Effect of Sulfated and Sulfonated Surfactants on the Intestinal Absorption of Heparin (33678)

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Intraduodenal administration of heparin in an emulsified form to rats or gerbils has been shown to result in the rapid appearance of clearing factor activity (lipoprotein lipase) in blood (1). In analogous experiments using insulin emulsions, substantial lowering of blood glucose was observed in the rat and gerbil (2). These results demonstrated intestinal absorption of heparin and insulin and suggested a possible means of effecting the absorption of other therapeutic biopolymers which are normally inactive when administered orally.

However, during the course of these experiments, it was observed that a number of sulfated or sulfonated surfactants used to stabilize the emulsions were capable of facilitating the absorption of heparin in the absence of an oil phase. The present study describes this effect and presents data on absorption and tissue distribution of heparin in the dog, following intraduodenal administration with sulfated or sulfonated surfactants.

Methods. Surfactants used were sodium lauryl sulfate (SLS, Mann), Aerosol OT (dioctyl sodium sulfosuccinate, American Cyanamid), G-3300 (an alkyl aryl sulfonate obtined from Atlas) and RE-610 (an anionic surface active phosphate ester obtained from General Aniline and Film). Heparin, sodium was the product of Lederle Laboratories, heparin-^{35}S was obtained from Calbiochem and Ediol, a commercial coconut oil emulsion, was the generous gift of Riker Laboratories.

Animals employed were Wistar (Royal-Hart) male rats (150–250 g) or Beagle (Lederle colony) dogs (5.0–8.6 kg), fasted 18 hr prior to experiment.

All preparations were administered intraduodenally. Heparin solutions were given at a dose of 100 mg of heparin/kg in a volume of surfactant solution (0.5% in water) of 5 ml/kg (25 mg/kg). Control animals received surfactant or heparin in aqueous solution. Heparin-^{35}S was administered at a dose of 7 or 14.4 μCi/kg to dogs or rats, respectively, and constituted 15–25% of the total heparin dose. All solutions were adjusted to pH 6.5 before administration.

Heparin emulsion was prepared as previously described (1) and contained heparin, 40 mg/ml, RE-610, 0.32%, and trioctanoin (Eastman), 8% (v/v). The emulsion was administered in a dose of 5 ml/kg (200 mg of heparin/kg). Controls received 200 mg of heparin/kg in 0.32% RE-610 solution.

Rats were anesthetized with 60 mg/kg of Diabutal (Diamond Laboratories), i.p. The duodenum was exposed through a midline incision and a loop of surgical thread was loosely placed 1 cm distal to the pyloric sphincter. The preparations were introduced via a blunt needle inserted into the duodenum between the pylorus and the loop. Before injection, the needle was advanced until the end was distal to the loop. The duodenum was held gently closed at the loop, the preparation was injected and the loop was pulled tight as the needle was withdrawn. This procedure prevented backflow of the preparation to the point of needle insertion with subsequent possible absorption via the damaged capillary bed. Gauze moistened with 0.9% saline was then placed over the incision. Blood samples were obtained by cardiac puncture.

Dogs were anesthetized with 60 mg/kg Diabutal, i.v. The thoracic duct was cannulated through a midline cervical incision using a polyethylene cannula (Clay-Adams, Intramedic, PE-90). The portal vein was exposed through a midline abdominal incision and cannulated with a flexible 16-gauge i.v. catheter needle (Jelco) cemented in place with a cyanacrylate tissue adhesive. The femoral vein was used as a source of systemic blood. Solutions were administered via a 70-cm catheter (Bard No. 305, size 8) insert-

ed orally through the esophagus and stomach. The distal end was guided through laporatomy into the duodenum to a position approximately 4 cm below the pyloric sphincter. The duodenum was constricted manually at the pylorus during injection of the test solution (30 sec) after which the catheter was withdrawn.

