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Intestinal Absorption of Heparin in the Rat and Gerbil (33422)

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The general impermeability of the gastrointestinal tract to aqueous solutions of heparin has long been recognized (1), and can be satisfactorily explained on the basis of the physical properties of the heparin molecule, namely, its high molecular weight and acidic nature. As a rule, water-soluble compounds of molecular weight greater than 200, penetrate the mucosal cell only with considerable difficulty, and ionized compounds are generally absorbed at a rate much lower than that of neutral molecules (2).

The present work demonstrates that absorption of heparin can occur when the polysaccharide is presented to the intestinal mucosa of rats and gerbils in the form of an oil-in-water (O/W) emulsion. The characteristics and chemical requirements for this phenomenon are herein reported and possible explanations are considered.

Materials and Methods. Materials. Emulsions were prepared with the following oils: corn or olive (Fisher), peanut (Welch, Holme and Clark), mineral, (Lederle) or trioctanoin (Eastman). Monoolein was obtained from Eastman. Water-soluble surfactants used were sodium taurocholate (Wilson), cholic acid or sodium glycocholate (Nutritional Biochemicals Corp.), polysorbitan monolaurate, "Tween-20" (Mann), and a phosphate ester surfactant, "RE-610" (Antara Chemicals, Division of General Aniline and Film). The water-soluble polysaccharides studied were heparin, sodium (Lederle) and mactin B, a heparin-like polysaccharide obtained from the clam, *Cyprina* (Arctica) *islandica* (3). "Ediol," a commercial coconut oil emulsion, was the generous gift of Riker Laboratories.

Preparation of emulsions. Prior to emulsification, heparin, sodium, and a water-soluble surfactant were dissolved in the aqueous phase. Oil-soluble components were dissolved in the oil phase. Emulsification was then carried out with either the Raytheon Sonic Oscillator Model DF-101 or the Branson Sonifier, Model S-125. The oil phase (4.5 ml) and its components were slowly added to the aqueous phase (31.5 ml) in 1-ml increments and emulsification continued until the oil phase was completely dispersed. This required about 5 min in the Raytheon Sonicator and a few seconds in the Branson Sonifier.

Preparation of micellar solutions. Micellar solutions (4) were prepared by dissolving 803 mg of monoolein in 50 ml of a solution containing sodium taurocholate (.02*M*), sodium glycocholate (.02*M*) and 30 mg heparin/ml. A clear solution was obtained upon shaking the mixture at 37° for 1.5 hr.

Administration. Animals employed were CFE male rats (*Carworth Farms*) (150–250 g) or male Mongolian gerbils (50–100 g) from our own colony. All preparations were administered intraduodenally at a dose of 100 mg heparin/kg body weight in volumes of 5.0 or 10.0 ml/kg body weight.

Animals were anesthetized with 60 mg/kg "Diabutal" (*Diamond Laboratories*). 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 preparation 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 prepara-

TABLE I. Effect of Various Emulsified Oils Prepared with Sodium Taurocholate on Heparin or Mactin B Absorption in the Rat and Gerbil.[a]

	Composition	Rat		Gerbil	
		Mean decrease in OD at 30 min ± SE	Clotting time (min)	Mean decrease in OD at 30 min ± SE	Clotting time (min)
1	Saline	0.02 ± 0.01 (3)	—	0.00 ± 0.01 (2)	—
2	Heparin	0.03 ± 0.00 (12)	—	0.02 ± 0.01 (6)	5.8 ± 0.5 (6)
3	Heparin + NaT	0.04 ± 0.00 (15)	3.8 ± 0.5 (4)	0.04 ± 0.02 (6)	5.2 ± 1.1 (6)
4	Monoolein + heparin + NaT + NaG	0.01 ± 0.01 (6)	—	—	—
5	Trioctanoin + heparin + NaT	0.56 ± 0.06 (7)	> 45 (5)	0.58 ± 0.02 (6)	> 45 (6)
6	Trioctanoin + NaT	0.01 ± 0.01 (3)	3.0 ± 0.2 (3)	−0.04 ± 0.01 (6)	5.3 ± 0.4 (6)
7	Trioctanoin + Mactin B + NaT	0.00 ± 0.01 (7)	—	—	—
8	Corn oil + heparin + NaT	0.23 ± 0.03 (24)	—	0.48 ± 0.06 (4)	> 45 (4)
9	Corn oil + NaT	0.04 ± 0.01 (11)	—	—	—
10	Peanut oil + heparin + NaT	0.17 ± 0.05 (5)	—	—	—
11	Mineral oil + heparin + NaT	0.07 ± 0.03 (6)	2.9 ± 0.4 (6)	0.01 ± 0.01 (6)	11.2 ± 2.5 (6)
12	Mineral oil + NaT	0.01 ± 0.01 (3)	2.5 ± 0.1 (3)	—	—

[a] NaT = Na taurocholate, NaG = Na glycocholate; final concentrations: heparin, 10 mg/ml (no. 4 contained 30 mg/ml); Na taurocholate, 0.035 *M* (no. 4 contained equimolar concentrations of Na taurocholate and Na glycocholate, total, 0.04 *M*); oil phase, 12.5%; number of animals tested in parentheses.

tion 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 intracardial or retrobulbar puncture.

Assays. The turbidimetric assay of clearing-factor activity (5) was carried out in 1-ml Coleman spectrophotometric 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 (pH 8.5, *Armour*), and 0.5 ml of rat or gerbil serum. The mixture was preincubated for 2 min at 37° and 0.1 ml of substrate (0.6% v/v "Ediol" in Tris buffer), was added at zero time. The optical density at 650 mμ was immediately recorded. The mixture was incubated at 37° and optical density readings taken at 15 and 30 min. 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. The results are reported in terms of the observed decrease in optical density at the times indicated.

Blood clotting times were estimated in rats by the capillary tube procedure of Jaques (6).

Results. Physical properties of emulsions. Emulsions were milk-white in appearance, of aqueous consistency, and completely miscible with water. Freshly prepared emulsions usually "creamed" within a few hours, but were easily resuspended by swirling. Approximately 70% of the oil phase was contained in particles below 0.8μ in diameter, as shown by Coulter counter estimation.

The data obtained with a number of heparin and sodium taurocholate-containing preparations tested in the rat and gerbil are presented in Table I. Neither heparin alone (Comp. 2), heparin in the presence of taurocholate (Comp. 3), nor heparin in a micellar solution of monoolein (Comp. 4) affected clearing-factor activity as compared with control intubations of saline (Comp. 1). However, when heparin was administered in a trioctanoin emulsion, stabilized with sodium taurocholate (Comp. 5) a strong clearing-factor response was elicited within 30 min (ΔOD = 0.56). Control emulsions (prepared in the

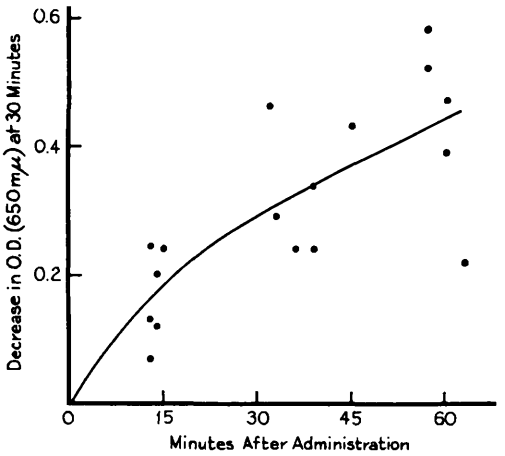

FIG. 1. Plot of rate of heparin absorption in the rat as measured by clearing-factor response. The emulsion contained heparin, 10 mg/ml; Na taurocholate, 0.035 *M*; and corn oil, 12.5%. Doses of 100 mg heparin/kg body weight were administered to 17 rats and blood withdrawn at the time intervals indicated. Each point represents 1 rat.

absence of heparin, Comp. 6), gave no response. Anticoagulant activity was also observed with Comp. 5; clotting times were extended to greater than 45 min.

Corn or peanut oil emulsions containing heparin (Comp. 8 and 10), caused significant clearing in the rat although not so much as emulsions prepared with trioctanoin. In the gerbil, heparin in both trioctanoin and corn oil emulsions elicited a strong clearing factor response. Mineral oil emulsions (Comp. 11) caused slight clearing activity in the rat and were inactive in the gerbil. Blood clotting time, however, was slightly increased in the latter. Mactin B in emulsion form (Comp. 7) showed no activity. Anticoagulant activity was observed in the gerbil with corn oil emulsions containing heparin (Comp. 8).

Rate of heparin absorption. As is shown in Fig. 1, clearing-factor activity was present as early as 13 min after administration. The increase in clearing-factor response appeared to be fairly linear up to 60 min. As the appearance of clearing factor in the blood after heparin injection appears to be instantaneous (7), it is reasonable to assume the response reflects the rate of heparin absorption.

Effect of adding heparin to control emulsion. Clearing-factor activity could be obtained by adding aqueous heparin to a previously prepared control emulsion immediately prior to administration (Table II, Comp. 3); i.e., it was not necessary for heparin to be present during the physical process of emulsification. Clearing-factor activity was also observed after intubation of "Ediol" to which heparin was previously added (Comp. 4). There was no significant difference in the activity of these heparin-containing preparations.

Effect of varying the surfactant. Clearing-factor activity was observed with heparin-containing emulsions prepared with sodium salts of taurocholate (Comp. 1), glycocholate (Comp. 2), or cholate (Comp. 3) as emulsifying agents (Table III); glycocholate emulsions gave slightly greater activity. Monoolein (Comp. 4) appeared to enhance the activity of taurocholate emulsions, but no significant clearing was noted in the absence of taurocholate (Comp. 5). Two commercial emulsifying agents, Tween-20 and RE-610, nonionic and anionic agents, respectively,

TABLE II. Activity in the Rat of Emulsions Prepared with Heparin vs Addition of Heparin after Emulsification.[a]

Composition	Mean decrease in OD ± SE	
	15 min	30 min
1 Corn oil + heparin + NaT	0.08 ± 0.01 (6)	0.15 ± 0.04
2 Corn oil + NaT	0.02 ± 0.00 (5)	0.02 ± 0.00
3 Composition 2 + aqueous heparin	0.08 ± 0.01 (6)	0.15 ± 0.03
4 "Ediol" + aqueous heparin	0.07 ± 0.02 (6)	0.11 ± 0.03

[a] NaT = Na taurocholate; final concentrations: heparin, 10 mg/ml; Na taurocholate, 0.035 *M*; oil phase, 12.5% v/v; number of animals tested in parentheses.

TABLE III. Activity in the Rat of Emulsions Prepared with Various Surfactants.[a]

Composition	Mean decrease in OD ± SE	
	15 min	30 min
1 Corn oil + heparin + NaT	0.07 ± 0.02 (6)	0.12 ± 0.05
2 Corn oil + heparin + NaG	0.12 ± 0.02 (6)	0.21 ± 0.04
3 Corn oil + heparin + NaC	0.06 ± 0.01 (6)	0.09 ± 0.01
4 Corn oil + heparin + NaT + Monoolein	0.14 ± 0.04 (6)	0.22 ± 0.04
5 Corn oil + heparin + monoolein	0.04 ± 0.01 (6)	0.05 ± 0.01
6 Trioctanoin + heparin + Tween-20	0.25 ± 0.09 (4)	0.32 ± 0.09
7 Heparin + Tween-20	0.07 ± 0.01 (9)	0.07 ± 0.01
8 Trioctanoin + Tween-20	0.03 ± 0.01 (4)	0.04 ± 0.01
9 Trioctanoin + heparin + RE-610	0.34 ± 0.10 (4)	0.46 ± 0.13
10 Heparin + RE-610	0.04 ± 0.00 (6)	0.04 ± 0.00
11 Trioctanoin + RE-610	0.04 ± 0.00 (6)	0.04 ± 0.00

[a] NaT = Na taurocholate, NaG = Na glycocholate, NaC = Na cholate; final concentrations: heparin, preparations 1–5, 10 mg/ml; preparations 6–11, 20 mg/ml; bile salts, 0.035 *M*; monoolein, 1% (v/v); Tween-20 or RE-610, 0.5% (w/v); oil phase, 12.5% (v/v); number of animals tested in parentheses.

were also studied. In both cases (Comp. 6 and 9, respectively) a strong clearing-factor response was observed when heparin was administered in an emulsified form. Slight activity was observed with solutions of heparin in Tween-20 (Comp. 7).

Dose response study. The results of a dose-response study in the rat is presented in Fig. 2. The clearing-factor response was dose-related with heparin (ED_{50} of 48 mg/kg).

Discussion. Loomis (8) has reported that a systemic anticoagulant effect is obtained when heparin is administered in the presence of an acid buffer (pH 4.0). It was proposed that absorption was the result of a partial neutralization of the carboxyl groups of the polysaccharide. Windsor and Cronheim (9) found that EDTA facilitated intestinal absorption of heparin, presumably by chelation of intestinal calcium and magnesium. It seems doubtful that either of these proposals can satisfactorily account for the absorption of heparin in the presence of a neutral emulsion.

Although the absorption of lipid-soluble agents, such as sulfonamides (10) and indoxole (11), can be enhanced by administration in an emulsified form, it seems unlikely that a lipid-insoluble polysaccharide such as heparin could be absorbed via this lipid-soluble route. Heparin adsorption at an oil surface is also difficult to visualize, and in any case, one would expect absorption to be prohibited by aqueous exposure of the acidic sulfate groups.

Any reasonable hypothesis must take into account both the necessity for a digestible oil phase and the rapid appearance of detectable clearing-factor activity after administration. The latter consideration might lend support to a particulate or pinocytotic route as this would not require significant lipolysis by pancreatic lipase (12). Further support for pinocytosis may be drawn from the work of Daugherty (13) who found that heparin markedly stimulated pinocytotic inclusion of India ink particles in cultured rat fibroblasts.

Arguing against a pinocytotic mechanism is the fact that mineral oil emulsions exhibit, at best, marginal activity. Absorption via pinocytosis does not seem to require a digestible substance. Latex (14), resin (15), or dye (16) particles are absorbed and, of more importance in this case, paraffin oils (17) and synthetic triglycerides normally resistant to hydrolysis by pancreatic lipase (18). The inability of mineral oil emulsions to effect absorption of heparin would indicate that some prior lipolysis was necessary.

It is also possible that heparin, in some association with the oil phase, is being absorbed in a manner incidental to two mechan-

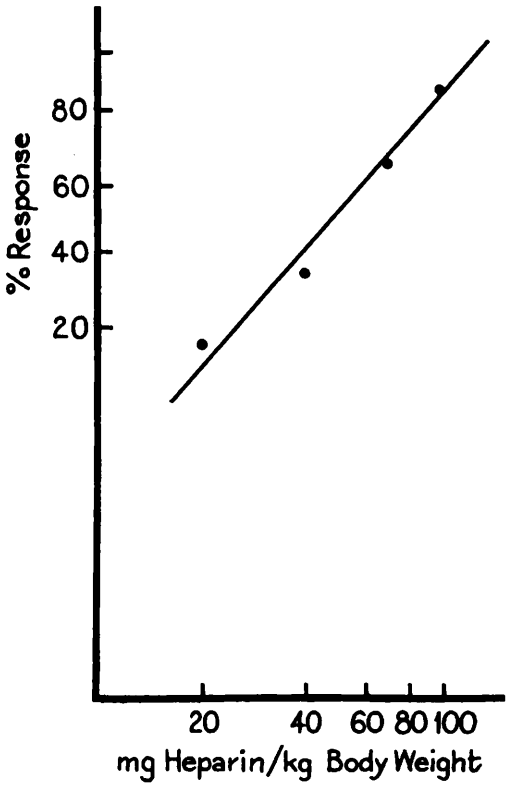

FIG. 2. Dose response of heparin absorption in the rat. The emulsions contained heparin, Na taurocholate, 0.008 *M*; trioctanoin, 12.5%; and heparin at 4.0, 8.0, 12.0, 16.0, and 20 mg/ml. Doses were administered to 6 rats/group. Percentage of response is the percentage of animals exhibiting a mean decrease in O.D. of 0.06 or greater at 30-min assay time.

isms of lipid absorption, one requiring lipolysis and another utilizing particulate material. The greater activity of trioctanoin in the rat is most interesting in this regard as this triglyceride, although attacked by pancreatic lipase, can also be easily absorbed across the intestinal epithelium of the rat without prior hydrolysis and emulsification (19).

Mactin B is very similar in analysis and structure to heparin and as active in eliciting anticoagulant and clearing-factor activity upon parenteral administration. Its inactivity upon intraduodenal administration may be a result of its higher molecular weight, (28,000 vs. 14,000 for heparin) (3).

The compositional requirements for the intestinal absorption of heparin appear relatively simple, i.e., a digestible oil emulsified with a suitable surfactant. The ease with which such preparations can be formulated suggest that the phenomenon may be useful in the preparation of an orally active heparin.

Summary. When heparin was administered intraduodenally in an emulsified form to rats and gerbils, a marked increase in clearing factor and anticoagulant activity was obtained. Vegetable oils were more effective than mineral oil, suggesting that an oil susceptible to lipolysis is necessary for activity. A number of emulsifying agents have been shown to be effective. Mactin B, a polysaccharide obtained from the clam, similar to heparin but of greater molecular weight, was not absorbed when introduced into the duodenum in an emulsified form.

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Development of Delayed Dermal Hypersensitivity in Guinea Pigs Immunized with Inactivated Respiratory Syncytial Virus Vaccines* (33423)

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Respiratory syncytial (RS) virus is an RNA containing virus which has been classified as a member of the myxovirus family on the basis of its nucleic acid composition, ether sensitivity, and ultrastructure (1). Many clinical studies have demonstrated that this virus is a major cause of serious respiratory disease in infants and young children (2). In addition, several investigators have indicated that RS virus may be a cause of significant respiratory disease in adults (3). The development of a safe and effective vaccine to prevent infection by this agent would be of great public health importance.

The recent epidemiologic studies by Chanock and associates have emphasized the fact that RS virus causes serious lower respiratory tract illness predominently in young infants under 6 months of age (4). During this period of time, almost all of the infected infants possess passively acquired maternal serum neutralizing antibody against RS virus. This temporal association of passive maternal RS neutralizing antibody and severe disease caused by RS virus suggested to these workers that there was immunologic enhancement of RS disease in young infants by the maternally transmitted antibody. The recent experience by several groups demonstrating that infants who received an antigenic, inactivated concentrated RS virus vaccine developed more extensive respiratory disease following natural RS infection than did comparable control groups has provided additional impetus for the investigation of the role of immunological reactions in the pathogenesis of RS virus infections (4).

Although delayed hypersensitivity has not been implicated by these investigations as a cause of these adverse vaccine reactions, this mechanism has been suggested as the etiology of the adverse reactions seen in individuals who received another inactivated myxovirus vaccine, measles vaccine (5). The present study was undertaken in an attempt to determine if delayed hypersensitivity could be demonstrated in animals which had received inactivated RS virus vaccines and to define the nature of the antigen or antigens responsible for such reactions.

Methods. Vaccines[1]. (1) *RS-monkey kidney-alum (RS-MK-A) vaccine.* The Burnett strain of RS virus was grown in vervet monkey kidney cells. The vaccine was inactivated with 1:4000 formalin, filtered, concentrated 25 times by Sharples centrifugation and precipitated and concentrated 4-fold with 4

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