Some side effects of heparin, heparinoids, and their antagonists

Effects of heparin, heparinoids, and their antagonists, other than actions on blood coagulation and lipemia clearing, are described. Many, if not all, of these properties are probably related to the unusual structure and high-charge density of these macromolecules. Some may have therapeutic utility.

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Since its discovery fifty years ago, heparin has been extensively used as an anticoagulant and, in recent years, as an agent to facilitate more rapid clearing of lipemic plasma. These functions of heparin have been adequately reviewed in the recent past. This monograph lists some of the side effects of heparin which are not so well known. Some may ultimately be shown to be directly related to the anticoagulant effect of heparin but, if so, this association has not yet been proved. Several of these effects have been observed only in vitro, and to our knowledge studies to document whether or not they persist in vivo have not yet been carried out. A few may have therapeutic applications. Knowledge of others may aid in the correct interpretation of extraneous effects which might occur during heparin therapy.

Formation of "complexes"

One of the earlier known side effects of heparin was demonstration of its ability to form stable salts with many proteins.[31, 54, 172] Over thirty years ago, Fischer and Astrup[56] demonstrated that these salts partially dissociate according to mass law as long as the product remains in solution; this has been confirmed by Jaques.[100] Complexing with heparin has been observed not only with proteins and peptides (silk peptone, gelatin, clupein, salmine, histones from thymus and blood, hemoglobin, egg albumin, casein, serum proteins, liver proteins, thromboplastins, enzymes, and so forth) but also with organic bases (benzidine, quinine, brucine, and piperidine) and basic dyes (thionine, toluidine blue, and others). Although optimal combination of heparin with protein occurs near the isoelectric point, it is possible for heparin to combine with proteins on the alkaline side of their isoelectric point. Dissociation of these compounds is markedly affected by pH and ionic strength.[100] Jaques has made reference to the difficulty of confirmation of reports by others regarding the inhibitory effect of heparin on certain proteins because reproduction of relative concentrations of heparin, the protein under study, of other proteins, pH, ionic strength, and so forth is often difficult. The early work on complex formation by heparin has been summarized by Jorpes.[106] Studies

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of charged polyglucose derivatives add to our understanding of the mechanism of effect of heparin and heparinoids. Macrocationic substances with enzymatic activity (hyaluronidase, ribonuclease, and lysozyme) are reversibly inhibited by synthetic sulfated polysaccharides; these enzymes can be reactivated by addition of other cationic agents such as sodium ion or protamine. Highly substituted sulfates are the most effective enzyme inhibitors, while sulfates of similar molecular size but lower charge density have less inhibitory activity. The product with highest branching and charge density is about five times as active as heparin in inhibiting ribonuclease. These derivatives also precipitate other basic proteins (protamine, an ACTH preparation, cytochrome C, and serum albumin) from aqueous solution at low pH and salt concentration.[151]

The electric charges of heparin preparations are proportional to their content of ester sulfate; cataphoric mobility is of the order of 17 to 19 $\times$ 10^{-5} cm.^{2} V^{-1} sec^{-1} at pH 2 to 8; heparin apparently has the strongest electric charge of any organic compound normally found in the human body.[219]

Macroglobulins isolated from patients with macroglobulinemia are precipitable by heparin but not by chondroitin sulfate.[149] Heparin and heparinoids precipitate fibrinogen, particularly at low temperatures.[9, 174, 194, 204] A stoichiometric relationship exists between heparin and fibrinogen[65] during the process of precipitation, as it does for other heparin-protein complexes.[56] Beta liproproteins are also precipitated by heparin.[25]

Heparin, along with other anionic polysaccharides, acts as an ion exchanger.[51, 182, 183, 211] The order of increasing cation affinity, when acid mucopolysaccharides are treated as ion exchangers, varies for heparin, heparin monosulfate, and chondroitin sulfate.[45] The order of increasing cation affinity for heparin is: sodium, potassium, magnesium, strontium, barium, and calcium; the differences in order of affinity between heparin and other anionic polysaccharides have been attributed to differences in structure of these several molecules. Their association with cations in solution is thought to be of electrostatic rather than covalent type. The affinity of acid mucopolysaccharides for calcium decreases in the order: heparin, chondroitin sulfate B, chondroitin sulfate A, and hyaluronic acid.[24]

The effect of heparin upon cellular calcium has been studied in vitro in Ehrlich ascites tumor cells labeled in vivo with isotopic Ca^{45}; no change in calcium content of cells was observed when cells were incubated in solutions containing up to 0.6 mg. heparin per milliliter.[205] Heparin does increase Rb^{86} outflow from the diaphragmatic muscle of rats, an effect similar to that produced by reduction in concentration of calcium ions.[28] Potassium exchange is affected in a similar manner.[86]

