Int. J. Exp. Path. (1992) 73, 147–155

Heparin enhances angiogenesis by a systemic mode of action*

Klas Norrby and Jan Sörbo
Department of Pathology, Göteborg University, Sahlgren Hospital, Göteborg, Sweden

Received for publication 8 May 1991
Accepted for publication 29 October 1991

Summary. A systemically-administered standard sodium heparin, but not an oligosaccharide fraction derived from the heparin, significantly potentiated angiogenesis induced by saline in normal rats, as assessed by the quantitative mesenteric window angiogenesis assay. This is the first unambiguous evidence that any single specific mast-cell product can potentiate angiogenesis in normally vascularized mammalian tissue. Whether systemic treatment with a heparin-like substance may be useful for stimulating neoangiogenic formation of collaterals in situations of relative microvascular insufficiency, such as coronary collaterals in patients suffering from ischaemic heart disease, is briefly discussed.

Keywords: angiogenesis, heparin, oligosaccharide, quantification, rat, mesentery

The mast cell is the sole site of heparin (Enerbäck & Norrby 1989; Rodén 1989). As yet, no physiological role for heparin has been clearly identified. The biological effects attributed to heparin-related polysaccharides generally involve the binding of other macromolecules, mostly proteins (Lindahl 1989); the injection of heparin also releases a number of proteins into the blood. Heparin binds avidly to endothelial cells, but the binding sites are not specific receptors for heparin (Barzu *et al.* 1986, 1987), and to a number of growth factors, probably by a different mechanism (Kurokawa *et al.* 1989). Fibroblast growth factor, FGF, is a potent heparin-binding angiogenic factor. When extracellular in form, the FGF is often bound to basement membranes and the matrix (Vlodavksy *et al.* 1987; Folkman *et al.* 1988; Vigny *et al.* 1988). Heparin can protect extracellular FGF from denaturation, degradation and enzyme digestion (Gospodarowicz & Cheng 1986; Rosengart *et al.* 1988; Barzu *et al.* 1989). Heparin-derived oligosaccharides interact with endothelial cells and FGF in ways similar to heparin and this interaction is independent of anticoagulant activity (Kurokawa *et al.* 1989; Sudhalter *et al.* 1989). Moreover, heparin and heparin-like molecules can rapidly release biologically-active FGF bound to extracellular matrices *in vitro* (Bashkin *et al.* 1989); how the release is effected is not fully understood.

The activity of heparin with regard to angiogenesis is thought to be bimodal; oligosaccharides and non-anticoagulant fractions of heparin appear to share this bimodal activity of heparin to a large extent (Folkman

Correspondence: Professor Klas Norrby, Department of Pathology, Sahlgren Hospital, S-413 45 Göteborg, Sweden.

*Part of this study was reported as an abstract at the International Symposium on Angiogenesis, St Gallen, Switzerland, 13–15 March 1991.

& Ingber 1989). In combination with limited quantities of different angiogenic factors, heparin potentiates the angiogenic response in the chorioallantoic membrane and the cornea assays; heparin alone appears unable to produce an angiogenic response. The way in which heparin potentiates angiogenesis in these assays is not known. When, on the other hand, heparin or a saccharide is present together with an angiostatic steroid, the anti-angiogenic effect can be augmented (Crum *et al.* 1985; Folkman *et al.* 1989).

The aim of the present investigation was to study quantitatively the effect of systemically-administered heparin and an oligosaccharide fragment thereof on angiogenesis induced in the normally vascularized mesenteric window of adult healthy rats. The i.p. injection of saline, which appears not to induce mast-cell heparin secretion in the test tissue (Norrby *et al.* 1989), was used as an angiogenesis effector (Norrby *et al.* 1990a).

Material and methods

Animals

Male Sprague-Dawley rats (Alab AB, Sollentuna, Sweden) weighing 210–240 g at the start of the experiments were used. They were acclimatized and kept under standard conditions (Norrby *et al.* 1986) and were fed standard pellets and water *ad libitum*. They were frequently weighed throughout the experiments. Two animals shared each cage.

