationic materials. No precipitate was observed in the in vitro experiment. A stability test showed dextran sulfate dissolved in simulated gastric fluid or simulated intestinal fluid had the same peak area as drug dissolved in mobile phase. No precipitate was seen, and the peak areas did not change when the solutions were held at ambient temperature for 4 h and reanalyzed. The solubility of dextran sulfate in vivo was not determined.

The formulation is diluted by secretions in the stomach, and further dilution may occur during intestinal transit. Muranishi et al. (1979a) and Taniguchi et al. (1980) have shown that absorption enhancement by MO-NaTC formulations is concentration-dependent. Muranishi (1985) has also shown that rapid intestinal transit occurs after oral administration, and this too may affect absorption.

Another barrier to drug absorption is the negatively charged mucous layer. It is thickest in the stomach and decreases distally (Rubinstein and

Tirosh, 1994). Enhancement of enteral absorption by lipid surfactant-mixed micelles also increases in the distal direction (Muranishi et al., 1979b; Taniguchi et al., 1980; Yoshikawa et al., 1981; Muranishi, 1985; Fukui et al., 1987; Hastewell et al., 1994; Swenson et al., 1994). A window of absorption or optimum absorption site has been reported for various drugs. No studies have been performed to determine whether an absorption window exists for dextran sulfate or structurally similar drugs.

Finally, our studies found the presence of intestinal contents decreased drug absorption from the small intestine. In the gavage experiments the fasted rats were not sacrificed, and the presence or absence of intestinal contents could not be determined.

3.4. Enteral administration and the effects of intestinal contents

In preliminary enteral experiments the intestines were not rinsed. The following intestinal regions were used in eight rats: the entire small intestine of two rats, the duodenum of one rat, the jejunum in two rats, and the ileum in three rats. In these rats, intestinal contents were present, and appreciable drug was found in the serum in only two of these rats. In one rat in which the entire small intestine was ligated, a maximum drug concentration of 9.5 μg/ml was found at 40 min. In one rat where the jejunum was used, the concentration of drug in serum reached 21.5 μg/ml at 10 min and remained between 14.3 and 19.5 μg/ml for 50 min. In the others, the drug concentration was below detectable limits.

The polyanionic dextran sulfate forms complexes with various cations, proteins and enzymes (Kimizuka et al., 1967; Larichev et al., 1983; Tankersley et al., 1983; Kikuchi et al., 1988). Many cations occur naturally in gastrointestinal fluids and dietary constituents. Therefore, an in vitro experiment was performed to determine whether the drug is bound to intestinal contents. Typical in vivo conditions exposed 0.35 ml of 40 mg/ml drug solution to $\sim$3 g of intestinal contents, giving a ratio of $\sim$8.3 g of intestinal contents per milliliter of drug solution. Ratios of 1, 5, 8.3 and 15 g/ml were prepared. The respective losses of dextran sulfate were 24, 22, 21 and 33%. These losses alone are not sufficient to account for the lack of absorption.

Complex formation and binding strength are affected by pH and ionic strength. Dextran sulfate may bind to constituents of intestinal contents under physiologic conditions and be displaced and react with the dye under the analytical conditions. Intestinal contents may disrupt the formulation, possibly by dilution. Micellar aggregates form various structures and may fluctuate to form different phases (Bourrel and Schechter, 1988). They have been shown to undergo a transition to lipid-bilayer vesicles upon dilution (Hjelm et al., 1988; Son and Alkan, 1989; Alkan-Onyuksel and Son, 1992). Additionally, the mucosal absorptive surface may be blocked by intestinal contents, a viscous semisolid which would impede drug diffusion. In some rats rinsing of the intestinal segment dislodged this material but did not remove it. Dextran sulfate was absorbed in these instances.

3.5. Administration to rinsed intestines

In all subsequent experiments the intestinal segment was rinsed. When intestinal contents were present, they were dislodged or removed by rinsing. A preliminary experiment utilizing the dispersion formulation in three rats indicated absorption enhancement occurred in each region of the small intestine. Delivery of drug to the duodenum gave a maximum serum concentration of 27.9 μg/ml at 40 min. Absorption from the jejunum resulted in a peak concentration of 21.5 μg/ml at 10 min. A maximum serum concentration of 25.6 μg/ml was found 30 min after drug administration to the ileum. The ileum was selected for additional experiments; 12 rats were used (Fig. 1). Variability was seen in both the maximum drug concentration (C_{max}) achieved and the time (t_{max}) at which maximum drug concentration occurred. C_{max} ranged from 25.6 μg/ml to 120.4 μg/ml; t_{max} ranged from 10 to 50 min. The elimination of dextran sulfate after intravenous administration to the rat has been reported to be biexponential with an initial half life of 20 min

and a terminal half life of 480 min (Hartman et al., 1990). In a study in humans the elimination of dextran sulfate after intravenous administration was monoexponential with a half life of 1.6 h (Lorentsen et al., 1989). Our studies show the drug has steady absorption during the first hour. In most cases drug concentration in serum increased with time then remained at a plateau.

When administered to rats in the MO-NaTC dispersion, dextran sulfate was absorbed well from the ileum. MO-NaTC enhancers are most commonly used enterally as a 40 mM equimolar formulation. Respective MO and NaTC concentrations of 10 and 20 mM were used in this study to reduce the possibility of mucosal irritation or damage. Although the rate and extent of absorption were variable, significant blood levels of dextran sulfate were found. The feasibility of enhancing the intestinal absorption of dextran sulfate has been demonstrated.

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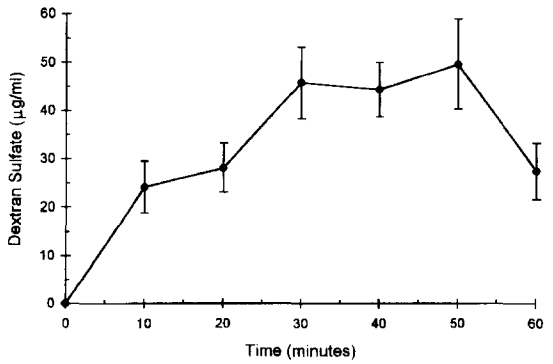

Fig. 1. Dextran sulfate levels in serum after administration to ileum of 12 rats in MO-NaTC dispersion. Error bars represent the standard error of the mean.

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