Heparin is a potent inhibitor in vitro of fumarase,[57] lysozyme,[115] and tissue ribonucleases and desoxyribonucleases.[128, 178, 207] Significant inhibition of acid and alkaline ribonucleases of hepatic origin has been obtained one hour after heparin injection in vivo in white mice.[129] Heparin, but not hyaluronic acid or polyglutamic acid, inhibits adenylic deaminase in vitro; no inhibition of phosphoenolpyruvic kinase, hexokinase, phosphoglyceric mutase, or enolase has been observed.[41] Heparin is a nonspecific and apparently competitive inhibitor of hyaluronidases of diverse origins.[77, 95] Both inhibitor and substrate compete for the same energy sites; an increase in ionic strength decreases the inhibitory effect of heparin and chondroitin sulfate B.[95] Serum beta-glucuronidase activity is decreased 43 to 64 per cent in human postheparin sera.[14] Beta-glucuronidase shows a two thousand fold greater affinity for heparin than for phenolphthalein beta-glucuronide.[27] Nonspecific stimulation of tyrosine-alpha ketoglutarate transaminase activity in vivo by heparin, chondroitin sulfate, and a number of other agents has been observed. This stimulation

is dependent upon intact adrenal glands or upon the presence of hydrocortisone.[138]

Heparin is thought to function in an ion-exchange capacity in its complexing with histamine. In vitro, heparin binds histamine[4, 12, 167] and also apparently inhibits release of histamine from cells to plasma.[44] Histamine is selectively bound by heparin in the presence of equivalent amounts of cadaverine, serotonin, or epinephrine but is displaced by multivalent ions in preference to univalent ions.[119] One mole of heparin will bind 21.5 to 22.0 moles of histamine.[119, 216] However, heparin, even in high doses, has little effect on the actions of histamine in vivo.[26, 132]

Heparin inhibits gastric secretion from Heidenhain pouches in dogs after stimulation by food or by exogenously administered histamine.[134] However, whether this suppression of gastric secretion is secondary to complexing with histamine in vivo is not yet known. Heparin, chondroitin sulfate, and sodium polyhydromannuronic acid sulfate, but not hyaluronic acid, inhibit pepsin in vitro. Chondroitin sulfate, which markedly reduces the number of gastric ulcers in Shay rats, has been shown to inhibit pepsin in vivo as well.[135]

The nonspecific complexing of heparin with cationic macromolecules has been used experimentally to protect mice from a lethal dose of polymyxin; intraperitoneal administration of heparin is more effective than an intravenous dose.[90] Sulfate and carboxyl derivatives of polyglucose have a similar protective effect against polymyxin and, to a lesser degree, neomycin and streptomycin.[152] Heparin apparently counteracts the acute toxic effects of neomycin without producing an equivalent loss in antibiotic activity.[89]

In recent years, further data on "complexing" by heparin in vitro have been reported. This complexing might have considerable physiologic significance if it were shown to occur in vivo as well. The anticurariform action of heparin and many heparinoids has been related both to magnitude of electronegative charge and to molecular size.[32] Heparin has been shown to dissociate the insulin complex in vitro, freeing insulin from its basic protein.[76] Heparin also complexes with kallidin; the complex may be split with compound 48/80.[217] Heparin and dextran sulfate accelerate plasma kinin formation in vitro, as reflected by increased oxytocic activity, possibly by blocking the effect of an inhibitor; protamine sulfate and hexadimethrine bromide but not toluidine blue block kinin formation both in the presence and absence of heparin but do not interfere with uterine response to preformed kinin.[6] Hexadimethrine apparently inhibits kinin formation by an inhibition of Hageman factor which normally plays a role in kinin formation by activating a kinin-forming enzyme.[47]

Heparin also complexes with trypsin; the heparin-trypsin complex may be dissociated by acidification to pH 3 for 30 minutes with complete recovery of tryptic activity. Heparin does not inhibit chymotrypsin.[94] Although heparin completely protects rats against LD_{99} doses of trypsin intravenously, this effect may be related to the anticoagulant action of heparin, blocking the coagulant properties of trypsin.[43]

In the past few years, the relationship of heparin to the plasmin-enzyme system has received a great deal of interest and attention. The current consensus of opinion is that, while large amounts of heparin inhibit plasmin in vitro and in vivo, smaller amounts stimulate fibrinolytic activity if a factor from the albumin fraction of plasma is also present.[8, 33, 78, 111, 112, 122, 199] Heparin[74] and a heparinoid (Ateroid)[121] increase fibrinolytic activity which has decreased in the presence of alimentary lipemia; Ateroid does not affect the plasmin-enzyme system in the absence of alimentary lipemia. Fibrinolytic activity in guinea pig serum is increased in vitro by heparin, hyaluronic acid and chondroitin sulfate, dextran sulfate, and several other synthetic sulfated polysaccharides; neutral polysaccharides have no effect. Total activ-

ity is dependent upon pH and concentration of acid polysaccharide. Some of the most potent stimulators of fibrinolytic activity have no anticoagulant activity. In higher concentrations, acid polysaccharides apparently precipitate inhibitors of fibrinolysis. Olesen[162] has suggested that the mechanism of action of these acid polysaccharides is polyanionic in character, and that they interact with an inactive complex in serum to release a plasminogen activator. The effects of these acid polysaccharides can be reversed by addition of cationic agents.