Induction of angiogenesis

Marked angiogenesis in the mesenteric windows follows a treatment protocol of two daily i.p. injections for 4.5 days of the highly selective mast-cell secretagogue Compound 48/80 in rats (Norrby *et al.* 1989), whereas a moderate angiogenesis follows a similar protocol of saline (0.9% NaCl) treatment (Norrby *et al.* 1990a). The saline used should contain a very small quantity of endotoxins, if any. The mechanism of saline-mediated angiogenesis still needs elucidation.

Compound 48/80 (Sigma), 'the archetypal histamine liberator' (Douglas 1985), which was dissolved in saline, is a condensation product of *p*-methoxy-*N*-phenylethylamine and formaldehyde. The animals were killed on days 14, 16 and 21 after the start of the i.p. treatment, i.e. in the midst of the expanding phase of the angiogenic response (Norrby *et al.* 1986).

Subcutaneous treatment with a standard heparin and a heparin-derived oligosaccharide fraction

Standard heparin. The molecular weight of the sodium heparin, derived from porcine intestinal mucosa, was approximately 15.0 kDa and its biological activity was 186 IU per mg. The heparin was dissolved in saline and administered in a volume of 0.1 ml per injection. The highest dose used, 0.50 mg/kg s.c., is of the same order as the dose used clinically in post-operative thromboprophylaxis (5000 IU = 0.40 mg/kg b.w.). At this dose, heparin affects the coagulation of plasma only marginally.

To test whether the mast-cell secretion in the test tissue following an i.p. injection of Compound 48/80 was affected by heparin, the heparin, at 0.5 and 5 mg/ml, was injected s.c. 1 hour before the 48/80. When the effect on angiogenesis was tested, heparin at 5 mg/ml was injected s.c. at 12-hourly intervals, starting on the first day of the i.p. treatment with 48/80 or saline and continuing until one day before the experiment was terminated.

Heparin-derived oligosaccharide

Two different fractions derived from the standard sodium heparin by enzymatic depolymerization followed by gel filtration and ethanol precipitation were used. In the experiments which were performed to elucidate whether the heparin fragment affected mast-cell secretion and mast-cell-mediated

angiogenesis in the test tissue, a mixture consisting mainly of octa, deca, and duodecasaccharides was used. Its mean molecular weight was approximately 3.3 kDa and its biological activity was 51 anti factor Xa IU per mg. In the subsequent experiment, which was performed to analyse the effect of the saccharide on saline-mediated angiogenesis, we used a closely related preparation that was mainly made up of a decasaccharide. This fraction had a mean molecular weight of about 2.4 kDa and a biological activity of 27 antifactor Xa IU per mg. The saccharides were dissolved in saline and administered in a volume of 0.1 ml per injection.

When testing whether the oligosaccharide affected mast-cell secretion in the mesenteric windows after an i.p. injection of 48/80, the saccharide at 0.5 and 5 mg/ml was injected s.c. 1 hour before the 48/80 was given. When the effect on angiogenesis mediated by 48/80 or saline was tested, the oligosaccharide at 5 mg/ml was injected s.c. at 12-hourly intervals, starting on the first day of i.p. treatment and continuing until one day before the experiment was terminated.

Quantification of the angiogenic response

The virtually two-dimensional vasculature in the extremely thin mesenteric window was quantified using two technically independent techniques; four windows per animal were analysed. There was no significant difference in the mean size of the windows examined in the three treatment groups. In animals receiving i.p. injection of Compound 48/80, the *number of vessel profiles per unit length* of the tissue was counted microscopically at ×400 in methacrylate-embedded sections cut perpendicular to the membranous surface (Norrby *et al.* 1986, 1989, 1990b). In saline-treated animals, the *vascularized area* (in %) in spreads of intact, unsectioned mesenteric windows was measured morphometrically (Norrby *et al.* 1990a); terminally i.v. infused ink–gelatin was used to visualize the vasculature. This variable was used since saline-mediated angiogenesis has been demonstrated in terms of vascularized area but not in terms of number of vessel profiles per unit length, as discussed elsewhere (Norrby *et al.* 1990a). *Photographic recording of spreads of whole windows* (Fig. 2) was done using a Wild Photomacroscope 420.

