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An evaluation of the oral administration of commercial and fractionated heparin samples in rats

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Summary

The oral absorption of the anticoagulant, heparin, has been studied in rats Heparin fractions from various commercial sources and with differences in molecular weight, anticoagulant activity and the type of counterions present have been tested. The results show that none of the heparin samples were absorbed into the blood system under the experimental conditions employed. Further work by administering heparins in non-toxic acids and complexed to spermine and lysine did not produce any absorbability of the drug. The experiments also revealed inherent changes in clotting times of rat plasma related to the animal's age between 8 and 16 weeks. For the activated partial thromboplastin time assay, clotting time (s) = 2.105 × age (weeks) + 7.18 ($P < 0.001$) and for the thrombin–fibrinogen time assay, clotting time (s) = 0.483 × age (weeks) + 8.67 ($P < 0.01$).

Introduction

Heparin, a naturally occurring polyanion, is used extensively as an anticoagulant for the treatment of pulmonary embolism and deep vein thrombosis. Heparin is a heterogeneous mixture (Horner, 1976), and its anticoagulant potency is directly related to the presence of sites on the molecule which interact with the plasma

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proteins, antithrombin III and thrombin (Abildgaard, 1968). Recently, many researchers have been directing their studies to determine the type of molecule and the identity of the species responsible for each of the variety of biological effects and biochemical interactions of heparin (Lindahl et al., 1979; Jordan et al., 1979).

Concurrent with these developments have been studies designed to assess and improve the absorption of heparin via oral administration. The gastrointestinal tract, although possessing a large surface area suitable for absorption, has been considered impermeable to such large, highly charged molecules, and parenteral administration has been favoured for drugs such as heparin. However, reports have recently appeared containing detailed formulations of heparin for oral administration, but these developments have yet to be extended into regular clinical use. Rectal absorption of heparin from safe delivery systems such as mixed micelles has recently been described (Taniguchi et al., 1980) and the acid salts of heparin, in contrast to the known neutral salts, have been shown to be absorbed through the walls of the intestinal tract and to exhibit some anticoagulant activity in the blood stream (Koh, 1969). Other researchers have used oil/water emulsions (Engel and Fahrenbach, 1978) and calcium-binding molecules (Reber and Studer, 1963; Windsor and Cronheim, 1961) to promote passage of heparin across the gastro-intestinal tract. However, prolonged exposure to some of these adjuvants affects the physical integrity of the tract, with resulting tissue damage, (Nadai et al., 1975) and therefore their prolonged use seems undesirable.

In the present study, a number of discrete heparin preparations have been tested for activity following oral administration, but the use of adjuvants such as detergents and calcium-sequestering agents to promote oral absorption has been avoided. The heparin preparations used vary in their commercial source, molecular weight, anticoagulant activity and the counterion present, and their possible in vivo activity in plasma has been determined after administration into the stomach of rats. Further, the effect of suppressing the ionization of heparin by simultaneous administration of acid and also by complex formation with lysine and spermine has been examined to attempt to improve the absorbability of the drug.

Because of the relatively large number of rats used in the study, the opportunity has been taken to investigate the changes in the plasma clotting time as a function of age of the animal.

Materials and methods

Materials

Sodium heparin was obtained from Diosynth, Morden, Surrey and Abbott Laboratories, North Chicago, IL; permanganate treated, second-crop, crude heparin from Abbott Laboratories; and lithium heparin, L-lysine dihydrochloride and spermine tetrahydrochloride from Sigma Chemicals. Topical thrombin was obtained from Parke Davies. Heparin porcine and cephalin were gifts from the W.H.O. International Laboratory for Biological Standards, Holly Hill, Hampstead, London, and from the National Reference Laboratory for Anticoagulant Control Reagents,

Withington Hospital, Manchester, respectively. All other reagents were of analytical grade and were obtained from B.D.H. Chemicals.

Preparation of heparin samples

Sodium heparin (1.25 g) (Diosynth) was dissolved in 1 M sodium chloride (18 ml) and was chromatographed on a column (120 × 2.9 cm) of Ultragel ACA 44 (L.K.B.) equilibrated with 1 M sodium chloride. The heparin solution was eluted with 1 M sodium chloride at a flow rate of 30 ml/h. The elute was monitored using acridine orange (2×10^{-5} M), and 5 distinct fractions were isolated (Fig. 1) (Diakun et al., 1979). The fractions denoted I, III and V, i.e. those of high, middle and low molecular weight, were precipitated by ethanol, centrifuged and dried in vacuo at ambient temperature.

The column was calibrated with heparin of known molecular weight previously determined by light scattering (Barlow et al., 1961). The elution volume of a sample of unknown molecular weight was compared to obtain an estimate of the molecular weight (Johnson and Mulloy, 1976).