Addition of heparin in low concentration to whole blood, serum, or oxalated plasma increases the rate of hydrolysis of benzoyl-l-arginine ethyl ester.[59] Since this ester is a substrate for plasmin and since larger amounts of heparin inhibit hydrolysis, this increase in enzymatic activity may be mediated through the effect of heparin in stimulating fibrinolytic (plasmin) activity. In vitro, in high concentration, heparin inhibits plasminogen activation by streptokinase or urokinase but not the fibrinolytic effect of *Aspergillus protease*.[8, 92]

Heparin has not been shown to have a demonstrable effect upon fibrinolytic activity in vivo. Heparin injections in healthy male volunteers previously given a bacterial pyrogen did not alter fibrinolytic activity.[93]

Anticomplementary effect; treatment of hyperimmune diseases

In vitro, in a sheep red-cell system, 0.04 to 0.06 mg. of heparin will inhibit one unit of complement from guinea pig serum; this inhibitory effect is considered to be on the third component of complement.[46] The anticomplementary effect of heparin and several heparinoids (dextran sulfate, chlorazol pink, and polyanethol sulfonate) does not correlate with relative anticoagulant potency.[127] Heparin treatment of guinea pigs has no effect upon synthesis of antibody induced by immunization with human serum.[96] Local or parenteral injection of heparin reduces the intensity of ocular inflammation produced by horse serum or tuberculin hypersensitivity.[18, 19, 210, 223, 224]

Heparin, heparinoids, and salts of a rare earth (neodymium) inhibit in vitro[197] and in vivo[198] hemolysis produced by antierythrocyte antibodies in rabbits. This effect is not related to anticoagulant activity since germanin and Treburon (a sulfonated pectin) which are weak anticoagulants have higher anticomplementary activity than heparin. Hemolysis induced by cobra venom is also inhibited, presumably by suppression of lysolecithinase. None of these agents has an effect on adsorption of amboceptor on the erythrocyte, on agglutination, or on hemolysis by simple lysins (saponin and digitonin).[197] Heparin has a beneficial effect in vitro and in vivo in acquired hemolytic anemia in man; the direct Coombs reaction decreases in titer and becomes negative, and plasma hemoglobin, serum bilirubin, and osmotic fragility decrease.[88, 164, 179, 181] Similar inhibition of hemolysis has been reported in patients with paroxysmal nocturnal hemoglobinuria.[60]

In the light of modern knowledge, the anticomplementary effect of heparin is not a convincing explanation for the beneficial effect of heparin in acquired hemolytic anemia.[180] Fifteen thousand units of heparin injected subcutaneously in normal subjects will depress complement activity about 40 per cent for less than 2 hours. Since 5,000 to 10,000 units per 24 hours will achieve a beneficial effect in the treatment of hemolytic anemia, and protamine which is a more potent anticomplementary agent has no effect, this cannot be the mechanism of action. Studies in vitro of the effect of heparin on autoantibodies and sensitized red cells from patients with acquired hemolytic anemia have shown that heparin acts on red blood cells and autoantibodies directly, and that complement plays no part.[180]

Pretreatment with heparin protects guinea pigs and rabbits from anaphylactic shock.[102, 103, 124, 220] Heparin pretreatment

also suppresses anaphylactic shock in sensitized pigeons[124] and the Arthus phenomenon in sensitized rabbits.[133] In anaphylactic shock, heparin prevents a decrease in platelets and an increase in 5-hydroxytryptamine in whole blood.[102, 103] Heparin may act by inhibiting the ability of platelets to absorb or release 5-hydroxytryptamine. Heparin also prevents the decrease in platelet count accompanying allergic reactions to a specific antigen and prevents most of the local response to intradermal injection of a specific antigen in man; this antiallergic effect of heparin has also been explained on the basis of inhibition of release of 5-hydroxytryptamine.[104] Heparin does not block histamine shock in the mouse, nor is the contracture of isolated ileal segments from sensitized animals elicited by antigen or histamine in vitro inhibited.[110]

Heparin has also been shown to prevent or partially suppress the manifestations of experimental nephrosis in rats,[177] nephritis in rabbits[79, 80, 118] and guinea pigs[191] produced by antikidney serum, and cortisone nephropathy in the rabbit.[176] Experimental nephrosis in dogs and rats is not affected by heparin treatment.[214]

Reversal of the L.E. (lupus erythematosus) test in vivo in several patients receiving injections of heparin has been reported; inhibition in vitro is also demonstrable and may be related to binding of complement.[85]

Effect upon ACTH and lymphocytic and granulocytic response

The possible antagonistic effects of heparin and ACTH have not been clearly defined. The inhibitory effect of ACTH on heparin appears to be spurious. Not all preparations of ACTH have an inhibitory effect.[16] The influence of ACTH on thrombin clotting time of heparinized plasma may be due to phenolic contaminants or additives in ACTH preparations which produce partial inactivation of heparin cofactor.[50] Heparin-neutralizing substances isolated from preparations of ACTH and heparin inhibitors (protamine, toluidine blue, methylene blue, fuchsin, and methyl violet) stimulate motor activity of the isolated guinea pig uterus; heparin has no direct effect on spontaneous motor activity of the uterus but inhibits the effect of these other agents.[17]

