Heparin: An Old Drug with a New Paradigm

Current discoveries are establishing the nature, action, and biological significance of this valuable drug.

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Summary. Recent studies have shown that heparin is a biochemical representative of a distinct class of compounds known as linear anionic polyelectrolytes. Members of this class are mixtures of individual highly negatively charged chains that show a wide spectrum of specific reactions with biologically active proteins. Upon administration, heparin chains enter a cellular pool and effectively prevent thrombosis by increasing the electronegative potential of the vessel wall. Anticoagulant activity is an unusual feature of a few heparin chains and appears to play a minor role in many clinical uses and in physiological and pathological responses.

For more than 40 years, heparin has been used as an essential drug in the diagnosis and treatment of diseases of the heart and blood vessels. It is prepared commercially from beef lung and pork intestinal mucosa. More than 900 billion units (6 metric tons), representing nearly 10 million patients, were used in the United States in 1976, and from 1974 to 1976 consumption increased by 300 billion units per year.

Heparin for clinical use was developed by research groups in Toronto and Stockholm (*1*, *2*). Its immediate acceptance for such use in 1938 was based on assumptions derived from Howell's theory of blood coagulation which postulated that considerable heparin was normally present in blood bound to prothrombin. However, the Howell theory has long been outdated and the related concepts of the clinical action of heparin are untenable. A body of work, summarized in reviews and symposia proceedings (*2*-*28*), now provides the basis for a new and useful paradigm.

Chemical Nature of Heparin

Heparin, an acidic carbohydrate with a positive optical rotation, is capable of forming salts with metals and has uronic acid, glucosamine, and sulfate components (*2*, *9*). The quantity of sulfur present, the slowness of heparin's reaction with periodate and nitrous acid, its very low reducing power and failure to dialyze, as well as its mean molecular weight range (7,600 to 19,700), indicate that the substance is polydisperse, consisting of polymers of alternate sulfoglucosamine and hexuronic acid molecules joined by glycosidic linkages (*11*, *12*, *18*, *22*). The amounts of these constituents in commercial heparins vary considerably (*7*, *19*, *22*). Trace quantities of heparin produce a metachromatic color change (blue to red) in the dye toluidine blue (*2*, *10*). The reaction of heparin with dyes demonstrates the ability of this substance to form complexes with many electrolytes (*10*, *19*).

By using the enzymes of *Flavorbacterium heparinum*, more than 90 percent of a commercial heparin has been obtained as the hexasaccharide (shown in Fig. 1) containing 2,6-disulfoglucosamine, 2-sulfoiduronic acid, and glucuronic acid in the proportion of 3 : 2 : 1 (*13*). The problem of polydispersity has been resolved by distinguishing individual members of the mixture with a combination of different separation techniques. For example, after electrophoresis on acrylamide gel containing LKB ampholyte and staining with toluidine blue, beef lung heparin was found to have at least 21 components with the same charge but molecular weights from 3,000 to 37,500 (2 to 22 hexasaccharides); pork lung heparin contained, in addition, dimers of each of the 21 components. Further differentiation achieved by examining individual slices of the gel with removal of ampholyte, and measuring anticoagulant activity, revealed that many of the chains had little or no activity, while some were two to three times more active than the parent material. Active chains were in the 12,000 to 20,000 molecular weight range, but this was not a uniform characteristic.

Heparin acts as an anticoagulant by reacting with a plasma protein, antithrombin-III (AT-III). About one-third of a given commercial heparin combines with AT-III fixed on an ion exchange column (*20*, p.431) and only about one-third of this heparin converts AT-III to the active anticoagulant (*29*). Different commercial heparin preparations, as well as fractions prepared from them, show different relative activities in various blood coagulation tests. There are corresponding differences in the distribution of various chains as shown by electrophoresis with LKB ampholyte (*22*, pp. 288-290). Lindahl (*30*), from studies of the biosynthesis of heparin, finds that single sugars in the chain can vary; for example, both sulfamino or acetylamino glucosamine may occur, and iduronic acid and glucuronic acid may occur in both unsulfated or sulfated forms. Lindahl concludes that inhibitory activity with respect to the blood coagulation system is possibly dependent on the presence of such variant sugars and that the binding site for AT-III involves a dodecasaccharide sequence with a variant sugar.

By sulfation of various polysaccharides, compounds such as dextran sulfate, Heparinoid G31150, and many others have been prepared. These heparinoids have many properties similar to heparin, as do the isolated chondroitins (sulfate) and heparitins (sulfate) (*4*, *10*, *11*, *28*). These substances have provided a wealth of information on the effect of fine variations in chemical structure and the importance of degree of sulfation on biological activities.