Samples of systemic and portal blood and lymph were collected at 15-min intervals. At 30 or 60 min, liver biopsies were taken using Gelfoam (Upjohn) to arrest bleeding. At the termination of the experiment, the whole liver was excised and biopsies of the rectus femoris muscle and perirenal adipose tissue were taken. The small intestine was excised and the contents collected with two 15 ml water washes. The intestine was opened and the mucosa scraped off with a glass slide. Aqueous 20% homogenates were prepared from all tissues. Tissue samples were homogenized for 1 min in a Waring blendor or by hand in all-glass homogenizers. Appropriate aqueous dilutions of each homogenate were made and 0.4-ml aliquots added to 25-ml counting vials containing 3.0 ml of NCS reagent (0.6 *N* surface active organic base in toluene obtained from Nuclear, Chicago). After 24 hr, 20 ml of scintillation solution, 4.0 g of 2,5-diphenyloxazole and 0.1 g of 1,-4-bis[2-(5-phenyloxzolyl)] benzene per liter of toluene, was added and radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer. Quenching was corrected for by addition of internal standards (heparin-^{35}S dissolved in NCS and scintillation solution). Tissue or serum concentration of heparin was calculated from specific activity of injected solutions, assuming negligible degradation of absorbed heparin.

Clearing factor activity (3) was determined via a turbidimetric assay on aliquots of serum or lymph in 1-ml Coleman spectrophotometer cuvettes. In the cuvette were placed 0.6 ml of 0.05 *M* tris(hydroxymethyl) aminomethane buffer (pH 8.5), 0.3 ml of 25% (w/v) bovine plasma albumin fraction V (Armour), pH 8.5, and 0.5 ml of serum or lymph. Substrate [0.1 ml of 0.6% (v/v) Edi-ol in Tris buffer], was added at zero time. The optical density at 650 mμ (approximately 0.75) was immediately recorded. The mixture was incubated at 37° and optical density readings were taken at 15 and 30 min. The results are reported in terms of the observed decrease in optical density at the times indicated.

Results. Intestinal absorption of heparin-^{35}S-emulsion in rats. The relationship between serum clearing factor activity (decrease in optical density) and serum heparin concentration in rats treated with an emulsion containing heparin-^{35}S is presented in Fig. 1. Serum clearing factor activity was observed in all rats treated with emulsion. Appearance of significant serum heparin levels was associated with optical density

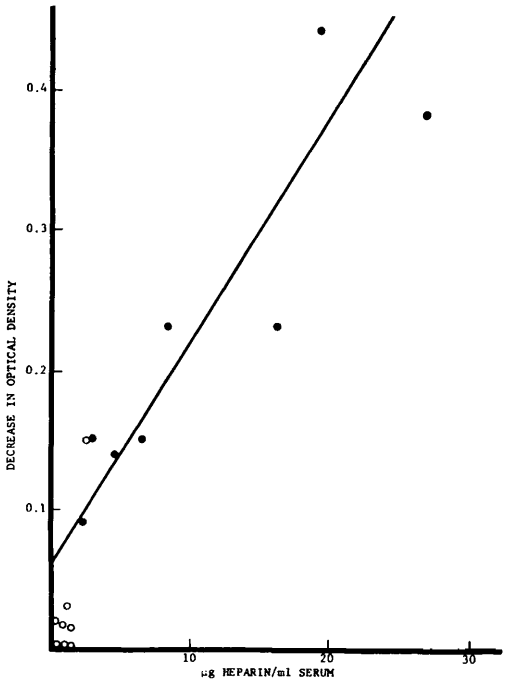

FIG. 1. Relationship between serum heparin concentration and clearing factor activity. Rats, previously fasted 18 hr, were dosed intraduodenally with heparin-^{35}S-containing emulsion (200 mg of heparin/kg) (●); or heparin-^{35}S in RE-610 solution, 0.32%, (○); and were bled 30 min later. Emulsion contained heparin, 40 mg/ml; RE-610, 0.32%; and trioctanoin, 8% (v/v). Points represent individual animals and are expressed as decrease in optical density at 650 mμ after 15 min of incubation.