The inhibitory effect of heparin on certain physiologic responses to ACTH may be valid. Heparin does not alter the magnitude of depletion of adrenal ascorbic acid produced by various stress-producing agents[154] but actually potentiates the decrease in thymic weight produced by ACTH.[37] Protamine sulfate, however, has been reported to suppress the effect of ACTH on depletion of adrenal ascorbic acid.[52] Heparin pretreatment prevents the decrease in total leukocyte and eosinophil counts in rats given sodium salicylate, epinephrine, or ACTH but, in moderate doses, has no effect on cortisone-treated animals.[82] Other investigators have shown an inhibitory effect of larger doses of heparin on cortisone-induced leukopenia as well.[81] Guinea pigs injected with heparin daily for 5 days develop a rise in eosinophil counts to several times basal values.[13] Heparin may mobilize eosinophils from the intestinal wall since perfusion of isolated dog intestine with heparinized blood results in a decrease of eosinophils in intestinal wall and an increase of these cells in the perfusate.[66] Heparin does not inhibit the degranulating effect of ACTH on gastric mucosal mast cells or its destructive effect on tissue eosinophilia.[169] A single intravenous injection of 20,000 I.U. of heparin produces in normal man an increase in eosinophils which lasts for more than 4 hours.[21] An intravenous injection of heparin in rabbits brings about a reduction in the number of circulating basophils.[168, 206] This phenomenon could not be reproduced in normal human males.[23, 206]

Intravenous heparin produces definite lymphocytosis in the calf; mobilization of lymphocytes from lymph nodes, rather than an actual increase in production, is favored

to explain this phenomenon.[99] Heparin injections in rats are followed by not only a significant increase in lymphocytes but in neutrophils as well; the lymphocytosis is predominant, however. When heparin and hydrocortisone are given simultaneously, heparin blocks the lymphopenic effect of hydrocortisone, and hydrocortisone inhibits the effect of heparin on the neutrophil count.[166]

Effect on the vascular system, blood flow, and oxygen consumption

Clinical observations from Germany of apparent beneficial effects of heparin in the treatment of hypertension have been followed by several experimental studies. Heparin in doses which do not appreciably affect clotting time lowers the blood pressure in DOCA-induced hypertension or nephrogenic hypertension in rats; the depression in blood pressure persists if heparin treatment is continued.[84, 114, 148] If, however, hypertensive rats are injected intraperitoneally with water to damage mast cells, the hypotensive effect of heparin is very transitory. Similar inhibition of heparin effect on blood pressure is observed after pretreatment with large doses of a histamine liberator (compound 48/80).[114] Although hypertensin is bound to heparin in vitro,[101] heparin-treated hypertensive rats respond like normal rats to injections of angiotensin amide, renin, or epinephrine.[83]

Some controversy exists concerning the effect of heparin on cardiac activity. Heparin inhibits the contraction of isolated frog hearts, apparently having a primary effect on heart muscle itself since atropine and epinephrine do not reverse this action. Since calcium, however, induces recovery, this effect may be related to the action of heparin as an ion exchanger in binding calcium. Inhibition of cardiac activity with a high concentration of calcium is reversed when heparin is added.[39] Heparin inhibits cardiac contractility even when desulfurated.[28] Glucuronic acid, but not glucosamine, has the same effect.[39] Protamine sulfate produces an inhibition of cardiac contraction which is not effectively reversed by calcium.[39]

Large doses of heparin increase myocardial contractile force.[73] Preparations of heparin which have a greater concentration of amino-containing compounds than that expected on the basis of the content of glucosamine cause a contraction of guinea pig ileum; this smooth muscle contractility, which could have been secondary to contamination with conjugated histamine, was different in type from that induced by histamine and was not prevented by an antihistamine.[72] This contraction of smooth muscle has been explained as probably related to contaminating polypeptides which have been shown to be present. Chondroitin sulfate and hyaluronic acid have no significant effect as smooth muscle stimulators.

Heparin inhibits serotonin-induced activity in an in vitro rat colon preparation and the vascular depressant effects of serotonin in cats.[193] However, heparin has no consistent effect on forearm blood flow in humans and does not block the vascular effects of serotonin injections.[126] These experiments do not exclude the possibility that heparin might prevent the release of serotonin from platelets or other cellular sources.

Conflicting studies have appeared concerning the effect of heparin on blood viscosity. Heparin in increasing amounts has been reported to decrease the apparent viscosity, pseudoviscosity, and yield value of whole blood, plasma, or serum.[35] However, the magnitude and significance of any decrease in viscosity is still controversial,[70] and recent studies do not confirm this finding.[61]

Intravenous injections of heparin in guinea pigs in doses comparable to or higher than those utilized in man have no effect on vasodilatation or capillary permeability. Vasodilatation is observed after intra-arterial heparin but not after the

intra-arterial injection of several heparinoids.[188] Heparin has no significant effect on cerebral blood flow, cerebral oxygen utilization, or cerebral vascular resistance in man.[202] In controlled studies of subjects with intermittent claudication of the lower extremities, heparin has brought about no improvement other than an anticipated "placebo effect" observed in controls as well.[158, 192]

