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Research Paper

Movement of Heparins Across Rat Gastric Mucosa is Dependent on Molecular Weight and pH

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Purpose. Movement of unfractionated (UFH) and low molecular weight heparins (LMWHs) through gastric mucosa was compared to determine effect of molecular weight on absorption.
Methods. Rat gastric mucosa, mounted in an Ussing chamber, was bathed in oxygenated Kreb's buffer, containing mannitol on the mucosal (lumen) at pH 7.4 or 4, and glucose on the serosal side (circulation) at pH 7.4. Heparins (10 mg/ml) were added to the mucosal side. Potential difference (PD), resistance, and short circuit current (Isc), were determined. Buffers and tissues were extracted to measure heparin by gel electrophoresis.
Results. PD increased on heparin addition and following a lag period, that was longer for UFH at pH 7.4 and LMWHs at pH 4.0, returned to baseline. Isc increased slightly for UFH at pH 4.0 but significantly for LMWHs at pH 7.4. More UFH or LMWHs were recovered from serosal buffers at pH 4.0 and pH 7.4 respectively. Results suggest UFH and LMWHs cross gastric mucosa faster, and active transport is involved, at pH 4.0 and pH 7.4, respectively.
Conclusions. Decreasing heparin size, increases movement through gastric mucosa at mucosal buffer pH 7.4 but not pH 4.0. The stomach environment may favor UFH absorption while the intestine environment favors LMWH absorption.

KEY WORDS: absorption; gastric mucosa; low molecular weight heparins; rat; unfractionated heparin.

INTRODUCTION

Heparins belong to a family of compounds called glycosaminoglycans (GAGs), which are derived from animal tissues. They are given as drugs of choice in the prevention and treatment of thrombo-embolic disorders by intravenous or subcutaneous routes and are believed to be ineffective when administered orally (1). However, evidence of oral absorption of heparins has repeatedly been reported in the literature. Unfractionated heparin (UFH) and low molecular weight heparins (LMWHs) were found to have antithrombotic effects after oral administration in a rat jugular vein (2), venous stasis (3) and rat carotid arterial model (4). Moreover, UFH and LMWHs were found with endothelium following oral administration with little in plasma similar to that observed with parenteral administration (2,5,6).

Only a few previous studies have provided evidence on the site and mechanism of oral heparin absorption. Heparin introduced into the gastrointestinal tract was found to increase the whole blood clotting time and plasma anti-factor Xa activity, with the anticoagulant effect being greater when heparin was placed in the stomach *versus* the small intestine (7). More chemical or [^{14}C] UFH was found in stomach tissue *versus* duodenum, jejunum, and ileum or colon tissue up to 24 h following administration by stomach lavage (8). As well, more UFH was found in the endothelium when heparin was placed in the rat stomach with the pyloric sphincter tied *versus* the small intestine (9). These findings suggest that stomach may be a site for heparin absorption. Most recently, we showed, using a vertical diffusion Ussing chamber, that UFH (10) and LMWHs (in press) cross rat gastric mucosa and that movement is dependent on the pH of the environment but differences in molecular weight have not been compared.

Decreasing the molecular size may facilitate the passage of heparin through the gastric mucosal membrane. The high negative charge and large molecular weight of heparin have been considered limiting factors to oral absorption (11). Natural heparin or UFH is polydisperse and has an average molecular weight of approximately 20,000 Da (12). The commercial LMWHs have an average molecular weight of approximately 3,000 Da. Moreover, depending on the type of fractionation method used, different LMWHs have different chemical structure. LMWHs are obtained by various methods of fractionation or depolymerisation of polymeric UFH (12,13) including oxidative depolymerisation with hydrogen peroxide or with Cu^{2+} and hydrogen peroxide, deaminative

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ABBREVIATIONS: APTT, activated partial thromboplastin time; GAG, glycosaminoglycan; Isc, short circuit current; LMWH, low molecular weight heparin; MWCO, molecular weight cut off; PD, potential difference; UFH, unfractionated heparin.

cleavage with isoamyl nitrite or nitrous acid, alkaline beta-eliminative cleavage of the benzyl ester and, beta-eliminative cleavage by the heparinase enzyme.

Antithrombotic activity also varies between UFH and LMWHs. Orally administered LMWHs, tinzaparin and reviparin, have antithrombotic activity at much lower doses than UFH in a rat jugular vein model of thrombosis. Single doses of 0.025, 0.1, and 7.5 mg/kg resulted in a 50% reduction in thrombosis incidence at 4 h for reviparin, tinzaparin and bovine lung UFH, respectively suggesting a faster or more complete absorption for LMWHs (4,6,8,14). Thus, the objective of the present study was to determine if LMWHs move across rat gastric mucosa faster than UFH. Two different LMWHs were studied.

