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Intestinal Absorption of Heparin: A Study of the Interactions of Components of Oil-in-Water Emulsions

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Abstract □ It was previously reported that intraduodenal administration of heparin in an emulsified form to rats and gerbils results in the intestinal absorption of heparin and appearance of serum clearing factor (lipase) activity. The present studies were undertaken to define the effects of the emulsion components on the absorption of heparin as measured by clearing factor activity and to determine optimum composition of the emulsion. Using a three-component composite design, the effects of varying heparin, surfactant (an anionic phosphate ester), and oil (trioctanoin) concentrations have been studied simultaneously and the characteristics of the interrelationships analyzed. Clearing factor activity was directly related to the concentration of each of the emulsion components. An inverse relationship was evident for heparin and oil such that the loss of activity resulting from a lowered heparin concentration can, within limits, be compensated for by an increase in the oil concentration. The data suggest that heparin absorption is directly related to and may vary with the particle size and total surface area of the oil droplets. The relationships presented may be unique for the particular surfactant and oil chosen.

Keyphrases □ Heparin absorption, effects of emulsion composition □ Emulsions, oil-in-water—interaction of components □ Clearing factor activity, emulsions

It was previously reported (1, 2) that the intestinal absorption of heparin could be effected by its intraduodenal administration in an emulsion containing vegetable oil and a suitable surfactant. A number of possible combinations were described (1). The present studies were undertaken to investigate the effects of emulsion composition on heparin absorption and to determine the concentrations of heparin, oil, and surfactant necessary to achieve maximum absorption of the polysaccharide. Combinations of emulsion components were varied in a factorial manner according to a three-factor composite design (3) to take into account possible interactions between components.

Table I—Concentrations Studied in Composite Design[a]

Heparin, mg./kg.	Trioctanoin, %	Surfactant, % 0.01	0.023	0.10	0.435	1.0
150	3.2					
	5.5					
	12.5			*9*[a]		
	28.6					
	50.5					
100	3.2					
	5.5		*1*		*5*	
	12.5					
	28.6		*6*		*2*	
	50.0					
55	3.2			*10*		
	5.5					
	12.5	*11*		*15*		*12*
	28.6					
	50.0			*13*		
30	3.2					
	5.5		*7*		*3*	
	12.5					
	28.6		*4*		*8*	
	50.0					
	3.2					
20	5.5					
	12.5			*14*		
	28.6					
	50.0					

[a] Numbers in italics correspond to points indicated in Fig. 1.

EXPERIMENTAL

Materials and Methods—Heparin, sodium (Lederle Laboratories), trioctanoin (Eastman), and a phosphate ester surfactant (RE-610, Antara Chemicals) were used. Preparation of the emulsions was carried out in a single step as previously described (1). Heparin in aqueous solution with the surfactant was used as control. Final concentrations of the surfactant and trioctanoin and doses of heparin are presented in Tables I and II. The volume of emulsion or solution administered was 5 ml./kg. body weight.

Animals used were male Wistar rats (175-250 g., obtained from

Table II—Clearing Factor Activity (Change in Optical Density) of Compositions Studied[a]

Heparin, mg./kg.	Trioctanoin, %	Surfactant, % 0.01	0.023	0.10	0.435	1.0	2.0	4.35
275	12.5				0.28 ± 0.06[b]			
190	12.5			0.31 ± 0.08			0.48 ± 0.06	
150	12.5			0.31 ± 0.08				
	5.5		0.10 ± 0.01		0.14 ± 0.03			
100	12.5				0.36 ± 0.06			0.54 ± 0.07
					0.32 ± 0.07			
	28.6		0.04 ± 0.02		0.19 ± 0.06			
55	3.2			0.08 ± 0.02				
				0.25 ± 0.06				
				0.24 ± 0.06				
				0.25 ± 0.08				
				0.19 ± 0.07				
	12.5	0.17 ± 0.05		0.33 ± 0.08		0.50 ± 0.04	0.31 ± 0.07	
				0.26 ± 0.08				
				0.12 ± 0.06				
				0.06 ± 0.03				
	50			0.07 ± 0.01				
30	5.5		0.04 ± 0.02		0.27 ± 0.06			
	12.5				0.23 ± 0.07			
	28.6		0.11 ± 0.02		0.07 ± 0.03			
20	12.5			0.10 ± 0.04				
				0.06 ± 0.01				
9.2	3.2			0.02 ± 0.00				
				0.03 ± 0.00				
				0.02 ± 0.01				
	12.5	0.03 ± 0.01		0.05 ± 0.01		0.08 ± 0.03		
				0.03 ± 0.01				
	50			0.03 ± 0.00				
55	0			−0.01 ± 0.00				