Quantification of mast-cell secretion. The reduction in the histamine content of the mesenteric window, in terms of ng histamine/μg protein (Norrby & Rammer 1983), 2 hours following an injection of 48/80 (1 μg/g b.w., 2 ml/100 g b.w.) was used as an established marker of mast-cell secretion since the mast cell is the main, if not the only, source of histamine in the test tissue.

Statistics. The difference between treatment groups was assessed using Mann–Whitney *U* rank sum test for unpaired observations (two-tailed); $P \leqslant 0.05$ was considered significant.

Table 1. Histamine content of mesenteric windows 2 hours after an i.p. injection of the highly selective mast-cell secretagogue Compound 48/80 in animals that had received a s.c. injection (0.1 ml) of saline, heparin at 0.5 and 5 mg/ml, or the heparin-derived oligosaccharide at 0.5 and 5 mg/ml 1 hour before the i.p. injection. The 48/80 treatment releases about 50% of the mast-cell histamine in the test tissue (Norrby *et al.* 1989). No statistically significant difference was observed

Subcutaneous treatment	Histamine concentration (ng/μg) (mean ± s.e.m.)
Saline (8)	0.87 ± 0.22
Heparin 0.5 mg (8)	0.93 ± 0.21
Heparin 5.0 mg (5)	0.79 ± 0.20
Oligosaccharide 0.5 mg (7)	0.94 ± 0.22
Oligosaccharide 5.0 mg (7)	0.97 ± 0.14

Figures in parentheses indicate the number of animals.

Results

Effect of subcutaneous heparin and oligosaccharide treatment on body weight

No haemorrhage or other side-effects were seen to result from the treatment with the heparin and the oligosaccharide, and the treatment had no effect on body weight compared with the controls receiving s.c. injections of saline. In fact, the difference in mean weight between the different treatment groups never exceeded 1% at any observation time (data not shown). Since the animals, although adult, normally gain in weight during the 2–3-week span of the angiogenesis experiments, and since any reduction in the rate of body weight increase appears to be a sensitive measure of adverse or toxic effects (Jakobsson *et al.* 1990), the present finding suggests that the saccharide treatment was atoxic.

Table 2. Number of vessel profiles per unit length of mesenteric window in animals treated with i.p. injections of Compound 48/80 for 4.5 days and s.c. injections of heparin, oligosaccharide or saline (treated controls) at 12-hourly intervals for 13 and 20 days; the animals were sacrificed on days 14 and 21 after the start of the i.p. treatment. Untreated controls were also included. Each group comprised 32 mesenteric window specimens. No statistically significant difference was observed between the animals which were given heparin, oligosaccharide or saline. The 48/80-treatment markedly increased the vasculature compared to untreated animals ($P \leqslant 0.025$ at both day 14 and day 21)

Treatment and day of sacrifice	Number of vessels/mm (mean ± s.e.m.)
Day 14	
48/80 + saline	0.527 ± 0.203
48/80 + heparin	0.585 ± 0.180
48/80 + oligosaccharide	0.398 ± 0.163
Nil	0.140 ± 0.054
Day 21	
48/80 + saline	1.040 ± 0.293
48/80 + heparin	0.710 ± 0.349
48/80 + oligosaccharide	0.939 ± 0.309
Nil	0.157 ± 0.079

Effect of subcutaneously administered heparin and oligosaccharide on mast-cell secretion and mast-cell-mediated angiogenesis

Neither the heparin nor the oligosaccharide significantly affected the Compound 48/80-induced mast-cell secretion (Table 1) or the 48/80-induced mast-cell-mediated angiogenesis (Table 2) compared with saline-treated controls. As expected, 48/80 treatment induced a marked angiogenic response compared with the controls which received no i.p. treatment (Table 2).