MATERIALS AND METHODS

Chemicals

Tinzaparin sodium (anti-Xa activity 90.7 IU/mg, peak maximum molecular mass of 5,600 Da), obtained from porcine mucosal heparin, was generously donated by Novo Nordisk, Denmark. Reviparin sodium (Batch W 49,522, average molecular mass of 4,300 Da, anti-Xa activity 130 IU/mg; anti-IIa activity 29 IU/mg) was from Knoll AG, Ludwigshafen, Germany. Bovine lung unfractionated heparin (156.2 U/mg) was obtained from Scientific Protein Labs, Division of Viobin Corporation, Wisconsin, USA. Materials for gel electrophoresis; petroleum ether, glacial acetic acid, and acetone were obtained from VWR Canlab, Mississauga, ON, Canada; sodium barbital, cetavlon (hexadecyltrimethylammonium bromide), toluidine blue, and HCl were from Sigma-Aldrich, ON, Canada; and agarose was from Bio-Rad, Mississauga, ON, Canada. Materials for Kreb's buffer, $MgCl_2$–$6H_2O$, $CaCl_2$–$2H_2O$, NaCl, KCl, Na_2 HPO_4, NaH_2PO_4–H_2O, $NaHCO_3$, mannitol, D-glucose, were from VWR Canlab. Molecular weight cut off (MWCO) 1,000 dialysis tubing was purchased from Spectrum Laboratories Inc., RanchoDominguez, CA, USA. Materials for LMWH extraction from mucosal tissues; protease from Streptomyces griseus was from Sigma-Aldrich; Tris, $CaCl_2$, isopropanol, and methanol were from VWR Canlab. Chemicals for anesthesia, chloral hydrate, sodium pentobarbital, magnesium sulfate, ethanol and propylene glycol were obtained from Sigma-Aldrich.

Gastric Mucosa Isolation from Rats

Animals were obtained from Charles River Canada Company, St. Constant, Quebec, Canada and were handled in accordance with the Principles of Animal Care set by the Canadian Federation of Biological Societies. All animal procedures were approved by the Animal Care Committee of the University of Saskatchewan and performed according to the guiding principles of the Canadian Council on Animal Care. Male Wistar rats (n=35, 250–300 g) were anesthetized by an intraperitoneal injection of Equithesin (chloral hydrate 4.2% *w*/*v*, sodium pentobarbital 0.98% *w*/*v*, magnesium sulfate 2.12% *w*/*v*, ethanol 10% *v*/*v*, propylene glycol 40% *v*/*v*, and sterile water to a volume of 100 ml: 1 ml/250 g rat). The stomach was removed from the abdominal cavity, the lumen washed several times with saline and an undamaged section of the glandular portion of gastric mucosa was separated from the submucosa as previously described (10).

Measurements of Movement of Heparins across Gastric Mucosa Using an Ussing Chamber

An EVC 4,000 voltage/current clamp (NaviCyte, Harvard Apparatus, Inc.) was used for transport studies across the gastric mucosa. Immediately after separation from the submucosa and serosa, the mucosa was mounted in the Ussing chamber. As a control, an additional portion of the mucosa was frozen for later GAG extraction.

The assembled chamber was placed in a block heater connected to a circulating water bath, maintained at 37°C. The hemi-chambers (1.5 ml) on each side of the mucosa were filled with warmed (37°C) oxygenated Kreb's Ringer bicarbonate buffer ($MgCl_2$–$6H_2O$, 1.1 mM; $CaCl_2$–$2H_2O$, 2.15 mM; NaCl, 113.96 mM; KCl, 5.03 mM; Na_2 HPO_4, 1.65 mM; NaH_2PO_4–H_2O, 0.30 mM; $NaHCO_3$, 25 mM) at pH 7.4 on the serosal side and pH 7.4 or 4.0 on the mucosal side. D-Glucose (40 mM) was added to the serosal buffer to provide an energy source. Mannitol (40 mM) was added to the mucosal buffer to provide an osmotic load equivalent to the serosal buffer. Buffers were added to each side of the chamber simultaneously to prevent hydrostatic pressure effects. Buffers in the hemi-chambers were circulated by gas lift (95% O_2/5% CO_2), controlled by valves (Precision Instrument Design, Los Altos, CA). The mucosal surface area exposed to buffers was 2.5 cm^2.

