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TRANSPORT AND METABOLISM OF DEXTRAN SULFATES AND THE EFFECT OF CALCIUM

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Abstract—The transport and metabolism of [^{35}S]dextran sulfates (DSs) of various molecular weights and sulfur contents in rat intestinal mucosa, and the effects of calcium thereon, were investigated. The *in vitro* desulfation of DSs was observed largely in the microsomal fraction and was not influenced by the chain length of the glucosidic bond of DSs. The optimal pH for the desulfation of DS was about 5.0. The depolymerization of DSs prior to the desulfation was not significant even in the lysosomal fraction. The transport of DSs by everted sacs of intestine was increased significantly by the reduction of Ca^{2+} or the addition of ethylenediamine tetraacetic acid (EDTA) to the medium. After the intraduodenal administration of 10 mg/kg of [^{35}S]DS of molecular weight 200,000 and sulfur content 18 per cent, the radioactivity in the lysosomal fraction was about 30 per cent of that in mucosal tissues, and decreased to 8 per cent with the simultaneous administration with EDTA (50 mg/kg). The radioactivity in the soluble fraction was also decreased. Lysosomal ATPase was activated by the DS or Ca^{2+}, and inhibited by the simultaneous administration of EDTA. These findings suggest that DSs are transferred by pinocytosis and other mechanisms and are then desulfated mainly in microsomes followed by depolymerization. Further, Ca^{2+} may be necessary for the access of DSs to lysosomes which are involved in the metabolism of acid polysaccharides.

It has been suggested that acid polysaccharides, such as heparin [1] or dextran sulfates (DSs) [2], may be poorly absorbed from the gastrointestinal tract. According to these reports when [^{35}S]-labeled chondroitin sulfate [3] or DSs [2] was orally administered, part of the radioactivity was recovered from urine, and about half of the radioactivity in the urine was detected as radioactive organic sulfate. We reported that, after the duodenal administration of [^{35}S]DSs of various molecular weights, neither their molecular weights nor their pK_a values were related to the absorption rate of the radioactivity, and suggested that DSs, especially those of high molecular weight, may be rapidly desulfated in the intestine, probably in the epithelium [2]. Reber and Studer [4] showed that the lipemia-clearing activity of heparin could be increased about 100-fold by the simultaneous administration of calcium-chelating agents. Thus, it appears that the absorption rate and the pharmacological effects of DSs probably depend on the catabolism of DSs. In addition, the possibility exists that calcium chelation may enhance the absorption or inhibit the catabolism of DSs in the intestinal epithelium.

Chondroitin sulfate sulfatase in rat liver acts only on substrates depolymerized previously by hyaluronidase [5]. Since chondroitin sulfate sulfatase has high substrate-specificity, it appears that the sulfatase attacking DSs may be another carbohydrate sulfate sulfatase.

The present report was carried out to examine the transport and metabolism of DSs in rat intestine.

MATERIALS AND METHODS

Female Donryu strain rats (about 200 g) were starved overnight prior to the experiments.

The composition of DSs used in the experiments was as follows; DS-L-1, intrinsic viscosity (IV) 0.027, average molecular weight (AMW) 3,000, sulfur content (S%) 18.3; DS-L-2, IV 0.038, AMW 5,000, S% 9.1; DS-M-1, IV 0.082, AMW 20,000, S% 18.1; DS-M-2, IV 0.083, AMW 20,000, S% 8.3; DS-H-1, IV 0.198, AMW 200,000, S% 18.1; and DS-H-2, IV 0.214, AMW 200,000, S% 8.5. IV, $[\eta]$, was determined in 2 M NaCl at 37°. [^{35}S]DSs of high sulfur content had a specific activity of about 1.5 μCi/mg, and that of low sulfur content had about 1.0 μCi/mg. All the DSs were dissolved in 0.9 per cent saline.

The administration of [^{35}S]DS (10 mg/kg) was by intraduodenal injection. In some cases, ethylenediamine tetraacetic acid (EDTA) (50 mg/kg) was administered duodenally in combination with DSs.

Serial blood and bile samples were obtained from the carotid artery and from the bile duct by polyethylene cannulation respectively. Urinary collection also was by cannulation of the urinary bladder.