[a] Rats previously fasted 18 hrs. were given the indicated compositions (5 ml./kg.) intraduodenally and blood samples obtained 30 min. later by cardiac puncture. [b] Values are arithmetic means ± *SE* of eight rats/group and are expressed as decrease in optical density of incubation medium. Initial optical densities were approximately 0.75. A − indicates an increase in optical density.

Royal-Hart Farms). All animals were maintained on Purina laboratory chow and fasted 18 hr. before use. Anesthesia was accomplished with 60 mg./kg. sodium pentobarbital[1] intraperitoneally. The duodenum was exposed through a midline abdominal incision and a loop of surgical thread loosely placed 1 cm. distal to the pyloric sphincter. Emulsions were injected into the duodenum between the pylorus and the loop *via* a blunt needle. The needle was advanced distal to the loop and the preparation injected while the duodenum was held closed. The loop was pulled tight as the needle was withdrawn. This procedure was used to prevent backflow of emulsion to the point of needle insertion with possible absorption *via* the damaged capillary bed. Blood samples were obtained by cardiac puncture 30 min. after dosing.

Clearing factor activity (4) was determined by a turbidimetric assay on serum in 1-ml. spectrophotometer (Coleman) cells. In the cell were placed 0.6 ml. 0.05 *M* tris(hydroxymethyl) aminomethane buffer (pH 8.5), 0.3 ml. 25% (w/v) bovine albumin Fraction V, pH 8.5 (Armour), and 0.5 ml. serum. Substrate (0.1 ml. of 6.0% v/v lipid emulsion[2] in Tris buffer) was added at zero time. The optical density at 650 mμ (approximately 0.75) was recorded, the mixture incubated at 37°, and optical density readings taken 30 min. later. Changes in optical density of 0.06 or greater were previously reported (2) to be indicative of significant clearing factor activity and to be directly related to intestinal absorption of heparin from emulsions. The results are reported as decrease in optical density and the percentage of animals (eight per group) responding with significant clearing factor activity following various treatments.

Experimental Design—Experimental situations often require a study of the effects of varying two or more factors simultaneously. In such cases, it is usually not sufficient to vary one factor at a time, holding the other constant, as possible interactions between factors are not revealed. However, these interactions can be evaluated if, after selecting the experimental limits for each variable, one or more experiments are performed with combinations of the variables chosen in a factorial manner (3). The effect of each factor can then be determined with the same accuracy as if only one factor at a time had been varied whenever there is no interaction, or the nature of the interaction is known.

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 effects of each variable and their interactions (3). This procedure has been used in the present study.

The experimental design chosen (3) is shown in Fig. 1. The block, consisting of Points 1-8, defines all first-order effects and all second-order interaction effects. These points were supplemented with the central point 15, and six axial points 9–14, which permit estimation of quadratic effects (3). Doses of heparin and concentrations of trioctanoin and surfactant in the emulsions were varied over the ranges indicated in Table I. Compositions corresponding to Points 1-8 and Point 15 were tested on the same day; Point 15 was tested in

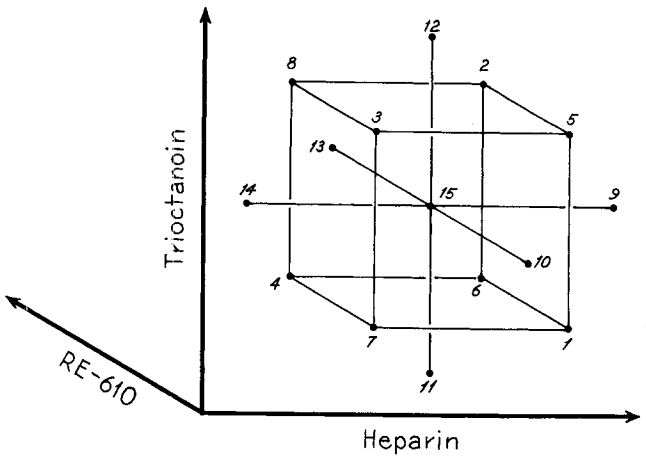

Figure 1—*Schematic representation of experimental design (see Table I).*

[1] Diabutal, Diamond Laboratories.
[2] Ediol, Riker Laboratories.

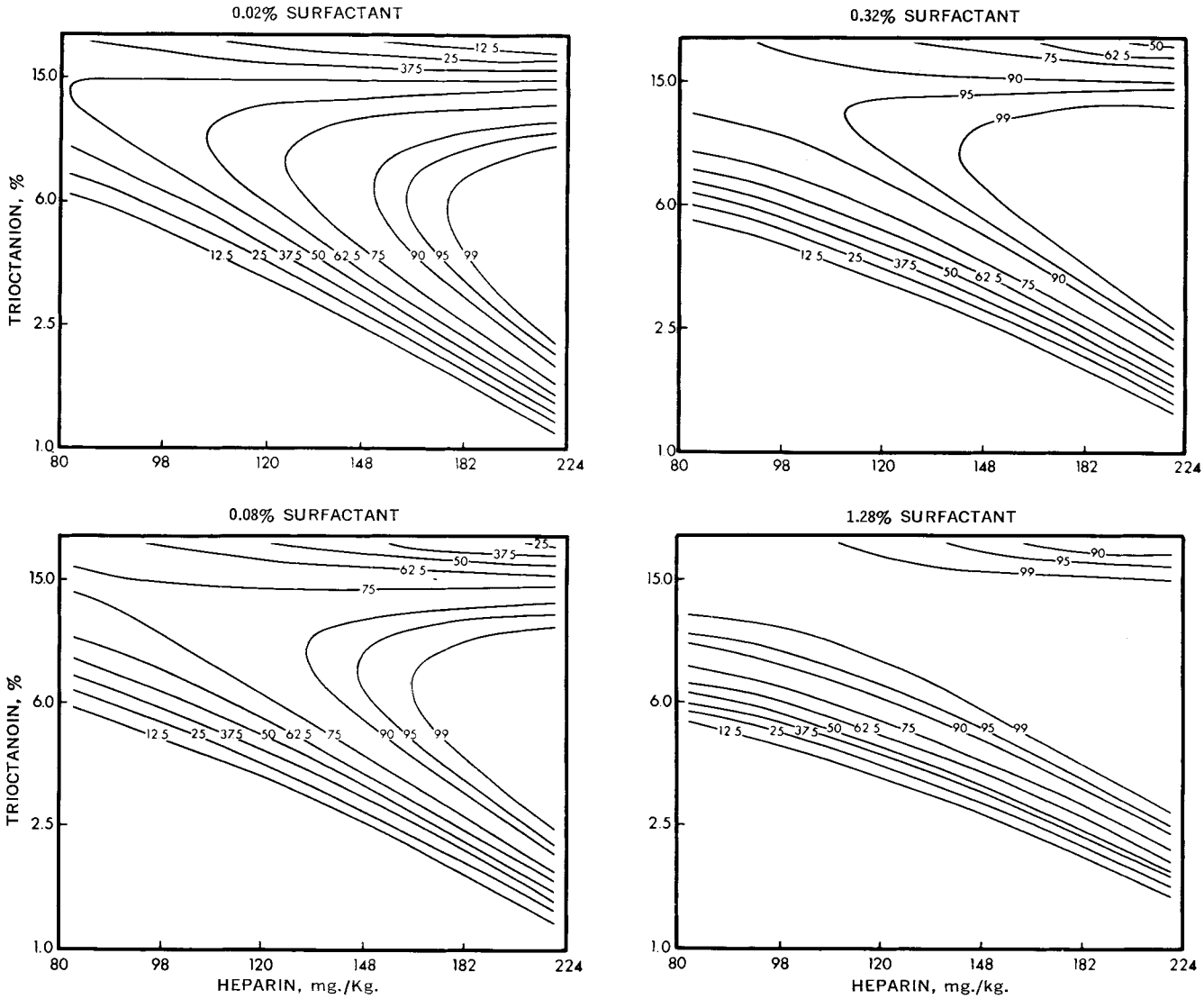

Figure 2—*Clearing factor activity profiles of heparin emulsions. Values interrupting the contour lines are arc sin means of percentage of animals (eight group) responding with significant clearing factor activity with various combinations of emulsion components.*

duplicate. On the second day, Points 9-14 were tested and Point 15 was repeated in quadruplicate. The percentage of animals responding with significant clearing activity following the various treatments was calculated and activity contours plotted using a computer (IBM-1130). The arc sin transformation was used. As the characteristics of the contours became clearer the technique of local exploration was used by testing additional axial points displaced toward higher heparin concentrations (Table II).

RESULTS AND DISCUSSION

Changes in optical density, *i.e.*, clearing factor activity, following treatment with various compositions are summarized in Table II. In agreement with previous studies (2) rats treated with a solution of heparin and surfactant (the absence of trioctanoin) had no clearing factor activity.

The interactions of the three emulsion components on heparin absorption are summarized in Fig. 2. The data indicate that for each concentration of surfactant a range of heparin doses and trioctanoin concentrations resulted in the same degree of clearing factor response. Thus, maximal activity (99% contour line) can be obtained with combinations of 2.5–14% oil and 148-220 mg./kg. heparin at 0.32% surfactant.

It is interesting to note that if surfactant concentration and heparin dose are held constant and trioctanoin levels raised, clearing factor activity increases to a maximum and then decreases. The explanation for this phenomenon is unknown but may be related to a preference of the absorptive mechanism for oil particles of a particular size and a varying of the conditions under which the maximum number of these are formed.

For a given dose of surfactant the optimum concentration of trioctanoin varies inversely as the dose of heparin. In other words, as the amount of heparin available for absorption is decreased, a greater fraction of this amount can be absorbed by increasing the oil concentration thus maintaining constant clearing factor activity. The degree of compensation for decreased heparin by increasing trioctanoin depends, however, on the extent of change in heparin dose. It was previously suggested (1) that in order for absorption to occur, heparin had first to be associated with emulsified trioctanoin. This inverse relationship may then represent a mass-action effect between heparin, trioctanoin, and the active heparin-trioctanoin complex.

The results also suggest that the total oil surface area generated affects heparin absorption. If, in addition to heparin, the concentration of trioctanoin is held constant, absorption of heparin continues to increase with increasing concentrations of surfactant. This could reflect a shift in the total oil surface, the additional surfactant allowing for the generation and stabilization of more surface area. The result would be a decrease in the average particle size and an increase in the total number of particles.

The relationships presented may be unique for the system containing trioctanoin and the surfactant used in the present study.

Whether or not combinations of other oils and surfactants will exhibit activity with different characteristics has yet to be investigated.