Effect of subcutaneous heparin and oligosaccharide treatment on saline-mediated angiogenesis

When saline injected i.p. was used as the angiogenesis effector in the mesenteric win-

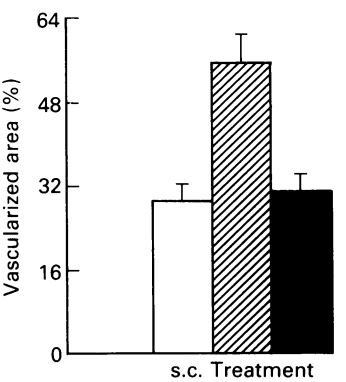

Fig. 1. Vascularized area as a percentage of mesenteric windows in animals treated i.p. with saline for 4.5 days and s.c. at 12-hourly intervals with saline, heparin or heparin-derived oligosaccharide for 15 days. The experiment was terminated on day 16 after the start of the i.p. and s.c. treatment. The □, saline and ▨, heparin groups each comprised 40 windows, whereas the ■, oligosaccharide group comprised 36 windows. The heparin treatment increased the angiogenic response compared with the saline treatment ($P \leqslant 0.001$) and the oligosaccharide treatment ($P \leqslant 0.001$). Mean ± s.e.m.

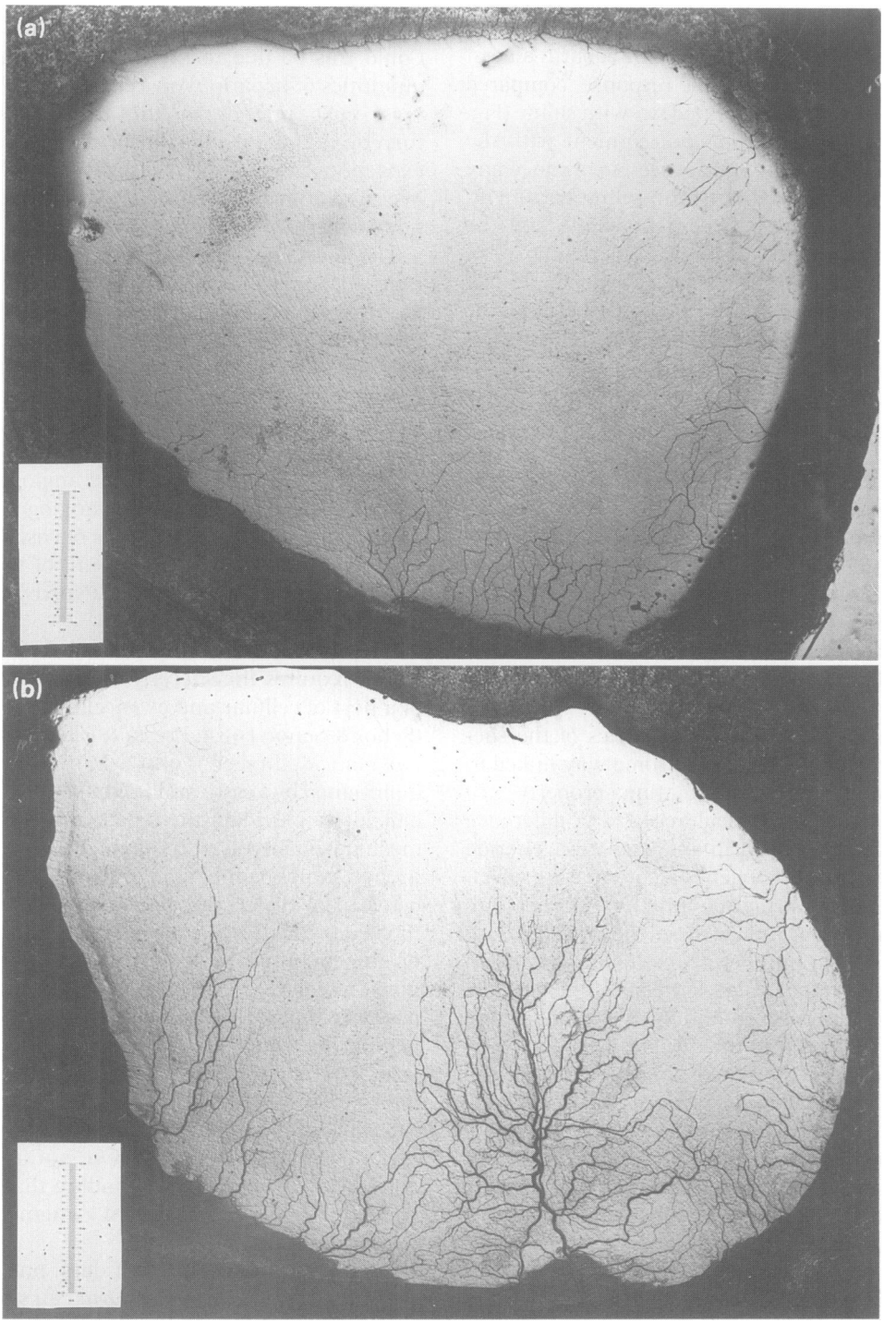

Fig. 2. Photomacrographs of spreads of intact, unsectioned mesenteric windows from animals treated i.p. with saline and receiving s.c. injection of a, saline and b, heparin. The microvasculature is visualized by intravasal infusion of ink-gelatin. In a, the window is 85.9 mm^2 and the vascularized area 28.9%; in b, the window measures 94.8 mm^2 in total area and the vascularized area is 57.2%. The vascularized area in both windows is close to the mean of the two populations of windows examined, as seen in Fig. 1. The bar inserted in the macrophotographs is 2 mm overall.

dows, the s.c. treatment with heparin almost doubled the angiogenic response, compared with the controls treated s.c. with saline (Fig. 1). In contrast, the s.c. treatment with the oligosaccharide was not seen to produce any effect, compared with the saline treatment. The difference between the saline and the heparin-treated animals (see photomacrographs in Fig. 2) was statistically highly significant, as was the difference between the oligosaccharide and heparin-treated animals.

Discussion

The finding that heparin augmented angiogenesis by a systemic mode of action in normally vascularized mammalian tissue is new. The fact that the heparin-derived oligosaccharide, which apparently had a degree of sulphation similar to that of the heparin, exerted no effect, suggests that features such as chain length, molecular weight, configuration, and chain flexibility, as well as the pharmacokinetic characteristics of the saccharide molecule, are in some way linked to its angiogenesis-stimulating property. To elucidate the critical molecular difference between the heparin and its oligosaccharide in terms of angiogenesis potentiating effect, molecular structure–function studies are required. As indicated in the introduction, there are a number of possibilities by which heparin might have exerted its angiogenesis-stimulating effect and further studies are needed to clarify the mechanism(s).