Heparin Complexes

Listed in Table 1 are many substances with which heparin and heparinoids form complexes. The formation of such complexes is indicated by a change in one or more properties of the substance. The

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reaction shows a mass-law relationship, with the dissociation constant affected by pH and inorganic electrolyte. Many of the complexes are insoluble (physically resembling a plastic), and the critical electrolyte concentration at which a sharp change occurs in the ratio of precipitated to unprecipitated substance is of theoretical value (*19*). The formation of the complex changes the polymer cation; for example, with cetyl pyridinium chloride, there is a transfer of energy from heparin to the alkylamine. The metachromasia of dyes (appearance of a new absorption band) is due to fixation of the dye molecules in dimer form with a decrease in π electron delocalization. Protein complexes show a shift of the isoelectric point; enzymes and hormones show activation or inhibition; polypeptides, a change of conformational form. Thermodynamic studies have shown that hydrogen bonds, donated by the sulfate groups, stabilize the complex even with the protein molecule electronegatively charged as a whole (*31*).

Thus heparin and heparinoids form firm ionic associations with many fixed structures. If one compares heparinoids and heparin in many systems it is evident that variations in the carbohydrate skeleton produce significant variations in affinity in a complex. In a mixture of substances with which heparin can combine, for example, blood plasma, the distribution of the heparin among the various constituents will be determined by their respective relative concentrations and affinities and by the concentration of the various heparin chains.

Effect of Heparin on Enzymes

Commercial heparin has been reported to change the activity of 50 enzymes (*5, 10, 25, 28*). The following effects have been observed: activation of the proenzyme, formation of adducts, stabilization of the enzyme, destabilization of the enzyme, inhibition of the enzyme, activation of an inhibitor, and release of the enzyme in vivo to the circulation.

The release by heparin of several lipoprotein lipases from endothelium, adipose tissue, and liver has been studied extensively. Heparin releases diamine oxidase (histaminase) from the intestine and several enzymes from lysosomes. It activates brain tyrosine hydroxylase, the synthesizer of brain serotonin. Inhibition has been shown for a wide range of enzymes in intermediary metabolism, DNA transformations, and in the respiratory and digestive tracts. The inhibitory effect of commercial heparin on blood coagulation is due to certain of the chains being able not only to combine with the plasma protein (AT-III) but also to catalyze (*20*) its conversion to an active inhibitor of serine proteases, notably the enzymes of the blood coagulation sequence—thrombin, factors Xa, XII, XI, IX, VIII, and plasmin, streptokinase, and urokinase (*15*, p. 66).

Inhibition of other enzymes has not been examined from the standpoint of activation of a specific inhibitor or distinguishing active and inactive chains in heparin, although the divergencies in the observations reported suggest that one or both of these variables may be significant. Reactivity of various commercial heparins and heparinoids with enzymes depends on a structural matching of the sulfated polysaccharide and enzyme, and no general rule can be made regarding relative effectiveness. A further complication is the demonstration by Pulver [see (*28*)] that the anticoagulant activity of a heparinoid is due to its release of the small amount of protein-bound heparin present in plasma, which then reacts with AT-III.

Effects of Heparin in vivo

The ability of heparin and heparinoids to form complexes with proteins and enzymes and change the activities of these substances contributes to heparin having a wide range of effects in vivo (Table 2). Many of these effects are produced with quite small amounts of heparin or heparinoid, sometimes less than the amount required for an anticoagulant effect. The formation of complexes with body constituents increases the electronegativity of cells.

Significance of Heparin and Heparinoids Being Linear Anionic Polyelectrolytes

The properties just described for heparin and heparinoids demonstrate an unusual order of biochemical reactivity (*10, 12, 14, 18, 28*). The key and lock analogy has been used for many years to illustrate the rule of specificity of biochemical reactions. The corresponding analogy for heparin and heparinoids must be a bag of skeleton keys that can fit many locks. This suggests a corresponding difference in chemical structure. This can be explained on the basis of current developments in the chemistry of linear anionic polyelectrolytes (*32, 33*). These consist of chain molecules of various lengths characterized by the presence of acid radicals at intervals along the chain. In aqueous solution, the strong negative charges along the chain create repulsion between the chains, so that there cannot be coiling and association (cross-linking)

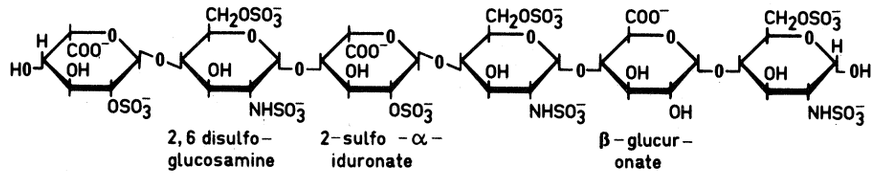

Fig. 1. Structure of heparin hexasaccharide. A chain of six sugars is shown; three molecules of 2,6 disulfoglucosamine alternate with the uronic acids, 2-sulfoiduronic and unsulfated glucuronic acid. The major constituents of heparin are represented by this hexasaccharide, 2 × [2,6-disulfoglucosamine-2-sulfoiduronic acid]-[2,6-disulfoglucosamine-glucuronic acid].