TABLE I. Clearing Factor Activity in the Rat Following Intraduodenal Administration of Heparin and Sulfated or Sulfonated Surfactants.[a]

Treatment	Decrease in optical density (650 mμ) 15 min	30 min
Heparin	0.03[b] ± 0.00	0.04 ± 0.00
Heparin + SLS	0.30 ± 0.06	0.43 ± 0.08
SLS	0.03 ± 0.00	0.03 ± 0.01
Heparin + Aerosol OT	0.24 ± 0.05	0.38 ± 0.05
Aerosol OT	0.03 ± 0.00	0.03 ± 0.00
Heparin + G-3300	0.24 ± 0.04	0.38 ± 0.05
G-3300	0.02 ± 0.00	0.03 ± 0.00

[a] Male rats previously fasted 18 hr were dosed intraduodenally with 100 mg of heparin/kg in 0.5% surfactant solution, (5 ml/kg) or aqueous heparin. Blood samples were taken 30 min after dosing.

[b] Values are means ± SE of 8 rats/group and are expressed as the change in optical density after 15 and 30 min of incubation. Initial mean optical density was 0.75.

changes of greater than 0.06 and the latter was directly related to the concentration of heparin. A decrease in optical density of 0.06 or greater was, therefore, considered significant in subsequent experiments.

Table I summarizes the data obtained following intubation in rat intestine of solutions containing heparin and sulfated or sulfonated surfactants. Serum from animals treated with heparin alone contained no significant clearing factor activity (decrease in optical density of less than 0.06). However, within 30 min after dosing, a strong clearing factor response (approximately a 46% decrease in optical density) was found in serum of animals treated with solutions containing heparin and SLS, Aerosol OT, or G-3300. Surfactant solutions alone elicited no significant activity.

Intestinal absorption of heparin in dogs in the presence of SLS. Blood and lymph concentration of heparin and serum clearing factor activity in dogs treated with heparin-^{35}S–SLS solutions are presented in Fig. 2. There was a rapid increase in clearing factor activity in both the venous systemic and portal blood which reached a maximum in 30–50 min. The increase in serum clearing factor was associated with a concomitant increase in heparin concentration as determined by ^{35}S activity in both systemic and portal blood. There was no significant difference in clearing factor activity or heparin concentration between the systemic and portal circulation. Based on the heparin concentration of systemic blood at 60 min and the calculated serum volume (5% of body wt.) approximately 1% of the total heparin dose was present in the circulation. These studies were not extended beyond 90 min as severe hemorrhage occurred from all cut surfaces of animals treated with heparin–surfactant solutions.

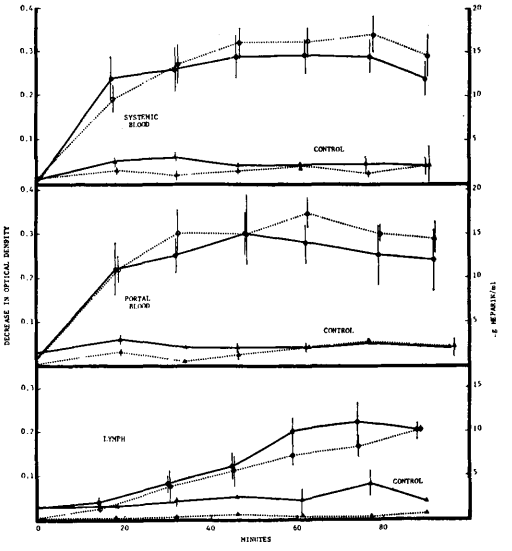

FIG. 2. Heparin concentration and clearing factor activity in blood and lymph of dogs. Dogs, previously fasted 18 hr, were dosed intraduodenally with heparin-^{35}S (100 mg/kg) in SLS (0.5% solution, (○); or an aqueous solution of heparin-^{35}S (100 mg/kg), (▲). Points are means with SE of four dogs and are expressed as change in optical density (—) at 650 mμ after 15 min of incubation or μg heparin (- - -).

TABLE II. Tissue Distribution of Heparin in Dogs Treated with Heparin-^{35}S or Heparin-^{35}S and SLS.[a]

Treatment	Intestine				Rectus femoris	Adipose tissue	Liver		
	Contents	Mucosa	Wall	Total			30 min	60 min	90 min
Heparin	62.2[b] ± 13.2 (4)	14.2 ± 2.7 (4)	3.8 ± 0.6 (4)	80.7 ± 12.5 (4)	None	None	None	None	None
Heparin + SLS	46.4 ± 15.2 (3)	13.4 ± 2.6 (4)	2.0 ± 0.5 (4)	63.1 ± 11.1 (3)	None	None	0.38 ± 0.01 (2)	0.47 ± 0.08 (3)	0.75 ± 0.07 (4)

[a] Dogs, previously fasted 18 hr, were dosed intraduodenally with heparin-^{35}S (100 mg/kg) in 0.5% SLS (5 ml/kg, 15 mg of SLS/kg) or aqueous heparin-^{35}S and tissue distribution determined 90 min later.