At 1 hr after the administration of DSs, the animals were killed by decapitation. The small intestine was quickly removed and washed with cold 0.9 per cent saline. The mucosa was scraped by spatula, and the scrapings were homogenized in 0.25 M sucrose and filtered once through muslin. The filtrates obtained were separated into subcellular fractions by differential centrifugation [6], with some modifications. A 10% homogenate was pelleted at 2,000 *g* for 10 min. The resulting pellet was washed once with sucrose and pelleted a second time. The resulting pellet constituted the nuclear fraction. The supernatant fractions from both washes were combined and centrifuged at 8,000 *g* for 10 min to produce the mitochondrial

pellet. The pellet was washed once with sucrose and pelleted by centrifugation. The supernatant fractions were centrifuged at 25,000 *g* for 20 min to produce the lysosomal pellet. The pellet was washed once with sucrose. The supernatant fractions were centrifuged at 105,000 *g* for 60 min. The pellet was washed once with 0.25 M sucrose and pelleted. The remaining supernatant fraction and the washings were combined and regarded as the soluble fraction, namely cytosol. Each of the pellets was suspended in distilled water for the counting of the radioactivity, or in incubation media for the assays of enzymatic activity. The purity of each fraction was confirmed by the assay of marker enzymes: succinate dehydrogenase in mitochrondria [7], acid *p*-nitrophenyl phosphate phosphatase in lysosomes [8], and glucose 6-phosphate phosphatase in microsomes [7]. In the present experiment, contamination by other fractions was less than 15 per cent.

In the *in vitro* experiment on DS absorption, according to the method of Crane and Wilson [9], everted sacs (0.5 g wet weight of tissue) of intestine containing 1 ml of Krebs–Ringer bicarbonate solution (pH 7.4) were incubated in the same solution containing 1 mg/ml of [^{35}S]DS. In some cases, the concentration of Ca^{2+} in the mucosal fluid was decreased to 0.4 mM instead of 2.2 mM of the normal solution, or 1 mM EDTA was added. After incubation for 1 hr, aliquots of these media and homogenates of the mucosa were used for the counting of the radioactivity.

Aryl sulfatase activity was assayed according to the method of Dodgson *et al.* [10]. ATPase activity was assayed according to the method of Quigley [11]. Protein was determined by the method of Lowry *et al.* [12].

In the case of subcellular fractions of intestinal mucosa, of serosal fluid in the experiment with everted sacs, and of blood, bile and urine, the radioactivity was analyzed by the ethanol method [2] after paper chromatography [13].

Radioactivity in all samples was measured by liquid scintillation counting using a toluene scintillator.

RESULTS

Metabolism of DSs in rat intestinal mucosa. Various DSs were incubated with subcellular fractions of intestinal mucosa of non-treated rats. In a preliminary experiment, when the fraction denatured by heating for 5 min at 50° was used, instead of each subcellular fraction, nonenzymatical or bacterial desulfation did occur. The inorganic [^{35}S]sulfate released from [^{35}S]DSs of high sulfur content was much more than from [^{35}S]DSs of low sulfur content after incubation (Table 1). High activity of the sulfatase against DSs was detected from the mitochondrial, lysosomal and microsomal fractions.

After incubation of [^{35}S]DS-H-1 with the lysosomal fraction (1 mg protein/ml) of intestinal mucosa for 24 hr at 37°, the amount of released inorganic [^{35}S]sulfate was 30 per cent of the added radioactivity at pH 4.0, 40 per cent at pH 5.0, 28 per cent at pH 6.0, and 3 per cent at pH 7.4. The optimal pH for DS sulfatase was observed at pH 5.0, and the optimal concentration of the substrate was 0.1 mg/ml, about 0.5 μM.

The metabolites of [^{35}S]DS-H-1 separated after incubation with the lysosomal fraction at pH 5.0 were, in large part, inorganic [^{35}S]sulfate migrating at R_f 0.7, and, in small part, a depolymerized but non-desulfated form of the DS at the origin (Fig. 1).

DS-L-1, DS-M-2 and DS-H-2 inhibited the desulfation of *p*-nitrocatechol sulfate by microsomal aryl sulfatase, but DS-H-1 enhanced the enzymatic activity (Table 2).

The relative activities of *p*-nitrocatechol sulfate sulfatase in subcellular fractions resembled those of the sulfatase for DS-M-2 and DS-H-2 (Table 3). These results suggest that DS of high molecular weight and low sulfur content may be desulfated, especially by aryl sulfatase.