Heparin-stimulated angiogenesis is the first unambiguous evidence that any single specific mast-cell product can potentiate angiogenesis in vascularized mammalian tissue. This conforms with previous reports of mast-cell-mediated angiogenesis in rats and mice (Norrby *et al.* 1986, 1988, 1989), and that protamine, a specific antagonist to heparin's anticoagulative effect, atoxically suppresses mast-cell-mediated angiogenesis (Jakobsson *et al.* 1990). We observed, however, no effect of heparin or its oligosaccharide on mast cell mediated angiogenesis. Could this be due to the fact that sizeable quantities of heparin were released from the mast cells present in the target tissue, thereby making it difficult for either of the exogenous saccharides to exert any effect over and above that of the released endogenous heparin?

The fact that different techniques were used for quantitating the vasculature in the experiments performed on mast-cell-mediated and saline-induced angiogenesis does not seem to render the interpretation of the results difficult. The two parameters employed—no. of vessel profiles per unit length of tissue and vascularized area—run in parallel in mast-cell-mediated angiogenesis whereas saline-mediated angiogenesis has been demonstrated in terms of vascularized area but not in terms of no. of vessel profiles per unit length of tissue (Norrby *et al.* 1990a).

Angiogenesis is a complex tissue reaction which requires the successful orchestration of a host of cellular and extracellular events (Schor & Schor 1983; Presta & Rifkin 1988; Folkman & Ingber 1989). Moreover, as maintained by Presta and Rifkin (1988), it is difficult to elucidate the detailed molecular mechanisms involved in angiogenesis since in-vivo assays cannot apparently distinguish between a 'direct' angiogenic factor, i.e. a molecule which is supposed to act primarily on the endothelial cells, an 'indirect' angiogenic factor, i.e. a molecule which is supposed to induce the release of a primary angiogenic factor from other cells, and a primary change induced in the extracellular matrix. The fact that the molecular mechanisms of saline-induced angiogenesis still await elucidation does therefore not invalidate the significance of the finding that the systemic heparin treatment augmented angiogenesis.