Although heparin does have an immediate effect on increasing coronary blood flow in dogs, intravenous injection of bishydroxycoumarin has a similar prompt effect. The mechanism of action of either of these agents has not been clarified; no change in blood viscosity was detected.[62]

An increase in survival time has been observed in heparinized rats maintained in an environment of pure nitrogen; no change in total oxygen consumption was noted.[109]

Following heparin administration the blood, brain, cerebrospinal fluid, and vitreous humor barriers become more permeable to P^{32} and to penicillin.[113] The erythrocyte content of lymph also increases.[226] The mechanism of this effect has not been defined. Saxl and associates[184] have shown that many polyelectrolytes with a negative charge disperse red blood cells, while those with a positive charge induce agglutination. Heparin is adsorbed on the surface of the erythrocyte, increasing electrostatic repulsion between red blood cells and thus increasing suspension stability of the blood.[184]

Effect upon growth of bacteria, yeasts, and viruses

Heparin in a protein-free medium is bacteriostatic but not bacteriocidal at concentrations of 100 parts per million or greater when tested against *Bacillus stewarti* or *Micrococcus pyogenes* var. *aureus*. Heparin failed to inhibit budding in yeasts at concentrations of 10,000 parts per million.[215] Heparin produces bacteriostasis in an organic medium only if blood from the region of an injury or pus is added to a culture of *Staphylococcus aureus* containing heparin.[196] Warren and Graham[215] have postulated that these findings support the premise that heparin contains more than one bacteriostatically active component, one not requiring a cofactor but rendered inactive by possible complexing with basic proteins, and the other requiring a cofactor but active in the presence of other proteins.

Protamine sulfate also has a bacteriostatic but not a bacteriocidal effect against many bacteria in neutral or acid media.[189, 215] Since added ribonucleic acid has been shown to block this bacteriostatic effect, Wolff and Brignon[221] have proposed that the effect of protamine may be to combine with ribonucleic acid, thus blocking cellular division.

Heparin and certain other sulfated mucopolysaccharides inhibit multiplication of some viruses in culture.[1, 156, 200, 201] Chondroitin sulfate has no effect on virus replication while dextran sulfate, heparin, and a sulfated agar polysaccharide all produce significant inhibition.[200] While dextran sulfate inhibits multiplication of encephalomyocarditis, Coxsackie A 9, and herpes viruses, the plaque-forming ability of attenuated types 1 and 2 poliovirus is actually enhanced.[201] Heparin has no effect on mumps, Newcastle disease, measles, vaccinia, adeno I and II, Coxsackie B 5, and ECHO 9 and 13 viruses.[209] Heparin decreases the titer of the infectious nucleic acid prepared from the lactic dehydrogenase agent but has no demonstrable effect on the intact virus.[160] The growth of fibroma virus-induced tumors in the rabbit is inhibited by heparin and polyanethol sulfonate.[91] Heparin therapy has also shown some promise in the clinical treatment of dermatitis herpetiformis.[3] Heparin is thought to inhibit virus multiplication by preventing the attachment of virus to cells;[209] dextran sulfate may have a different mechanism of action.[200] Various polyanions with strong electronegative charges produce viral inhibition; molecular

size and the degree of sulfation appear to influence the magnitude of the effect.[157]

Effect upon other cells and collagen

The development of the fertilized eggs of certain marine organisms is inhibited by heparin, toluidine blue, and Thrombocid (a heparinoid).[87, 150]

Several investigators have reported that heparin has a strong growth-inhibitory effect on both fibroblasts[175] and chondroblasts[55] in tissue culture although this finding could not be duplicated with strain L fibroblasts.[117] No inhibition of growth of three strains of human cells (one from a nonneoplastic source), grown directly on glass in a fluid medium, was observed after exposure to high concentrations of heparin; Lisnell and Mellgren[137] have proposed that other reports regarding growth-inhibiting action of heparin in vitro may have resulted from differing tissue culture techniques. Other investigators have grown cells in a plasma coagulum; the effect of heparin may have been related to the stimulation by heparin of fibrinolytic destruction of the coagulum, causing the cells to lose support and thus a suitable medium for growth.

Incubation of rabbit bone marrow cells in concentrations of heparin from 0.025 to 1.0 mg. per milliliter is not followed by a significant decrease in synthesis of desoxyribonucleic acid. Phenol in low concentration does depress DNA synthesis. Lochte, Ferrebee, and Thomas[139] speculate that phenol added as a preservative in heparin solutions may be one factor influencing prior reports of the inhibitory effects of heparin. This effect of phenol is only observed, however, when the concentration of phenol is greater than 0.12 mg. per millimeter. Although heparin does not appear to have an effect upon synthesis of desoxyribonucleic acid, there is considerable evidence that it does produce solubilization of nucleoprotein. Decreases in mitotic activity in hanging-drop cultures of embryonic chick heart after the addition of heparin have been related to the histochemical demonstration of granules of ribonucleoprotein which accumulate in the cytoplasm of these cells.[165] Heparin in a low concentration increases the viscosity of a rat liver brei. This increased viscosity is prevented by removal of the nuclear fraction from the brei; heparin increases the viscosity of nuclear suspensions, and DNA can be demonstrated in the centrifugate. Similar experiments with mitochondria have shown that heparin causes the release of ribonucleic acid from this fraction. Anderson and Wilbur[5] propose, on the basis of these experiments, that heparin displaces nucleic acids from basic proteins and that this may be the mechanism of the inhibitory effect of heparin upon cell division.