Harvard/Navicyte Micro-Reference voltage measuring electrodes (2.5 mm×5.0 cm) and electrodes for passing current were placed on either side of the mucosa as previously described (10). Potential difference (PD) or voltage difference across the mucosa (ΔV) in mV, resistance (R) in mΩ, and short circuit current (Isc) in mA (a measure of the net active ion transport across the mucosa) were determined. To determine R, a current of 15 mA was passed across the mucosa using the pulse generator and ΔV was recorded. The R was then calculated using Ohm's law: $R=\Delta V_t\ I^{-1}$, where ΔV_t is voltage difference across the mucosa at a specific time and I is the current of 15 mA. Finally, the transmucosal current was clamped to zero and ΔV_t was measured. Since R of the tissue is known, the short circuit current (Isc) can then be calculated: $Isc=\Delta V_t R_t^{-1}$

The tissue was stabilized in buffer for 40 min with electrical measurements taken every 5 min. Heparins were then added to the mucosal buffer by adding 0.1 ml of the stock solutions of 150 mg/ml to obtain a final concentration of 10 mg/ml. Electrical measurements were continued every two min for an additional 84 min. Mucosal and serosal buffers as well as mucosal tissues were then collected and frozen at −4°C for later extraction and analysis. Electrical parameters were normalized to the value taken just prior to addition of heparins. Changes in PD, R, and Isc were then determined by subtracting the value from this time.

Determination of Heparins in Buffers and Tissues

Mucosal and serosal buffers were dialyzed in distilled water for 48 h using MWCO 1,000 dialysis tubing. The dialyzed buffers were then dried and used for analysis of

chemical heparin. The GAGs were extracted from mucosal tissue by a published method with some modifications (15). Agarose gel electrophoresis was used to identify and measure heparins in extracts. Dried powders were dissolved in suitable volumes of water and applied to agarose gel slides, along with the administered LMWH as a reference. Gels were fixed in 0.1% hexadecyltrimethylammonium bromide and air-dried. Slides were stained with 0.04% toluidine blue in 80% acetone, and background color was removed using 1% acetic acid. The heparins were identified by electrophoretic migration as compared to reference material and amounts were determined by densitometry.

Data Analysis and Statistical Procedures

All data are expressed as mean±standard error of the mean (SEM). A one-tailed unpaired *t* test was used to determine significant differences in the degree of negativity on addition of heparins, the lag period, and the time period for PD to return to the baseline in different environments. A one-tailed *t* test was also used to measure differences in heparin concentrations in serosal buffers and experimental mucosa under different conditions and in the rate of movement across gastric mucosa.

Differences in Isc were calculated by subtracting the average of the first five values obtained at 40–48 min following addition of heparins to the mucosal buffer, from the average of the last five values recorded at 116–124 min. A one-tailed *t* test was used to compare differences in Isc between groups. Values were considered significant at $P<0.05$.

RESULTS

Changes in Electrical Parameters of Rat Gastric Mucosa Following Addition of UFH or LMWHs to the Mucosal Buffer at Different pH

Mucosal Buffer at pH 7.4

The mucosal tissue was stabilized in the Ussing chamber for 40 min before drug addition. When heparins were added to the mucosal buffer, the PD increased and became more negative by 1.6±0.3, 1.8±0.4 and 1.6±0.5 mV upon addition of UFH, tinzaparin and reviparin respectively (Fig. 1A, and a, and Table I), which was similar when LMWHs were compared to UFH ($P=0.4$, one-tailed *t* test). This was followed by a lag period where no changes in PD were