Table 1. Substances with which heparins and heparinoids form complexes (*10, 11, 12, 19, 27*).

Inorganic ions	*Alkylamines and alkaloids*	*Dyes*	*Biogenic amines*
Na^+	Cetylpyridinium chloride*	Methylene blue	Histamine
K^+	Cetavlon* (hexadecyl trimethyl ammonium bromide)	Toluidine blue*	5-Hydroxtryptamine
Ca^{2+}*	Brucine	Azure A*	Hypertensin
Ba^{2+}*	Choline	Thionin	
Cl^-		Neutral red	
SO_4^{3}*		Pyronin	
CH_3COO^-		Acriflavine*	
Plasma proteins		*Basic proteins*	*Drugs*
α-Acid glycoprotein	Fibrinogen*	Protamines*	Chloroquine
α-2-Globulin	Antithrombin-III*	Histones*	Chlorpromazine
Post-albumin†	Cryofibrinogen*	Globins*	Neomycin
γ-Globulins	Waldenstrom's macroglobulin*		Streptomycin
β-Lipoproteins*	Platelet factor 4*		

*Substances that have a high affinity for heparin. †A plasma protein fraction occurring after albumin upon electrophoresis.

of parts of the chain. Hence, in aqueous solution, the chains must be extended. On neutralization of the negative charges by combination with cations, particularly polycations, strongly bound complexes are formed and "catalysis" sometimes results (*34*).

These various properties are exhibited to a high degree by heparins and heparinoids. The flexibility of the carbohydrate chain allows the sulfated mucopolysaccharide to fit many relatively fixed chemical configurations which are electropositive (cationic dyes) or less electronegative (proteins). The presence in the heparin or heparinoid of a number of different chains (with relative concentrations in a non-normal distribution) increases the probability of fitting a particular polymer of opposite charge. With the anionic groups occurring along the chain at short intervals, there is a sharing of charges to produce a shell of electronegativity surrounding the carbohydrate core with loci of greater or less charge density. This results in a strong electrical interaction with surrounding small ions such as the "counter-ions," Na^+ and K^+, and the co-ions, Cl^-, CH_3COO^-, and SO_4^{2-}.

Three types of ion association can be distinguished—site binding, atmospheric trapping, and free association. Ions held in the first two types of association are nondialyzable. Thus, 12 percent of the sulfate of commercial heparin is inorganic but is not removed by dialysis. With increasing molecular size, linear polyelectrolytes show entanglement characterized by a rapid increase in viscosity with moderate increase in molecular size. This is observed with the macromolecular heparins. It is evident, then, that heparin and related compounds constitute a new class of biochemical compounds—biological linear anionic polyelectrolytes. Besides heparins and heparinoids, probably heparitins, hyaluronic acids, and carrageenins are members of this class.

Clinical Pharmacology

On the basis of the belief that heparin served as an anticoagulant preserving the blood in the circulation, heparin was administered for many years by intravenous injection and monitored by measurements of the decreased coagulability of frequently taken blood samples (*2, 3*). This procedure is now being replaced. Subcutaneous injections given every 8 hours in a quantity only sufficient to cause minimal changes in coagulation of blood samples (low-dose heparin) are effective in prevention of venous thrombosis (*17, 27*). The incorporation of ionizable heparin in the surface of plastic catheters and prostheses is as effective as or more effective than injecting the patient with heparin in preventing formation thereon of thrombi (*14, 28, 35*). The effects of different modes of administration of heparin on the resulting coagulability of the blood is shown in Fig. 2. When a large quantity of heparin is administered by way of the lung, a minimal change in blood coagulability persists for 10 to 15 days. A single intravenous injection renders the blood incoagulable for a short time. Continuous intravenous injection in suitable dosage can maintain a moderate decrease in blood coagulability for as long as the injection is continued. What is remarkable is that when doses for the different routes of administration are matched to produce the same moderate decrease in blood coagulability, approximately the same total amount of drug is required.