The 3 fractions and sodium heparin (Abbott Laboratories) were then converted to their calcium salts. An ion exchange Amberlite resin IR-120 (H) column (90 × 2.9 cm) was washed with calcium chloride (2 M) followed by distilled water and the heparin samples (200 mg in 2 ml of water) were added to the column and eluted with distilled water. The heparin solution was then passed down another similarly treated Amberlite column in order to ensure the completion of the ion-exchange process. The calcium solution was freeze-dried, and after acid hydrolysis the calcium content of the heparin preparation was determined by titration against ethylenediamine-

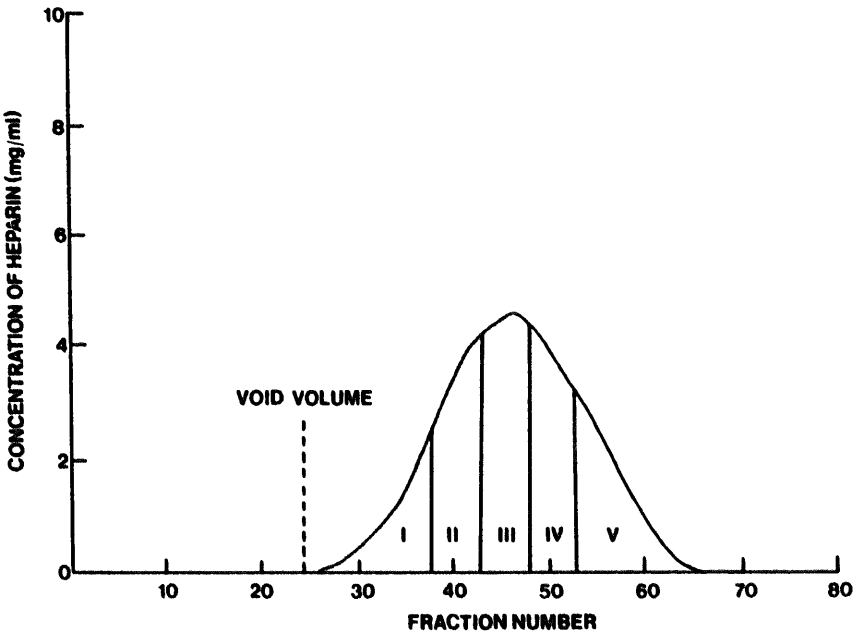

Fig. 1. Elution profile of sodium heparin (Diosynth) on Ultragel ACA 44 chromatography column (120×2.9 cm) previously equilibrated with 1 M sodium chloride. Heparin (1.25 g dissolved in 1 M sodium chloride (18 ml)) was eluted with 1 M sodium chloride at a flow rate of 30 ml/h.

tetraacetic acid (EDTA) to establish the extent of ion-exchange. At least 80% of each sample was converted to the calcium salt using the above technique.

Animals and oral testing

Male and female rats of known age and weight (weighing 200–250 g) of the Lister Hooded/Ola strain were used. The rats were starved for 24 h prior to the tests but had free access to water. Heparin was fed at two dose levels, viz. 20 mg and 100 mg total dose per rat, and was administered in 1 ml of distilled water through a polyethylene tube (diameter 2 mm) that had been carefully passed down the oesophagus into the stomach. Control rats were fed on distilled water (1 ml). Complexes of sodium heparin (Abbott)–lysine in which only 40% of the heparin's charge remained were prepared by mixing a calculated amount of lysine with heparin (20 mg), dissolving both in distilled water (1 ml). This was then administered to the rats as described. The charge equivalent weight (i.e. molecular weight/anionic sites) of sodium heparin (Abbott) was previously calculated to be 185 g/equivalent (Diakun et al., 1978). Similar heparin complexes were prepared with spermine.

Fifty minutes after the administration of heparin the animal was anaesthetized with an interperitoneal injection of sodium pentobarbitone (60 mg/kg). When the rat was under the anaesthesia (approximately 10 min later) an incision was made in the abdominal wall, the aorta exposed and whole blood collected from the base of the aorta into a syringe containing a 3.13% solution of trisodium citrate dihydrate in 0.9% sodium chloride, to give a final blood : citrate ratio of 9 : 1.

Analytical methods

The collected blood sample (10 ml) was cooled over ice for 2 min before being centrifuged at 1800 *g* for 20 min to give a platelet poor plasma. This was kept at 4°C until assayed using the activated partial thromboplastin time (APTT) (Thomson, 1970) and the thrombin fibrinogen time test (TFT) (Dacie and Lewis, 1970). All the glassware for these assays was previously siliconized by soaking in 2% dichlorodimethylsilane in 'Analar' carbon tetrachloride, dried, and then rinsed in distilled water.

The anticoagulant activity of the heparin samples was determined using the British Pharmacopoeia (BP) 1973 method.

Acid stability

The heparin samples to be tested for oral activity were added to hydrochloric acid (0.1 M) and the mixture incubated at 37°C; aliquots were removed at 0, 0.5, 1, 2 and 24 h. These were adjusted to pH 7.4 using dilute sodium hydroxide, made up to a designated volume with distilled water and the APTT measured using freshly collected platelet-poor plasma. This plasma was obtained from healthy human males who had not received any medication for the previous 14 days.