On the basis of the present data, one can speculate about whether heparin can stimulate collateral blood vessel formation in conditions of insufficient microvasculature such as may be the case in the ischaemic myocardium and in some types of impaired

wound healing. It is, indeed, known that wounds and ischaemic myocardium release angiogenic factors (Kumar *et al.* 1983; Galloway *et al.* 1984). It is, furthermore, interesting to note that s.c. injections of heparin in dogs increase the myocardial blood circulation following experimentally induced myocardial ischaemia (Fujita *et al.* 1987), suggesting that the heparin treatment accelerated the development of coronary collaterals. No clear distinction between the vascular expansion of small pre-existing vessels, which commonly occurs in the formation of collateral blood vessels, and de-novo angiogenesis was made, however. Indirect evidence that heparin can accelerate exercise-induced coronary collateral development in patients with coronary heart disease has also been presented (Fujita *et al.* 1988).

In this context, it is worth noting that heparin as well as anticoagulant and non-anticoagulant heparin fragments markedly inhibit arterial smooth muscle cell and myointimal proliferation (Clowes & Karnowsky 1977; Guyton *et al.* 1980; Hoover *et al.* 1980; Castellot *et al.* 1981, 1984; Dryjski *et al.* 1988). Although the molecular basis of this inhibitory effect is not yet clear, it has been suggested that heparin may be a possible therapeutic anti-atherogenic agent in states of accelerated atherosclerosis, especially after procedures accompanying endothelial injury such as angioplasty and vascular surgery (Edelman *et al.* 1990). In passing, it is worth remembering that heparin is also an established prophylactic and therapeutic agent in the case of venous thromboembolism (Kakkar & Hedges 1989; Ware & Salzman 1989).

In conclusion, we hypothesize that systemically administered heparin, which significantly potentiated angiogenesis in normally vascularized rat tissue in the present study, can favourably affect patients with coronary heart disease by stimulating the neoangiogenic development of microvascular collaterals and providing protection from the acceleration of atherosclerosis. Further studies are required to elucidate this area and to demonstrate whether a heparin-like non-anticoagulant substance, that combines a high angiogenesis-stimulating effect with few complications in the clinical setting, can be found.

Acknowledgements

This study was supported by the Swedish Medical Research Council (project no. 5942). It was decided that the saccharide treatment and subsequent analyses should be performed blindly. The solutions of saline, heparin and oligosaccharides were provided in coded vials by Dr Per Ostergaard, Novo Nordisk A/S, Gentofte, Denmark, and the code was opened by Dr Ostergaard after the analyses were completed. We are, hence, much indebted to Dr Ostergaard. We also thank Ms Gunvor Jefferth and Ms Ann Nehlmark for their excellent technical assistance.

References

BARZU T., LORMEAU J.-C., PETITOU M., MICHELSON S. & CHOAY J. (1989) Heparin-derived oligosaccharides: affinity for acidic fibroblast growth factor and effect on its growth-promoting activity for human endothelial cells. *J. Cell. Physiol.* **140**, 538–548.

BARZU T., VAN RIJN J.L.M.L., PETITOU M., MOLHO P., TOBELEM G. & CAEN J.P. (1986) Endothelial binding sites for heparin. Specificity and role in heparin neutralization. *Biochem. J.* **238**, 847–854.

BARZU T., VAN RIJN J.L.M.L., PETITOU M., TOBELEM G. & CAEN J.P. (1987) Heparin degradation in the endothelial cells. *Thrombosis Res.* **47**, 601–609.

BASHKIN P., DOCTROW S., KLAGSBRUN M., SVAHN C.M., FOLKMAN J. & VLODAVSKY I. (1989) Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparanase and heparin-like molecules. *Biochemistry* **28**, 1737–1743.