When heparin was administered by instillation into the lungs of rats, it was found that although 99 percent of the heparin could be recovered 1 minute after instillation, only 50 percent was present in the lung after 1½ hours; the other 50 percent could be recovered from the rest of the body. Only a moderate decrease in blood coagulability was observed. Hence, there was rapid transfer of the heparin to other parts of the body with only small concentrations being evident in blood (*23*). The heparin leaves the lung by way of the blood, lymph, and macrophages, and is taken up by the endothelium and cells of the reticuloendothelial system. This also occurs with subcutaneous administration. The differences in the coagulation response shown in Fig. 2 with different modes of administration correspond to differences in the proportion taken up by the different cellular compartments. Any heparin entering the blood is removed rapidly by the endothelium against a concentration gradient. Hence, the half-life of heparin in the circulation after a single intravenous injection (*15*, p. 45) is very short, 56 to 152 minutes depending on the dose. As shown in Fig. 3, chains that are inactive with antithrombin can be returned to the circulation as active chains. Hence, coagulation tests on blood samples do not measure solely the injected heparin.

Table 2. Some of the biological effects of commercial heparin and heparinoids (*10, 11, 13, 28*).

Effects on cells

Markedly increase negative electric potential of vascular wall
Bind red blood cell initiation factor
Decrease platelet count
Inhibit osteoblasts
Displace DNA from isolated nuclei
Produce lymphocytosis
Increase B-lymphoctye migration
Inhibit both T- and B-lymphocytes
Activate macrophages

Effects on hormones

Release: thyroxin
Suppress: somatotropin; aldosterone production
Activate: parathormone
Inhibit: adrenocorticotropic hormone and cortisone in Thorn test; pitressin antidiuresis; angiotensin production

Inhibition of sensitivity reactions

Complement system: inhibit binding of C1q, C1s, and C2; potentiate C1 inhibitor; inhibit generation of amplification convertase

Glomerulonephritis: rats with antiserum to rat kidney; rabbits with a nephrotoxic serum; rabbits with heterologous antiserum to kidney; rabbit with γ-globulin; Masugi nephritis

Anaphylaxis: in pigeons, guinea pigs, isolated Dale preparation; bronchospasm; anaphylatoxin; reversed anaphylactic shock

Antigen-antibody reactions: Arthus phenomenon; lupus erythematosis test; Coomb's test; Rh antibodies

Cells: lymphocyte-triggering; lymphocyte release of mononuclear cell exudation; activation of globulin PG_x

Protective actions against

Toxic bases: Congo red; compound 48/80; 5-hydroxtryptamine; mellitin
Drugs: curare; digitalis; ouabain; neomycin; polymyxin
Toxins: peritonitis; burns; Russell viper venom; tissue thromboplastin
Trauma (inhibition): pulmonary resuscitation; bends; tourniquet shock; hypoxia; hypotension
Stress (blockage): lymphopenia; spontaneous hemorrhage; general adaptation syndrome

Toxicity

Very few side reactions or allergic manifestations have been reported with the clinical use of heparin, although the drug has been administered intravenously to patients for 40 years in every possible variation of dosage pattern with other drugs (*8, 9, 28*). This is a unique record and must be due to the protective actions of heparin in blocking toxic agents and antigen-antibody responses (see Table 2). The rapid uptake of the drug by body cells also helps to diminish immediate side reactions. The main untoward effect of heparin is hemorrhage. Spontaneous hemorrhage results when more than one of the main physiological factors involved in hemostasis—blood coagulation, platelets, vessel wall, blood flow, and blood pressure (*9*)—are changed adversely, such as when a decrease in blood coagulability is brought about by an anticoagulant at the same time that a decrease occurs in vascular integrity because of stress (*7*). Although heparin blocks the effects of stress (Table 2), its ability to compete with myosin adenosine triphosphatase in the vessel wall (*36*) explains the hemorrhagic effect observed when the blood vessels of a heparinized individual are cut or punctured. Protamine is used as an antidote for heparin. An excess of protamine must be avoided as it is a histamine-liberator and thrombocytopenic agent.

Therapeutics

Heparin is used to prevent or limit clotting and thrombus formation in cardiovascular diagnostic and surgical procedures, as with in-dwelling venous catheters, cardiopulmonary catheters, surgery of the heart and vessels, metal and plastic prostheses (replacements), extracorporeal circulations, artificial organs, and transplants (*14, 28*). It is used for treatment of postoperative and postpartum thromboembolism (*2, 3, 14, 15, 17, 23*), in hypercoagulable states (*23*), and for disseminated intravascular coagulation (*17, 28*).

From the development of artificial surfaces for use in the circulation, much new information has been obtained on why clots and thrombi do not form in normal blood vessels (*35*). Porous prosthetic grafts in the circulation, and blood vessels with their inner lining removed, maintain a negative electrokinetic wall charge and are resistant to thrombosis as long as blood pressure and flow are maintained. This charge is due to the maintenance of an electrical double layer with a higher rate of flux of HCO_3^-, Ca^{2+} and K^+.