Addition of heparin before or after freeze-thaw–induced structural alterations of Littré ascites tumor cells brings about dramatic fading of nuclei. No effect is seen after addition of heparin to unfrozen control samples. After injury of the plasma membrane by freezing and thawing, heparin is able to enter the cell and produce solubilization of nucleoprotein. Similar effects are seen with fresh nuclei isolated from chick erythrocytes. The gellike viscosity of the medium surrounding frozen-thawed preparations of ascites tumor cells or chicken erythrocytes is immediately liquefied by desoxyribonuclease. Chondroitin sulfate A has no effect in this system.[190]

Heparin is taken up by cells in tissue culture, producing various cytologic effects on hamster sarcoma, depending upon its concentration in the culture medium. At 100 and 1,000 gamma per milliliter, it stimulates the appearance of cytoplasmic microvilli. At 1,000 gamma per milliliter an alteration in neutral-red staining is observed; at 2,000 gamma per milliliter vital staining disappears. Cytoplasmic and nucleolar vacuolation and abnormal mitotic figures are also observed.[36] These effects may be related to other observations concerning the effect of heparin upon solubilization of ribonucleoprotein and desoxyribonucleoprotein.

Heparin is a potent inhibitor of the uptake of labeled serum-bound lipids by human and animal epithelial cells. This appears to be due to a physicochemical effect of heparin upon the cell membrane since other anions or cations produce similar or opposite results, depending exclusively upon their charges.[131] This finding may be of considerable importance in pointing to a potential action of heparin and other anionic macromolecules in influencing mechanisms of cellular transport. Heparin also stimulates pinocytosis in mouse fibroblasts[42] and induces the formation of pseudopodia in *Amoeba proteus* when a fine pipette containing 3×10^{-5} molar heparin is held near an ameba. Heparin apparently depolarizes the membrane of the ameba; this is thought to be the basis for stimulation of pseudopod formation.[15]

Conflicting reports have appeared concerning the effect of heparin upon mitotic activity of cells. Heparin injected intraperitoneally in rats reduces the number of mitoses in gastric epithelium but not in duodenal epithelium.[170] Intraperitoneal injection of heparin results in marked stimulation of mitotic activity and increase in desoxyribonucleic acid synthesis in parenchymal liver cells of normal rats; heparitin sulfate has a similar effect. Chondroitin sulfate B displays lesser activity while chondroitin sulfate A, chitin sulfate, and polystyrene sulfonate have no stimulatory effect.[229] This action of heparin may be responsible for contradictory reports concerning the presumed stimulatory effect of plasma from hepatectomized rats on mitosis of liver cells in normal rats, since this effect is not observed when plasma is collected in citrate rather than heparin solutions.[228]

Collagen fibers are formed immediately in vitro by the action of heparin (1:80,000) or Paritol (a heparinoid, 1:40,000) on solutions of collagen. Glucuronic acid, glucosamine, and N-acetyl glucosamine have no effect.[153] Other investigators have reported that low concentrations of heparin retard fibril formation in solutions of acid-soluble collagen at pH 7. Chondroitin sulfates A and C accelerate fibril formation. Chondroitin sulfate B and hyaluronic acid have no effect.[222]

Effect upon the growth of transplantable tumors

Since heparin is most effective if given prior to and again immediately after tumor inoculation, it may act to inhibit successful implantation of tumor cells.[69] This effect of heparin may be related to its anticoagulant effect in suppressing fibrin formation, but documentation of this fact, as far as inhibition of the growth of a primary tumor inoculum is concerned, has not yet been established.

Preliminary exposure of sections of rat carcinoma to a solution of heparin for 22 hours prior to transplantation has completely suppressed tumor development.[67] Zakrzewski,[227] studying thousands of animals with rat and mouse sarcoma, has shown that heparin injected intravenously, intraperitoneally, or into tumor tissue is effective in reducing the rate of tumor growth and in prolonging survival of the animals. Confirmatory studies have demonstrated increased longevity in mice with Ehrlich[11] or Krebs[69] ascites tumor treated with either heparin or a heparinoid. Other investigators have not been able to reproduce these findings,[123, 218] although significant decreases in mitotic index[136] or in percentage of tumor "takes" have been reported.[2, 105] The subcutaneous administration of heparin has no effect on the primary growth of sarcoma T-241 and sarcoma DBA49 in mice; metastases are increased markedly in heparin-treated mice with the former neoplasm, while metastasis formation decreases in mice with sarcoma DBA49.