Results

Heparin samples were selected for the oral testing programme on the basis of their anticoagulant activity, the type of counterion present and their molecular

weight distribution. Fig. 1 shows the elution profile of one commercial heparin preparation (sodium heparin, Diosynth) following gel filtration on Ultragel ACA 44. The fractions denoted I, III and V were isolated and subsequently used in this experimental programme. The fractions were known to be in the molecular range 12,000–13,000; 8000–9000 and 5000–6000 daltons, respectively.

After their isolation, the anticoagulant activities of these fractions were determined using the British Pharmacopoeia Method, and Table 1 includes these together with the values obtained for the commercial heparins used in this study, as well as the activities determined by the manufacturers.

Since these samples were to be administered directly into the rats' stomachs, the effect of acid on the activity of the samples was determined. Fig. 2 shows the variation in the APTT clotting times of 10 heparin samples following treatment with hydrochloric acid (0.1 M). After a 2-h incubation, the heparin samples had lost about 10–20% of their activity and after 24 h the loss of activity was nearly complete. Human plasma with no added heparin gave APTT values of 38–45 s. When the same acid-hydrolyzed heparin samples were assayed using the acridine orange titrimetric technique (Diakun et al., 1979) no change in the concentration of heparin was detectable after 2 h.

Table 2 shows the results of administering samples at two doses (20 mg and 100 mg per rat) and then assaying the plasma using the two standard haematological assays: APTT and TFT. Clotting times similar to the control values were obtained and a similar lack of effect was observed when the ionization of heparin was suppressed with non-toxic acids (Table 3). The charge of heparin was also neutralized (partly) by complex formation with spermine and lysine. Table 4 shows the APTT and TFT clotting times 1 h after the rats were fed these complexes. Again the results show that no oral absorption occurred. Additional experiments were performed with the rats being left for periods longer than 1 h (2, 3, 6, 8 and 16 h) after the administration of heparin. Heparin was also administered orally at a dose of 250 mg/animal. In all these experiments no increased plasma clotting time was detectable.

TABLE 1

THE ANTICOAGULANT ACTIVITY OF THE HEPARIN FRACTIONS AND SAMPLES AGAINST W.H.O. 3RD INTERNATIONAL STANDARD

Sample	Activity (BP units/mg)	Manufacturer's value (USP units/mg)
Sodium heparin (Abbott)	162.8	157.0
Calcium heparin	180.0	–
Lithium heparin (Sigma)	160.9	160.0
Heparin crude—permanganate-treated (Abbott)	42.3	49.8
Sodium heparin (Diosynth) Fraction V	178.6	–
Sodium heparin (Diosynth) Fraction III	165.0	–
Sodium heparin (Diosynth) Fraction I	84.0	–

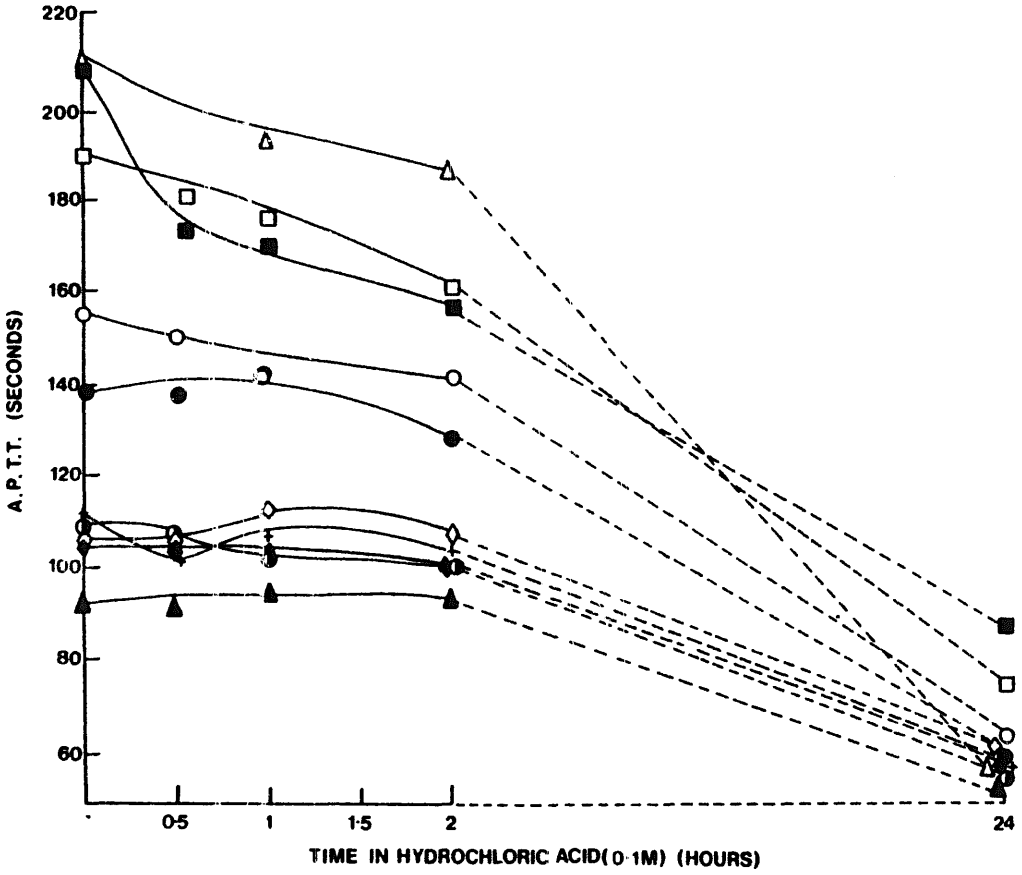

Fig. 2. APTT clotting times for heparin samples after treatment with hydrochloric acid (0.1 M) at 37°C. ◇, calcium heparin, Fraction I; △, calcium heparin, Fraction III; ▲, calcium heparin, Fraction V; +, sodium heparin, Fraction I; ○, sodium heparin, Fraction III; ◆, sodium heparin, Fraction V; □, sodium heparin; ■, calcium heparin; ●, lithium heparin; ○, crude heparin.

The individual clotting times are presented in Tables 2 and 3 in order to emphasize the prolonged clotting times obtained for some rats with two heparin samples (lithium heparin and sodium heparin fraction V) and with sodium heparin in 0.1 M HCl. These anomalous results were substantiated by both the APTT and TFT tests.

The sex, age and weight of all the rats used were noted and possible correlation between these and clotting times were explored. When the APTT and TFT clotting times were plotted against the age of the rat, the trends noted in Figs. 3 and 4, respectively, were obtained. In order to identify the most significant relationships between age and clotting times, the data have been grouped as shown in Table 5. The control samples (no. of observations ~20) were divided into two groups, one with and one without acid added. The variation of correlation coefficients for the total controls and the two groups is not considerable since the total number of observations is small, and in this instance, the correlation between age of rats and clotting time using APTT and TFT assays does not appear to be significant (Bailey, 1959).

However, with an increased number of observations, when the data from the

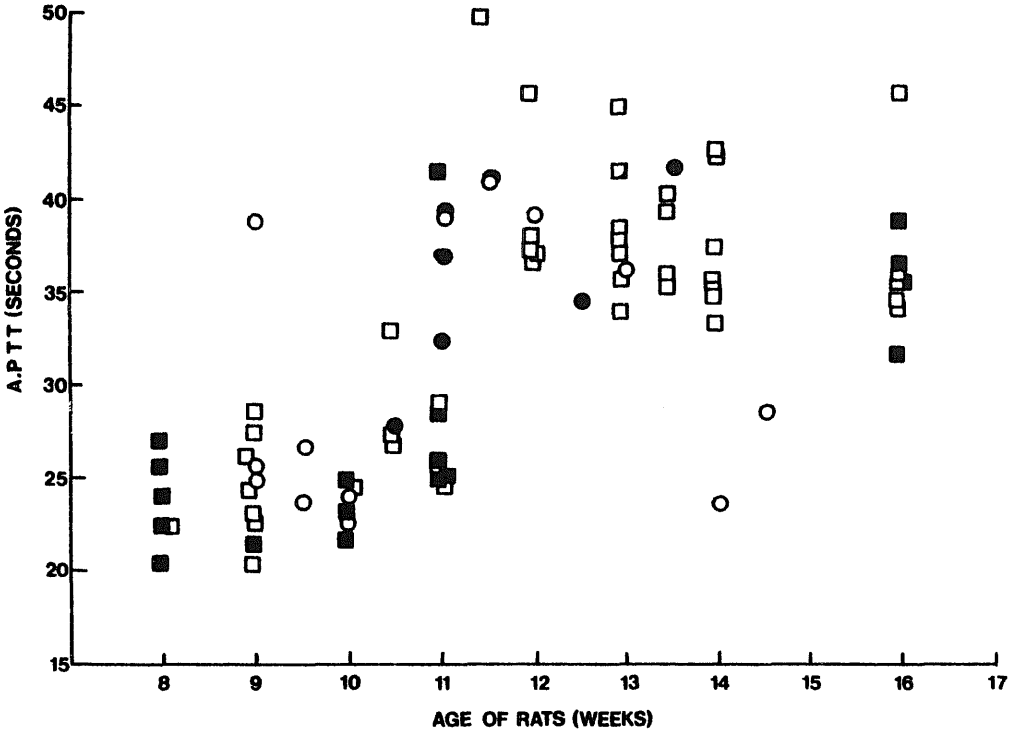

Fig. 3. Relationship between the APTT values and age of rats following oral administration of heparin. Control no acid (●); control acid data (○); all heparin fractions except lithium heparin and sodium heparin, fraction V (□); lithium heparin and sodium heparin, fraction V (■).

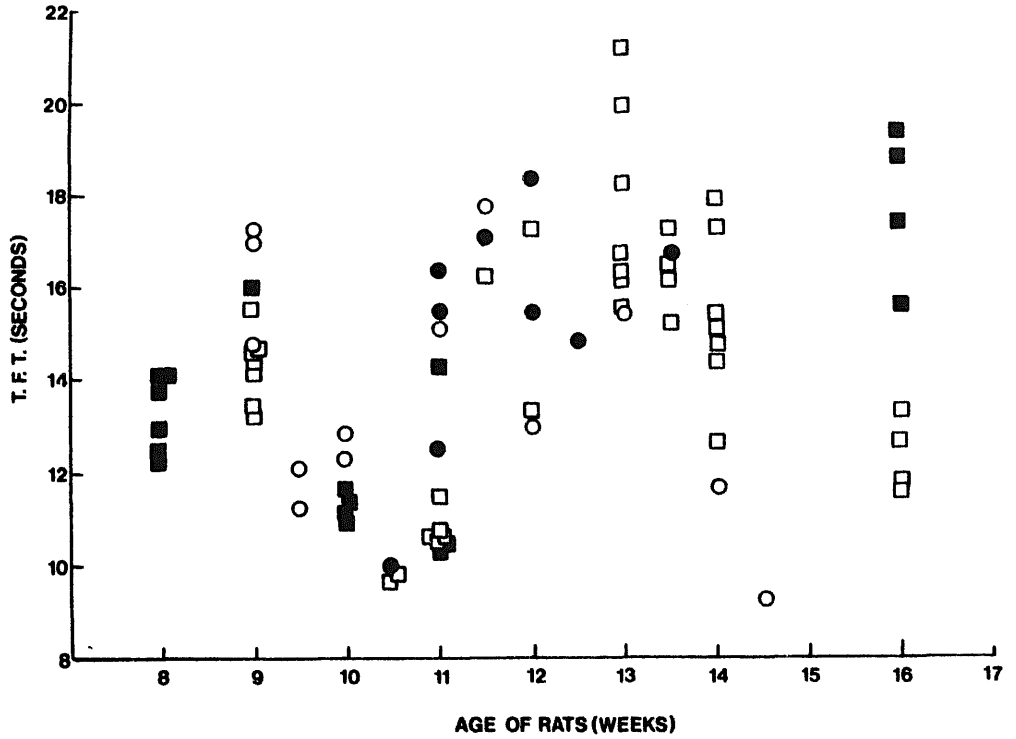

Fig. 4. Relationship between the TFT values and age of rats following oral administration of heparin. Control no acid (●); control acid data (○); all heparin fractions except lithium heparin and sodium fraction V (□); lithium heparin and sodium heparin fraction V (■).

TABLE 2

ANTICOAGULANT EFFECTS OF VARIOUS HEPARINS AFTER ORAL ADMINISTRATION INTO RATS

Sample	Dose	APTT [a] (s)	Mean ± S.D. [b]
Sodium heparin (Abbott)	20 mg	45.6, 37.9, 37.9, 33.8, 41.4	39.5 ± 4.2
	100 mg	28.8, 35.6, 44.9, 38.2, 37.0, 37.2	37.0 ± 4.8
	control	41.8	
Calcium heparin	20 mg	35.8, 35.2, 40.3	37.6 ± 2.4
	100 mg	39.2	
	control	42.0	
Lithium heparin (Sigma)	20 mg	38.9, 36.1, 35.4, 31.8, 25.3, 26.9	32.4 ± 5
	100 mg	57.8, 134.3, 36.7, 23.9, 22.1, 20.3, 22.4, 21.2	42.35 ± 37.7
	control	38.9	
Heparin crude—permanganate-treated (Abbot)	20 mg	42.3, 33.0, 35.6, 37.3	37.1 ± 3.6
	100 mg	42.3, 35.3, 34.9	37.5 ± 3.7
	control	34.6	
Sodium heparin, Fraction I	20 mg	35.5, 45.7, 34.1	37.4 ± 5.1
	100 mg	34.3	
	control	37.0	
Sodium heparin, Fraction III	20 mg	22.4, 22.6, 26.9	29.85 ± 9.9
	100 mg	28.5, 49.7	
	control	27.9	
Sodium heparin, Fraction V	20 mg	24.7, 21.5, 22.8, 24.0, 41.4	26.9 ± 7.6
	100 mg	140, 25.3, 26.8	72.1 ± 58.7
	control	27.9	
Calcium heparin, Fraction I	20 mg	24.7, 24.7, 25.8	25.0 ± 0.7
	100 mg	24.7	
	control	32.5	
Calcium heparin, Fraction III	20 mg	26.5, 32.8	28.8 ± 3.2
	100 mg	27.1	
	control	27.9	
Calcium heparin, Fraction V	20 mg	20.0, 24.4	23.9 ± 3.2
	88 mg	27.2	
	control	23.8	

[a] Each clotting time represents the average of 3 determinations on each plasma.