[b] Values are means ± SE of number of animals in parentheses and are expressed as percentage of administered heparin-^{35}S recovered.

Figure 2 also shows that clearing factor activity and heparin concentration were significantly increased in lymph 30 min after dosing. Clearing factor activity in lymph approached blood levels 60 min after administration, while heparin-^{35}S concentration remained significantly lower than in blood throughout the study. The appearance of heparin or clearing factor in lymph after 40 min was associated with gross contamination with blood. The volume of lymph collected during the 90-min period was 14.7 ± 3.0 or 22.1 ± 4.7 ml in heparin or heparin–SLS-treated animals, respectively. Recovery of administered radioactivity in lymph was 0.02% in heparin–SLS-treated dogs.

Tissue distribution of administered heparin is summarized in Table II. In terms of the percentage of dose administered there was no significant difference between the two treatment groups in content of radioactivity in intestinal contents, mucosa, or wall. Significant counts were found in the liver of the heparin–SLS-treated group reaching a maximum level of 0.75% of administered radioactivity. On the basis of the 90-min value, liver contained about 4.5 mg of administered heparin. No detectable radioactivity was found in the muscle, perirenal adipose tissue of both groups, or liver of the control group.

Discussion. The present studies in the rat using heparin-^{35}S-containing oil-in-water emulsions confirm previous observations (1) on the ability of such emulsions to effect heparin absorption. The correlation of radioactivity and clearing factor activity in both rats and dogs treated with emulsion or solutions of labeled heparin, indicates actual absorption of the polysaccharide rather than possible activation of an endogenous heparinoid.

Parenteral administration of a number of compounds including sulfates of amylopectin and cellulose (4) and the negatively charged inorganic ions, phosphotungstate, silicotungstate, or phosphomolybdate (5) have been reported to initiate clearing factor activity upon parenteral administration. Surfactants used in the present studies are highly charged anions, and might be expected to behave similarly. However, no clearing factor activity was elicited following intraduodenal administration of surfactant solutions in the absence of heparin. In a separate experiment, intravenous administration of SLS to rats at a dose of 5 mg/kg did not result in detectable clearing factor activity.

It was previously reported (1) that intraduodenal administration of heparin in solution with the surfactants sodium taurocholate, nonionic Tween-20 or the anionic phosphorylated RE-610 did not result in detectable levels of circulating clearing factor activity in the rat or gerbil. In the light of the present results, the use of sodium taurocholate (a sulfated surfactant) was reinvestigated. Pre-

liminary data indicated that Wistar rats treated with heparin (93 mg/kg) in 0.4% sodium taurocholate solution exhibited highly significant clearing factor activity, whereas a 0.2% sodium taurocholate solution was without effect at a heparin dose of 64 or 135 mg/kg. Therefore, although the phenomenon, at present, appears to be limited to sulfated or sulfonated surfactants, the possibility of other surfactant classes being active at different concentrations cannot be ruled out.

Partial loss of clearing factor activity was reported following passage of the circulating enzyme through the intact liver of dogs or man (6), and the perfused liver of rats (7). It was also demonstrated that clearing factor is inactivated by liver mammalian heparinase (7), indicating that heparin functions as a prosthetic group on the enzyme. In the present study, the deposition of approximately 1% of the heparin dose in the liver is consistent with these findings.

The rapid appearance of both clearing factor and radioactivity (labeled heparin) in portal and systemic blood and their slow appearance in thoracic duct lymph indicate rapid absorption via the portal circulation, with lymphatic absorption playing a minor role, if any. Activity in lymph was associated with gross contamination with blood suggesting extravasation at the tissue level.