Increases in tumor growth have been reported after treatment with glucuronic acid or glucosamine, components of the heparin molecule.[105] For this reason heparin inhibitors have been evaluated; partial inhibition of growth of Ehrlich ascites tu-

mor has been reported after treatment with mixtures of toluidine blue and protamine or toluidine blue, thionine, and ammonium chloride but not after treatment with a single heparin antagonist.[38, 40]

The effect of heparin upon suppression of tumor metastasis is probably secondary to the anticoagulant effect of heparin.[2, 34, 58, 120] In a study of the incidence of hepatic metastases in rats after intraportal injection of Walker carcinoma cells, an appreciable decrease in incidence of metastatic lesions was seen only when heparinization preceded tumor injection and when heparin was continued for 4 to 7 days after injection.[58] Heparin has also been reported to decrease the number of tumor cells circulating in the blood stream,[34] although this finding has not been confirmed by other investigators.[120, 144]

Effect upon wound healing and bone repair

Conflicting reports have appeared concerning effects of heparin upon wound healing. Daily injections of heparin bring about an increase in the tensile strength of wounds in rats 5 days after wounding. This increase in tensile strength was over 50 per cent in each of four experiments, and the wounds healed more rapidly than in control animals. A further increase in tensile strength was achieved when alternating injections of histamine and heparin were given.[53] Healing of small burns in dogs has been accelerated by heparin treatment[143] although the experimental design was such that the more rapid healing may have been due to the influence of secondary wounds on acceleration of healing rather than to the effects of heparin per se. In well-controlled studies, heparin has increased the survival time of dogs with lethal burns and no supportive treatment.[48] Vascular healing is not significantly altered by heparin treatment.[10]

Heparin treatment of scorbutic guinea pigs has been associated with an acceleration of the scorbutic process, producing a further decrease in the tensile strength of wounds and increasing the incidence of nonunion of fractures.[161] Histologic evidence of a deleterious effect of either heparin or oral anticoagulants upon bone repair in rabbits and dogs[195] is in support of the premise that inhibition of wound healing or bone repair by these agents may be related to their anticoagulant effect. Since oral anticoagulants appear to have an effect similar to that of heparin, competitive inhibition by heparin of mucopolysaccharide synthesis seems a less likely explanation than the suppression by anticoagulants of the development of the fibrin matrix upon which fibrous tissue or bone is laid down. Daily heparin injection produces no demonstrable histologic effect upon osteogenesis in mice.[125] However, rabbits receiving a high fat diet and given heparin for a prolonged period frequently develop spontaneous fractures.[63] Rats on a low calcium, high phosphorus diet and vitamin D develop rickets, osteoporosis, and show a marked increase in tissue mast cells.[208] When the calvaria of Swiss mice are maintained in tissue culture, the addition of small amounts of heparin or a heparinoid (Treburon) to the medium markedly enhances the amount of bone resorption obtained with suboptimal concentrations of parathyroid extract, vitamin A, or vitamin D_2, all of which stimulate bone resorption; no effect is seen when any of these agents is added singly at the same concentration. Goldhaber[68] speculates that heparin functions as a cofactor in stimulating bone resorption. The collagenolytic activity of rat bone cell homogenates is increased two- to fourfold in animals receiving large doses of heparin for 10 or more days.[7]

Several clinical reports have appeared, describing spontaneous fractures of vertebrae and ribs in patients receiving 15,000 units or more of heparin per day for periods greater than 6 months.[75, 97] No deleterious effects have been observed in a large series of patients receiving 10,000 units or less per day for 1 to 15 years.[75] These patients have had no alteration in parathyroid

function, a normal serum calcium, low urinary calcium, and normal excretion of hydroxyproline. Griffith and associates[75] have suggested that, on the basis of preliminary observations relating heparin effect to a decrease in stability of lysosome-like bodies in bone cells,[225] the primary effect of heparin may be at this locus, and the bone abnormality seen in mast cell disease may be secondary to increased systemic levels of heparin.

Effect upon diuresis

Several isolated clinical observations of an apparent diuretic effect following heparin injections, the earliest being that of Raynaud,[171] stimulated subsequent documentation of this phenomenon. Heparin and several heparinoids (N-formyl-chitosan polysulfuric acid and xylan polysulfuric acid) produce diuresis in humans characterized by an increase in urinary sodium and a slight decrease in potassium excretion.[146, 185] The heparinoids are less potent as diuretics than heparin. The diuretic effect of these agents appears only after at least 36 hours of therapy and continues for several days after heparin is discontinued. The similarities between this pattern of response and that induced by amphenone or spirolactones has been noted.[185] Measurement of aldosterone excretion in patients receiving a heparinoid revealed a reduction in aldosterone excretion (no effect on 17-hydroxycorticoid excretion) in conjunction with an increase in sodium output[30, 145, 147, 186, 212]; addition of spirolactone to this regimen produces an appreciable further increase in natriuresis.[30] Several highly sulfonated heparinoids have natriuretic properties but little anticoagulant activity.[186] Schlatmann and associates[186] have speculated that the effect of heparin on natriuresis and aldosterone suppression may be mediated by inhibition of the renin-angiotensin system which is thought to stimulate aldosterone secretion. In support of this theory is the finding that heparin complexes with angiotensin[101] and that, although heparin or heparinoids have no effect on aldosterone secretion from isolated perfused adrenals, they do suppress secretion when administered to the intact animal.[49, 64, 213]

A different pattern has been observed in both intact and adrenalectomized dogs which, within 15 minutes after intravenous injection of heparin, develop an appreciable increase in urinary potassium without a comparable change in sodium output; these were short-term experiments lasting for 2 hours or less.[155] A comparable effect is not seen in humans.[187] In longer-term studies in dogs, sodium output in urine is increased significantly.[186]

Heparin appears to inhibit the antidiuretic effects of vasopressin when the effects of vasopressin alone and heparin followed by vasopressin are compared in water-loaded human subjects.[163]

Miscellaneous effects

Injection of heparin into normal rats produces a decrease in nitrogen excretion of a magnitude equivalent to the effect of 2.5 units of growth hormone; protamine produces an increased nitrogen excretion and inhibits the action of heparin.[71] This anabolic effect of heparin could not be confirmed in short-term studies (5 days).[116]

Heparin activates in the globulin fraction of a serum a substance which inhibits the action of human or rat melanocyte-stimulating hormone on frog skin.[108]

The color intensity of sulfobromophthalein dye is increased in postheparin serum and the absorption peak shifts from 5,800 to 5,950 Angstrom units; studies were carried out 3 minutes after intravenous injection of 2 to 50 mg. of heparin intravenously in normal human males.[203]

During the induction of hypothermia in dogs, administration of heparin lowers "terminal temperature" (the rectal temperature to which an animal can be cooled by surface cooling before the advent of ventricular fibrillation or cardiac arrest).[29] This effect may be secondary to an antithrombotic effect, alteration of suspension stability of the blood, or, less likely, to

lipoprotein lipase activity in prevention of fat embolism. The antithrombotic effect seems most likely.[140]

Heparin alone or heparin in combination with nicotinic acid raises serum iron levels in patients with "infectious hyposideremia"; this has been explained on the basis of inhibition by heparin of the uptake of free iron by the reticuloendothelial system.[22] Phagocytosis of colloidal particles and bacteria is also inhibited by heparin and other negatively charged macromolecules.[98, 159]

Injection of a heparinoid (Treburon) reduces the period of anesthesia from pentobarbital in dogs, is partially effective (significant reduction in time of return of righting reflex) in pigeons, and has no significant effect on anesthesia in rabbits; it has no demonstrable analeptic effect in unanesthetized animals.[107]

Injection of heparin in rabbits is accompanied by a highly significant rise in fasting blood sugar which can be prevented by prior injection of tolazoline. Heparin does not alter hyperglycemic response to glucagon or epinephrine but does decrease the magnitude of the hypoglycemia induced by insulin. The authors postulate that heparin stimulates endogenous release of epinephrine.[20] Administration of heparin to normal males produces a mean rise in blood glucose over that in control subjects of 30 mg. per cent with a peak effect at 4 hours.[142]

Pulmonary edema produced in dogs by intracarotid infusion of massive amounts of physiologic saline is prevented by pretreatment with heparin but not pretreatment with oral anticoagulants.[141]

When rats are pretreated with one of a number of heparinoids, but not heparin, a significant anti-inflammatory effect is demonstrable as measured by the zymosan-edema test.[130]

Comment

The multitude of ancillary effects of a pharmacologic agent has seldom evoked the interest or documentation that heparin has. This, in itself, is of both theoretical and practical interest as a demonstration of the many unsuspected side effects which may be produced by a drug administered to achieve a solitary therapeutic objective.

In the 30 years of extensive clinical use of heparin, reports of *undesirable* side effects have been remarkably few. Occasional hemorrhagic complications related to its anticoagulant action are inevitable. Very rarely, an anaphylactoid response following repeated injections has been observed. In recent years, interest has developed concerning potential deleterious effects upon bone metabolism of long-term administration of relatively large doses of heparin. The possibility of development of osteoporotic changes after chronic administration of heparin for 6 months or more seems real.

Several other pharmacologic effects of heparin deserve further investigation because of their potential therapeutic usefulness in other areas of human disease. The complexing of heparin with many cationic macromolecules may be more of theoretical than practical value since most of the effects described have been observed only in vitro. The apparent reduction of toxicity of neomycin in vivo without equivalent loss in antibiotic activity may have practical utility. The role of heparin and heparinoids in reducing experimental peptic ulcers is of considerable interest; whether this effect is related to complexing with pepsin or histamine has not yet been clarified.

The therapeutic value of heparin in certain autoimmune diseases and allergic states is also receiving current attention.

The natriuretic properties of heparin are well documented. Since heparin and related compounds without appreciable anticoagulant activity potentiate the effects of other diuretics as well, this action may also have therapeutic value.

If heparin's antiviral action is secondary to an inhibition of attachment of virus to cell membrane, then this function at an extracellular locus provides some ground

for hope that it might be effective against some viruses in vivo. Its apparent beneficial effect in the treatment of dermatitis herpetiformis would support this premise.

The effect of heparin and plasmin in inhibiting tumor implantation and metastasis has been under investigation in several laboratories; this inhibition is most likely secondary to the antithrombotic actions of these agents.

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