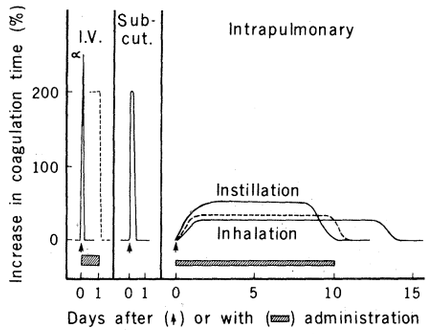

Fig. 2. Changes in coagulation of blood samples after administration of heparin. Results of single intravenous injection (I.V.), single subcutaneous injection (subcut.), and intrapulmonary administration (instillation, inhalation) indicated by solid lines. Results of continuous intravenous injection for the time shown by cross-hatching is indicated by the dotted lines. The symbol for infinity indicates the blood samples do not clot. [From Jaques and Mahadoo (*23*), courtesy of Stratton Intercontinental Medical Book Corporation]

Athrombogenesis of the normal vascular wall has been shown to be due to the maintenance of an electronegative charge on the surface by both metabolic and physicochemical processes (such as blood flow). The endothelium contains a multisulfated mucopolysaccharide biopolymer which is antithrombogenic, and it has a negative potential of 160 millivolts relative to the normal hydrogen electrode. In the venous system the valve cusps lack capillaries, and oxygen tension falls immediately in venous valve pockets when pulsatile flow stops, venous wall metabolism being 5 to 10 times the arterial rate; this explains why thrombosis occurs much more frequently in the venous system (*28*).

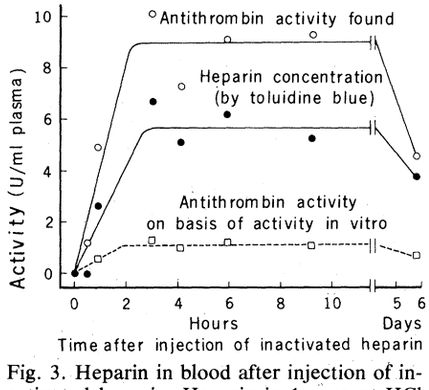

Fig. 3. Heparin in blood after injection of inactivated heparin. Heparin in 1 percent HCl was heated for 5 minutes at 100°C. Antithrombin activity was reduced to 20 percent of the original heparin. The inactivated heparin (100 mg) was injected subcutaneously into a rabbit at zero time. Blood samples were taken at the times shown and the heparin was extracted and tested. Symbols: •, total heparin present in plasma according to the toluidine blue test; □, antithrombin activity of acid-inactivated heparin equivalent to heparin assessed with toluidine blue; ○, actual antithrombin activity of the heparin present in plasma. Because the heparin in the blood showed more than ten times the antithrombin activity of the heparin injected, the latter must have been activated in vivo. [From Levy and Jaques (*40*), courtesy of Pergamon Press Ltd.]

Commercial heparin prevents both the formation of thrombi and intravascular coagulation. Although the injuries produced in vascular endothelium and intima by various techniques are demonstrably different according to their interface potentials, and when they are examined with the scanning electron microscope, heparin prevents and reverses the thrombotic changes (*35*). Injected heparin becomes bound to endothelium (Fig. 4B); direct measurement shows that the concentration in the endothelium is more than 100 times that in the plasma. This confers on the vessel wall a supranegative charge (or reverses a relatively positive charge because of metabolic defect). Additional actions of heparin at the endothelial surface reinforce its antithrombotic action (*28*). It increases adsorption of α_1-acid glycoprotein in adsorbed plasma layers on surface. It reduces rigidity or torque values of fibrinogen surface layers (clotting of fibrinogen without thrombin). It displaces lipoprotein lipase. Together, AT-III and heparin inhibit accumulation of platelets. Some chains activate AT-III to inhibit serine proteases; for example, thrombin, blood factors XII, XI, X, and IX, kallikrein, and plasmin.

Other clinical uses in myocardial infarction and inflammatory and allergic conditions (*6, 25, 28*) are based on the effect of heparin in enzyme release (lipoprotein lipases) and inhibition, and on its ability to block toxic agents (chemicals, viruses, histamine) and reduce inflammation (Table 2). These uses require higher doses than usual of the anionic polyelectrolyte, and for this a heparin of low anticoagulant activity in vitro would probably be best. This is because it is evident that the anticoagulant activity of heparin is a minor part of its clinical and biological actions and, in fact, is undesirable in commercial heparin because it increases the hemorrhagic tendency. The conversion of heparin chains to chains that combine with AT-III may represent a catabolic step, since desulfation of heparin combined with AT-III appears to be the main pathway for disposal of this powerful drug and autopharmacologic agent. As discussed above, anticoagu-

lant activity depends on the almost fortuitous presence of variant sugars in the heparin chains bound.