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A Simple Dilution Analog Computer for Simulation of Drug Distribution Processes

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Abstract □ A simple technique is described which provides plots of simulated tandem first-order processes much like the analog computer. The exponential change in concentration of a solution undergoing dilution at constant volume provides the basis for simulation of first-order processes. A UV-absorbing indicator is introduced into the first of a series of connected containers and the solution is rapidly pumped through the system. The continuously changing indicator concentration in any container can be measured and continuously monitored by a spectrophotometer equipped with a flow cell and recorder. Several pharmacokinetic models, as in delayed-release formulation and in molecular modification, were studied with this device and the resulting plots were found to be accurate and reproducible. The validity of dilution analog simulation of first-order processes is shown mathematically. One of the uses of the dilution analog simulator is in teaching and visualizing tandem first-order reactions such as pharmacokinetic models.

Keyphrases □ Analog computer simulation—drug distribution processes □ Model, three compartment—drug distribution □ Kinetic equations—drug distribution model □ Diagrams—models, drug distribution □ UV spectrophotometry—distribution monitoring

The analog computer has been used widely to simulate and analyze drug distribution models (1–3). This report illustrates how first-order dilution techniques with the aid of a spectrophotometer can be used in place of an electronic analog computer to simulate drug transport and distributional systems. In essence, the dilution technique consists of addition of diluent at a constant rate to a well-stirred aqueous solution of a UV-absorbing indicator in which constant volume is maintained by means of an overflow vent. The concentration of indicator decreases exponentially in such a system and additional compartments can be added, the indicator concentrations of which are also governed by exponential laws.

Nonelectronic analog simulation for the purpose of teaching and illustration has been achieved by several ingenious but complicated methods. Krüger-Thiemer (3) used the principle of gas diffusion through evacuated compartments separated by porous membranes. Wendell (4) used a hydrodynamic analog in which the exponential decline of hydrostatic pressure produced a first-order emptying process. Both methods require special equipment and are not easily monitored.

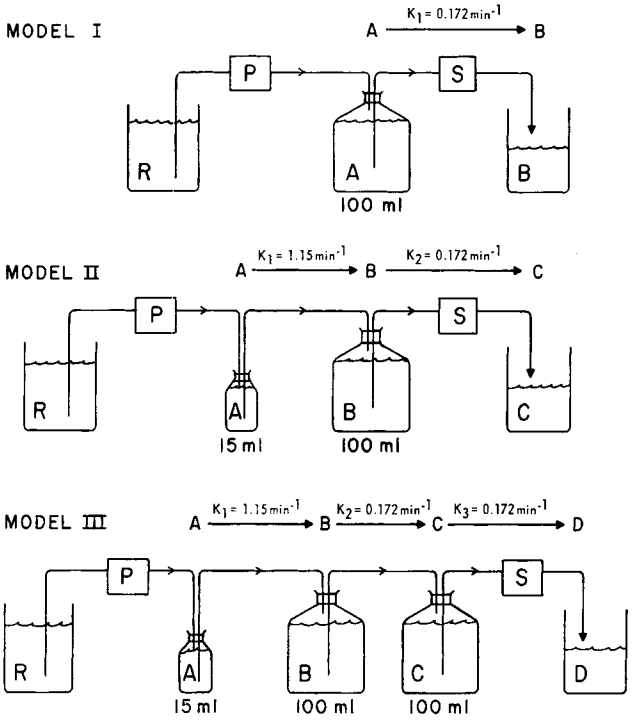

Figure 1—*Models for tandem first-order processes.* P *is a pump which delivers fluid from reservoir* R *to compartment* A *forcing solute solution through the other compartments (flow rate = 17.2 ml. min.*$^{-1}$*).* S *is a spectrophotometer with a flow cell.*

Dilution analog simulation has few disadvantages compared with these systems. The equipment required, including a spectrophotometer, is readily available in most laboratories. While this study deals mainly with systems of tandem first-order processes, the dilution analog technique is not necessarily restricted to such simple systems. Variations in the techniques should permit the simulation of more complex cases such as those involving simultaneously both forward and re-