Previous studies showed that intraduodenal administration of SLS and insulin does not result in the absorption of insulin (2). This indicates that the surfactants studied are not inducing a nonspecific alteration in intestinal impermeability and that the phenomenon has some degree of specificity.

At this time, one can only speculate on the mechanism of absorption. One possibility is a micellar route. The critical micellar concentration of SLS is approximately 0.16% (8). and, in the concentration used in this study, would exist essentially in the micellar state. It is difficult to envision a negatively-charged hydrophilic molecule such as heparin associated with either the hydrophobic interior of the SLS micelles or the negatively-charged exterior surface. It appears more likely that absorption is due to some effect of the surfactant on the intestinal mucosa.

As sulfated and sulfonated surfactants have been shown to bind to proteins such as bovine serum albumin (9), it is possible that similar binding may occur with certain mucosal proteins. It has also been demonstrated that heparin, which complexes very readily with proteins (10), is dialyzable in their absence (11). Perhaps sulfated or sulfonated surfactants compete with heparin for specific mucosal proteins which normally render heparin nondialyzable. The binding of these proteins by surfactant might then permit the passive diffusion of heparin across the mucosa.

The present study may have implications for the clinical treatment of atherosclerosis. Numerous investigators (12, 13) have reported that the parenteral administration of heparin to cholesterol-supplemented animals results in a lowering of total blood lipids and a substantial reduction in the incidence of subsequent atherosclerosis. In the rabbit, heparin has been shown to arrest development of preestablished aortic atheroma (14). It has also been demonstrated that a statistically significant negative correlation exists between the incidence of atherosclerosis and the levels of circulating heparin or heparinoid substances in the blood (15). Engelberg *et al.* (16) studied the effect of continuous heparin therapy for 2 years in individuals with a prior history of myocardial infarction and concluded that heparin retarded the progress of coronary atherosclerotic disease. Further investigation of the use of sulfated or sulfonated surfactants to effect the intestinal absorption of heparin is necessary to clarify their possible utility in the treatment of atherosclerosis.

Summary. The intraduodenal intubation of heparin-^{35}S in solution with sulfated or sulfonated surfactants in both the rat and dog has been shown to result in the appearance of circulating clearing factor (lipoprotein lipase). The increase in enzymic activity was paralleled by a proportional increase in serum radioactivity. The clearing factor response occurred within 15 min in both species and

reached a maximum in the dog within 30–40 minutes. The relatively slow appearance of both clearing factor and radioactivity in the thoracic duct lymph of the dog indicated that absorption was proceeding via the portal circulation. Approximately 2% of the total dose was absorbed, 50% of the absorbed heparin accumulating in the liver. Negligible radioactivity was found in the muscle and perirenal fat.

Grateful acknowledgement is made of the invaluable technical assistance of Mr. Theodore Van Trabert and Miss Anne Greening.

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Received Sept. 27, 1968. P.S.E.B.M., 1969, Vol. 130.

Effect of Carbohydrate Source on Serum and Hepatic Cholesterol Levels in the Cholesterol-Fed Rat (33679)

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Modified food starches are used widely in the food industry because of their special cold stability and resistance to syneresis. This stability is achieved through the selection of starches high in amylopectin content (usually waxy maize or sorghum) which are then chemically treated to cross-link the starch polymer.

Several review articles emphasized that the consumption of complex carbohydrates in both man and experimental animals is usually accompanied by a slight but statistically significant decrease in the concentrations of cholesterol and lipid in the blood whereas a high intake of simple sugars has the opposite effect (1–3). There is not complete agreement, however, on the comparative effects of starch vs sucrose feeding since there are reports that starch feeding is not followed by reductions in serum cholesterol in man (4) and that starch-fed rats actually had higher serum and hepatic cholesterol levels than sucrose-fed controls (5). No published information is available on the influence of modified food starches on serum or hepatic lipid levels in man or experimental animals. The purpose of the present study was to evaluate the comparative effects of sucrose and complex carbohydrates in the form of modified food starches on serum and hepatic lipids in the cholesterol-fed rat.

Methods. Six groups of 10 male weanling rats each of the Harlan-Wistar strain were fed the basal ration shown in Table I. The carbohydrate portion of the various rations was supplied from the following sources: