Intestinal Absorption of Heparin Facilitated by Sulfated or Sulfonated Surfactants

R. H. ENGEL* and S. J. RIGGI†

Abstract □ The intraduodenal administration of heparin in combination with selected sulfated or sulfonated surfactants results in the appearance of circulating clearing factor activity (lipoprotein lipase). High levels occur within 30 min., indicating rapid absorption of heparin. The characteristics of absorption have been studied using a factorial statistical design. Both sodium lauryl sulfate and dioctyl sodium sulfosuccinate show a simple dose response relationship for surfactant and heparin, while an alkyl aryl sulfate and sodium taurocholate reveal more complex characteristics not easily resolved. The administration of heparin and dioctyl sodium sulfosuccinate in enteric-coated capsules to the dog results in the appearance of significant clearing factor activity 3 hr. after dosing.

Keyphrases □ Heparin, intestinal absorption—surfactant effect □ Intraduodenal administration—heparin, surfactants □ Surfactants, sulfated, sulfonated—heparin absorption □ Turbidimetric analysis—spectrophotometer

Gastrointestinal absorption of heparin has been recently demonstrated in the rat and gerbil following intraduodenal administration of the polysaccharide in an emulsified form (1, 2). These emulsion compositions required the presence of a metabolizable oil and stabilizing surfactant. It was subsequently shown that a number of these surfactants (those containing sulfate or sulfonate groups) were also capable of facilitating heparin absorption when administered in solution (2). The finding that the presence of an oil phase was not necessary to achieve absorption suggested that the active components, heparin and surfactant, might be easily formulated into a therapeutic composition offering the combined effects of an oral anticoagulant and antilipemic agent.

The present investigation was undertaken in order to examine the effect of selected sulfated and sulfonated surfactants on the intestinal absorption of heparin and to determine the quantitative effect of any possible interactions occurring between these two components. The experimental approach utilizes a factorial statistical design which allowed the simultaneous varying of both components.

EXPERIMENTAL

Materials and Methods—The surfactants used were sodium lauryl sulfate, SLS (Mann Res. Labs.), dioctyl sodium sulfosuccinate, and the corresponding benzoate, DSS and DSS-B, respectively (American Cyanamid Co.), an alkyl aryl sulfate, AAS (G-3300, Atlas Powder Co.), and sodium taurocholate, NAT (Calbiochem). Heparin (American Cyanamid Co.) and a commercial coconut oil emulsion[1] were also used.

Animals employed were Wistar (Royal-Hart) male rats (150–250 g.) and male beagles from the Lederle colony (7–9 kg.). All animals were fasted 16 hr. prior to dosing. Anesthesia, when required, was accomplished with sodium pentobarbital[2] (60 mg./kg.) intravenously in dogs and intraperitoneally in rats.

The following procedure was used for intraduodenal administration of solutions. Rats were anesthetized, the duodenum was exposed through a midline incision, and a loop of surgical thread loosely placed around the duodenum about 1 cm. distal to the pylorus. The solution was then introduced *via* a blunt needle inserted into the duodenum between the pylorus and the loop. Before injection, the needle was advanced until the end of the needle was distal to the loop. The duodenum was then held gently closed at the loop, the solution (5 ml./kg.) injected, and the loop 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 intracardiac puncture at appropriate times.

Dry materials were administered to dogs intraduodenally in gelatin capsules. Intraduodenal administration was accomplished by performing a laparotomy and inserting a glass tube through the pyloric sphincter *via* a gastric fistula. Gelatin capsules (size 00) were then passed through this tube directly into the duodenum, the tube withdrawn, and the gastric fistula closed surgically. Blood samples were taken from the femoral vein at appropriate intervals.

Two surfactants SLS and DSS-B, in combination with heparin were administered orally to dogs in enteric-coated capsules. DSS-B was used rather than DSS because its granular consistency allowed for easier formulation. Capsules (No. 5 hardshell) were enterically coated with repeated applications of a solution containing cellulose acetate phthalate 200 g., diethyl phthalate, 100 g., methylene chloride, 1 l., and isopropyl alcohol, 1 l. Between applications the capsules were dried in hot air (50°). Heparin and surfactant were given in the ratios, heparin–SLS = 4:1 and heparin–DSS–B = 3.4:1, the latter corresponds to a heparin–DSS ratio of 4:1. Empty capsules and capsules containing heparin were used as controls. Capsules were administered to each of eight adult male beagles (each treatment in duplicate) according to a balanced crossover design at a dose of 100 mg. heparin and 25 mg. of surfactant/kg. This dose required 19 to 38 capsules per dog (contained in a single gelatin capsule No. 11), a number large enough to minimize any differences that might have occurred in the disintegration time of the capsules (4 min. in simulated intestinal juice) or effects of intestinal localization. At least 3 days were allowed to elapse between treatments. Blood samples were withdrawn from the jugular vein at 0, 0.5, 1 through 7, and 24 hr. after dosing.

The turbidimetric assay of serum clearing factor activity (3) was carried out in 1-ml. spectrophotometric cells.[3] In the cell 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[4] (pH 8.5), and 0.5 ml. of serum. The mixture was incubated for 2 min. at 37° and 0.1 ml. of substrate (0.6% v/v coconut oil emulsion in tris buffer), was added. The optical density at 650 mμ was immediately recorded (the initial optical density averaged 0.75). Optical density readings were taken at 15 and 30 min. after substrate addition. Mixtures containing control serum showed a decrease in optical density of 0.02 ± 0.01 SE. A drop in optical density of 0.06 units was considered significant (2).

Statistical Design of Heparin-Surfactant Profiles—Experimental situations often require a study of the effects of varying two or more components simultaneously. In such cases, it is usually not sufficient to vary one component at a time, holding the other constant, as possible interactions between components may not be revealed. In addition, it is possible that there may be more than one combina-

[1] Ediol, Riker Labs.

[2] Diabutal, Diamond Laboratories.

[3] Coleman.

[4] Fraction V, Armour Industrial Chemical Co., Chicago, Ill.

Table I—Experimental Design and Dosage Combinations Studied

Heparin, mg./kg.	SLS or DSS, mg./kg. 0.38	0.82	5.3	34.4	75.0	Heparin, mg./kg.	AAS, mg./kg. 0.5	1.1	7.1	45.8	100.0	Heparin mg./kg.	NAT, mg./kg. 15.8	22.2	50.0	113.0	158.0
50.0	—	—	*10*[a]	—	—	50.0	—	—	*10*	—	—	79.0	—	—	*10*	—	—
61.2	—	*5*	—	*3*	—	61.2	—	*5*	—	*3*	—	93.0	—	*5*	—	*3*	—
100.0	*8*	—	*1,6,7,11*	—	*9*	100.0	*8*	—	*1,6,7,11*	—	*9*	135.0	*8*	—	*1,6,7,11*	—	*9*
163.3	—	*4*	—	*2*	—	163.3	—	*4*	—	*2*	—	196.0	—	*4*	—	*2*	—
200.0	—	—	*12*	—	—	200.0	—	—	*12*	—	—	229.0	—	—	*12*	—	—

[a] Numbers in italics are dosage combinations studied (eight animals per combination) and are arranged according to the design of Box and Wilson (4).

tion of the two components that will give the desired effect and, while varying each component in a linear fashion will result in describing a single optimum composition, the existence of other optimum compositions, giving perhaps greater response would not be revealed.

The experimental design described by Box and Wilson (4) involves the simultaneous varying of two components over the area defined by the dosage range of each component. These doses are chosen in a factorial manner. The effect of each component can then be determined with the same accuracy as if only one component at a time had been varied. In addition, such a study also permits the analysis of any possible interactions between the variables as well as the determination of any number of optimum conditions that may occur for maximum activity.

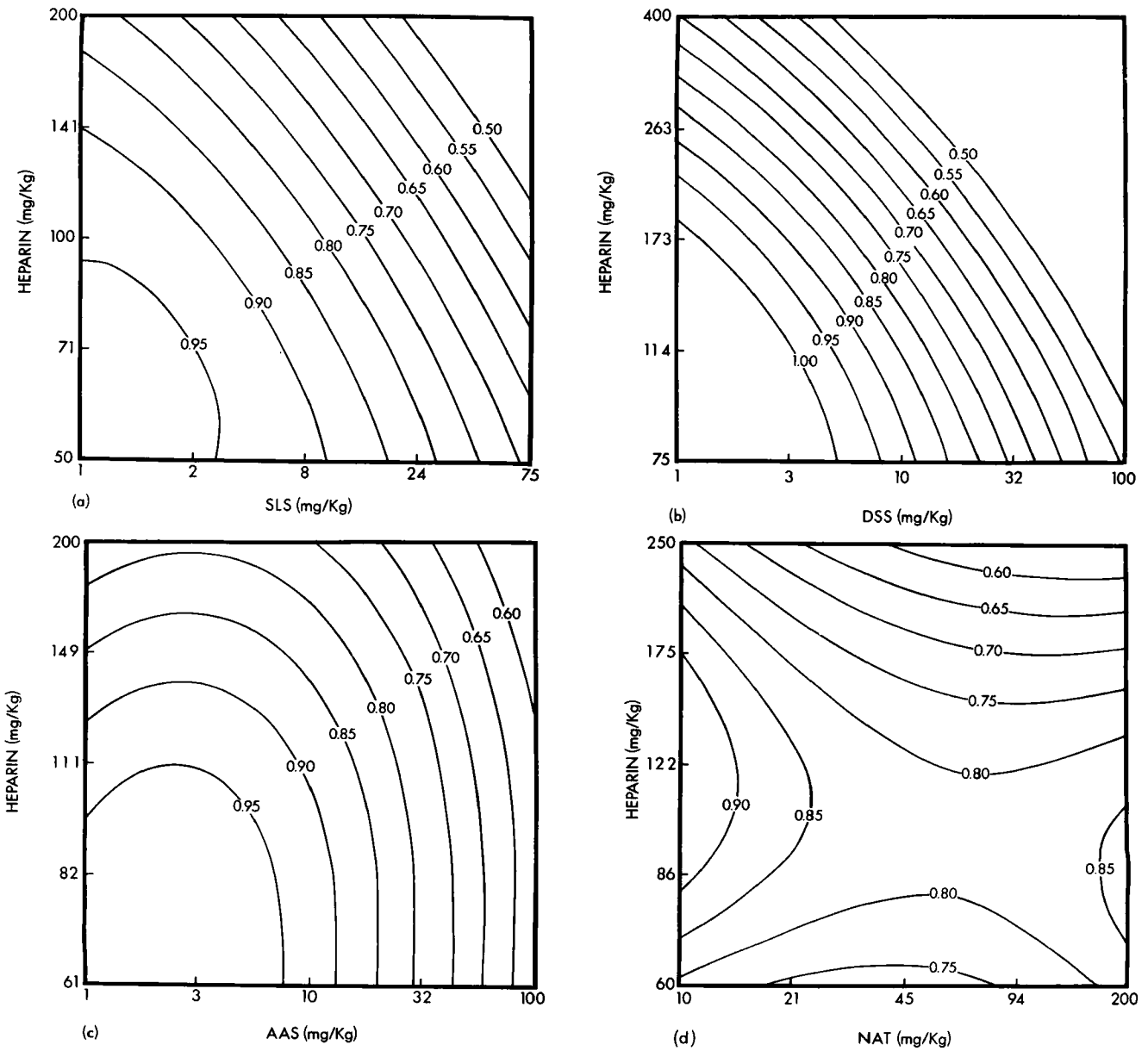

Figure 1—*Clearing factor activity profiles for heparin in combination with various surfactants: (a) SLS, (b) DSS, (c) AAS, (d) NAT. Heparin-surfactant combinations in solution were administered intraduodenally to rats, 5 ml./kg. (eight rats per group). Blood samples were withdrawn 30 min. later and the serum assayed for clearing factor activity. Profiles were calculated by using a computer and activities are expressed as the ratio of the optical densities at 30 min. versus 0 min. (assay time).*

Table II—Effect of Heparin-Surfactant Compositions Administered Orally to the Dog in Enteric-Coated Capsules[a]

Treatment	Geometric Average	% of Heparin Treatment
Placebo	0.927	99
Heparin	0.937	100
		87
Heparin + SLS	0.814	(65–116)[b]
		59[c]
Heparin + DSS-B	0.555	(44–79)

[a] Treatments were administered to eight dogs per group in a balanced crossover design. Blood samples withdrawn between 0 and 24 hr. Values are geometric average of minimum optical density ratios regardless of time of effect in individual dogs. [b] 95% confidence limits. [c] $p \leq 1001$.

If the resulting factorial design is too large to lend itself to convenient experimental study, the proposed plan may be divided into smaller representative blocks in a particular manner which describes the main effect of each variable and their interactions. This procedure has been used in the present study.

A dosage range was chosen for heparin and each surfactant. Within each of these ranges five doses of the components were selected in a factorial manner resulting in 25 possible combinations for each heparin-surfactant mixture (Table I). The area described by these concentration ranges was studied by administering compositions corresponding to Points 1 through 12. Points 1 through 6 were studied on the first day and Points 7 through 12 on the second day. The center point was studied twice on each day, permitting an evaluation of day-to-day variations. Blood samples were withdrawn 30 min. after administration and the serum assayed for clearing factor activity.

Prior to statistical analysis, transformations of these data were required in order to stabilize the variances. The relationships between response and dose were found to be highly significant ($p < 0.001$) for the log of the ratio, initial optical density/final optical density, and were analyzed using this transformation. The data were calculated and the activity profiles plotted using a computer.[5] Probit analysis was used in the calculation of ED_{50}'s.

RESULTS

Heparin-surfactant profiles for SLS, DSS, AAS, and NAT in the rat are shown in Fig. 1. A ratio of 0.92 or less represents significant activity. When heparin is administered with either SLS (Fig. 1a) or DSS (Fig. 1b) a dose-response relationship is evident for both heparin and surfactant ($p = 0.001$). In each case, the changes in heparin and surfactant doses are compensatory so that a decrease in heparin dose can be offset by a corresponding increase in surfactant dose.

A different picture is seen with heparin and AAS (Fig. 1c). At any given heparin dosage, the clearing factor response initially decreases as the surfactant dose is increased from 1 mg./kg. to about 3 mg./kg. At higher surfactant doses the clearing factor response increases. Thus with AAS, a minimum response is obtained at about 3 mg. surfactant/kg. and the contours indicate an increased response could be found if the dosage range of surfactant were extended to lower levels. The AAS-heparin relationship is very significant with $p < 0.001$.

A fairly complex relationship is indicated for heparin and NAT combinations (Fig. 1d); however, the response was not significantly related to the dose of heparin or NAT ($p = 0.09$). The activity profile may be interpreted to indicate that at an NAT dose of 50 mg./kg. there is moderate response up to about 100 mg. heparin/kg. and a more marked change in response with increasing amounts of heparin.

The effect of oral administration of heparin and surfactants was studied in dogs with enteric-coated capsules containing heparin and SLS or DSS-B. Following clearing factor assay, the ratio of initial to final optical density at 30 min. assay time was calculated. Analysis of the data indicated that expression of the results in this form was sufficient to remove any individual day, dog, or carryover effects. The ratios for each dog on a given day were summarized by taking the minimum ratios (maximum effect) regardless of the time (0 to 24 hr. after dosing) at which it occurred. The geometric averages of those ratios for each treatment are shown in Table II.

The combination of heparin and DSS-B caused a significant increase in clearing factor activity compared to heparin alone ($p < 0.001$). The time of maximum effect ranged from 1 to 7 hr. with an average of 3.2 hr. The combination of heparin and SLS did not appear to be effective over a 24-hr. period.

Heparin and SLS were clearly effective when administered in uncoated gelatin capsules directly into the duodenum *via* a glass tube inserted through the stomach. These results are shown in Fig. 2. Compared to a heparin control, the combination of heparin and SLS resulted in a significant increase in clearing factor activity within 30 min., which reached a peak at 2 hr. and remained at that level for at least another 2 hr.

It was also desired to study the time course of the clearing factor response with combinations of heparin and various surfactants, as these combinations were also to be used in dose response studies and determination of relative potencies of heparin-surfactant mixtures. The activity profiles obtained in rats (Fig. 1) were used to select heparin-surfactant combinations of equivalent activity. A standard dose of 100 mg. heparin/kg. was arbitrarily chosen and the amount of each surfactant required to give an optical density ratio of 0.75, obtained from the profiles. These doses (14 mg. SLS/kg., 31 mg. DSS/kg., 38 mg. AAS/kg., and 120 mg. NAT/kg.) in combination with 100 mg. heparin/kg. were administered intraduodenally to anesthetized rats, blood samples were withdrawn at various intervals, and assayed for clearing factor activity. The results are shown in Fig. 3. With heparin and DSS, activity was greatest 15 min. following administration. All other combinations showed greater activity at 30 min. or no significant difference between 15 and 30 min. In each case, at 1 hr., activity was greatly decreased from the maximum. Dose-response studies were accordingly carried out 15 min. following administration of heparin and DSS and 30 min. with all other combinations. The results are summarized in Table III. The heparin-DSS and heparin-AAS appear to be the most effective compositions ($ED_{50} = 46$). However, as the 95% confidence limits overlap in all cases, it is difficult to make a quantitative evaluation of the best composition. The results support the previous data indicating a requirement for substantially higher concentrations of NAT than the other surfactants.

DISCUSSION

Absorption of heparin across the intestinal mucosa has been reported previously. Loomis (5) noted significant anticoagulant

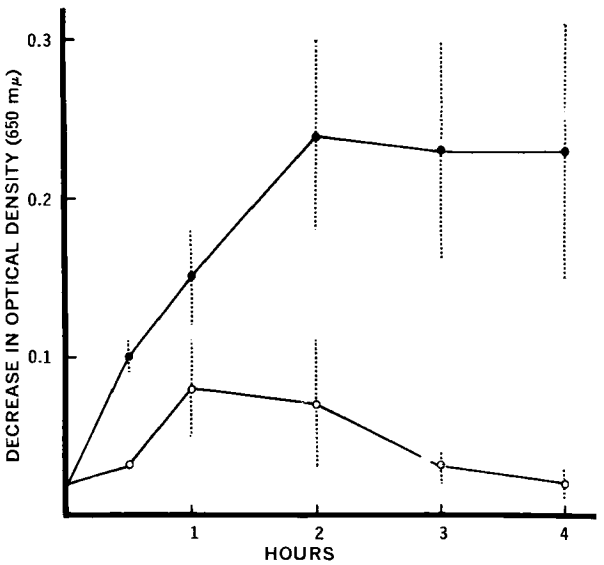

Figure 2—*Clearing factor activity in the dog following intraduodenal administration of heparin and SLS. Heparin (100 mg./kg.) or heparin and SLS (25 mg./kg.) were administered intraduodenally in uncoated gelatin capsules by the way of gastric fistula (four dogs per group). Blood samples were withdrawn at various intervals and the serum assayed for clearing factor activity. Key: ●, heparin and SLS; ○, heparin control. The errors are expressed as SE.*

[5] IBM.

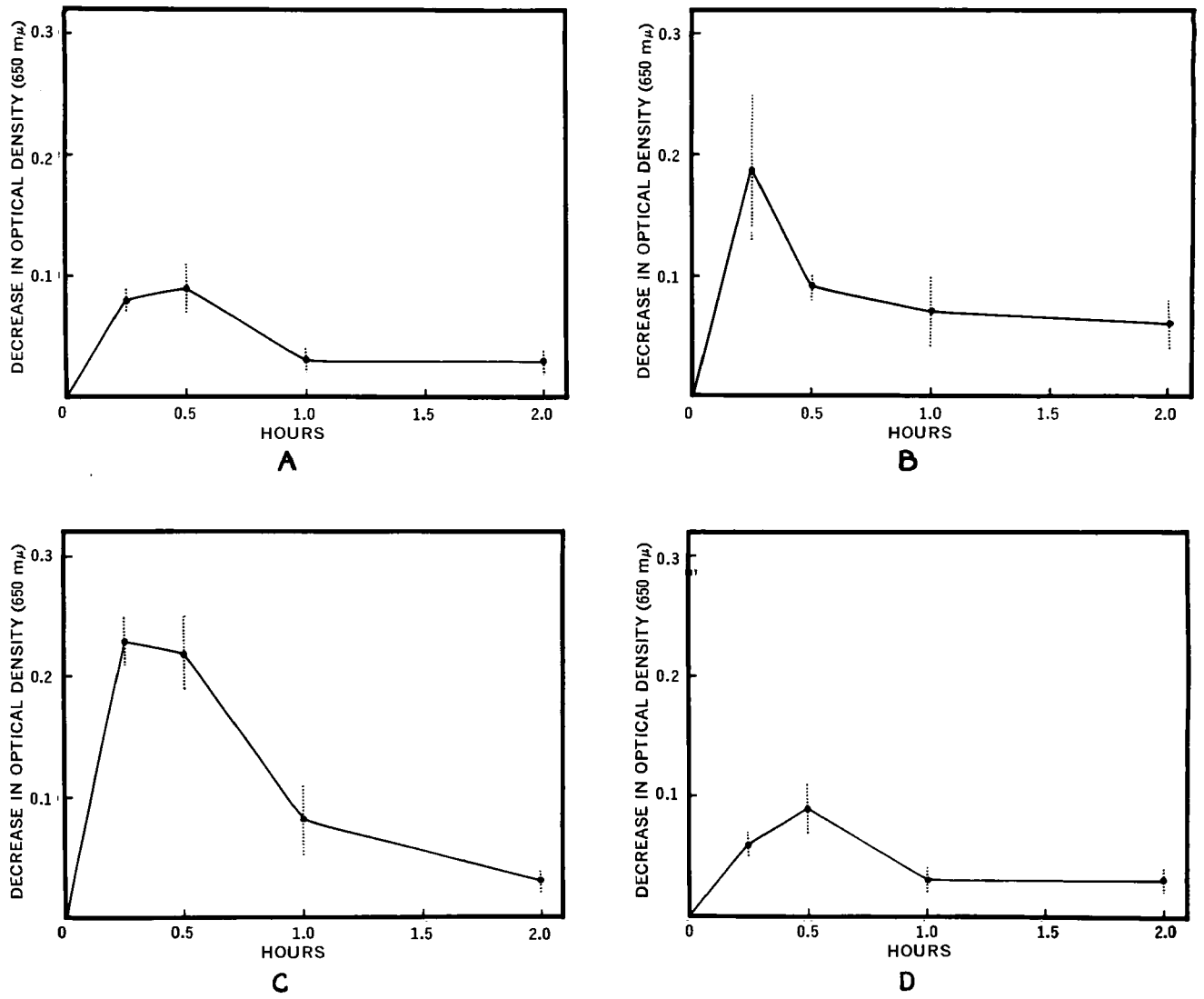

Figure 3—*Time course of clearing factor response in the rat following intraduodenal administration of various heparin-surfactant combinations. Test solutions were administered intraduodenally (5 ml./kg., eight rats per group) at dosages of 100 mg. heparin/kg. and 14 mg. SLS/kg. (a), 31 mg. DSS/kg. (b), 38 mg. AAS/kg. (c), or 120 mg. NAT/kg. (d). Blood samples were withdrawn at appropriate intervals and the serum assayed for clearing factor activity. Results are expressed as decrease in optical density at 650 mμ at 15 min. of assay time ± SE.*

activity following intestinal intubation of heparin in an acid buffer (pH 4.0) and proposed that absorption was due to the partial neutralization of carboxyl groups on the polysaccharide. However, this would not explain absorption in the presence of a neutralized surfactant. Windsor and Cronheim (6) found that EDTA (ethylene diaminetetraacetic acid) facilitated heparin absorption and suggested that the chelation of intestinal calcium and/or magnesium was involved. This was supported by the inability of calcium or magnesium salts of EDTA to enhance absorption. Sulfated and sulfonated surfactants may operate in an analogous manner, forming calcium and magnesium soaps, most of which would be insoluble, thereby removing the barrier to heparin absorption.

Table III—Dose Response of Combinations of Heparin and Surfactants in the Rat

Surfactant	Sampling Time, min.	ED_{50}[a] Heparin + Surfactant, mg. combination/kg.	ED_{50}[a] Heparin, mg./kg.
DSS	15	46 (16–98)[b]	35 (14–75)
SLS	30	108 (52–211)	95 (46–185)
AAS	30	46 (23–86)	33 (17–62)
NAT	30	149 (59–369)	71 (28–176)

[a] ED_{50} values were calculated from five doses with eight animals per dose. [b] 95% Confidence limits.