[b] The mean was calculated from all the determinations measured on each plasma sample.

[c] The means of the combined controls were compared by Student's *t*-test with the mean of each set of

heparin-containing doses were included (case 2), the value of r increased and the correlation is significant ($P < 0.001$) for the APTT assay only. Further increase in the correlation coefficient is observed when the number of observations is increased to 67 (case 3) (Table 5). For case 3, the correlation is significant for both assays and

t-test [c]	TFT [a] (s)	Mean ± S.D. [b]	t-test [c]
N.S.	15.4, 16.2, 20.0, 21.3	17.8 ± 2.7	$P<1\%$, Sig.
N.S.	11.5, 16.8, 15.6, 16.3, 18.3, 17.3 16.8	16.0 ± 2.25	N.S.
N.S.	16.2, 17.4, 16.6 15.3 16.8	16.4 ± 0.8	$P<0.1\%$, Highly sig.
N.S.	18.9, 17.5, 19.5, 13.8, 14.2	16.8 ± 2.5	N.S.
$P<0.1\%$, Highly sig.	22.3, >10 min, 15.7, 14.2, 13.0, 12.4, 12.3, 16.1 14.7		$P<0.1\%$, Highly sig.
N.S.	18.0, 12.7, 14.8, 17.4	15.7 ± 2.2	N.S.
N.S.	14.4, 15.2, 15.5 14.8	15.0 ± 0.6	N.S.
N.S.	12.7, 11.9, 13.4 11.7 15.5	12.4 ± 0.8	$P<0.1\%$, Highly Sig.
N.S.	14.2, 13.4, 14.6 14.7, 16.3 15.5	14.6 ± 1.0	N.S.
$P<0.1\%$, Highly sig.	11.2, 11.0, 11.7, 11.3, 14.3	11.9 ± 1.3	N.S.
$P<0.1\%$, Highly sig.	>20 min, 10.5, 10.6 10.0		$P<0.1\%$, Highly sig.
$P<0.1\%$	10.6, 10.8, 10.7 10.7 12.5	10.7 ± 0.2	$P<0.1\%$
N.S.	9.8, 10.0 9.9 10.0	9.9 ± 0.3	$P<0.1\%$
N.S.	13.3, 14.2 15.6 11.2	14.4 ± 1.0	N.S.

data. Where only a few clotting times were recorded, the 20 mg and 100 mg data were combined to find the mean.

N.S. = not significant.

if y is the clotting time (seconds) and x the age of rat (weeks), the regression equation for the APTT assay is $y = 2.105x + 7.18$ and for the TFT assay in case 3, $y = 0.483x + 8.67$.

TABLE 3

ANTICOAGULANT EFFECTS OF VARIOUS HEPARINS IN NON-TOXIC ACIDS AFTER ORAL ADMINISTRATION INTO RATS

Sample	Dose	APTT [a] (s)	Mean ± S.D. [b]	*t*-test [c]	TFT [a] (s)	Mean ± S.D. [b]	*t*-test [c]
Sodium heparin (Abbott) in 0.1 M HCl (1 ml)	20 mg 100 mg 0.1 M HCl (1 ml)	29.6, 30.3 33.3, 25.4, 219 23.7	67.6 ± 7.9	N.S.	11.2, 12.3 12.4, 11.3, 135 11.7	29.4 ± 45.1	N.S.
Sodium heparin (Abbott) in tartaric acid (30 mg/ml)	20 mg 100 mg 1 ml tartaric acid (30 mg/ml)	24.8, 26.0 32.7, 57.9, 36.4, 26.7, 26.7, 32.7, 28 28.9, 23.2, 27.7	32.4 ± 9.9 26.8 ± 2.35	$P \simeq 5\%$, Prob. sig.	9.0, 9.2 9.5, 10.4, 9.1, 11.9 11.7, 12.2, 12.5 9.3, 11.2, 12.1	10.9 ± 1.4 11.2 ± 1.2	$P < 0.1\%$, Highly sig.
Sodium heparin (Abbott) in citric acid) (30 mg/ml)	20 mg 100 mg 1 ml citric acid (30 mg/ml)	26.6, 25.4 37.7, 29.2, 29.2, 63.1, 32.3, 30.2, 25.7 25.8, 24.8	33.3 ± 11.4 25.3 ± 0.6	$P \simeq 5\%$, Prob. sig.	13.3, 13.4 15.4, 14.1, 14.3, 27.6, 16.7, 17.3, 15.7 17.2, 17.0	16.4 ± 4.3 17.1 ± 0.4	$P < 1\%$, Sig.
Sodium heparin (Abbott) in 0.5 M NaCl containing 30 mg/ml citric acid	100 mg 1 ml 0.5 M NaCl citric acid (30 mg/ml) 0.5 M NaCl (1 ml)	26.1, 39.3, 27.9, 25.1 22.6 24.2	29.6 ± 5.95	N.S.	12.1, 11.9, 12.2, 11.7 12.8 12.3	12.0 ± 0.3	$P < 0.1\%$, Highly sig. (lower)
Sodium heparin, Fraction V in citric acid (30 mg/ml)	20 mg 100 mg 1 ml citric acid (30 mg/ml)	40.8, 48.2, 54.0 69.4, 70.2, 44, 37.1 59.7 39.2	52.9 ± 12	$P < 0.1\%$, Highly sig.	12.2, 13.5, 15.0, 14.6, 20.0, 14.5 15.9 16.8 15.1	15.3 ± 2.3	N.S.
Sodium heparin, Fraction III in citric acid (30 mg/ml)	20 mg 100 mg 1 ml citric acid (30 mg/ml)	42.1, 40.9, 40.3 41.0 41.0	41.1 ± 0.75	$P < 0.1\%$, Highly sig.	16.3, 21.2, 16.1 19.0 17.8	18.1 ± 2.2	N.S.

[a] Each clotting time represents the average of 3 determinations on each plasma.

[b] The mean was calculated from all the determinations measured on each plasma sample.

[c] The means of the combined controls were compared by Student's *t*-test with the mean of each set of data (combining the 20 mg and 100 mg data).

N.S. = not significant.

TABLE 4

ANTICOAGULANT EFFECTS OF HEPARIN COMPLEXES FOLLOWING ORAL ADMINISTRATION INTO RATS

Sample	Dose	APTT (s)	TFT (s)
Sodium heparin (Abbott) with spermine	20 mg Hep/7.1 mg spermine (100% : 75%)	47.2, 48.6, 40.2	13.7, 13.8, 13.8
	20 mg Hep/2.4 mg spermine (100% : 25%)	35.5	12.2
	spermine 7.1 mg	48.3	13.8
	sodium chloride (1 ml of 0.9%)	39.2, 38.9	13.0, 14.7
Sodium heparin (Abbott) with lysine	20 mg Hep/14.7 mg lysine (100% : 40%)	42.7, 41.2, 59.8	15.1, 15.9, 16.3
	lysine (4.7 mg)	48.7	15.3
	sodium chloride (1 ml of 0.9%)	36.3	15.5

Each clotting time represents the average of 3 determinations in each plasma.

TABLE 5

CORRELATION COEFFICIENTS FOR THE RELATIONSHIP BETWEEN ACTIVATED PARTIAL THROMBOPLASTIN TIME AND THROMBIN FIBRINOGEN TIME AND AGE OF RAT

Case	APTT			TFT		
	No. of observations	Correlation coefficients [a] (r)	Significance (P)	No. of observations	Correlation coefficients [b] (r)	Significance (P)
(1) Controls (a) no heparin or acid added	7	0.5481	Not significant ($P>0.10$)	9	0.5428	Not significant ($P>0.05$)
(b) acid data controls	13	0.1984	Not significant ($P>0.10$)	13	−0.3883	Not significant ($P>0.10$)
(c) all controls (i.e. 1(a)+1(b))	20	0.3048	Not significant ($P>0.10$)	22	−0.0843	Not significant ($P>0.10$)
(2) Controls 1(a)+heparin (all times except NaCl/acid/Li Hep and Na Hep, Fraction V)	48	0.6276	Significant ($P<0.001$)	50	0.2059	Not significant ($P>0.10$)
(3) 2+low Li Hep and Na Hep, Fraction V data	67	0.6778	Significant ($P<0.001$)	68	0.3672	Significant ($P<0.01$)
(4) 3+all Li Hep and Na Hep, Fraction V values	70	0.3421	Significant ($P<0.01$)	71	0.0854	Not significant ($P>0.10$)

[a] Correlation between age of rats and the activated partial thromboplastin time (APTT) test.

[b] Correlation between age of rats and the thrombin fibrinogen time (TFT) test.

Discussion

The safe and effective oral administration of drugs such as heparin has been a major objective of pharmacologists and clinicians, and this present work was initiated in order to provide definitive information on the oral absorption of a variety of heparins and heparin subfractions in rats. Although some heparin preparations have been shown to be orally active, this activity is dependent on the use of adjuvants or calcium-binding agents. It has generally been assumed that heparin per se is not absorbed from the gastro-intestinal tract, although some claims have been made about the absorbability of discrete molecular fractions of heparin (Lasker, 1975) and heparin in conjunction with acids and buffers (Loomis, 1959; Sue et al., 1979). These studies have been performed mainly with mice and/or with parts of larger animals.

A densely charged and large heterogeneous substance such as heparin does not appear to be a likely candidate for wholesale passage across the gastro-intestinal tract. Overall absorbability might be increased by using lower molecular weight fractions of heparin or by suppressing the charge on the molecule by simultaneously administering acids. Koh and Bharucha have shown that heparinic acid, which is readily absorbed from the intestine, can be stabilized by forming complexes with a number of weak nitrogeneous bases or amino acids, without loss of absorbability. In the present work, complexes between heparin and spermine and lysine have been prepared with a known percentage of the polyanion's charge neutralized (Koh and Bharucha, 1972).

Another feature that influences the absorbability of heparin is the type of counterion present. A recent report (Thomas et al., 1976) showed that lower blood levels of heparin were achieved after a subcutaneous injection of calcium heparin than after the same number of units of sodium heparin injected subcutaneously. Two other recent studies have shown that, regardless of possible variation depending on the counterion, no differences in the haematological systems were detected after administration of sodium heparin and calcium heparin (Bender et al., 1980; Vaugher et al., 1979).

In order to examine the possible effects of these features on the absorbability of the drug, heparin samples that varied in their anticoagulant potency, molecular weight range and counterion were used. These samples were administered directly into the rat's stomach by instillation, and caused much less trauma to the animal compared with intraduodenal administration under anaesthesia. The effect of the stomach acid on the stability of heparin was examined. The chemical assay using acridine orange fluorescence did not reveal any significant decrease in heparin concentration following acid treatment for 2 h whilst the APTT assay showed a 10–20% decrease in activity. These results underline the greater sensitivity of the haematological test to the features of the heparin molecule responsible for anticoagulancy; in contrast, the fluorimetric test assays the total polyanionic groups of the heparin molecule but takes no account of some of the physical changes, such as depolymerization, that influence biological potency.

The time taken for the passage of heparin along the gastro-intestinal tract was

also investigated. When Dextran Blue (0.1 g in 1 ml) was fed to a rat, it was noted in the post mortem that the material reached the lower extremities of the intestine after 1 h. In view of this observation and the acid stability tests, it is unlikely that the activity of the heparin would have decreased by mixing with stomach acids. Nevertheless, in the subsequent experiments heparin was administered at two dose levels, 20 mg and 100 mg, in order to ensure efficient delivery to any potential sites of absorption.

Two complementary haematological tests were used to assay for plasma heparin after administration: the thrombin fibrinogen time (TFT) which is sensitive to very low heparin levels (0.01 units/ml–0.09 units/ml), and the activated partial thromboplastin time (APTT), which is sensitive to slightly higher heparin levels (0.08 units/ml–0.5 units/ml).

The results (Tables 2, 3 and 4) show that no significant absorption occurred. Although two unusually high values were obtained from both tests for lithium heparin and the low molecular weight fraction (fraction V), this data was not reproduced in subsequent experiments. Again, slightly prolonged clotting times were obtained when the heparin was administered with some of the non-toxic acids. However, these results were obtained after the rats were fed with a total dose of 100 mg or approximately 16,000 units. The prolongation of clotting time in the results represents an increase as a result of approximately 0.4 units/ml plasma being absorbed, i.e. some 0.03% of the total heparin administered.

Our data, showing the non-absorption of heparin in rats are different from preliminary data presented by other researchers. Lasker (1975) found a significant increase in the clotting time after administration of a low molecular weight preparation in mice, but no increase in the clotting time was observed after the same preparation was administered to two anaesthetized rhesus monkeys. Sue et al. (1976) administered defined molecular weight fractions into the stomachs or duodeunum of mice in doses of about 250 mg/kg and observed a slight anticoagulant effect. Our study was performed using a large number of rats and although increased clotting times were obtained with some rats, it is our conclusion that these are statistical aberrations rather than evidence for heparin absorption.

During this work, it was noted that the APTT clotting times apparently varied with the age of the rat population (Figs. 3 and 4) and the linear correlation coefficients (r) were calculated for the cases noted in Table 5. Although the experiments were not designed per se to explore the relationship between clotting times and age an interesting relationship does emerge. As a result of the age distribution and the sample number, the controls either collectively, or when divided into two groups (based on the experimental procedure), did not show any correlation. However, with a wider distribution of age and an increased sample number a definite correlation appears (cases 2 and 3 for APTT and case 3 for TFT).

Clearly, these data suggest a possible relationship between age and clotting times in the rat and further confirms that orally administered heparin was not absorbed by the animals. However, the correlation coefficients (r) between age and the results from the two haematological assays vary for most of the cases examined, e.g. the correlation coefficients for the TFT assay are consistently lower than for the APTT

data and only becomes significant ($P < 0.01$) in case 3. These differences arise as a result of different sensitivities and bases of the tests. However, for case 3, strong evidence exists for a positive correlation between both APTT and TFT values and the age of the rat.

In summary, we feel it inappropriate to draw a firm conclusion for the relationship between clotting time and age. However, the data presented here does show that this relationship deserves further examination, since the age of the rat could have an important bearing on the control values obtained in future studies.

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