CASTELLOT J.J., ADDONIZIO M.J., ROSENBERG R.D. & KARNOVSKY M.J. (1981) Cultured endothelial cells produce a heparin-like inhibitor of smooth muscle cell growth. *J. Cell Biol.* **90**, 372–379.

CASTELLOT J.J., BEELER D.L., ROSENBERG R.D. & KARNOVSKY M.J. (1984) Structural determinants of the capacity of heparin to inhibit the

proliferation of vascular smooth muscle cells. *J. Cell. Physiol.* **120**, 315–320.

CLOWES A.W. & KARNOWSKY M.J. (1977) Suppression by heparin of smooth muscle cell proliferation in injured arteries. Nature **256**, 625–626.

CRUM R., SZABO S. & FOLKMAN J. (1985) A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. *Science* **230**, 1375–1378.

DOUGLAS W.W. (1985) Histamine and 5-hydroxytryptamine (serotonin) and their antagonists. In *The Pharmacological Basis of Therapeutics*. Eds A.G. Gilman, L.S. Goodman, T.W. Rall & F. Murad. New York: Macmillan. p. 613.

DRYJSKI M., MIKAT E. & BJORNSSON T.D. (1988) Inhibition of intimal hyperplasia after arterial injury by heparins and heparinoid. *J. Vasc. Surg.* **8**, 623–633.

EDELMAN E.R., ADAMS D.H. & KARNOVSKY M.J. (1990) Effect of controlled adventitial heparin delivery on smooth muscle cell proliferation following endothelial injury. *Proc. Natl Acad. Sci. USA* **87**, 3773–3777.

ENERBÄCK L. & NORRBY K. (1989) The mast cells. In *Cell Kinetics of the Inflammatory Reaction*. Ed. O.H. Iversen. Current Topics in Pathology. Volume 79. London: Springer Verlag. pp. 169–203.

FOLKMAN J. & INGBER D.E. (1989) Angiogenesis: regulatory role of heparin and related molecules. In *Heparin. Chemical and Biological Properties. Clinical Application*. Eds D.A. Lane & U. Lindahl. London: Edward Arnold. pp. 317–333.

FOLKMAN J., KLAGSBRUN M., SASSE J., WADZINSKI M., INGBER D. & VLODAVSKY I. (1988) A heparin-binding angiogenic protein—basic fibroblast growth factor—is stored within basement membrane. *Am. J. Pathol.* **130**, 393–400.

FOLKMAN J., WEISZ P.B., JOULLIÉ M., LI W.W. & EWING W.R. (1989) Control of angiogenesis with synthetic heparin substitutes. *Science* **243**, 1490–1493.

FUJITA M., MIKUNIYA A., TAKAHASHI M., GADDIS R., HARTLEY J., MCKNOWN D. & FRANKLIN D. (1987) Acceleration of coronary collateral development by heparin in conscious dogs. *Jap. Circul. J.* **51**, 395–402.

FUJITA M., SASAYAMA S., ASANOI H., NAKAJIMA H., SAKAI O. & OHNO A. (1988) Improvement of treadmill capacity and collateral circulation as a result of exercise with heparin pretreatment in patients with effort angina. *Circulation* **77**, 1022–1029.

GALLOWAY A.C., PELLETIER R. & D'AMORE P.A. (1988) Ischemic hearts stimulate endothelial cell growth. *Surgery* **96**, 435–438.

GOSPODAROWICZ D. & CHENG J. (1986) Heparin protects basic and acidic FGF from inactivation. *J. Cell. Physiol.* **128**, 475–484.

GUYTON J.R., ROSENBERG R.D., CLOWES A.W. & KARNOVSKY M.J. (1980) Inhibition of rat arterial smooth muscle cell proliferation by heparin. I In vivo studies with anticoagulant and non-anticoagulant heparin. *Circ. Res.* **46**, 625–634.

HOOVER R.L., ROSENBERG R.D., HAERING W. & KARNOVSKY M.J. (1980) Inhibition of rat arterial smooth muscle cell proliferation by heparin. II In vitro studies. *Circ. Res.* **47**, 578–583.

JAKOBSSON A., SÖRBO J. & NORRBY K. (1990) Protamine and mast-cell-mediated angiogenesis in the rat. *Int. J. Exp. Path.* **71**, 209–217.

