ORG 10172: A low molecular weight heparinoid anticoagulant with a long half-life in man

I. D. BRADBROOK¹, H. N. MAGNANI², H. C. T. MOELKER², P. J. MORRISON¹, J. ROBINSON¹, H. J. ROGERS¹, R. G. SPECTOR¹, T. VAN DINTHER² & H. WIJNAND²

¹Guy's Drug Research Unit, United Medical and Dental Schools, Guy's Hospital, London SE1 9RT and

²Scientific Development Group, Organon International B.V., Oss, The Netherlands

1 ORG 10172 is a heparinoid with mean molecular weight 6500 daltons. Intravenous bolus injections of ORG 10172 were compared with placebo and heparin injections in 91 separate studies in 83 healthy male subjects.

2 6400 units ORG 10172 produced a mean maximum change of 14.7 s in kaolin cephalin time (c.f. > 120 s for 5000 units heparin).

3 Changes in prothrombin time were minimal (1.6 s for 6400 units ORG 10172 and 4.5 s after 5000 units heparin).

4 A dose-related increase in bleeding time occurred after ORG 10172 and at high doses (> 3200 units) some secondary bleeding, which was never serious, occurred at between 1 and 4 h after incision.

5 A dose-dependent reduction in *ex vivo* platelet adhesiveness was found at 10 min after ORG 10172 injection.

6 ORG 10172 promoted a much smaller release of lipoprotein lipase as compared with heparin.

7 The effect of ORG 10172 on plasma factor X_a activity (one measure of its action) was described by a biexponential decay with a mean distribution half-life of 2.34 (s.e. mean 0.16) h and mean disposition half-life of 17.6 (s.e. mean 1.1) h. It thus has a much longer duration of effect than heparin.

8 There was a linear relationship of plasma anti-X_a response to increasing dose although there was some variability only partly explained by differences in body weight or surface area.

9 ORG 10172 administration by bolus intravenous injection was well tolerated and there was no evidence of adverse effects on clinical chemistry or haematology tests.

Keywords ORG 10172 heparinoid heparin anticoagulant

Introduction

Heparin is an effective anti-thrombotic agent, but its main side-effects are bleeding and wound haematoma. The incidence of major haemorrhages have been reported to vary from 1–33%

depending upon route of administration and population at risk although no route appears to be entirely free of this problem (Salzman *et al.*, 1975; Mant *et al.*, 1977; Wilson *et al.*, 1981).

Correspondence: Professor H. J. Rogers, Department of Clinical Pharmacology, United Medical and Dental Schools, Guy's Hospital, London Bridge, London SE1 9RT

Bleeding with heparin has been assumed to be directly related to its anticoagulant effect mediated by antithrombin III and consequently unavoidable. However, the evidence for a relationship between anticoagulant response and the risk of bleeding is conflicting (Mant *et al.*, 1977; Norman & Provan, 1977) and possibly the anti-thrombotic and haemorrhagic properties of heparin could be separated by modifying the heparin molecule. Heparin is a linear polymeric mucopolysaccharide of heterogeneous composition with an average molecular weight of 10,000–15,000 daltons but ranging from 3,000–45,000 daltons (Jaques, 1979). The antithrombin III binding site resides in a specific sequence of monosaccharides (Hook *et al.*, 1981). Carter *et al.* (1981) demonstrated that a low molecular weight heparin fraction with a high affinity for anti-thrombin III has a smaller influence on platelet function than high molecular weight heparin or low molecular weight heparin with low affinity for anti-thrombin III. These investigators suggested that high molecular weight heparin possesses both platelet and anti-thrombin III binding sites whilst low molecular weight heparin has lost the platelet binding site (Holmer *et al.*, 1980). Since part of the haemorrhagic effect of heparin is related to its effect on platelet function, loss of the platelet binding site might reduce its haemorrhagic tendency. Although, as is well known, heparin augments the inhibitory activity of anti-thrombin III on factors II, IX, X, XI and XII (Wessler & Gitel, 1979) it can also interact with these factors independently of its co-factor by directly disrupting the calcium- and phospholipid-dependent assembly of factor X and prothrombin activator complexes. This may explain why certain low molecular weight heparin fractions have a greater inhibitory effect on the X_a clotting time than the thrombin clotting time (Ofosu *et al.*, 1982). Thus heparins with a greater anti- X_a to anti-II $_a$ ratio retain their anti-thrombotic properties but have less haemorrhagic effects.