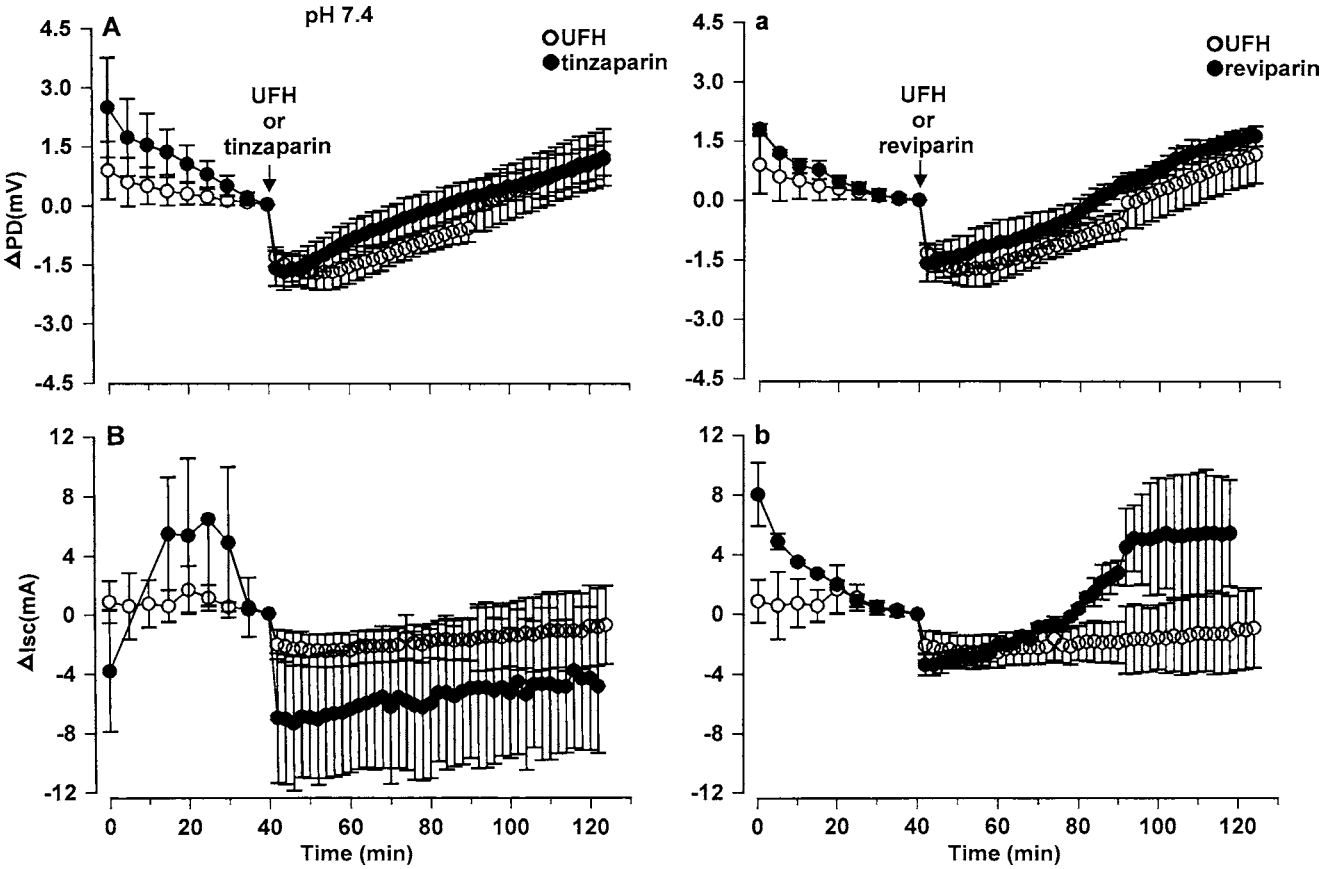

Fig. 1. Changes in electrical parameters across rat gastric mucosa on addition of UFH or LMWHs (10 mg/ml) to the mucosal buffer at pH 7.4. The potential difference (*PD*) became more negative when the mucosal side was compared to the serosal side immediately after addition of heparins to the mucosal buffer. After a lag period, PD returned to the resting level with time (**A** and *a*). The lag period was significantly greater for UFH *versus* LMWHs (one-tailed *t* test). Short Circuit Current (Isc) did not change during the experimental period after UFH addition while it increased significantly after tinzaparin or reviparin addition when LMWHs were compared to UFH (**B** and *b*). Results are shown as mean±SEM of eight experiments for UFH, seven experiments for tinzaparin, and four experiments for reviparin.

Table I. Changes in Electrical Parameters (PD, R, and Isc) Across Rat Gastric Mucosa in an Ussing Chamber Following Addition of UFH or LMWHs to the Mucosal Buffer

		UFH	Tinzaparin	Reviparin	LMWHs[d]
At pH 7.4					
Changes in PD	Negativity (mV)	−1.6±0.3	−1.8±0.4	−1.6±0.5	−1.7±0.3
	Lag Period (min)	18.8±4.9	7.0±1.0[a]	4.0±0.0[a]	5.7±0.8[a]
	Time to reach resting level after lag period (min)	39.3±17.6	35.0±9.5	36.7±4.3	35.7±5.0
Changes in Isc	Change in baseline (mA) (124–40 min)	0.0±0.0	2.7±0.5[b]	23.0±12.4[b]	11.4±6.2[b]
At pH 4.0					
Changes in PD	Negativity (mV)	−2.2±0.1	−2.3±0.8	−2.0±0.2	−2.2±0.4
	Lag Period (min)	9.2±2.8	16.4±9.4	15.0±2.4	15.8±5.0
	Time to reach resting level after lag period (min)	40.5±7.9	47.6±1.4	46.0±3.7	46.9±5.9
Changes in Isc	Change in baseline (mA) (124–40 min)	4.2±1.5	0.9±0.5	0.5±2.3	0.7±1.1[c]

[a] Significantly less than UFH, one-tailed *t* test
[b] Significantly greater than UFH, one-tailed *t* test
[c] $P=0.05$, UFH *versus* LMWHs combined, one-tailed *t* test
[d] Results combined for tinzaparin and reviparin

observed. The lag periods were 18.8±4.9 min for UFH, 7.0±1.0 min for tinzaparin, 4.0±0.0 min for reviparin, and 5.7±0.8 min for LMWHs combined and were significantly greater for UFH *versus* tinzaparin, reviparin, and LMWHs combined ($P=0.04$, $P=0.03$, and $P=0.005$ respectively, one-tailed *t* test, Fig. 1A, and a, Table I). After the lag period, the PD began to decrease and reached its previous resting level at similar periods of time; 39.3±17.6, 35.0±9.5 and 36.7±4.3 min for UFH, tinzaparin and reviparin respectively ($P=0.4$, one-tailed *t* test, when LMWHs were compared to UFH; Table I).

The Isc did not change during the experimental period after UFH addition to the mucosal buffer while it did increase by 2.7±0.5 and 23.0±12.4 mA after tinzaparin or reviparin addition respectively and by 11.4±6.2 mA for LMWHs combined. This Isc change was significantly less for UFH when compared to tinzaparin, reviparin and combined values for LMWHs ($P=0.0001$, $P=0.004$, and $P=0.04$ respectively, one-tailed *t* test) (Fig 1B, and b, and Table I).

Heparins were extracted from the serosal buffer and mucosal tissue 84 min after addition of heparins to the mucosal buffer (Table II). Recovery of UFH from serosal buffer was 65.0±18.8 μg and was significantly less than 214.1±61.6 μg for reviparin ($P=0.01$, one-tailed *t* test) and 190.3±48.1 μg for LMWHs combined ($P=0.04$, one-tailed *t* test), but not for tinzaparin 176.8±70.1 μg ($P=0.09$, one-tailed *t* test). Heparin recovered from mucosal tissue was significantly greater for UFH (51.9±19.9 μg) than LMWHs combined (24.8±1.1 μg; $P=0.04$, one-tailed *t* test) but not for tinzaparin (25.0±1.5 μg, $P=0.08$, one-tailed *t* test) or reviparin (24.4±1.6 μg, $P=0.2$, one-tailed *t* test). The calculated rate of movement of heparins across the gastric mucosa, based on the chemical recovery of heparins from the serosal buffer at 84 min, was significantly less for UFH (0.5±0.1 μg cm^{-2} min^{-1}) compared to reviparin (1.5±0.5 μg/cm^2/min, $P=0.003$) and LMWHs combined (1.4±0.3 μg cm^{-2} min^{-1}, $P=0.03$), but not for tinzaparin where there was a trend which did not reach significance (1.3±0.5 μg cm^{-2} min^{-1}, $P=0.06$; Table II).

Mucosal Buffer at pH 4.0

The electrical properties of the membrane were recorded when pH of the mucosal buffer was 4.0, the average pH of the stomach in rats fed a normal diet (16). The same pattern of changes in PD was observed at pH 4.0 as noted at pH 7.4.

Table II. Recovery of Heparins from Buffers and Tissues 84 min after Addition of Heparin to the Mucosal Buffer

Treatments	Mucosal buffer (μg)	Serosal buffer (μg)	Control Mucosa (μg)	Experimental mucosa (μg)	Rate[d] (μg cm^{-2} min^{-1})
At pH 7.4					
UFH ($n=6$)	3,917.7±271.3	65.0±18.8	0.0±0.0	51.9±19.9	0.5±0.1
Tinzaparin ($n=7$)	2,266.7±584.2	176.8±70.1	0.0±0.0	25.0±1.5	1.3±0.5
Reviparin ($n=4$)	2,875.0±953.8	214.1±61.1[a]	0.0±0.0	24.4±1.6	1.5±0.5[a]
LMWHs[e] ($n=11$)	2,510.0±442.3	190.3±48.1[a]	0.0±0.0	24.8±1.1[b]	1.4±0.3[a]
At pH 4.0					
UFH ($n=5$)	2,400.0±597.0	111.3±36.3	0.0±0.0	26.9±5.7	0.8±0.1
Tinzaparin ($n=6$)	3,250.0±845.6	51.5±2.3[c]	0.0±0.0	34.2±4.7	0.4±0.0[b]
Reviparin ($n=4$)	3,500.0±333.3	47.5±4.3	0.0±0.0	35.0±7.4	0.3±0.0[b]
LMWHs[e] ($n=10$)	3,350.0±484.6	49.9±2.2[b]	0.0±0.0	34.5±3.8	0.3±0.0[b]

[a] Significantly greater than UFH, one-tailed *t* test
[b] Significantly less than UFH, one-tailed *t* test
[c] $P=0.05$, tinzaparin *versus* UFH, one-tailed *t* test
[d] Rate of heparin movement across the gastric mucosa based on the chemical recovery of heparins from the serosal buffer
[e] Results for tinzaparin and reviparin combined

After tissue stabilization in the Ussing chamber for 40 min, changes in PD increased and became more negative upon addition of heparins to the mucosal buffer (Fig. 2A and a, and Table I). The PD became more negative by 2.2±0.1, 2.3±0.8 and 2.0±0.2 mV when UFH, tinzaparin or reviparin were added to the mucosal buffer respectively and was similar between groups when LMWHs were compared to UFH (P=0.2, one-tailed t test). After similar lag periods of 9.2±2.8 min for UFH, 16.4±9.4 min for tinzaparin and 15.0±2.4 min for reviparin (P=0.2, one-tailed t test when UFH was compared to LMWHs), the PD began to decrease in negativity. The PD returned to baseline in similar time periods; which were 40.5±7.9 min for UFH, 47.6±1.4 min for tinzaparin and 46.0±3.7 min for reviparin (P=0.1, one-tailed t test when UFH was compared to LMWHs). The Isc increased by 4.2±1.6 mA following UFH addition *versus* 0.9±0.5 mA after tinzaparin and 0.5±2.3 mA after reviparin addition (P=0.05, one-tailed t test when UFH was compared to LMWHs; Fig. 2B, b, and Table I).

Heparins were chemically recovered from serosal buffer and the mucosal tissue after addition of heparins to the mucosal buffer for 84 min (Table II). Recovery of UFH from the serosal buffer was 111.3±36.3 μg and significantly greater when compared to LMWHs combined 49.9±2.2 μg (P=0.01), with a trend toward an increase compared to tinzaparin 51.5±2.3 μg (P=0.05) but not reviparin 47.5±4.3 μg (P=0.08, one-tailed t test). Moreover, 26.9±5.7 μg UFH was recovered from the mucosal tissue, which was similar to 34.2±4.7 μg for tinzaparin (P=0.2, one-tailed t test), 35.0±7.4 μg for reviparin (P=0.2, one-tailed t test), and 34.5±3.8 μg for LMWHs combined (P=0.2, one-tailed t test). The calculated rate of movement of heparins across the gastric mucosa at 84 min based on their chemical recovery from serosal buffer was 0.8±0.1 μg cm^{-2} min^{-1} for UFH, 0.4±0.0 μg cm^{-2} min^{-1} for tinzaparin, 0.3±0.0 μg/cm^2/min for reviparin, and 0.3±0.0 μg cm^{-2} min^{-1} for LMWHs combined (Table II). Rate of movement was significantly less for LMWHs combined and for tinzaparin and reviparin compared to UFH (P<0.0001, P=0.0001, P<0.0001 respectively, one-tailed t test).

DISCUSSION

Heparins have been administered by intravenous and subcutaneous routes for more than 70 years. They are believed not to be effective when administered orally (1). These assumptions are based on the observations that little or no change is seen in anticoagulant activity following oral administration of heparins (17) or heparins are too highly

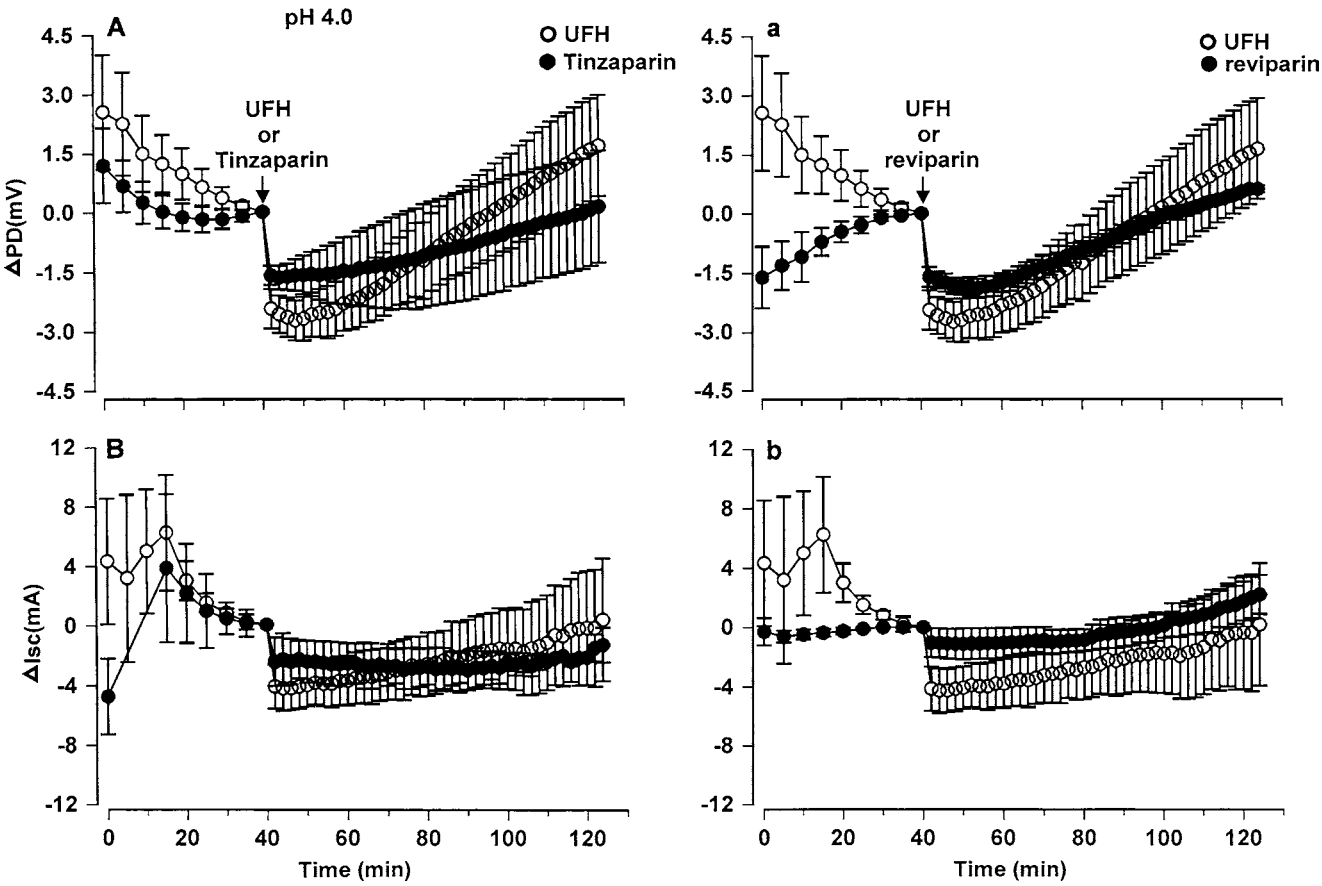

Fig. 2. Changes in electrical parameters across rat gastric mucosa on addition of UFH or LMWHs (10 mg/ml) to the mucosal buffer at pH 4.0. The potential difference (*PD*) became more negative when heparins were added to the mucosal buffer. After a lag period, *PD* returned to the resting level with time (**A** and *a*). The lag period was similar for LMWHs *versus* UFH at pH 4.0. Short Circuit Current (*Isc*) increased during the experimental period when heparins were added to the mucosal buffer. Increase in Isc was similar when UFH was added to the mucosal buffer *versus* LMWHs (**B** and *b*). Results are shown as mean±SEM of six experiments for UFH, six experiments for tinzaparin, and four experiments for reviparin.

charged or too large to be considered candidates for gastrointestinal absorption (11). Based on these assumptions, considerable effort has been spent on increasing oral heparin absorption by addition of a simple organic chemical *N*-(8-(2 hydroxybenzoyl)amino)caprylate (SNAC) (18), using adjuvants (19), surfactants (20), or combining heparin with diamine complexes (21), and biodegradable and non-biodegradable polycationic polymers (22). Despite all these efforts, a great deal of evidence exists in the literature showing that heparins are effective when administered by the oral route without addition of other compounds or use of delivery agents. Both UFH and LMWH in drinking water, given to spontaneously hypertensive rats, returned systolic blood pressure to normal (23). Thrombosis was prevented by oral UFH and LMWHs in the rat jugular vein (6,8,9,14), carotid artery (4), and venous stasis models (3,24).

Although the effectiveness of orally administered heparins on thrombosis in the rat model has been thoroughly studied, there is little known about the site of heparin absorption. Tissue distribution studies showed that orally administered heparin was found in gut and non-gut tissues. Much was recovered from the stomach tissue (8). As well, more heparin is recovered from endothelium when UFH is placed in the stomach *versus* that of the duodenum for 15 min (9). These studies suggested that the stomach may be a site for heparin absorption when administered orally. Our recent *in vitro* studies using a vertical diffusion Ussing chamber with rat gastric mucosa showed that UFH and LMWHs cross rat gastric mucosa and that movement is dependent on the pH of the environment (9,10). In the present study using this model we show that movement across rat gastric mucosa is affected by molecular weight.

Changes in electrical parameters following heparin addition suggest that heparins cross rat gastric mucosa. Changes in PD increased and became very negative the moment heparins were added to the mucosal buffer, when the mucosal side was compared to the serosal side (Figs. 1 and 2, and Table I). There was a lag period when the PD remained negative for some time after which PD returned to the resting level. Heparins were chemically recovered from the serosal buffer and the mucosal tissue. Although changes in the electrical parameters of rat gastric mucosa after addition of UFH or LMWHs (tinzaparin or reviparin) to the mucosal buffer followed the same pattern, there were some important differences (Table I). UFH or LMWHs responded differently to pH changes in mucosal buffer. At pH 7.4, the lag period was significantly shorter for LMWHs compared to UFH, while the opposite was seen at pH 4.0. These results suggest that LMWHs may cross the mucosa easier in a basic environment while an acidic environment favours the movement of UFH. This is supported by chemical recovery of heparins from serosal buffers (Table II). The LMWHs were found in significantly higher concentrations than UFH in serosal buffer at pH 7.4. However, the concentration of UFH in serosal buffer was significantly higher than that of LMWHs at pH 4.0.

It is likely that prevention of ionization plays some role in facilitating the movement of UFH across the gastric mucosa at acidic pH. As UFH is a very large and highly acidic molecule, an acidic environment may prevent ionization and thus facilitate movement through the gastric mucosa. The LMWHs on the other hand, are smaller in size and therefore may be less affected by acidic conditions. Stomach absorption of UFH is also supported by previous reports of significant increases in whole blood clotting time following administration of heparin dissolved in diluted acids (7) and increased endothelial heparin following tying of the rat pyloric sphincter and administration of heparin into the stomach *versus* the duodenum (9).

Other factors may also determine the site and rate of heparin absorption. When UFH or LMWHs are introduced into the mucosal buffer of the Ussing chamber, they interact with the mucosa and are then released into the serosal buffer. UFH has high nonspecific binding to proteins and cells (25–27). The UFH can bind to a variety of plasma proteins such as lipoproteins, fibrinogen, fibronectin, vitronectin and histidine-rich glycoproteins, to proteins secreted by platelets like platelet factor 4 and von Willebrand factor, and to endothelial cells (25). Thus, UFH interactions with components of the mucosal membrane, when added to the mucosal buffer, is likely much more complicated than LMWHs. UFH may remain attached to the luminal mucosal membrane for some time before it can cross to the serosal side. Furthermore, some of the UFH that is internalized may remain associated with the mucosal cells for some time before it is released to the serosal side. This is supported by previous observations showing that UFH remained inside endothelial cells for 5 days after uptake as determined by the presence of toluidine blue stained metachromatic inclusions (28). LMWHs on the other hand, may pass through the mucosa easier since they have less protein binding capacity and smaller size. Our results imply that LMWHs are likely absorbed at a faster rate than UFH particularly in a neutral environment. This agrees with previous results showing that orally administered LMWHs were effective as anti-thrombotic drugs at lower doses than UFH and thus may be absorbed faster than UFH. In the rat jugular vein thrombosis model, ED_{50}s of 7.5, 0.1 and 0.025 mg/kg were seen for UFH, tinzaparin and reviparin, respectively (6,8,29). Thrombosis was also prevented at 0.1 and 7.5 mg/kg for tinzaparin and bovine UFH respectively in a rat carotid artery model (4).

The Isc results also support the concept that LMWHs move across the mucosa at a higher pH where a lower pH favors movement of UFH (Table I). Change in Isc, an indicator of active transport across the mucosal membrane (30), increased noticeably after addition of LMWHs to the mucosal buffer under neutral conditions. Contrary to this, Isc increased when UFH was added to the mucosal buffer at pH 4.0. The Isc results suggest that an active transport mechanism becomes activated and facilitates the transport of LMWHs and UFH across the mucosal membrane under neutral and acidic conditions respectively.

In conclusion, this study supports the observations that heparins are absorbed by the gastrointestinal tract. Results suggest that UFH and LMWHs require different environmental pH conditions for optimal absorption. While the acidic environment seems to help passage of UFH through the gastric mucosa, LMWHs are transported better in a basic environment. This suggests that LMWHs may be better absorbed in the intestine while UFH may be preferentially absorbed in the stomach. The LMWHs may pass through the mucosa faster at lower levels of the gut, since they have less

protein binding compared to UFH. Further studies are needed to better understand the movement of heparins across the gut and the mechanisms involved.

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