KAKKAR V.V. & HEDGES A.R. (1989) Use of heparin as a prophylactic agent against venous thromboembolism. In *Heparin. Chemical and Biological Properties. Clinical Application*. Eds D.A. Lane & U. Lindahl. London: Edward Arnold. pp. 455–473.

KUMAR S., WEST D., SHAHABUDDIN S., ARNOLD F., HABOUBIN N., REID H. & CARR T. (1983) Angiogenesis factor from human myocardial infarcts. *Lancet ii*, 364–368.

KUROKAWA M., DOCTROW S.R. & KLAGSBRUN M. (1989) Neutralizing antibodies inhibit the binding of basic fibroblast growth factor to its receptor but not to heparin. *J. Biol. Chem.* **264**, 7686–7691.

LINDAHL U. (1989) Biosynthesis of heparin and related polysaccharides. In *Heparin. Chemical and Biological Properties. Clinical Application*. Eds. D.A. Lane & U. Lindahl. London: Edvard Arnold. pp. 159–189.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1986) Mast-cell-mediated angiogenesis: a novel experimental model using the rat mesentery. *Virchow's Arch. B Cell Pathol.* **52**, 195–206.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1988) Age-dependent mast-cell-mediated angiogenesis. *APMIS* **96 (Suppl. 2)**, 251–261.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1989) Mast-cell secretion and angiogenesis, a quantitative study in rats and mice. *Virchow's Arch. B Cell Pathol.* **51**, 251–256.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1990a) Quantitative angiogenesis in spreads of intact rat mesenteric windows. *Microvasc. Res.* **39**, 341–348.

NORRBY K., JAKOBSSON A. & SÖRBO J. (1990b) On mast-cell-mediated angiogenesis in the rat mesenteric-window assay. *Agent Actions* **30**, 231–233.

NORRBY K. & RAMMER L. (1983) Normal mitotic reactivity of fibroblasts and mesothelial cells in thrombocytopenic rat. *Virchow's Arch. B Cell Pathol.* **42**, 185–192.

PRESTA M. & RIFKIN D.B. (1988) New aspects of blood vessel growth: tumor and tissue-derived angiogenesis factors. *Haemostasis* **18**, 6–17.

RODÉN L. (1989) Highlights in the history of heparin. In *Heparin. Chemical and Biological Properties. Clinical Application.* Eds. D.A. Lane & U. Lindahl. London: Edward Arnold. pp. 1–23.

ROSENGART T.K., JOHNSON W.V., FRIESEL R., CLARK R. & MACIAG T. (1988) Heparin protects heparin-binding growth factor-1 from proteolytic inactivation *in vitro*. *Biochem. Biophys. Res. Commun.* **152**, 432–440.

SCHOR A.M. & SCHOR S.L. (1983) Tumour angiogenesis. *J. Pathol.* **141**, 385–413.

SUDHALTER J., FOLKMAN J., SVAHN C.M. & BERGENDAL K. (1989) Importance of size, sulfation, and anticoagulant activity in the potentiation of acidic fibroblast growth factor by heparin. *J. Biol. Chem.* **264**, 6892–6897.

VIGNY M., OLLIER-HARTMANN M.P., LAVIGNE M., FAYEIN N., JEANNY J.C., LAURENT M. & COURTOIS Y. (1988) Specific binding of basic fibroblast growth factor to basement membrane-like structures and to purified heparan sulfate proteoglycan of the EHS tumor. *J. Cell. Physiol.* **137**, 321–328.

VLODAVSKY I., FOLKMAN J., SULLIVAN R., FRIDMAN R., ISHAI-MICHAELI R., SASSE J. & KLAGSBRUN M. (1987) Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix. *Proc. Natl Acad. Sci. USA* **84**, 2292–2296.

WARE J.A. & SALZMAN E.W. (1989) Use of heparin in treatment of thromboembolic disorders. In *Heparin. Chemical and Biological Properties. Clinical Application.* Eds D.A. Lane & U. Lindahl. London: Edward Arnold. pp. 475–494.