It was previously reported that intestinal absorption of heparin (1) occurred following intraduodenal administration of the polysaccharide in an emulsified form. The chemical nature of the surfactant was not a critical factor and although anionic materials were preferred, anionic, cationic, or nonionic surfactants were all effective. As in the present work, absorption was very rapid, with significant circulating clearing factor being detected within 15 min. of intraduodenal administration. It appears that the presence of the oil phase serves to increase the clearing factor response at any given concentration of heparin and sulfated surfactant. Whereas 100 mg. heparin/kg. administered in a solution of NAT (188 mg./kg.) results in an optical density change of 0.11, in the presence of corn oil or trioctanoin, the optical density change was 0.23 and 0.56, respectively (1). This effect has also been noted with SLS.

The present study may have implications for the clinical treatment of atherosclerosis. In recent years, numerous investigators have reported the blood lipid-lowering effects of heparin (7) and its ability to retard the progress of coronary atherosclerotic disease (8).

Heparin is infrequently used in this regard due primarily to the necessity for parenteral administration. This situation could possibly be changed by formulation of heparin with a nontoxic sulfated surfactant.

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Structural Studies on Complexes IV: Crystal Structure of a 1:1 5-Chlorosalicylic Acid and Theophylline Complex

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Abstract □ Single crystal X-ray diffraction methods were utilized in obtaining the crystal and molecular structure of a 1:1 association complex between 5-chlorosalicylic acid and theophylline. The crystals are monoclinic (space group P $2_1/c$) with cell parameters of a = 7.845 Å.; b = 9.636 Å.; c = 21.185 Å.; and β = 92.20°. The crystals contain a significant fraction of the impurities 3,5-dichloro- and 3-chlorosalicylic acid. Hydrogen bonds are the major attractive forces between the components of the complex. A relatively strong hydrogen bond exists between the carboxyl group of 5-chlorosalicylic acid and N(9) of theophylline (2.682 Å.). The theophylline molecules are in a dimeric arrangement by virtue of centrosymmetrically related hydrogen bonds between N(7) and TO(10). The packing arrangement of the molecules suggests that the stacking forces are similar to those in a caffeine:5-chlorosalicylic acid complex.

Keyphrases □ Complexes—structural studies □ 5-Chlorosalicylic acid-theophylline complex—crystal, molecular structure □ Diffractometry—5-chlorosalicylic acid-theophylline complex □ Hydrogen bonding—5-chlorosalicylic acid-theophylline complex

Spectroscopic, kinetic, and a variety of other physical chemical methods have been used successfully to obtain valuable information on the nature and strength of intermolecular association complexes between a wide variety of biological compounds (1). However, these methods are generally unable to provide detailed descriptions of the complex at the atomic level. For this, one may in some instances resort to the direct method of X-ray diffraction. Though this technique requires crystalline complexes, whose structures may be somewhat different from those in solution, the results are quite useful when correlated to solution data. It is through such correlations that a clear picture of various molecular association complexes involving pharmaceutically important molecules may emerge.

The interactions of salicylates and other biological materials containing *pi* systems with xanthine derivatives have been studied quite extensively in solution. The thermodynamic parameters of such complexes obtained by Higuchi *et al.* (2, 3) suggest that "hydrophobic" forces aside from hydrogen bonding play a significant role. Donbrow and Jan (4) have indicated that xanthine–hydroxybenzoic acid complexes may involve a donor-acceptor-type mechanism. In a crystallographic study on the 1:1 complex between 5-chlorosalicylic acid and caffeine, the idea of "polarization bonding" was put forth (5). Although the primary intermolecular force in that solid-state complex is hydrogen bonding, some evidence was found for a localized interaction between the α-β unsaturated ketone portion of the xanthine and the *pi* system of the salicylic acid molecule. Even with the wide variety of experimental data now available on such complexes and postulated models for the interactions (for a theoretical model see reference (6)), the interactions in these complexes have not been elucidated with sufficient detail to draw definitive conclusions on their molecular nature.

The present report concerns a structural study on a 1:1 complex formed between theophylline and 5-chlorosalicylic acid. This structure was determined with the hope of providing further insight into the molecular nature of the "polarization" forces, and also to learn more about the role hydrogen bonds play in stabilizing such complexes.

EXPERIMENTAL

Prism-shaped crystals of the 1:1 complex were obtained by dissolving equal molar quantities of theophylline (Matheson, Coleman and Bell, Inc.) and 5-chlorosalicylic acid (Eastman Kodak practical grade) in an alcohol-water solution and allowing the solution to