Effects of calcium on the absorption and metabolism of DSs. The effect of a calcium-chelating agent on the absorption of [^{35}S]DS-H-1 of a higher average molecular weight and higher sulfur content than heparin has, was examined. After simultaneous administration of [^{35}S]DS-H-1 with EDTA, the blood level of radioactivity was two to three times higher than after the DS alone, and the radioactivity in bile also considerably increased (Fig. 2). In contrast to the high levels of the radioactivity in blood and bile, the excretion of the radioactivity into urine was markedly inhibited by simultaneous administration with

Table 1. Subcellular distribution of the activity of dextran sulfate sulfatase in rat small intestine mucosa*

Substrates	Activity of dextran sulfate sulfatase† (inorganic [^{35}S]sulfate released/mg protein/24 hr)				
	Nuclei	Mitochondria	Lysosomes	Microsomes	Cytosol
DS-L-1	6.9 ± 2.4	43.2 ± 6.4	30.8 ± 1.7	45.0 ± 9.9	5.9 ± 1.0
DS-L-2	8.4 ± 2.9	12.2 ± 3.7	24.1 ± 9.8	8.6 ± 3.1	2.1 ± 0.5
DS-M-1	10.8 ± 3.5	25.9 ± 4.3	28.0 ± 6.5	46.9 ± 7.2	7.9 ± 0.9
DS-M-2	4.9 ± 1.9	9.4 ± 1.5	5.1 ± 1.9	12.0 ± 1.3	3.9 ± 0.5
DS-H-1	7.2 ± 3.1	41.6 ± 9.2	38.5 ± 3.3	59.2 ± 3.5	8.7 ± 1.5
DS-H-2	3.3 ± 2.5	15.5 ± 3.7	10.0 ± 5.4	19.3 ± 2.6	5.1 ± 0.3

* Incubations were carried out in 2 ml of 0.1 M acetate buffer (pH 5.0) containing about 2 mg protein of each fraction and 1 mg of [^{35}S]DS for 24 hr at 37°. Each value represents the mean and standard deviation of four experiments.

† Per cent of the radioactivity added.

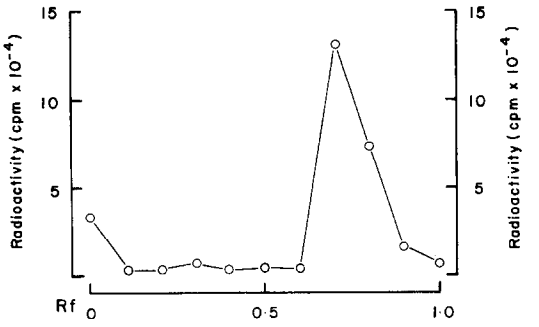

Fig. 1. Paper chromatographic pattern of the radioactivity of the products of incubation of [^{35}S]DS-H-1 and the lysosomal fraction of small intestinal mucosa in rats. The incubation system was the same as is Table 1. The unchanged DS was previously removed by the addition of 10% cetylpyridinium chloride solution to the medium after the incubation, and centrifugation. The supernatant fraction was combined with 95 per cent (final 80 per cent) ethanol in 2 per cent Na_2SO_4 and then centrifuged. All the radioactivities in this solution were recovered from the precipitate obtained. Filter paper: Toyo No. 51A. The developing system: 25 per cent n-propanol in 67 mM phosphate buffer (pH 6.4) for 24 hr at 4°.

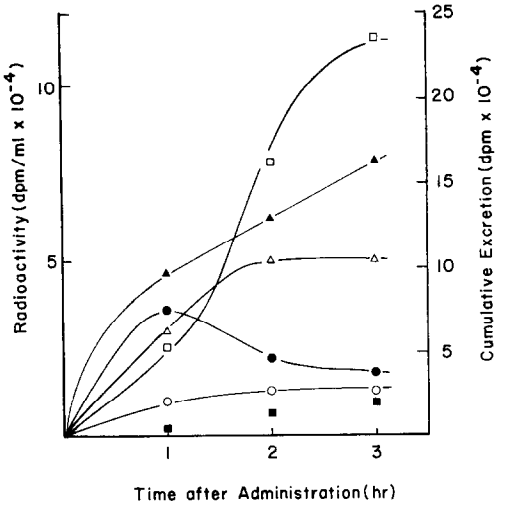

Fig. 2. Effects of EDTA on the absorption and excretion of [^{35}S]DS-H-1 (10 mg/kg) after duodenal administration to rats. EDTA was administered duodenally at a dose of 50 mg/kg. The concentration of the radioactivity in blood (DS alone, ○, with EDTA, ●) and in bile (DS alone, △, with EDTA, ▲), and the cumulative radioactivity in urine (DS alone, □, with EDTA, ■) were plotted vs time. Each point represents the mean from four experiments.

Table 2. Effects of dextran sulfates on microsomal aryl sulfatase activity of rat small intestine mucosa*

	Aryl sulfatase		
	Additions	Activity†	Per cent of control
None		138 ± 7.8	100 ± 5.7
DS-L-1	10 μg	80. ± 7.7	58 ± 5.6
	100 μg	33 ± 2.8	24 ± 2.0
	1 mg	43 ± 6.4	31 ± 4.6
DS-L-2	100 μg	148 ± 9.4	107 ± 6.8
	1 mg	78 ± 6.8	57 ± 4.9
DS-M-1	100 μg	126 ± 7.9	91 ± 5.7
	1 mg	78 ± 6.8	57 ± 4.9
DS-M-2	10 μg	76 ± 7.4	55 ± 5.4
	100 μg	37 ± 5.9	27 ± 4.3
	1 mg	20 ± 14.5	14 ± 10.5
DS-H-1	1 mg	181 ± 7.5	131 ± 5.4
DS-H-2	100 μg	108 ± 15.6	78 ± 11.3

* Incubations were carried out in 2 ml of 0.1 M acetate buffer (pH 5.0) containing 2.5 mM *p*-nitrocatechol sulfate and about 2 mg protein of microsomal fraction for 1 hr at 37°. Each value represents the mean and standard deviation of four experiments.

† Expressed as nmoles sulfate released/mg of protein/hr.

EDTA. Examination of the paper chromatographic pattern of blood and bile revealed that the amount of inorganic [^{35}S]sulfate decreased slightly after the simultaneous administration of EDTA.

The subcellular distribution of the radioactivity after the simultaneous administration of [^{35}S]DS with EDTA was examined. After duodenal administration of each [^{35}S]DS alone, the radioactivity in the soluble fraction was about half of the radioactivity taken up by intestinal mucosa (Table 4). In the case of DS-H-1, about 30 per cent was detected in the lysosomal fraction. As compared with the administration of DS alone, the simultaneous administration of high molecular weight DS with EDTA appeared to increase the radioactivity in the soluble fraction and, conversely, to decrease the radioactivity in the lysosomal fraction.

The effect of calcium on DS transfer was examined by the everted sac method, using rat small intestine. With all the DSs used in these experiments, especially DS-M-1, DS-M-2 and DS-H-1, transfer of the radioactivity to the tissue and the serosal fluid was enhanced by the fall of Ca^{2+} concentration or the addition of EDTA to the incubation medium (Table 5). In the case of DS of low sulfur content, more of the radioactivity accumulated in the tissue than was transferred to the serosal fluid. The concentration ratio of the radioactivity in serosal to mucosal fluid was elevated by the addition of EDTA to the medium. In the case of DS-H-1, the ratio was significantly increased also by the decrease of Ca^{2+} concentration in the mucosal fluid.

Four radioactive peaks were observed in the paper chromatographic pattern of the serosal fluid after the incubation of [^{35}S]DSs. These peaks were at R_f 0.65 to 0.75 as inorganic sulfate, at R_f 0.90 to 1.00 as the depolymerized form, at R_f 0.20 to 0.30 as the desulfated form, and at the origin as the unchanged form of [^{35}S]DSs (Fig. 3). The greater part of the radioactivity, however, was inorganic sulfate. EDTA added to the mucosal fluid did decrease the amount of the radioactivity as inorganic sulfate in the serosal fluid after the incubation of DS-H of high or low sulfur content. EDTA, however, did not cause a remarkable change in the paper chromatographic pattern of the serosal fluid after the incubation of DS-L-1.

The fact that, after duodenal administration of high molecular weight [^{35}S]DS, high radioactivity was observed with the lysosomal fraction of intestinal mucosa may suggest pinocytotic uptake of the DS by the epithelium. Pinocytosis is thought to

Table 3. Comparison of the subcellular distribution of aryl sulfatase and dextran sulfate sulfatase*

Substrates	Subcellular fractions	Specific activity†	Relative activity‡
p-Nitrocatechol sulfate	Mitochondria	98 ± 15.0	0.71 ± 0.109
	Lysosomes	49 ± 2.1	0.36 ± 0.015
	Microsomes	138 ± 7.8	1.00 ± 0.057
	Cytosol	22 ± 0.1	0.16 ± 0.001
[^{35}S]DS-M-2	Mitochondria	9.4 ± 1.5	0.78 ± 0.125
	Lysosomes	5.1 ± 1.9	0.43 ± 0.158
	Microsomes	12.0 ± 1.3	1.00 ± 0.108
	Cytosol	3.9 ± 0.5	0.33 ± 0.042
[^{35}S]DS-H-2	Mitochondria	15.5 ± 3.7	0.80 ± 0.192
	Lysosomes	10.0 ± 5.4	0.52 ± 0.280
	Microsomes	19.3 ± 2.6	1.00 ± 0.135
	Cytosol	5.1 ± 0.3	0.26 ± 0.016

* Each value represents the mean and standard deviations of four experiments.

† Specific activity for the *p*-nitrocatechol sulfate substrate is expressed in nmoles sulfate released/mg of protein/hr; specific activity for the [^{35}S]DS-M-2 and [^{35}S]DS-H-2 substrates is expressed as per cent of added radioactivity.

‡ Ratio of the specific activity to that in microsomes of the same group.

Table 4. Subcellular distribution of radioactivity in small intestinal mucosa after duodenal administration of [^{35}S]dextran sulfates to rats*

DS	Treatment with EDTA	Per cent of radioactivity taken up by mucosa				
		Nuclei	Mitochondria	Lysosomes	Microsomes	Cytosol
DS-L-1	−	16.3 ± 2.86	7.3 ± 4.08	16.9 ± 4.70	15.1 ± 5.92	44.4 ± 9.02
	+	19.7 ± 6.65	9.4 ± 5.00	14.2 ± 6.25	16.6 ± 6.15	40.1 ± 9.72
DS-M-1	−	13.3 ± 2.25	17.5 ± 6.55	10.3 ± 3.52	13.3 ± 4.70	45.6 ± 8.40
	+	9.8 ± 2.15	15.2 ± 2.80	10.4 ± 2.13	12.2 ± 5.80	52.4 ± 9.38
DS-H-1	−	12.1 ± 1.79	8.5 ± 2.30	27.0 + 11.45	15.1 ± 7.65	37.3 ± 8.35
	+	14.0 ± 0.40	6.3 ± 0.32	7.5 ± 0.85†	9.3 ± 2.65	62.9 ± 10.85‡

* Rats received 10 mg/kg of [^{35}S]DS simultaneously with or without 50 mg/kg of EDTA-Na_2, and 2 hr later were killed. Each value represents the mean and standard deviation of four experiments.

† Significant difference from the group: with no administration of EDTA, $P < 0.05$.

‡ $P < 0.01$.

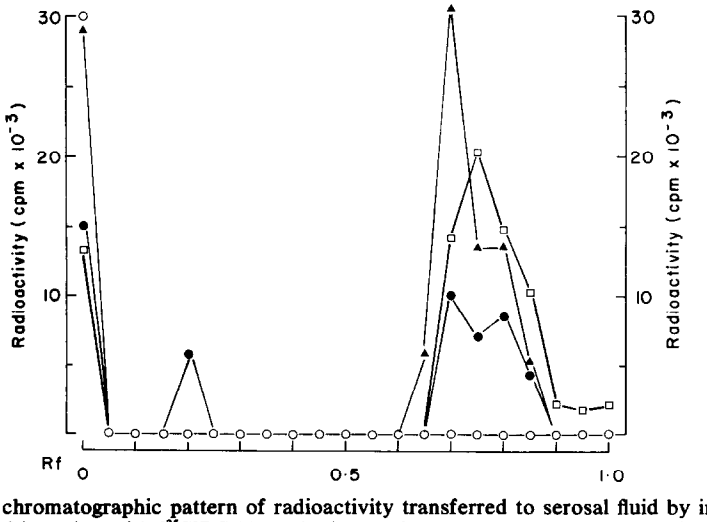

Fig. 3. Paper chromatographic pattern of radioactivity transferred to serosal fluid by incubation of everted sacs of intestine with [^{35}S]DS-H-1. The incubation system was the same as in Table 5. The everted sacs were incubated in a medium containing 2.2 mM $CaCl_2$ (□), 0.4 mM $CaCl_2$ (▲), or 1 mM EDTA (●). Distribution of standard [^{35}S]DS-H-1 is shown by the symbol ○.

Table 5. Effects of calcium and EDTA on transfer of [^{35}S]dextran sulfates by everted sacs of small intestine in rats*

	Concn. (mM)	Radioactivity in: Mucosal fluid (% added)	Serosal fluid (% added)	Ratio†	Intestinal tissue (% added)
DS-L-1	$CaCl_2$ 2.2	83.2 ± 1.21	6.4 ± 0.31	0.50 ± 0.024	10.4 ± 1.34‡
	$CaCl_2$ 0.4	80.2 ± 1.49‡	6.7 ± 0.42	0.53 ± 0.027	13.1 ± 1.18‡
	EDTA 1	78.7 ± 1.36§	7.5 ± 0.92	0.60 ± 0.063‡	13.8 ± 1.41‡
DS-L-2	$CaCl_2$ 2.2	85.9 ± 2.01	5.1 ± 1.85	0.42 ± 0.122	9.1 ± 1.04
	$CaCl_2$ 0.4	84.3 ± 1.78	6.7 ± 1.69	0.54 ± 0.119	9.0 ± 1.18
	EDTA 1	83.0 ± 3.84	9.7 ± 2.53‡	0.77 ± 0.063§	7.3 ± 1.39
DS-M-1	$CaCl_2$ 2.2	81.8 ± 6.24	7.5 ± 4.18	0.61 ± 0.293	10.8 ± 1.28
	$CaCl_2$ 0.4	74.2 ± 5.13	11.1 ± 4.28	0.90 ± 0.301	14.7 ± 1.41§
	EDTA 1	69.0 ± 5.21‡	19.3 ± 3.04§	1.47 ± 0.212§	11.8 ± 1.14
DS-M-2	$CaCl_2$ 2.2	85.2 ± 7.41	6.9 ± 1.85	0.50 ± 0.128	7.9 ± 0.94
	$CaCl_2$ 0.4	80.2 ± 5.22	8.5 ± 2.85	0.61 ± 0.201	11.4 ± 1.41§
	EDTA 1	74.3 ± 3.14‡	10.6 ± 1.75‡	0.86 ± 0.125§	15.1 ± 1.34‖
DS-H-1	$CaCl_2$ 2.2	80.3 ± 2.41	11.2 ± 4.16	0.95 ± 0.293	8.5 ± 3.21
	$CaCl_2$ 0.4	74.6 ± 3.29‡	17.9 ± 1.95‡	1.41 ± 0.137‡	7.5 ± 2.22
	EDTA 1	67.4 ± 4.18§	23.5 ± 0.66§	1.79 ± 0.42§	9.1 ± 2.49
DS-H-2	$CaCl_2$ 2.2	87.8 ± 8.41	4.0 ± 1.96	0.29 ± 0.136	8.2 ± 0.81
	$CaCl_2$ 0.4	81.3 ± 7.42	5.1 ± 0.20	0.41 ± 0.014	13.6 ± 0.71‖
	EDTA 1	80.5 ± 7.14	6.4 ± 0.15	0.48 ± 0.011‡	13.1 ± 0.67‖

* Everted sacs of intestine (0.5 g wet weight of tissue) containing 1 ml Krebs–Ringer bicarbonate solution (pH 7.4) were incubated in 7 ml of the same solution containing 1 mg/ml of [^{35}S]DS for 1 hr at 37° under aeration. In some cases, 0.4 mM $CaCl_2$ or 1 mM EDTA-Na_2 was added to the incubation medium on the mucosal side instead of 2.2 mM $CaCl_2$ in the normal solution. Each value represents the mean and standard deviation of five experiments.

† Concentration ratio of radioactivity in serosal to mucosal fluid/0.5 g of tissue.

‡ Significant difference from group of 2.2 mM $CaCl_2$, $P < 0.05$.

§ $P < 0.01$.

‖ $P < 0.001$.

Table 6. ATPase activity of subcellular fractions in small intestinal mucosa after duodenal administration of dextran sulfates with or without EDTA to rats*

Treatments	ATPase activity (μmoles P_i/mg protein/20 min): Mitochondria	Lysosomes	Microsomes
Non-treated	53 ± 3.8	5 ± 3.8	40 ± 23.3
DS-L-1 alone	9 ± 5.1†	4 ± 0.3	53 ± 4.3
with EDTA	7 ± 4.6	9 ± 4.3	49 ± 3.6
DS-M-1 alone		49 ± 2.9†	24 ± 3.9
with EDTA		33 ± 3.4†	36 ± 6.6‡
DS-H-1 alone	4 ± 2.9†	167 ± 34.4†	3 ± 1.1‡
with EDTA	4 ± 2.2	63 ± 6.1§	24 ± 8.0§

* Rats received 10 mg/kg of DSs with or without 50 mg/kg of EDTA, and were killed 2 hr afterward. The incubations were carried out in 2 ml of 100 mM Tris–HCl buffer (pH 7.4) containing 67 mM NaCl, 3 mM KCl, 4 mM $MgCl_2$, 2 mM ATP-Na_2 and 2 mg protein of each fraction for 20 min at 37°. Each value represents the mean and standard deviation of four experiments. For the DS-alone group, significant difference from the non-treated group is shown; and for the group receiving DS with EDTA, significant difference from the DS-alone group is shown.

† $P < 0.001$.

‡ $P < 0.05$.

§ $P < 0.01$.

be an energy-requiring process and to consume ATP [14]. Furthermore, since part of the lysosomal membranes is probably derived from plasma membranes in the process of pinocytosis [15], the activity of lysosomal ATPase may be enhanced by the occurrence of pinocytotic uptake. Therefore, ATPase activity in subcellular fractions of intestinal mucosa after the administration of DSs was determined. The activity of ATPase in mitochrondria was significantly inhibited by the

Table 7. Effects of *in vitro* dextran sulfate-H-1 and calcium on activity of lysosomal ATPase in small intestinal mucosa of rats*

Additions		ATPase activity (μmoles P_i/mg protein/20 min)
None		4.7 ± 3.8
Ca^{2+}	1.16 mM	52.3 ± 9.6
DS-H-1	10 μg/ml	36.3 ± 3.1
	100 μg/ml	33.7 ± 3.5
	1 mg/ml	45.0 ± 4.8

* Lysosomal fractions of non-treated rats were used. Incubation system was the same as in Table 5. Each value represents the mean and standard deviation of four experiments.

administration of DS-H-1 or DS-L-1 (Table 6). No significant change in ATPase activity of microsomes was caused by DS-L-1 or DS-M-1, whereas DS-H-1 significantly inhibited the activity. However, the activity of lysosomal ATPase was markedly enhanced after the administration of DS-H-1 or DS-M-1, and these enhanced activities were slightly decreased by the simultaneous administration with EDTA.

The effect of *in vitro* calcium and DS-H-1 on the ATPase activity of the lysosomal fraction from non-treated rats was examined. The ATPase activity was stimulated more than 10-fold by the addition of 1.16 mM Ca^{2+} (Table 7). DS-H-1 also increased the activity of lysosomal ATPase.

DISCUSSION

The metabolism and transport of DSs and the effect of calcium were studied in rat intestinal mucosa. DSs are thought to be metabolized in two ways, desulfation by sulfatase and depolymerization by α-glucosidase, i.e. dextranase. In the present experiments, the highest activity of DS sulfatase in subcellular fractions of the intestinal mucosa was observed in the microsomal fraction. Further, as the molecular weight and sulfur content of DS increase, more inorganic sulfate is released after the incubation of DSs with the microsomal fraction (Table 1). Based on this finding, the desulfation of DSs in the microsomal fraction appeared not to be influenced by the differences in glucosidic bond or side chain of DSs.

Tudball and Davidson [5] reported that chondroitin sulfates are depolymerized by hyaluronidase at first, and then the depolymerized products are desulfated by chondroitin sulfate sulfatase. From this report, it is considered that the sulfatase has high affinity for acid polysaccharides of low molecular weight. Thus, the desulfation of DS is probably not due to chondroitin sulfate sulfatase. Further, DSs may not need to be depolymerized by α-glucosidase prior to desulfation.

As far as the optimal pH for DS sulfatase present in lysosomes is concerned, it is thought that the desulfation of DS-H-1 may be due not to the sulfatase active against chondroitin sulfate A, C or hyaluronic acid with an optimal pH of 3.9, but rather to heparitin sulfate sulfatase with an optimal pH of 4.8 [16].

After the incubation of DS-H-1 with the lysosomal fraction, the metabolites of DS which we expected, the depolymerized and the desulfated form, were scarcely detected (Fig. 1). This suggests that dextran produced by the desulfation of DSs may be rapidly depolymerized by dextranase in lysosomes, or that this enzyme cannot hydrolyze the glucosidic bond in DSs unless previously sulfate groups are released from DSs. On the other hand, the relative activity of aryl sulfatase against the substrate, *p*-nitrocatechol sulfate, resembled that of DS-M-2 and DS-H-2 sulfatase (Table 3). In addition, aryl sulfatase was inhibited by these DSs (Table 2). The subcellular localization of aryl sulfatase of intestinal mucosa in these experiments was much the same as its localization in rat liver [17]. These findings suggest that DS of high molecular weight and low sulfur content may be desulfated by aryl sulfatase.