ORG 10172 is a heparinoid isolated from animal mucosa with a mean molecular weight of approximately 6500 daltons. In animal studies it shows anti-thrombotic activity without a tendency to induce bleeding and probably acts by interference with fibrin formation having little action on platelets (Meuleman *et al.*, 1982). It has been used in a small number of patients undergoing haemodialysis (Henny *et al.*, 1983), with haemorrhagic stroke (ten Cate *et al.*, 1984) and is currently undergoing extensive safety and efficacy studies in a variety of clinical conditions. The present paper summarises data from the early Phase I studies in normal volunteers.

Methods

Subjects

This paper reports a series of 91 separate studies of placebo, ORG 10172 and heparin injections in normal subjects carried out over a period of 4 years. As some subjects received more than one injection these studies employed a total of 83 healthy young male volunteers who were all aged between 18 and 35 years and whose body weights were within 10% of their ideal weight as given by actuarial tables. Body surface area was derived from a nomogram (Documenta Geigy, 1971). Pre-study physical examinations and ECGs were in all cases normal and routine haematology, clinical chemistry, clotting tests and urinalysis were performed before and after each injection. All subjects were blood grouped and their serum saved in case of necessity for transfusion. A urine sample was tested for common over-the-counter drugs and abused substances on the day prior to drug administration. Subjects refrained from use of aspirin or other non-steroidal anti-inflammatory drugs for 14 days prior to each study and abstained from alcohol for 48 h before and during each study.

The protocols were approved by the Committee on Ethical Practice of Guy's Hospital and Medical School. All subjects were fully informed of the nature of ORG 10172 and heparin and understood the purpose of the studies. They gave written consent and were free to withdraw at any stage.

Materials

ORG 10172 was supplied by Organon International BV, Oss, Netherlands as a sterile, pyrogen-free solution in isotonic distilled water (800–1250 anti- X_a units ml^{-1}). It contained 1.5 mg ml^{-1} sodium bisulphite. During the course of these studies six batches of ORG 10172 were studied which helped to validate the isolation procedure. Within the production limits used to define the chemical composition of ORG 10172 the proportions of chondroitin sulphate, dermatan sulphate, heparan sulphate the high affinity anti-thrombin-III binding sub-fraction of ORG 10172 varied slightly. This however did not appreciably alter the properties of the different batches.

Heparin USP (Thromboliquine®) and the diluent for ORG 10172 which was used as placebo were obtained from Organon International BV, Oss, Netherlands.

Drug administration and blood sampling

Following an overnight fast and the measurement of baseline clinical observations drug (placebo, heparin as a standard dose of 5000 i.u. or ORG 10172) was given as an intravenous bolus by direct venepuncture. Blood samples for pharmacokinetic studies and clotting studies were taken at appropriate times from an indwelling venous cannula (Venflon 21 G) inserted into a convenient vein of the arm opposite to that which received the injection. The cannula was kept patent by flushing with small boluses of physiological saline.

During the course of the investigation the subjects were closely observed. They remained supine for at least 2 h following drug administration and were then allowed limited mobility around the unit. Smoking was not allowed during the studies. Subjects received normal meals at 4 and 10 h after drug dosing and were discharged after overnight observation at 24 h returning to have later blood samples taken when indicated by the protocol. Urine samples were collected at 4 hourly intervals up to at least 24 h and tested for the presence of haemoglobin and the centrifuged deposit examined microscopically.

Because not all of these studies were performed for the same purpose, some included different combinations of measurements and analyses.

Measurements

(i) Prothrombin time (PT)—Quick's method.

(ii) Kaolin cephalin time (KCT)—(Hall & Malia, 1984).

(iii) Bleeding time was determined by the method of Mielke *et al.* (1969) using the Simplate II apparatus (General Diagnostics Inc, Morris Plains, New Jersey, USA). The procedure was slightly modified from its usual implementation by exerting very firm pressure on the skin to gain maximum skin-cutting blade contact. This gave a painless test which could demonstrate increased bleeding times following injection of 5000 u heparin. All tests were performed in these studies by the same investigator to increase their uniformity.

(iv) Anti- X_a activity in frozen plasma was measured by spectrophotometric determination of the rate of hydrolysis of the chromogenic substrate S 2222 (Kabi Diagnostics, Stockholm, Sweden) based on the amidolytic method of Teien & Lie (1977).