Autopharmacology of Heparin

Heparin is extracted commercially from tissues of cattle and swine. Material of higher anticoagulant activity has been prepared from the tissues of dogs and baleen whales, and of lower activity from sheep tissues (*19*). From tissues of these and other species are extracted sulfated polysaccharides that differ from commercial heparins in chain structure but resemble them in their properties as linear anionic polyelectrolytes (*18, 27*). These sulfated polysaccharides may or may not show anticoagulant activity. Recent studies have identified these various types of tissue heparin as heparins with molecular weights of 1.2 to 30 $\times$ 10^3 resembling commercial heparins, macromolecular heparins (10^5 to 10^6 daltons), heparitins A, B, and C, and multisulfated chondroitins. Because heparin was defined and measured in terms of the pharmacopeia definition by anticoagulant activity, many of these observations in the past have gone unpublished and probably much heparin in tissue has been missed. The presence of heparin in tissues can be established on the basis of the linear anionic polyelectrolyte properties of heparin and related compounds. A complete system for the identification and quantitation of heparin and related mucopolysaccharides has been developed (*19*), with microelectrophoresis in agarose being used as a quantitative procedure. The classes of linear anionic polyelectrolytes can be distinguished in this system by the action of specific enzymes and specific acid polyelectrolyte properties. This procedure, which can be applied to the analysis of small tissue samples of biological studies, makes possible investigations of the distribution of heparins in biopsy and autopsy material and thus the determination of the significance of these substances in human and animal tissues in health and disease.

The different linear anionic polyelectrolytes extracted from tissues for heparin as described above are characterized by a high degree of sulfation and are generally not covalently bound to protein. Glycosaminoglycans are covalently bound to proteins in proteoglycans (*37*). Both groups of compounds appear to be synthesized through sulfation with 3'-phosphoadenine-5'-phosphosulfate (PAPS) while they are attached to protein. The separation from protein of the more highly sulfated compounds converts them to linear anionic polyelectrolytes which then have the properties of drugs, that is agents which specifically change body reactions and responses. Macromolecular heparins and the dimer in commercial pig mucosal heparin are dissociated by ascorbic acid. Interconversion of chains apparently occurs in vivo (see Fig. 3), and enzymes have been described that depolymerize macromolecular heparins. This is probably important in controlling biological activities (*18*, p. 38). The patterns for the chains of linear anionic polyelectrolytes vary with the species (*27*). In man, low-molecular-weight heparins are relatively sparse and the linear anionic polyelectrolytes appear to be multisulfated chondroitins, heparitins, and macromolecular heparins.

Mast Cell Heparin

Mast cells contain large numbers of granules (Fig. 4c) that give distinctive metachromatic (purple) colors with toluidine blue (*2*). This is due to the presence of heparin, heparitins, and multisulfated chondroitins. Mast cells also contain histamine. The anionic polyelectrolytes constitute 25 percent, and histamine 9 percent, of the total organic content of the mast cell. The attachment of a strong base, histamine, and strong acids and heparin to a basic protein in insoluble granules that fill the cell makes the mast cell an effective ion exchanger (*16*) for inorganic ions and proteins (antigens and antibodies) in tissue fluid. When mast cell granules enter tissue fluid, the histamine and heparin are dissociated by the Na^+ ion and become prime movers in the local tissue response (*6*). Histamine increases permeability and lymph flow, for example, while heparin activates macrophages and forms complexes with toxic agents. Hence, the mast cell can be considered an emergency packet of drugs. Further, secretion of histamine by mast cells in contact with muscle and myoepithelial cells occurs in response to postganglionic neurons, hormones, and tissue-fluid osmotic pressure (*24, 28*). Basophilic leucocytes also contain metachromatic granules and histamine, but the linear anionic polyelectrolytes present are polysulfated chondroitins (*16, 27*). There are correspondingly considerable differences in polyelectrolyte composition between mast cells of different tissues and species, suggesting differences in responses and functions.

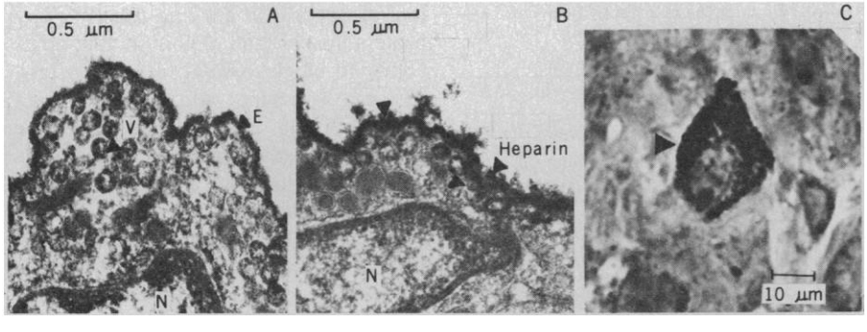

Fig. 4. Histological appearance of heparin and related sulfated mucopolysaccharides. (A) Electron photomicrograph of normal endothelial cells of rat aorta. The tissue was fixed in glutaraldehyde and osmium with ruthenium red, and stained with uranyl acetate and lead citrate (*E*, endothelium sheath showing the normal sulfated mucopolysaccharide; *N*, nucleus of endothelial cell), ×45,000. (B) The same cells 5 minutes after injection of heparin. Note the much greater amount of sulfated mucopolysaccharide in endothelium sheath indicated by arrows. (C) Typical mast cell (arrow) that is found throughout the pig intestine and the source of most commercial heparin. Note the appearance of a tightly packed package of granules as in an ion-exchange column. Tissue fixed with glutaraldehyde, mounted in epon, and stained with toluidine blue. The cell is cut to reveal the nucleus. [(A) and (B) are from Hiebert and Jaques (*41*), courtesy of Pergamon Press Ltd.]

Non-Mast Cell Heparin

Non-mast cell heparin has been demonstrated in dog liver, beef muscle, and rat gastrointestinal tract (*24, 27*). Like non-mast cell histamine, it appears to be manufactured on demand close to the reaction site. In the ovarian cycle, heparin may be responsible for shut-down of follicular steroid production at ovulation by inhibiting coupling between hormone receptor and adenylyl cyclase (*38*). In fat absorption, the release of heparin triggers the release of diamine oxidase which destroys the histamine previously released by the fat (*27*). As the histamine increases lymph flow and fat transport, heparin serves as "keeper of the gate" (*28*). Such a modulator role of non-mast cell histamine activity may be a general role of heparin in the body economy. In

addition, heparin in the lymph that is transported from the intestine after fat ingestion, on contacting endothelium, will displace lipoprotein lipase to activate the major metabolic pathway for chylomicrons.

Conclusion

On the basis of new knowledge of the nature and actions of heparin and related compounds, the following paradigm can be presented. Heparin and similar compounds are linear anionic polyelectrolytes. They consist of mixtures of polysaccharide chains relatively free of protein, with multiple acidic groups that give them a highly negative charge. As such, they form specific stable complexes and change the activity of biologically active proteins. They thus increase the negative charge of cell surfaces. On administration, heparins are rapidly taken up by endothelium, and also by cells of the reticuloendothelial system and by macrophages. The increase in negative charge of the vessel wall accounts for a large part of their clinical effectiveness in preventing thrombosis. The heparins attach to cells rather than circulating in plasma and can be transformed by cellular enzymes. The linear anionic polyelectrolytes occurring biologically are the lower-molecular-weight heparins (6,000 to 30,000 daltons), macromolecular heparins (up to 10^6 daltons), heparitins, and multisulfated chondroitins. These compounds make up a large proportion of mast cells and basophilic leucocytes. Like histamine that is present in mast cells, these compounds are also found in other locations where they are synthesized on demand. Anticoagulant activity is a property of only a few chains of commercial heparin (and possibly of other linear anionic polyelectrolytes). It will take many years to investigate all the ramifications of this new paradigm (*39*), but it promises to be more amenable to investigation than the previous one found so unsatisfactory by so many workers (*18*, p. 58).