It is reported that dextranase is located in lysosomes of rat liver [18]. In the present experiments, some of the depolymerized forms of DS-H-1 were detected after the incubation of the DS with everted sacs of intestine (Fig. 3), but very few were detected after incubation with the lysosomal fraction of intestinal mucosa (Fig. 1). Therefore, the depolymerization of DSs of high sulfur content appears to be slow unless the DSs are desulfated previously, as described above. We have observed a considerable amount of the depolymerized form of DS from liver after intravenous administration of DS.* These observations suggest that there is a significant difference in the activities of the dextranases in intestinal mucosa and liver.

Reber and Studer [4] have reported that, after oral administration of heparin, lipemia-clearing activity was enhanced by simultaneous administration of calcium-chelating agents. This enhanced pharmacological effect may be due to increased transport of heparin. After duodenal administration of [^{35}S]DS-H-1 with EDTA, the radioactivities in blood and bile were two to three times higher than that of DS alone, whereas the excretion into urine was decreased (Fig. 2). At this time, the radioactivity in the soluble fraction of intestinal mucosa increased and the proportion of inorganic [^{35}S]sulfate decreased (Table 4). The metabolism of DSs of low molecular weight was not affected by the administration of EDTA. The increase of DS transport induced by EDTA was shown also in the *in vitro* experiments (Table 5). Furthermore, EDTA inhibited markedly the desulfation of DSs (Fig. 3). These findings suggest that calcium chelation does not influence the enzymatic activities which are involved in the metabolism of DSs, but inhibits the access of DSs of high molecular weight to the metabolic sites of acid polysaccharides, microsomes and lysosomes.

After duodenal administration of DS of high molecular weight, one-third of the radioactivity taken up in the intestinal mucosa was detected in the lysosomal fraction (Table 4). This distribution of radioactivity appears to result from the pinocytotic uptake of the DS by intestinal epithelium, because pinocytotic vesicles are probably re-

* Unpublished observations.

covered from the lysosomal fraction by subcellular fractionation [19].

On the other hand, it could be considered that, after the administration of [^{35}S]DS-H-1, the DS was distributed to the cytosol by membrane transport rather than by pinocytotic uptake, and was then absorbed non-specifically to the particulate fractions. This possibility can be discarded because over 80 per cent of the radioactivity added to homogenates of intestinal mucosa of non-treated rats was recovered from the soluble fraction.

It appears that pinocytotic vesicles are formed by the infolding of the sites of plasma membrane to which are adsorbed certain materials, and that then the vesicles fuse with lysosomes containing various acid hydrolases [20]. Therefore, the activity of ATPase in lysosomes is thought to be an index of pinocytotic uptake [15]. In the present report, DSs of high molecular weight enhanced this activity of lysosomes, which was activated also by Ca^{2+} (Table 7). In fact, the enhanced ATPase activity was inhibited by EDTA *in vivo* (Table 6) and *in vitro* (Table 7). It is reported that Na^+–K^+-dependent ATPase is inhibited by Ca^{2+} [21]. These findings suggest that lysosomal ATPase enhanced by DS of high molecular weight may be Ca^{2+}-activated ATPase. Ca^{2+} may be necessary to initiate pinocytosis, and to activate the ATPase. The activation of this enzyme is thought to change the conformation of the plasma membrane [14]. EDTA increased the radioactivity in the soluble fraction (Table 6). This result may be due to the fragility of the plasma membrane which is caused by EDTA, and then to the increased diffusion of DSs across the membrane. This phenomenon may result in the increase of the radioactivity of blood and bile after simultaneous administration of [^{35}S]DSs with EDTA.

From the present report, it is suggested that some fractions of DSs, especially of high molecular weight, are transported probably by pinocytosis, and other fractions of DSs may be transferred by unknown mechanisms, for example the acid transport system, facilitated diffusion, etc. It also is considered that DSs taken up by intestinal epithelium are desulfated by aryl sulfatase in lysosomes and/or in microsomes, and then are depolymerized by α-glucosidase in lysosomes.

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