(v) Platelet adhesion to glass beads *ex vivo* was studied using the standardised Adeplat S test (Simmelweis s.r.l., Milan, Italy). This is based upon Hellem's (1970) method where the

reduction in platelet count of whole blood is determined following the passage of a fixed volume of blood through a standard column of glass micro-beads at a given rate using an infusion pump (Adeplat pump system).

(vi) Lipoprotein lipase (triacylglycerol lipase, EC 3.1.1.3) was determined by the titrimetric method of Tietze & Fiereck (1966).

Pharmacokinetic analysis

A standard two compartment open pharmacokinetic model was found to give the best fit to plasma anti- X_a activity. The parameters of the biexponential function characterising the model were estimated using the iterative non-linear least squares optimisation program ELSFIT implemented on a Hewlett-Packard 85 computer.

Results

Effects on KCT and PT

Figure 1 shows the dose-related increase (Δ KCT) in KCT which occurred at the time of the peak plasma anti- X_a level after various doses of ORG 10172. This was small however by comparison with the changes following 5000 u heparin which increased KCT by > 120 s. Placebo injections produced no significant prolongation of KCT. As can be seen from Figure 1 the changes in PT after ORG 10172 were minimal (1.6 ± 0.3 s,

Figure 1 Mean changes over baseline in KCT (○) and PT (x) with dose of ORG 10172.

$n = 28$ after 6400 units, c.f. 4.5 ± 0.6 s, $n = 6$ after 5000 u heparin).

Bleeding time

The modified procedure used in these studies produced a uniform incision of depth 1 to 1.5 mm and longer baseline bleeding times than have been usually reported with this procedure. For 92 studies the baseline bleeding time was 7.7 (s.e. mean 0.20) min. This may be compared with a range of 2.5–5.5 min for 19 normal males found by Young *et al.* (1980) using the Simplate device. However, this procedure allows demonstration of a dose-related increase in bleeding time with ORG 10172 (Table 1). At high doses, as with heparin, some subjects showed bleeding time prolongations greater than 20 min following ORG 10172.

In addition to bleeding time prolongation the use of this modified bleeding time test revealed a phenomenon of secondary bleeding or oozing from the incisions used to test bleeding time which occurred between 1 and 4 h after the cuts were made and primary bleeding had stopped. This continued for between a few minutes to 30 h and occurred with all batches of ORG 10172, rarely with heparin and never with placebo. The incidence with doses of 3200 and 6400 (combined) was 30.8% ($n = 68$) but it decreased progressively with each succeeding batch from 90% to 7.5%.

Platelet adhesion to glass beads

This test, although simple, is difficult to standardise and is influenced by many factors including exercise and stress, e.g. participation in the study. The results (Table 2) suggest a possible dose dependent reduction in platelet adhesiveness at 10 min after drug administration was found as compared to the pre-dose adhesion value. Paradoxically subjects showing secondary bleeding/oozing from bleeding time incisions (see above) showed the smallest reduction in platelet adherence.

Lipoprotein lipase

Serum for this estimation was taken at 10 min after the i.v. bolus drug ingestion. The results are shown in Table 3. The test has a relatively high variance, partly due to interindividual variability, but ORG 10172 provoked a much smaller enzyme release than heparin. No correlation was noted between peak plasma anti-X_a activity at any dose level and serum lipolytic

Table 1 Changes in bleeding time (BT, mean $\pm$ s.e. mean) with dose of ORG 10172 and peak anti-Xa levels in plasma

Drug administered	Number	Dose (units)	Mean peak plasma anti-Xa activity (anti-Xa units ml^{-1} plasma)	Bleeding time			Percentage of subjects with BT		
				Baseline (min)	At peak [anti-Xa] (min)		> 5 min	> 10 min	> 15 min
Placebo	8	—	—	8.1 ± 0.5	7.9 ± 0.6		6	2	0
Heparin	8	5000	0.99 ± 0.03	8.3 ± 0.4	21.4 ± 2.3		90	80	40
ORG 10172	8	1600	0.44 ± 0.01	7.3 ± 0.8	$9.6 \pm 0.9^*$		13	0	0
	40	3200	0.89 ± 0.03	7.1 ± 0.3	12.1 ± 1.0		38	18	8
	28	6400	1.62 ± 0.05	8.3 ± 0.4	$13.2 \pm 0.7^{**}$		52	33	16

*1 result, **4 results recorded as > 15 min hence excluded from mean.

Table 2 Platelet adhesion to glass beads at 10 min after injection of placebo, heparin or ORG 10172 expressed as mean $\pm$ s.e. mean change over pre-treatment percentage of platelets adhering

Treatment	Number (total)	Dose	Change over pre-treatment percentage adhering		
			Re-bleeding occurred	No re-bleeding occurred	All results
Placebo	4	—	—	—	-3 ± 8
Heparin	4	5000 units	—	—	-7 ± 15
ORG 10172	4	1600 units	$+7 (2)^*$	$-8 (2)$	-1 ± 8
	12	3200 units	$-6 \pm 9 (5)$	$-11 \pm 15 (7)$	-9 ± 9
	16	6400 units	$-12 \pm 5 (10)$	$-29 \pm 11 (6)$	-19 ± 5

*Figures in parentheses = number of subjects.

Table 3 Release of serum lipoprotein lipase activity expressed as mean $\pm$ s.e. mean percentage change over baseline (LPL %) at 10 min following placebo, heparin or ORG 10172 injection

Treatment	Dose	Number of observations	LPL (%)
Placebo	—	10*	-2.4 ± 4.4
Heparin	5000 units	4	56.3 ± 12.3
ORG 10172	800 units	6	-3.8 ± 6.5
	1600 units	6	13.0 ± 5.7
	3200 units	18	11.6 ± 2.1
	6400 units	22	12.5 ± 5.3

*Additional placebo data are included from other studies performed for different purposes during this period.

activity presumably because maximal release of lipoprotein lipase had occurred at a dose of 1600 units ORG 10172.

Relationship of plasma anti- X_a response to dose and body size

As shown in Figure 2 there is a linear relationship between the plasma anti- X_a activity response at 10–15 min after an i.v. bolus injection of ORG 10172 and the administered dose. At any given dose level there is great variability and Figure 3 demonstrates the relationship at various dose levels of the plasma anti- X_a activity measured 10 min after a bolus i.v. injection related to body surface area. The wide scatter suggests that other factors must determine the relationship.

Pharmacokinetics of plasma anti- X_a data

All results at all doses were consistent with a biexponential time course of anti- X_a effect in plasma after intravenous bolus administration of ORG 10172 (Figure 4). Data characterising the

Figure 2 Relationship between maximum observed anti- X_a response in plasma and dose of ORG 10172 given by intravenous bolus injection (see Table 4 for number of subjects at each dose).

fitted model are summarised in Table 4. There was excellent inter-study agreement for parameters at different dose levels, but within and inter-individual variation in kinetic parameters was great over the whole dose range. A large part of this variance was constituted by the assay which has a coefficient of variation of 15–20% at very low plasma levels, and it is on these low levels that estimation of $t_{1/2}$ elimination depends. Neither $t_{1/2}$ of distribution nor elimination were dose-related. The elimination half-life in a total number of 63 healthy volunteers now on record (including the 42 presented in Table 4) follows a log-linear distribution with an arithmetic mean of 19.2 h (s.d. 8.0 h).

The detailed effect kinetics of ORG 10172 will be the subject of a separate paper.

Figure 3 Relationship between body surface area and peak anti-X_a response in plasma at 10 min after intravenous doses of 1600 u (●, *n* = 14*), 3200 u (△, *n* = 40) and 6400 u (X, *n* = 28) ORG 10172. Additional data (○) from other studies performed by ourselves have been added.

Figure 4 Representative profile of anti-X_a response (u ml⁻¹) vs time following bolus intravenous injection of 6400 u ORG 10172.

Adverse effects

None of the subjects noted any untoward symptoms during or following any of the studies. Secondary bleeding occasionally required arrest by pressure bandage or local adrenaline packs but was never profuse.

During routine laboratory monitoring only minor degrees of change were observed: all were clinically insignificant and were considered to represent random fluctuations.

Discussion

The results obtained show that ORG 10172, 6400 anti-X_a units (approximately 640 mg), produced a plasma anti-X_a activity following an i.v. bolus injection which was double that following i.v. bolus injection of 5000 units of standard heparin (Table 1). This was accomplished with no evidence of intolerance and complete safety from bleeding in normal young males. Unlike heparin, a relatively small, but dose-related increase in KCT occurs following ORG 10172 although this is of no practical value in monitoring its levels as an alternative to anti-thrombotic activity, anti-coagulant activity or plasma concentration. This effect on multiple-factor clotting tests such as KCT is a known feature of low molecular weight heparin fractions (Barrowcliffe *et al.*, 1979).

The most important safety test carried out was the modified bleeding time. As usually performed this is a platelet function test but with additional pressure producing deeper cuts and thus severing larger dermal blood vessels the test possibly measures the more complex interaction between platelets, coagulation factors and blood vessels walls. This type of test is probably a better model for what happens in routine surgery than the conventional bleeding time which shows poor correlation with intra/post-operative bleeding. As a consequence it also relates to the dose of anticoagulant as shown in the present study. It should be noted that the baseline values for

Table 4 Distribution and elimination half-lives of plasma anti-X_a activity following ORG 10172

Dose ORG 10172 (units)	Number of observations	t _{1/2} distribution (h)		t _{1/2} elimination (h)	
		Mean	s.e. mean	Mean	s.e. mean
800	3	1.8	0.67	23.3	4.1
1600	7	1.76	0.16	13.6	1.4
3200	16	2.81	0.22	19.1	3.7
6400	16	2.41	0.25	16.9	1.3
Overall	42	2.34	0.16	17.6	1.1

bleeding time obtained by this modified procedure are longer than by the conventional method. The bleeding time prolongations even with 6400 anti- X_a units of ORG 10172 were always less than those seen with 5000 units of standard heparin despite the higher plasma anti- X_a activity produced by the former. Our data suggest that treatment with i.v. doses of ORG 10172 less than 1600 units (i.e. producing plasma anti- X_a levels $< 0.5 \text{ u ml}^{-1}$) should not importantly increase the bleeding risk in patients. However, the significance of the secondary bleeding phenomenon noted in these studies will require further evaluation in patients requiring surgery and/or high doses of ORG 10172. This seems to be a dose-related effect and possibly reflects the sizes of vessels cut and the widened scope of the haemostatic processes required to seal them. Further studies concerning the physical properties of the clot formed after ORG 10172 are also in progress.

Unlike heparin, ORG 10172 has little apparent effect on platelet function. Mikhailidis *et al.* (1984) have demonstrated that whereas standard heparin markedly enhances platelet aggregation by ADP and inhibits the anti-aggregatory effect of epoprostenol, ORG 10172 has neither of these effects. Some effects were however found in the present study on the adhesion of platelets to glass beads and further investigation of these properties will be required. This technique yields very variable results and its conclusions must be treated with caution. Table 2 however suggests that paradoxically subjects showing secondary bleeding/oozing from their bleeding time incisions showed the smallest reduction in platelet adherence following ORG 10172. This may indicate that this phenomenon is related not so much to platelet reactivity as to the interaction between the coagulation system and the walls of relatively large dermal vessels.

ORG 10172 activated less lipoprotein lipase than heparin suggesting that it is less absorbed to the endothelium since it is believed that heparin binds to lipases at the endothelial cell surface to protect them against inactivation (Olivecrona *et al.*, 1977). This could be because heparin has a higher charge density than ORG 10172 and is thus able to bind to this site. The lack of activity found in our studies makes it unlikely that its anticoagulant activity is mediated by release of endogenous heparin since the lipoprotein lipase system is highly sensitive to low heparin concentrations (Negus *et al.*, 1971) and the amount of endogenous heparin required to produce the observed changes on X_a activity would lead to much more than the observed lipoprotein lipase activation.

Figure 2 shows the linear relationship of the plasma anti- X_a response to increasing dose although there is some variability in response which is only partly explained by differences in body weight or surface area (Figure 3). Even data from the same subject studied on up to six occasions showed large variation. This may in part reflect the different times of year (i.e. different seasons) on the level of physical fitness of the subjects (most of the subjects were athletic), mental stress (e.g. student examinations) and diet when these studies were conducted since all of these factors are known to influence the results of coagulation tests. Nevertheless, the observed responses were linear overall. The plasma anti- X_a responses to standard heparin showed similar variability, but the peak level occurred some 5–10 min following the i.v. bolus than that for ORG 10172. One interesting contrast is that nonlinear pharmacokinetic behaviour is now well established for heparin and an increasing half-life with increasing dose has been demonstrated in several species including man (Estes, 1980). Our data indicate that the ORG 10172 anti- X_a response in plasma is linearly dose-related and the kinetics of response to this material are apparently linear. This should greatly aid dose adjustment in clinical practice. Although strictly speaking our study has not determined the pharmacokinetics of ORG 10172, having investigated instead one response to its administration, we have repeatedly demonstrated that this response can be adequately described by a biexponential function with half-lives of distribution and elimination. The elimination half-life characterises the decline in the extent to which factor X_a in the coagulation cascade is inhibited by ORG 10172 and was found to be about 4–5 times longer than the mean of four subjects given 5000 i.u. heparin i.v. and approximately 10 times the half-life reported by Estes in 1980 for heparin in normal subjects. This long half-life of effect should increase the clinical utility of ORG 10172 allowing once daily injection in the prophylaxis of venous thrombosis. It is however important to exercise caution in extrapolation of the kinetic data since our subjects were normal healthy young males and the elimination of heparin is known to be slower in females (Cipolle *et al.*, 1981) and in patients with pulmonary embolism (Hirsh *et al.*, 1976). In addition, circadian and ultradian changes have been demonstrated in the effects of heparin (Decousus *et al.*, 1985).

It is assumed that low molecular weight heparin, while maintaining anti-thrombotic potency because of its high anti- X_a effect will have the advantage of a reduced effect on overall clotting. This suggests that such fractions may have an

advantageous risk benefit ratio over conventional heparin which is true for animal models (Meuleman *et al.*, 1982). This advantage applies particularly to prophylaxis of thrombosis and may be less important when thrombin generation has already occurred as in progressive thrombosis. It should be noted however that the high anti-X_a activity of low molecular weight heparins does not necessarily predict antithrombotic activity (Ockelford *et al.*, 1982) and other aspects of the pharmacology of such fractions are presumably important. Our human Phase I studies after intravenous administration have confirmed some of the properties of ORG 10172 found in animals. Two uncontrolled clinical studies utili-

sing i.v. dose regimens (Henny *et al.*, 1983; ten Cate *et al.*, 1984) have shown that it does not exacerbate haemorrhage in patients. Further controlled clinical studies, particularly using the subcutaneous route of administration, will be required to demonstrate that it is possible to dissociate the anti-thrombotic and bleeding-inducing properties of heparin and to delineate the place of ORG 10172 in practice. While the i.v. route was necessary in these studies, to relate the different responses to ORG 10172 injection to the plasma anti-X_a response, clinical experience and healthy volunteers studies suggest that it could be even safer when administered by the subcutaneous route.