References and Notes

1. D. W. G. Murray, L. B. Jaques, T. S. Perrett, C. H. Best, *Surgery* **2**, 163 (1937).
2. E. Jorpes, *Heparin: Its Chemistry, Physiology and Application in Medicine* (Oxford Univ. Press, London, 1939).
3. Th. Koller and W. R. Merz, *Thrombosis and Embolism: Proceedings of the First International Conference, Basel, 1954* (Schwabe, Basel, 1955).
4. R. Pulver, *Chemotherapia* **3**, 388 (1961).
5. H. Engelberg, *Heparin: Metabolism, Physiology and Clinical Application* (Thomas, Springfield, Ill., 1963).
6. T. F. Dougherty and D. A. Dolowitz, *Am. J. Cardiol.* **14**, 18 (1964).
7. L. B. Jaques, *Anticoagulant Therapy: Pharmacological Principles* (Thomas, Springfield, Ill. 1965).
8. W. W. Coon and P. W. Willis, *Clin Pharmacol Ther.* **7**, 379 (1966).
9. L. B. Jaques, in *Proceedings of the Third International Pharmacology Meeting, 1966* (Pergamon Press, New York, 1968), vol. 6, p. 25).
10. ———, *Prog. Med. Chem.* **5**, 139 (1967).
11. J. E. Jorpes, in *Heparin, Handbuch der Experimentale Pharmakology, Neue Serie*, F. Markwardt, Ed. (Springer-Verlag, Berlin, 1971), vol. 27, p. 143.
12. J. Ehrlich and S. S. Stivala, *J. Pharm. Sci.* **62**, 517 (1973).
13. C. P. Dietrich and S. C. M. Dietrich, *Cienc. Cult. Sao Paulo* **26**, 55 (1974).
14. R. A. Bradshaw and S. Wessler, Eds., *Heparin Structure, Functions and Clinical Implications. Advances in Experimental Medicine and Clinical Implications* (Plenum, New York, 1975), vol. 52.
15. A. Silverglade, *Curr. Ther. Res. Clin. Exp.* **18**, 1 (1975).
16. L. B. Jaques, *Gen. Pharmacol.* **6**, 235 (1975).
17. V. V. Kakkar and D. R. Thomas, Eds. *Heparin Chemistry and Clinical Usage* (Academic Press, New York, 1976).
18. E. R. Simon, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 9 (1977).
19. L. B. Jaques, *Methods Biochem. Anal.* **24**, 203 (1977).
20. R. D. Rosenberg, *Semin. Hematol.* **14**, 427 (1977).
21. T. C. Laurent, Ed., *Uppsala J. Med. Sci.* **82**, No. 2 (1977).
22. L. B. Jaques and N. M. McDuffie, *Semin. Thromb. Hemostasis* **4**, 277 (1978).
23. L. B. Jaques and J. Mahadoo, *ibid.*, p. 298.
24. L. B. Jaques, *ibid.*, p. 326.
25. H. Engelberg, *Monogr. Atheroscler.* **8**, 1 (1978).
26. ———, *Pathobiol. Annu.* **8**, 85 (1978).
27. N. M. McDuffie, Ed., *Heparin: Structure, Cellular Functions and Clinical Applications* (Academic Press, New York, 1979).
28. L. B. Jaques, *Pharmacol. Rev.*, in press.
29. R. D. Rosenberg, presentation at a symposium on Heparin: Structure, Cellular Functions and Clinical Applications, Saskatoon, 6 to 8 July 1979.
30. U. Lindahl, *Uppsala J. Med. Sci.* **82**, 167 (1977); in *Heparin, Structure, Cellular Functions, and Clinical Applications*, N. M. McDuffie, Ed. (Academic Press, New York, 1979).
31. M. A. Rozenfel'd, K. L. Erzinkyan, L. A. Piruzyan, *Izv. Akad. Nauk. SSSR, Ser. Biol.* **3**, 419 (1975).
32. S. A. Rice and M. Nagasawa (with a contribution by H. Morawitz), *Polyelectrolyte Solutions* (Academic Press, New York, 1961).
33. J. Overbeek, *Pure Appl. Chem.* **46**, 91 (1976).
34. N. Ise, in *Charged and Reactive Polymers*. vol. 2, *Polyelectrolytes and Their Applications*, A. Rembaum and E. Sélégny, Eds. (Reidel, Boston, 1975), p. 71.
35. P. N. Sawyer, B. Stanczewski, A. Pomerance, T. Lucas, G. Stoner, S. Srinivason, *Surgery* **74**, 263 (1973).
36. W. O. Cruz and C. P. Dietrich, *Proc. Soc. Exp. Biol. Med.* **26**, 420 (1967).
37. U. Lindahl and M. Höök, *Annu. Rev. Biochem.* **47**, 385 (1978).
38. H. R. Lindner, A. Amsterdam. Y. Salomon, A. Tsafiri, A. Nimrod, S. A. Lamprecht, U. Zor, Y. Koch, *J. Reprod. Fertil.* **51**, 215 (1977).
39. Many of the investigations on the physiology and pathobiology of heparin have centered on lipoprotein lipase and atherosclerosis. Engelberg (*26*), in a review of these studies, proposed related parts of the new paradigm. The ion exchange aspect was first proposed by R. D. Higginbotham [*Fed. Proc. Fed. Am. Soc. Exp. Biol.* **16**, 58 (1957)].
40. S. M. Levy and L. B. Jaques, *Thromb. Res.* **13**, 429 (1978).
41. L. M. Hiebert and L. B. Jaques, *ibid.* **8**, 195 (1976).

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