References

- Barrowcliffe, T. W., Johnson, E. A., Eggleton, C. A., Kenshall-Cook, G. & Thomas, D. P. (1979). Anticoagulant activities of high and low molecular weight heparin fractions. *Br. J. Haematol.*, **41**, 573-583.
- Carter, C. J., Kelton, J. G., Hirsh, J. & Gent, M. (1981). Relationship between the antithrombotic and anticoagulant effects of low molecular weight heparin. *Thromb. Res.*, **21**, 169-174.
- Cipolle, R. J., Seifert, R. D., Neilan, B. A., Zaske, D. E. & Haus, E. (1981). Heparin kinetics: variables related to disposition and storage. *Clin. Pharmac. Ther.*, **29**, 387-393.
- Decousus, H. A., Croze, M., Levi, F. A., Jaubert, J. G., Perpoint, B. M., De Bonadona, J. F., Reinberg, A. & Queneau, P. M. (1985). Circadian changes in anticoagulant effect of heparin infused at a constant rate. *Br. med. J.*, **290**, 341-344.
- Documenta Geigy (1971). Eds Dien, K. & Lentner, C., p. 537. Macclesfield: Geigy Pharmaceuticals.
- Estes, J. W. (1980). Clinical pharmacokinetics of heparin. *Clin. Pharmacokin.*, **5**, 205-230.
- Hall, R. & Malia, R. G. (1984). *Medical Laboratory Haematology*, pp. 564-566, edition 1. London: Butterworths.
- Hellem, A. J. (1970). Platelet adhesiveness in von Willebrand's disease. A study with a new modification of the glass bead filter method. *Scand. J. Haematol.*, **7**, 374-382.
- Henny, C. P., ten Cate, H., ten Cate, J. W., Surachno, S., van Bronswijk, H., Wilmink, J. M. & Ockelford, P. A. (1983). Use of a new heparinoid as anticoagulant during acute haemodialysis of patients with bleeding complications. *Lancet*, **i**, 890-893.
- Hirsh, J., Van Aken, W. G., Gallus, A. G., Dollery, C. T., Cade, J. F. & Jung, W. L. (1976). Heparin kinetics in venous thrombosis and pulmonary embolism. *Circulation*, **53**, 691-695.
- Holmer, E., Lindahl, U. & Backstrom, G. (1980). Anticoagulant activities and effects on platelets of a heparin fragment with high affinity for antithrombin. *Thromb. Res.*, **18**, 861-869.
- Höök, M., Thunberg, L., Reisenfeld, J., Backstrom, G., Pettersson, I. & Lindahl, U. (1981). Structure of the antithrombin-binding sequence in heparin. In *Chemistry and biology of heparin*, eds. Lundblad, R. L., Brown, W. V., Mann, K. G. & Roberts, H. R. pp. 271-279. New York: Elsevier/North Holland.
- Jaques, L. B. (1979). Heparins-anionic polyelectrolyte drugs. *Pharmac. Rev.*, **31**, 99-106.
- Mant, M. J., O'Brien, B. D., Thong, K. L., Hammond, G. W., Birtwhistle, R. V. & Grace, M. G. (1977). Haemorrhagic complications of heparin therapy. *Lancet*, **i**, 1133-1134.
- Meuleman, D. G., Hobbelen, P. M. J., van Dedem, G. & Moelker, H. C. T. (1982). A novel antithrombotic heparinoid (ORG 10172) devoid of bleeding inducing capacity. A survey of its pharmacological properties in experimental animal models. *Thromb. Res.*, **27**, 353-363.
- Mielke, C. H., Kaneshiro, M. M. & Maher, I. A. (1969). The standardised normal Ivy bleeding time and its prolongation by aspirin. *Blood*, **34**, 204-210.
- Mikhailidis, D. P., Barradas, M. A., Mikhailidis, A. M., Magnani, H. & Dandona, P. (1984). Comparison of the effect of a conventional heparin and a low molecular weight heparinoid on platelet function. *Br. J. clin. Pharmacol.*, **17**, 43-48.
- Negus, D., Pinto, D. J. & Slack, W. W. (1971). Effect of small doses of heparin on platelet adhesiveness and lipoprotein lipase activity before and after surgery. *Lancet*, **i**, 1201-1205.
- Norman, C. S. & Provan, J. L. (1977). Control and complications of intermittent heparin therapy. *Surg. Gynaec. Obst.*, **145**, 338-342.
- Ockelford, P. A., Carter, C. J., Mitchell, L. & Hirsh, J. (1982). Discordance between the anti-X_a activity and antithrombotic activity in an ultra-low molecular weight heparin fraction. *Thromb. Res.*, **28**, 401-409.
- Ofusu, F. A., Modi, G., Cersleus, A. L., Hirsch, J. & Blajchman, M. A. (1982). Heparin with low affinity to antithrombin III inhibits the activation of prothrombin in normal plasma. *Thromb. Res.*, **28**, 487-497.
- Olivecrona, T., Bengtsson, G., Marklund, S., Lindahl, U.

- U. & Höök, M. (1977). Heparin-lipoprotein lipase interactions. *Fed. Proc.*, **36**, 60–65.
- Salzman, E. W., Deykin, D., Shapiro, R. M. & Rosenberg, R. (1975). Management of heparin therapy: controlled prospective trial. *New Engl. J. Med.*, **292**, 1046–1050.
- ten Cate, H., Henny, C. P., Buller, H. R., ten Cate, J. W. & Magnani, H. N. (1984). Use of a heparinoid in patients with haemorrhagic stroke and thromboembolic disease. *Ann. Neurol.*, **15**, 268–270.
- Teien, A. N. & Lie, M. (1977). Evaluation of an amidolytic heparin assay method: increased sensitivity by adding purified antithrombin III. *Thromb. Res.*, **21**, 169–173.
- Tietze, N. W. & Fiereck, E. A. (1966). A specific method for serum lipase. *Clin. Chim. Acta*, **13**, 352–358.
- Wessler, S. & Gitel, S. N. (1979). Heparin: new concepts relevant to clinical use. *Blood*, **53**, 525–544.
- Wilson, J. E., Bynum, L. G. & Parkey, R. W. (1981). Heparin therapy in venous thromboembolism. *Am. J. Med.*, **70**, 808–816.
- Young, V. P., Giles, A. R., Pater, J. & Corbett, W. E. N. (1980). Sex differences in bleeding time and blood loss in normal subjects following aspirin ingestion. *Thromb. Res.*, **20**, 705–710.

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